Dr. Anu Sukhdev
Prof. Kapilesh Jadhav

ARCHIVES OF INDUSTRIAL MICROBIOLOGY

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CHAPTER 1

EXPLORATIVE STUDY ON INDUSTRIAL MICROBIOLOGY

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Abstract:

Industrial microbiology is a branch of biotechnology that applies microbial sciences to create industrial products in mass quantities. There are multiple ways to manipulate a microorganism to increase maximum product yields. Introduction of mutations into an organism many be accomplished by introducing them to mutagens. The medical application to industrial microbiology is the production of new drugs synthesized in a specific organism for medical purposes.

Keywords:

Industrial Microbiology, Biotechnology, Food Technology, Probiotics, Yeast.

Introduction

More recent progress includes the ability to produce monoclonal antibodies for analytical, diagnostic, therapeutic and purification purposes, pioneered by Milstein and Kohler in the early 1970s. However, many of the greatest advances have followed the massive developments in genetic engineering (recombinant DNA technology) over the last 20 years. This technology has had, and will continue to have, a tremendous influence on traditional, established and novel fermentation processes and products. It allows genes to be transferred from one organism to another and allows new approaches to strain improvement. The basis of gene transfer is the insertion of a specific gene sequence from a donor organism, via an expression vector, into a suitable host. Hosts for expression vectors can be prokaryotes such as the bacterium Escherichia coli; alternatively, where post-translational processing is required, as with some human proteins, a eukaryotic host is usually required, e.g. a yeast[1], [2].

The producer microorganism

Key factors relating to this aspect are: the strategy for initially obtaining a suitable industrial microorganism, strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a reliable inoculum and the continuing development of selected strains to improve the economic efficiency of the process. For example, the production of stable mutant strains that vastly overproduce the target compound is often essential.

The fermentation medium

The selection of suitable cost-effective carbon and energy sources, and other essential nutrients, along with overall media optimization are vital aspects of process development to ensure maximization of yield and profit. In many instances, the basis of industrial media are waste

products from other industrial processes, notably sugar processing wastes, lignocellulosic wastes, cheese whey and corn steep liquor.

The fermentation.

Industrial microorganisms are normally cultivated under rigorously controlled conditions developed to optimize the growth of the organism or production of a target microbial product. The synthesis of microbial metabolites is usually tightly regulated by the microbial cell. Consequently, in order to obtain high yields, the environmental conditions that trigger regulatory mechanisms, particularly repression and feedback inhibition, must be avoided.

Fermentation products

The overall economics of fermentation processes are influenced by the costs of raw materials and consumables, utilities, labour and maintenance, along with fixed charges, working capital charges, factory overheads and operating outlay. Fermentation products can be broadly divided into two categories: high volume, low value products or low volume, high value products. Examples of the first category include most food and beverage fermentation products, whereas many fine chemicals and pharmaceuticals are in the latter category.

Food, beverages, food additives and supplements

A wide range of fermented foods and beverages have been produced throughout recorded history. They continue to be major fermentation products worldwide and are of vast economic importance. Fermented dairy products, for example, result from the activities of lactic acid bacteria in milk, which modify flavour and texture, and increase long-term product stability. Yeasts are exploited in the production of alcoholic beverages, notably beer and wine, due to their ability to ferment sugars, derived from various plant sources, to ethanol. Most processes use strains of one species, *S. cerevisiae*, and other strains of this yeast are used as baker's yeast for bread dough production.

Health-care products

In terms of providing human benefit, antibiotics are probably the most important compounds produced by industrial microorganisms. Most are secondary metabolites synthesized by filamentous fungi and bacteria, particularly the actinomycetes.

Microbial enzymes

Microbial enzymes, particularly extracellular hydrolytic enzymes, have numerous roles as process aids or in the production of a wide range of specific food and nonfood products. Proteases, for instance, are extensively used as additives to washing powders, in the removal of protein hazes from beer and as microbial rennets for the production of cheese.

Industrial chemicals and fuels

Industrial feedstock chemicals supplied through fermentation include various alcohols, solvents such as acetone, organic acids, polysaccharides, lipids and raw materials for the production of plastics. Some of these fermentation products also have applications in food manufacture.

Literature Review

Skovgaard *et al.* discussed that a major factor in the diversity of traffic is the queuing process at controlled intersections, which is crucial to many transportation issues. There is a need for understanding of the stochastic and dynamic behaviour of traffic at controlled intersections notwithstanding the proliferation of queuing models during the last 50 years. The current models' application validity and interpretation potential are often constrained by heuristic, simplifying assumptions that were used in their development. By presenting the queue process as probabilistic, this essay seeks to close this gap. This is accomplished at both stationary and vehicle-actuated controls. The probability distribution of queues at fixed control signals may be calculated on the assumption of a certain distribution of arrivals and departures. The likelihood of seeing many cars in a line has a dynamic nature, even though these distributions are considered to be stationary. The length of green periods may be adjusted based on this dynamic process using vehicle-actuated controls, in theory. In these systems, vehicle delays and queues continue to be random processes as a result of signal timings becoming dynamic stochastic variables. In this article, we examine these two control mechanisms' performance in terms of network dependability. The models created in this study are simple, have short calculation times, and are shown to be compatible with the more complex models utilised in programmes for microscopic simulation[3].Since many years ago, microorganisms have been employed to digest food and feed as well as to produce a wide range of biochemicals on a big scale, from alcohols to antibiotics. The term "Industrial Microbiology or Microbial Biotechnology" refers to the use of microorganisms to produce a product or service with significant economic value. Industrial microbiology is identical with "fermentation" in terms of its scope, goals, and activities since fermentation refers to any process mediated by or involving microbes in which a good or service of economic value is produced.

Abdel-Aziz *et al.* carried out a study in which they suggested that current scientific and applied research is needed to help develop functional foods that will benefit public health, and the regulatory structure has to be changed to make it easier to examine novel functional components and their health claims. There is an urgent need for more biomarkers that indicate changes in health status, and it is crucial to be able to explain how such changes relate to a specific health problem. The validated indicators of health status need to be expanded via research, which also needs to examine the relationship between genes and gene products and illness risk[4].

Lagarda*et al.* carried out an investigation in which they reported that the biological management of insects, bacteria, and fungi that harm various crops is discussed in this article. These significant issues may be biologically controlled by using various microorganisms including bacteria, viruses, and fungi. In this article, we go into great depth on the various microorganisms' modes of action in controlling insects and plant diseases. The invention and fabrication of many formulations to be utilised in the fields for the biological control of specific plant diseases are also shown, along with unique methods to increase the effectiveness of these microbes against their targets[5].

In a study by Beloqu*et al.* they demonstrated that since microorganisms make up the vast majority of current biotechnological applications, it is widely acknowledged that microbes make

up the biosphere's highest proportion of biodiversity. Numerous initiatives are being made globally to introduce new products of microbial origin to the market due to their biotech impact. Direct isolation of microbes, however, has consistently shown that the majority of them cannot be cultured, and few representatives of many important phyla of microbes have been identified to date. Therefore, the understanding of novel microbes and/or their genomic information, or from their communities, will present a tremendous opportunity to offer industry novel products and processes based on the use of microbial resources, as well as contribute to and extend the basic mechanistic knowledge on the functioning of organisms. The exploration of the genetic reservoir of (un)cultured microbes for industrial applications is highlighted in this review with some examples and developments [6].

In a study by Demain *et al.* it was reported that the fields of industrial microbiology and molecular genetics were wed thirty years ago. When the inaugural Symposium on the Genetics of Industrial Microorganisms was held in Prague, the incident occurred. Numerous genetically modified substances that are produced in microbial, mammalian, or insect cells are now on the market. The area is expanding thanks to new technologies including PCR, site-directed mutagenesis, combinatorial biosynthesis, gene therapy, antisense, and abzymes, as well as high-throughput screening, monoclonal antibodies, and transgenic plants and animals. Agriculture biotechnology has advanced significantly, but regrettably political debate is holding it back[7].

Intriguing findings like the discovery that yeast is a living organism and is responsible for the fermentation of beer and wine were the forerunners of early biotechnology (BT). One of the leading sciences was the field of "New Biotechnology." Despite having historical roots, biotechnology continues to have an impact on many different industrial sectors, including those that produce food, feed, and other commodities as well as polymers, biofuels, and energy, as well as services like environmental protection and the development and manufacturing of many of the most potent pharmaceuticals. The creation of unique goods and effective, environmentally friendly manufacturing techniques is made possible by our ability to grasp life at the molecular level[8].

Estevinho *et al.* discussed about genomes, transcriptomics, and proteomics, which made it feasible to understand microbial biogeochemical processes and their interactions with macroorganisms in both health and illness, the relevance of microbiology has increased tremendously. The field of applied microbiology, which examines the use of microorganisms in certain activities, is receiving special attention. It is predicted that its economic worth would surpass USD 675.2 billion [9].

Investigations are made into the function of bioscience and biotechnology in biochemical processes. Review of the uses of fermentation in various biological processes. Najafpour *et al.* investigated about how beneficial substances like food, medication, chemicals, antibiotics, and protein are biologically transformed into live cells. Our everyday lives utilise a variety of biobased goods that may be produced via fermentation methods. Readers who have a thorough grasp of bioconversion processes may be better able to create cutting-edge technologies for producing a large number of items. This Chapter presents schematic flow diagrams for several fermentation processes. In these biological pathways, fermentative processes are employed to

produce amino acids, enzymes, proteins, and antibiotics from carbon sources such maize, potato starch, molasses, and whey[10].

Discussion

One of the most innovative and promising methods for cost-cutting, resource conservation, and pollution control is industrial biotechnology. It's often referred to as the third biotechnology wave. Industrial biotechnology may have a greater global influence than medical and agricultural biotechnology if it is fully developed. It gives companies a means to save costs, open up new markets, and preserve the environment. Additionally, the road to the market is faster and simpler since many of its goods do not need to go through the extensive review processes that medication companies do. Today, as opposed to up to a decade ago, innovative industrial processes may go from lab investigation to commercial use in two to five years.

Vitalists and chemists engaged in heated debates that led to the reversal of beliefs and paradigms but also spurred more inquiry and advancement. By creating pure monoculture in sterile media and collaborating with Robert Koch's work to recognise that a single pathogenic organism is the causal agent for a certain illness, Pasteur's work contributed to the development of the science of microbiology. Pasteur also made advances in the production of beer, wine, and alcohol, which are very relevant to the economy. Many years later, Buchner proved wrong the idea that living cells needed more than just pure chemical rules to function. This was known as the "vis vitalis" theory. The chemical underpinning of bioconversions was discovered to be enzymes. Citric acid, chemical components needed for explosives, notably in times of war, acetone and butanol, and other products were produced via fermentation as a consequence of research on the production of products in microbial fermentations. The Second World War's penicillin shortage prompted industrial production of the drug, ushering in the age of antibiotics and the availability of other drugs like streptomycin. Then came a brand-new category of high-value goods, mostly secondary metabolites, such steroids produced by biotransformation. The establishment of courses in the biological sciences departments of various universities coincided with the mid-20th century's acceptance of biotechnology as a legitimate field of study. Beginning in the 1970s and 1980s, BT came to the notice of governmental organisations in Germany, the UK, Japan, the USA, and other countries as an area with the potential for innovation and economic development, which drove the field's spread. The study of genes and their relationships throughout evolution has greatly broadened and united the area of life sciences as a result of basic studies in biochemistry and molecular biology. Accessible goods and services now cover a wider range of needs. By stimulating and funding the creation of new techniques, apparatus, and businesses, economic input hastened research and development.

The integration of biotechnology into industrial processes is changing not just how we produce goods but also bringing us new goods that were unthinkable only a few years ago. Because industrial biotechnology is so new, neither industry nor policymakers nor consumers fully comprehend its advantages.

Industrial biotechnology has always combined product enhancements with pollution control. The application of industrial biotechnology to address the phosphate water contamination issues

brought on by the usage of phosphates in laundry detergent in the 1970s is the best example of how this was accomplished. Enzymes created by biotechnology businesses outperformed phosphates at removing stains from clothes. This allowed for the substitution of a polluting component with a non-polluting biobased addition while boosting the performance of the final product. With lower wash water temperatures and corresponding energy savings, this invention significantly decreased phosphate-related algae blooms in surface waterways all around the world while also enabling customers to wash their garments more effectively.

In fact, crude industrial biotechnology has existed since at least 6000 B.C., when Neolithic tribes utilised microbial yeasts to manufacture beer and fermented grapes to make wine. As humankind gained more understanding of fermentation, cheese, yoghurt, vinegar, and other food items could be made. Louis Pasteur demonstrated that fermentation was a product of microbial activity in the 1800s. Sir Alexander Fleming then succeeded in removing penicillin from mould in 1928. Massive-scale fermentation procedures were created in the 1940s in order to produce this miracle medicine in large numbers. However, the biotechnology revolution that gave birth to contemporary industrial biotechnology did not start until after World War II.

Since then, industrial biotechnology has created enzymes for use in both the manufacturing industry and our everyday lives. To eliminate stubborn protein deposits, certain contact lens cleaning solutions include enzymes, as doe’s meat tenderizer. Industrial biotechnology mostly entails the synthesis of enzymes, which are specialised proteins, by microorganisms. These enzymes have naturally developed into high-performance biocatalysts that facilitate and accelerate intricate biological processes. Industrial biotechnology is such an innovative new technology because of these incredible enzyme catalysts.

Utilizing natural processes to enhance and improve biochemical pathways that may be employed in production is known as industrial biotechnology. The genomes, proteomics, and bioinformatics disciplines of research of finely detailed information produced from cells are at the forefront of the industrial biotechnology revolution. As a consequence, researchers may now use novel methods to study a wide variety of microorganisms, including marine diatoms, yeasts, and fungus, bacteria, and yeasts.To discover and enhance nature's enzymes, industrial biotechnology firms use a wide range of specialised approaches. The rich genetic variety seen in microbial communities is being taken advantage of by researchers thanks to data from genomic studies on microorganisms. Prior to using DNA probes to seek for genes that create enzymes with particular biocatalytic capabilities at the molecular level, researchers first look for enzyme-producing bacteria in the natural environment. After being separated, these enzymes may be recognised and examined for their suitability for use in certain industrial processes. They may be enhanced using biotechnology methods if required.

The recent and significant advancements in biotechnology methods have resulted in a fast increase in the number of biocatalytic instruments that are readily accessible for industrial applications. Many chemical engineers and product development experts in the commercial sector are unaware of the availability of the biocatalysts or whole-cell procedures since they are so new. This is an excellent illustration of a "technology gap," or the time it takes for a new technology to become widely used. To move forward more quickly with the integration of

biotechnology into industrial processes that are more affordable and sustainable, this gap must be closed. Dramatic examples of what these potent new tools may achieve are provided in "New Biotech Tools for a Cleaner Environment." The goal of the paper is to increase interest in this potent technology, reduce the technological gap, and speed up the transition to a more sustainable future.

Fungi (yeasts, moulds, and similar organisms) and certain prokaryotes, particularly members of the genus Streptomyces, are the main organisms utilised in industrial microbiology. Industrial microorganisms are metabolic experts that can synthesise one or more compounds in high yield. Industrial microbiologists often use traditional genetic techniques to select high-yielding microbial variations with the intention of boosting the product yield to the point where an economically viable procedure is achievable. As a result, the behaviour of the production strain may be quite different from that of the original wild-type strain.

A plenary lecture, "The Marriage of Genetics and Industrial Microbiology - After a Long Engagement, a Bright Future," covered the industrial uses of mutants, the failure of genetic recombination, controlling branched and unbranched pathways, and ideas for the future, such as locating the biochemical sites of advantageous mutations, utilising recombination, and using genetic means to increase enzyme production. The Symposium took place three years before recombinant DNA technology became widely available, which is rather astonishing. The inaugural Genetics and Molecular Biology of Industrial Microorganisms (GMBIM) meeting was held in Orlando, Florida, after this significant conference in 1976. All six GMBIM conferences that followed were held in Bloomington, Indiana. The pharmaceutical and agriculture industries are benefiting greatly from the number of biotechnology businesses that are operating today.

Industrial microbiology refers to the utilisation of microorganisms to produce goods or services with a financial value. Fermentation refers to any process in which a product of commercial value is produced and which is mediated by or involves microbes. In terms of their scope, goals, and actions, industrial microbiology and fermentation are essentially interchangeable. The microbial product can include microbial cells (either living or dead), microbial biomass, and components of microbial cells, as well as microbial metabolites, intracellular or extracellular enzymes, or chemicals produced by microbes using the substrate or other components of the medium, and/or modified compounds that have undergone microbiologic transformation. It can also include recombinant products made using DNA recombinant technology.

The services provided by microorganisms include the following: degradation of organic wastes, detoxification of harmful substances and industrial wastes, management of oil spills, etc. Industrial microbiology also includes tasks like making biocontrol agents, biofertilizer inoculants, biofuel, etc.Industrial microbiology operations start with the isolation of microbes from nature, their screening for product production, enhancement of product yields, maintenance of cultures, mass culture utilising bioreactors, and often finish with the recovery and purification of products.

Features of an Industrial Microorganism That Is Useful

A microorganism that is employed in an industrial process has to possess more qualities than only the capacity to generate the desired material in high yield.

1. The organism must be able to grow and produce products in large-scale cultivation in the first place.
2. In order to be readily inoculated into the enormous containers needed to develop the generating organism on an industrial scale, it should generate spores (if it is a fungus or yeast) or some other kind of reproductive cell form.
3. It must also develop quickly and provide the necessary output in a little amount of time.
4. It must also be able to grow in a liquid culture media that is inexpensive and available in large numbers. Waste carbon from other industries is used in several industrial microbiological operations as a primary or supplementary component of large-scale growth medium. These include whey and maize steep liquor, a byproduct of the corn wet-milling industry that is high in growth-promoting nutrients like nitrogen (a waste liquid of the dairy industry containing lactose and minerals).
5. Industrial microbes shouldn't be harmful to humans or other animals.
6. Animals or plants that are significant commercially. A pathogen would offer potentially devastating issues because of the large cell densities in industrial microbial operations and the near impossibility of preventing contamination of the environment outside the growing vessel.
7. Finally, an industrial microbe should be susceptible to genetic modification because mutation and conventional genetic selection methods are often used to boost production. An industrial process clearly benefits from a microbe that is genetically stable and simple to develop.

Industrial microbial processes can cause problems

1. Choosing the least costly media for the microbe's growth in order to increase production and revenues. Frequently, this is a byproduct of another industrial operation, such as whey, corn steep liquor, or waste from the manufacturing of sugar.
2. Preserving strain purity and creating improved strains to increase yield. o A single mutation might significantly reduce the yield or lead to the production of unwanted chemicals. For the development of their product, the industrial research labs are continually looking for superior strains.
3. Preventing contamination from viruses (phage) and other bacteria as well as from those that reside on the relevant bacterium. Purity must be maintained throughout the manufacturing process and the medium must be disinfected before being inoculation with the appropriate organism. An enzyme that can destroy the product in millions of litres of media may be produced by a little amount of a contaminant. Viruses are a persistent threat to many microorganisms since they may infect and kill the targeted microbe in an entire tank with only one infection. Vast containers and large amounts of media need both engineering and microbiological challenges to be successfully sterilised.

4. Creating quick and effective procedures for obtaining the needed product in a reliable, safe-to-use form. If they are not immediately purified, the byproducts of many fermentations are often unstable in the IMPURE FORM or prone to unintended changes. The intended product has to be isolated from potentially harmful elements that may be present in the final growing mixture. The hunt for more effective purification processes is ongoing since every stage in the purifying process results in a loss of the product.
5. Constantly looking for methods to increase production by altering the strain, the nutrients, or the environment. Product yields are regularly checked since they are susceptible to minute changes in the environmental factors and nutrient levels. During the manufacturing process, variables like pH, oxygen concentration, nitrogen/phosphorous ratio, etc. may be changed.
6. The huge amounts of waste materials that remain after the product is manufactured may be disposed of safely and affordably. Due to their abundance in organic matter, the waste products of these big fermentations provide significant waste management challenges since they are extremely polluting if discharged into the environment untreated. The expense of the treatment, however, reduces the profit margin and drives up the price of the product.

History of Microbes: For thousands of years, people have used microbes to create products like bread, wine, and other foods, but these processes were entirely artistic:

1. Industrial microbiology is a relatively recent field of study—about 150 years.
2. Anthony Leeuwenhoek reported his first findings of microbes in 1677.
3. In addition to proving the theory of spontaneous creation of microbes false, Spallanzani's and Schwan's experiments from 1799 and 1837, respectively, also established a method for sterilising liquids and air via the use of heat.
4. According to Schwan's research, yeast, a fungus or mould, is what causes alcoholic fermentation, and inoculation sped up the process.
5. However, the scientific foundation for industrial microbiology started with a paper published in 1857 by Louis Pasteur that detailed his research on lactic acid fermentation, including the microscopic characteristics of the microorganisms and a suitable medium for the process. This paper is widely regarded as marking the beginning of microbiology.
6. Pasteur reported the first artificial microbe medium in 1860 and used it to research alcohol production.
7. Luis Pasteur demonstrated in 1861 that the presence or absence of CO2 affects the development and physiology of yeast (and, therefore, the buildup of the fermentation product, alcohol). The Pasteur Effect is a phenomena that also applies to other microbes.
8. Lister outlined the dilution method in 1878 in order to produce the first pure microbiological culture of the lactic acid bacteria.
9. Robert Koch proposed a method in 1881 for generating pure cultures from separated distinct colonies grown on solidified media; this method is still commonly used today.

In 1876, Cohn demonstrated that bacterial spores are very heat resistant and devised the "intermittent sterilisation" method to render them inactive. - In 1897, Buchner used cell-free

yeast juice to show alcohol fermentation, and he proposed that a proteinaceous enzyme was in charge of the process. - Vitamins are still employed in fermentation today as a result of Wildiers' 1901 demonstration that yeast needed growth factors (vitamins) for growth, particularly at low inoculum levels. - Penicillin was unintentionally found in 1929 by Alexander Fleming cultivating Penicillium as a contaminant on a Petri plate containing Staphylococcus. Fleming invented the method for testing penicillin's antibacterial activity using bacteria and demonstrated the drug's minimal toxicity to humans and animals. After that, during the Second World War, there was a vigorous hunt for antibiotics that resulted in the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later research led to the introduction of microbes with metabolic pathways inhibited, which accumulate a lot of metabolic intermediates.

Conclusion

Since before the Bible, industrial microbiology has improved human existence by producing fermented foods and drinks. The quantity of microbial products for food, industry, research, and medicine multiplied throughout the antibiotic era. The discipline of molecular genetics is entering a new growth phase as a result of revolutionary discoveries that hold the potential of providing answers to pressing global issues. Since before the Bible, industrial microbiology has improved human existence by producing fermented foods and drinks. The quantity of microbial products for food, industry, research, and medicine multiplied throughout the antibiotic era. The discipline of molecular genetics is entering a new growth phase as a result of revolutionary discoveries that hold the potential of providing answers to pressing global issues.

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CHAPTER 2

INDUSTRIAL APPLICATIONS OF MICROORGANISMS

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Abstract:

Evidence for human use of microorganisms in the production of valuable goods extends back thousands of years. Over the ensuing centuries, the use of microbes in industrial processes has grown steadily, and continues today. This work provides information on the diversity of industrial processes that utilize microbes, including examples of microbe-dependent products.

Keywords:

Microorganisms, Bacteria, Strains, Genetic Engineering.

Introduction

Traditionally, microbes used in fermentation processes have been naturally occurring strains of bacteria and molds that carry out a specific metabolic reaction on a substrate. In the last 25 years, industrial microbes have increasingly been mutant strains engineered to selectively synthesize maximized amounts of various metabolic intermediates. The products of an industrial microbial process can be divided into two broad classes: primary metabolites (produced within the microbes' major metabolic pathways and essential for microbes' function), and secondary metabolites (byproducts of metabolism that may not be critical to the microbes' function).

Desirable properties

Desirable qualities for effective microorganisms in industrial use include the following:

1. Ability to grow in culture
2. Genetic stability
3. Ability to efficiently produce a target product in a short time period
4. Limited need for additional growth factors
5. Utilization of a wide range of low-cost and readily available carbon sources
6. Amenability to genetic engineering
7. Non-pathogenicity
8. Readily harvested from the fermentation process
9. Limited production of byproducts to simplify purification
10. Production of spores or other reproductive cells (to allow inoculation into large fermenters)
11. Ability to protect itself from contamination by other microbes
12. Relatively large cell size, to allow faster settling or simpler filtering

Microbe types

Industrial microorganisms generally fall into one of the following categories: yeasts, fungi (other than yeast), algae, bacteria, archaea and viruses as demonstrated in Figure 1.

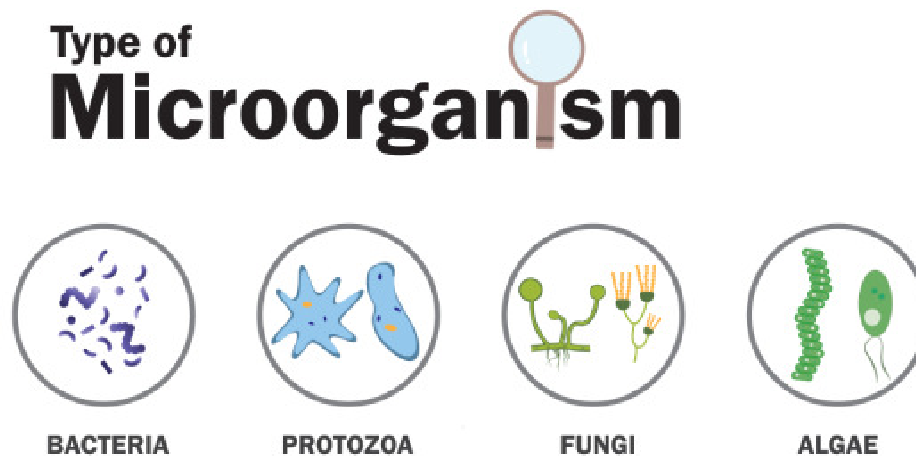

Figure 1: Illustrating the types of Microorganisms.

Algae. The term algae refers to an extremely diverse group of eukaryotes (organisms with defined cellular nuclei), strains of which are used industrially in the production of biofuels, such as biodiesel, and in wastewater treatment, among other uses. Bacteria. Among the earliest life forms, bacteria are prokaryotes (lacking defined nuclei) with staggering metabolic diversity. Industrially, many different bacterial species are used in a wide range of processes from biofuels to pharmaceuticals [1], [2].

Archaea. Similar in many ways to bacteria, archaea constitute a separate class of organisms with distinct metabolic pathways and unique biochemistry. Archaea find limited industrial use, but are used in biogas production, sewage treatment and as a source of heat-tolerant enzymes.

Fungi. A family of eukaryotes that includes yeasts, molds and mushrooms, fungi have long been used to produce antibiotics in industry [3], [4].

Yeast. Likely the oldest domesticated microorganism, yeast is a single-celled eukaryote best known industrially for producing ethanol from sugars by fermentation.

Viruses. Viruses can be used as delivery vectors for introducing genetic material into other cells.

Key industrial microbe species

A. *Saccharomyces cerevisiae* (yeast for brewing and bread-making)

B. *Escherichia coli* (bacteria for recombinant proteins and others)
C. *Aspergillus niger* (fungus for manufacturing citric acid and enzymes)
D. *Clostridium butyclicum* (bacteria used in soured milk and cheeses)
E. *Xanthomonas campestri* (bacteria that produces xanthan gum)
F. *Deinococcus radiorans* (bacteria for soil and water remediation)

Development and strain selection

The removal of troublesome bacteria from their native environments is the first step in creating a producer strain. Alternative sources for microorganisms include organisations that maintain culture collections, such as the American Type Culture Collection (ATCC) in Rockville, Maryland, the Commonwealth Mycological Institute (CMI) in Kew, Surrey, England, the Fermentation Research Institute (FERM) in Tokyo, Japan, and the U.S.S.R. Research Institute for Antibiotics (RIA) in Moscow, U.S.S.R.

Bacteria, actinomycetes, fungus, and algae are the most common microorganisms of economic interest. These microorganisms may be found practically everywhere, including in the soil, water, air, and tissues of both plants and animals as well as on their surfaces. However, mud from lakes and rivers, as well as soils, are the most typical sources of industrial microorganisms. The properties of the product required from the desired microbe, as well as the process development, will often determine the biological environment from which a desired microorganism is more likely to be separated. The obvious place to seek, for instance, is in hot water springs if the goal is to find a source of enzymes that can endure high temperatures. There are several intricate isolation techniques available, however no one technique can identify every bacterium found in a sample. Utilizing specialised enrichment methods, such as soil treatment (UV irradiation, air drying or heating at 120°C, filtration or continuous percolation, washings from root systems, treatment with detergents or alcohols, pre-inoculation with toxic agents), selective inhibitors (antimetabolites, antibiotics, etc.), nutritional (certain C and N sources), pH and temperature changes, aeration, etc., it is possible to isolate a wide variety of microorganisms. Only a small subset of the microorganisms contained in a sample are intended to be multiplied selectively using the enrichment procedures. However, these methods demand a lot of work and money, as well as a lengthy period of time (20–40 days). The primary techniques for consistently isolating organisms from soil samples are sponging (soil directly), dilution, gradient plate, aerosol dilution, flotation, and differential centrifugation. Frequently, an enrichment approach is employed in combination with these strategies.

Primary screening is a group of much specialised techniques that enables the identification and isolation of microorganisms that are capable of generating the desired metabolite. Primary screening should ideally be quick, cheap, predictive, specific, yet effective for a wide variety of substances, and usable on a large scale. Since a lot of isolates need to be screened in order to find a few viable ones, primary screening takes a lot of time and effort. However, this may be the most important stage since it gets rid of the vast majority of undesired, worthless isolates that are either non-producers or producers of recognised chemicals. Computer-based databases play a significant role by instantly giving in-depth details on the previously recognised microbial antibiotic compounds. For a number of microbial products, quick and efficient screening

methods have been developed. These methods either use the product's own characteristics or those of its metabolic pathway to find isolates that are of interest. For extracellular enzymes and enzyme inhibitors, for instance, certain screening approaches are rather straightforward. But for the majority of highly valuable microbiological goods, the screening process is often time-consuming and difficult, and it frequently requires two or more phases, as is the case with antimicrobials. Focusing on a group of organisms that are predicted to produce novel goods may be advantageous in certain circumstances. For instance, the hunt for novel antibiotics is now concentrated on uncommon Actinomycetes, or Actinomycetes different than those found in the genus

Inoculum development describes the process of bringing a population of microorganisms from a dormant stock culture to an active state of growth that is appropriate for inoculation in the last step of manufacturing. Taking inoculum from a functional stock culture to start growing in an appropriate liquid medium is the initial stage in the formation of inoculum. Commonly floating in sterile tap water before being added to the broth are bacterial vegetative cells and spores. The hyphae are broken up and then added to the broth in the case of actinomycetes and non-sporulating fungi. Flask cultures are often employed for inoculum generation; flask sizes may range from 50 millilitres to 12 litres, and their numbers can be adjusted as necessary. Small fermenters may be used if necessary. To raise the volume to the appropriate level, inoculum development is often done step by step. An increase in inoculum volume of 20–200 times is possible at each phase by using inoculum at a rate of 0.5–5% of the medium volume. The inoculum typically employed during the production stage makes up around 5% of the medium volume.

Microorganisms are widely employed to produce a wide variety of goods and services. They have shown to be very helpful due to the simplicity of mass culture, rapid pace of development, utilisation of inexpensive substrates (which are often wastes), and variety of possible products. Their ease with which they may be genetically altered has also created almost endless additional opportunities for new goods and services from the fermentation industries.

Traditional fermentations, such as certain food and alcoholic beverage fermentations, were once carried out (and are still carried out in many situations) by a combination of wild microorganisms originating from the raw materials or the surrounding environment. Less than 120 years ago, the first efforts to modify the microorganisms involved were made when they were initially separated from these processes as pure cultures, from which the most beneficial strains were then chosen. Most of the fermentation techniques created during the first 80 years of the 20th century relied on monocultures. The particular microorganisms used were often isolated from the environment, requiring the random screening of several isolates. As an alternative, appropriate microbes were obtained from culture collections. Regardless of where they came from, the majority of these microbes underwent further modifications via breeding or mutagenesis programmes to enhance their characteristics for industrial application. Recombinant microorganisms have been employed in a number of processes created in the last 20 years, and genetic engineering techniques are increasingly being applied to enhance current commercial strains.

When selecting microorganisms for industrial usage, regulatory issues are often of the utmost significance. In the fermentation industry, especially when producing food goods and components, proven GRAS (Generally Regarded as Safe) microorganisms are often preferred. This is due to the stricter standards and much greater expenses involved with process and product certification employing a "novel" microbe. There must be extra safety precautions taken when pathogens and/or certain GMMs are utilised as the producing organism. It may be conceivable to utilise altered ("crippled") strains that cannot live outside the fermenter environment and special containment facilities are used.

Isolation of appropriate microorganisms from their surroundings the two kinds of strategies used to isolate a suitable industrial microbe from the environment are "shotgun" and objective techniques. The shotgun method involves gathering samples of free-living microorganisms, biofilms, or other microbial communities from soil, water, waste streams, animal and plant matter, sewage, and odd man-made and natural settings. Then, these isolates are examined for desired characteristics. The alternative is to sample from specified locations where organisms with the required traits are thought to be probable constituents of the natural microflora in order to adopt a more objective approach. Sites that are known to be polluted by a particular target drug, for instance, may be sampled while seeking to identify an organism that can breakdown or detoxify it. Environmental factors like these could favour microbes that can break down this substance.

Selecting the growth medium and culture conditions that should be employed to isolate the target microorganism after the samples have been collected is a significant challenge (s). Often, killing or suppressing the development of common species while promoting the growth of unusual ones is the first step. Then, enrichment cultures may be carried out in batch cultures or, often, continuous systems are more appropriate. Prior to isolation and screening, this raises the amount of the target organisms and promotes the development of those with the required features. However, this mechanism of selection is only effective when the targeted characteristic gives the organisms a competitive advantage.

Selecting or creating the ideal selective medium and growth conditions is required for subsequent isolation as pure cultures on solid growth media. Each must be tested for the required characteristic, production of a certain enzyme, inhibitory substance, etc. after being isolated as pure cultures. However, as strain development can often be used to significantly enhance performance, the degree of activity or concentration of the intended product per se is not of great importance at this point. Additionally, it is required to test chosen isolates for other crucial characteristics including stability and, if necessary, non-toxicity.

The hunt for a single bacterium is easier to apply these isolation and screening techniques to. However, it is significantly more challenging to identify consortia whose members may change over time and who collectively possess the desired talent or attribute. When it comes to the capacity to break down a complicated resistant chemical, such groupings may be more effective. Microorganisms of historical, current, and possibly future importance may be found in abundance in microbial culture collections. Around the globe, there are roughly 500 cultural collections; the majority of them are small, specialised collections that only provide cultures or

other related services by special arrangement. Others, most notably national collections, provide comprehensive services to academic and industrial organisations in addition to publishing catalogues that identify the organisms they house. For instance, the National Culture Collection (UKNCC) in the UK is composed of many collections. They are kept in different facilities and often focus on bacteria, yeasts, filamentous fungus, or algae of industrial or medicinal significance; in contrast, the American Type Culture Collection (ATCC), which is the country's major centralised collection, houses all varieties of microorganisms.

A culture collection's key duties include maintaining the current collection, continuing to collect new strains, and providing authenticated, pure culture samples of each organism. The creation and use of cryopreservation and freeze-drying (lyophilization) techniques, together with miniaturised storage techniques, have helped with problems of culture maintenance. One practical technique is the adsorption of cells to glass beads (2 mm in diameter) that may be stored frozen, allowing for the removal of individual beads without thawing the whole sample.

Literature Review

Because cellular productivity is crucial to economic success, sustaining it is necessary for the industrial fermentation of microorganisms that produces biochemicals. Static engineering can be used to create high-productivity microbial strains, but since culture conditions and cell states are constantly changing, these strains may not stay at their peak productivity for the duration of the culture. Min *et al.*addressed metabolic flux fine control by dynamic regulators in response to metabolites or extracellular stimuli, reliable production systems, and auto-induction systems employing quorum sensing in this article as we present dynamic regulators of industrial microorganism optimization[5].

Evolutionary engineering is a commonly used technique in biological research to enhance the features of microorganisms, such as high environmental tolerance and increased product production. Zhu *et al.* listed the typical evolutionary tactics used by industrial microbes in this paper and talk about how evolutionary engineering may be combined with other biotechnologies like systems biology and inverse metabolic engineering. Finally, we discuss the significance and potential uses of evolutionary engineering, particularly in the improvement of industrial microbial cell factories[6].

Liu *et al.* provide an in-depth analysis of the cofactor engineering approaches for addressing the troublesome redox imbalance in metabolism modification, along with information on their advantages, applicability, and current developments. In vitro biocatalysis typical examples are also provided. Additionally, we quickly go over how cofactor engineering may benefit from the tools and techniques used in synthetic biology. Finally, cofactor redox engineering's future objectives and problems are discussed[7].

Omura *et al.*provide results of 99% of S. avermitilis' genome's sequence analysis. The linear chromosome, the biggest bacterial genome sequence with at least 8.7 million base pairs, sheds light on the inherent variety of Streptomyces' synthesis of secondary metabolites. The S. avermitilis genome has 25 different types of secondary metabolite gene clusters. Four of them deal with the biosynthesis of melanin pigments, whereby two clusters encode tyrosinase and its

cofactor, another two encode an ochronotic pigment made from homogentiginic acid, and another cluster encodes melanin made from polyketides. Seven and five genes, respectively, make up the gene clusters for the biosynthesis of carotenoids and siderophores. For the biosynthesis of type-I polyketide compounds, there are eight different types of gene clusters, and two of these clusters are also engaged in the biosynthesis of type-II polyketide-derived chemicals. In addition, a polyketide synthase with similarities to phloroglucinol synthase was found. Nonribosomal peptide synthetases produce peptide molecules via the biosynthesis of eight clusters. These clusters of secondary metabolites are dispersed across the genome, but half of them are close to both ends. These clusters take up around 6.4% of the genome in overall length[8].

In a study by Papini*et al.*, investigation is carried out to mimic the phenotypes brought on by the various metabolic engineering methodologies, mathematical modelling is used together with high-throughput analytic technologies including transcriptome, proteome, and metabolome analysis. In fact, it is anticipated that systems biology will significantly advance the development of cell factories, and as a result, we suggest the term "industrial systems biology" to describe how systems biology will advance the advancement of industrial biotechnology for the production of sustainable chemicals[9].

Ikeda *et al.* carried out an investigation in which Streptomyces species produce a wide range of bioactive secondary metabolites, which makes them of significant pharmacological relevance. The linear chromosome of Streptomyces avermitilis has had its whole nucleotide sequence identified. A category of antiparasitic medications called avermectins, which are produced by S. avermitilis, are utilised in both human and veterinary medicine. At least 7,574 possible open reading frames are encoded in the genome's 9,025,608 nucleotides, which has an average GC content of 70.7%. (ORFs). 721 paralogous families are made up of 35 percent of the ORFs (2,664). Thirty gene clusters that make up 6.6% of the genome are involved in the manufacture of secondary metabolites. The internal 6.5-Mb portion of the S. avermitilis genome was substantially preserved with regard to gene order and content when compared to Streptomyces coelicolor A3 (2), and included all known critical genes while displaying flawlessly asymmetric organisation at the oriC centre. Instead of being conserved, the terminal sections tended to include nonessential genes[10].

Discussion

Microorganisms have been employed extensively in the industry to produce a variety of biochemical products. Evolutionary engineering in conjunction with other biotechnologies has garnered greater interest recently in an effort to better integrate the activities of microbial cells. It has been shown that traditional laboratory evolution works well at allowing more advantageous mutations to develop in various genes, but it also has certain intrinsic drawbacks, such as a lengthy evolutionary time and unregulated mutation rates. Recent research, however, suggested that certain fresh approaches could eventually get over these constraints.

Additionally, for commercial chemical and biofuel production, cost considerations restrict the expression of heterologous proteins utilising inducible promoters to reduce metabolic load. The

design of genetic circuits, which precisely regulate gene expression or influence genetic behaviour toward a particular phenotype, has recently been aided by synthetic and systems biology. The creation of dynamic regulators can keep the cellular phenotypic at its highest level of production in response to variables including cell concentration, oxygen, temperature, pH, and metabolites. In all biological species, NAD and NADP play a crucial role as electron donors or acceptors, driving significant catabolic and anabolic processes. Additionally, they are essential for maintaining intracellular redox equilibrium. However, numerous metabolic engineering initiatives in industrial microorganisms aimed at changing or introducing metabolic pathways, particularly those involving the consumption, generation, or transformation of NAD/NADP, frequently induce fluctuations in redox state, which severely obstruct cellular metabolism and lower growth performance and biosynthetic capacity. A soil bacterium called Streptomyces avermitilis performs complicated morphological differentiation as well as the generation of secondary metabolites, one of which, avermectin, has significant economic value in both human and animal medicine. The variety of this genus Streptomyces' synthesis of secondary metabolites as an industrial microorganism is of particular interest. The fact that it has several metabolic routes for biosynthesis plays a significant role in its significance as a generator of a wide range of secondary metabolites.

As the chemical industry strives to produce chemicals that may be used as fuel or as building blocks for the manufacturing of solvents and materials, industrial biotechnology is growing quickly. Designing and developing effective cell factories that can guarantee the cost-effective conversion of the raw material into the desired chemical is a significant problem in conjunction with the development of sustainable bioprocesses. This is accomplished via the process of metabolic engineering, in which the cell factory's metabolism is modified to ensure a successful conversion of sugars—the usual raw materials used in the fermentation industry—into the desired output. However, the intricate regulation that has developed in conjunction with the adaptation of the various microbes to their biological niches makes engineering of cellular metabolism often difficult. Systems biology technologies may be used to map these regulatory structures, further deregulate them, and find creative metabolic engineering solutions that satisfy mass balance restrictions.When opposed to environmental isolation, using microorganisms chosen from a culture collection certainly offers considerable cost savings, and it also has the benefit that some characterization of the microbe will have already been completed. The drawback is that rivals have access to the same microbes, however.

Improvement of industrial strains and strains

1. Regardless of where a particular microbe came from, it should ideally display:
2. Genetic constancy.
3. Effective production of the intended product, whose biosynthetic pathway is ideally well described.
4. Minimal or no need for supplementary vitamins and growth factors.
5. Making use of a variety of inexpensive and easily accessible carbon sources.
6. Capacity for genetic modification.

7. Unless this is the goal product, safety, non-pathogenicity, and should not manufacture hazardous agents.
8. The fermentation's ready for harvesting.

If the intended product is intracellular, ready breakage occurs.

The primary goal of biotechnological procedures is to enhance or optimize the specific qualities desired in an organism. By obtaining improved organisms via the use of screening and selection processes, advancements have been made in this field. All uncommon or innovative strains develop while the others do not under a selection system. All strains grow in a screening system, but certain strains or cultures are selected because they exhibit the necessary traits needed by the relevant industry.

In order to create microorganisms with novel and desired traits, genetic modification is performed. The creation of cultures for industrial microbiology relies heavily on the traditional techniques of microbial genetics.Once a suitable culture has been identified, it is possible to enhance the culture using a number of methods, such as chemical mutagenesis and UV radiation. However, these techniques often only result in the elimination of unwanted features or an increase in output as a result of the lack of control mechanisms. Rarely has it resulted in the emergence of a brand-new function or feature. So, an organism having a desirable trait will be chosen from the natural environment, reproduced, and exposed to a mutational programme, after which the best offspring will be chosen by screening. For instance, Penicillium notatum's first cultures, which could only be cultured in static environments, produced little amounts of penicillin. P. chrysogenum strain NRRL 1951, which was enhanced by mutation, was discovered in 1943. (Using X-ray treatment, UV and mustard gas the yield was increased from 120 IU to 2 580 IU). P. chrysogenum is cultivated in aerobic agitated fermenters now, where it delivers 55 times more penicillin than the first static cultures did.

Protoplast and cell fusion and recombinant DNA technology are two novel methods of DNA manipulation that have become more important in industrial genetics in recent years (genetic engineering).Nowadays, yeasts and moulds are often utilised in protoplast fusion. Since most of these microbes are asexual or only have one sort of mating, the likelihood of random mutations that might cause strain degeneration is reduced. Protoplasts are created by cultivating the cells in an isotonic solution while subjecting them to enzyme treatments, such as cellulose and ß-galacturonidase, to conduct genetic investigations with these microorganisms. Osmotic stabilisers like sucrose are subsequently used to rebuild the protoplasts. Selective plating procedures are used to find the desired recombinants if fusion results in hybrids. The novel protoplasm fusion product may be employed in further research after cell wall regeneration.

The ability to combine protoplasts from various microbial species, even those that are not closely related taxonomically, is a significant benefit of the protoplast fusion approach. For instance, P. chrysogenum and Penicillium roquefortii protoplasts have been combined. Erythrocytes and yeast protoplasts may also be united. The production of monoclonal antibodies includes a kind of mammalian cell fusion, which is one of the most fascinating and financially lucrative fields of biotechnology. Pure monoclonal antibodies were created in 1975 from a hybridoma formed by ß-

lymphocytes and myeloma tumour cells. With the use of the monoclonal antibody method, cells that normally secrete antibodies but have a short lifespan are transformed into immortal cells with the ability to develop indefinitely. Today, monoclonal antibodies are often used in several diagnostic procedures that need a high level of specificity. Diagnostic kits containing specific monoclonal antibodies have been developed for use in medicine, plant and animal husbandry, and food production.

Genetic modification

Reassortment of genetic material happens during normal sexual reproduction as a consequence of the breaking and rejoining of chromosomal DNA molecules, however this is only possible amongst closely related taxa. There are many possibilities for combining genes thanks to genetic engineering or recombinant DNA technologies. These methods enable the splicing of DNA molecules from a variety of sources, and when used in conjunction with genetic transformation methods, they make it easier to introduce foreign DNA into other species (Fig. 3.2). It is possible to separate DNA into groups of one or more genes after obtaining it from plants, animals, or microbes (the donors). Once coupled to another DNA strand (the vector), such pieces may be transferred into the host or recipient cell and added to the genetic makeup of the new host. The host cell may then be multiplied in large quantities to develop unique genetic traits and chemical capabilities that could not be achieved by standard breeding or mutation techniques.

By using a procedure called site-directed mutagenesis, short DNA sequences that have been chemically produced may be introduced into recipient microbes. This may result in minor genetic changes that modify one or more amino acids in a target protein. These seemingly insignificant alterations in amino acids have been discovered too often result in unanticipated modifications in the properties of proteins, giving rise to novel enzymes with increased environmental resistance and the ability to catalyse specific processes. These methods fall under the umbrella of protein engineering. It is possible to produce bioactive peptides and enzymes with distinctly varied properties (stability, kinetics, and activity). Additionally, a greater understanding of the molecular underpinnings of how these changed products work is possible. The creation of enzyme-active sites to encourage the alteration of "unnatural substrates" is one of the most intriguing fields. This strategy could enhance resistive material transformation or perhaps result in the breakdown of substances that were previously resistant to biological processing.

Maintenance of microorganisms

Once a bacterium or virus has been chosen or produced to fulfil a particular need, it must be kept in its original state for usage and research in the future. However, this may result in mutations and phenotypic changes in microorganisms, which is why periodic transfers of cultures have historically been performed. A range of culture preservation approaches may be utilised to retain desirable cultural traits in order to prevent these issues. With microorganisms, lyophilization, or freeze-drying, and storage in liquid nitrogen are widely used.

Media for culture maintenance

Key industrial strains are stored and subcultured using these medium. They are made to decrease the potential for genetic variation and maintain optimal cell viability. They must especially cut down on the creation of hazardous metabolites that might cause strain destabilisation. If a strain has a tendency to become unstable spontaneously, it should be kept alive using medium chosen for that characteristic's preservation.

Conclusion

The genetics of industrial microbes is the primary topic of this chapter. There are two main categories of mutagenic processes that cause mutation in microorganisms: (1) mistakes that are directly caused by base mispairing, and (2) errors that are introduced via different repair systems. Excision, postreplication, and error-prone repair processes operating on single-strand gaps produced directly or indirectly in DNA are generally responsible for the bulk of mutations. The industrial microbiologist is now interested in the detection of mutagenic substances, which is the opposite of mutation induction. The pH concentration, treatment time, and phase of cellular development all interact to optimise mutation induction; these interactions are often organism-dependent. Rates of mutation may also be impacted by adding or removing DNA precursor nucleotides. By plating dense populations of mutagenized organisms on solid substrate containing a hazardous chemical, resistance mutants may be identified. Placing simply on a minimum medium is all that is necessary to separate prototrophic reversions from auxotrophs.

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CHAPTER 3

PRIMARY AND SECONDARY MICROBIAL METABOLITES

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Abstract:

The total number of biochemical processes that an organism engages in is known as its metabolism. Metabolites, which are often limited to tiny molecules, are the metabolic intermediates and products. While main metabolites are present in every live cell capable of division, the word "secondary," first used by A. Kossel in 1891, denotes that secondary metabolites are present just incidentally and are not crucial for an organism's survival. Despite coming from primary metabolism, the organism's fundamental molecular structure is not made up of secondary metabolites. Contrary to main metabolite, its absence causes an organism's viability to be more severely compromised rather than instantly ending. In this work microbial metabolites primary and secondary are discussed.

Keywords:

Biochemical,Metabolites, Microbial Metabolism, Microorganisms.

Introduction

Primary metabolites are the compounds that are directly involved in the metabolic pathways of an organism necessary for its growth, development, and reproduction. These metabolites are associated with the physiological processes occurring in the organism. Primary metabolites are produced in the organism during the growth phase, as a result of the growth mechanism. The growth phase associated with the production of primary metabolites is termed as 'trophophase'. The production of primary metabolites is initiated when the nutrients necessary for the body are available in the medium [1]–[3].

These are found in most cells throughout the body and are also termed central metabolites. Primary metabolites are crucial for various metabolic activities as some act as a substrate for these processes, while others act as catalysts. Some primary metabolites like amino acids are common throughout the organisms, whereas some are restricted to some cells or some organisms. Even though primary metabolites play an essential role in the growth and development of an individual, these do not have pharmacological actions or effects against other factors. The production of primary metabolites usually occurs at a high rate as these are constantly required for the body. These can also be extracted easily through simple extraction procedures[4].

Primary metabolites are divided into two groups; primary essential metabolites and primary metabolic end products. Primary essential metabolites include compounds like proteins and carbohydrates that make up the structural and physiological organization of the organism. In

contrast, primary metabolic end products include products like lactic acid and ethanol that are the end products of various metabolic pathways. Examples of primary metabolites include proteins, enzymes, carbohydrates, lipids, vitamins, ethanol, lactic acid, butanol, etc.

Secondary metabolites are the organic compounds that are produced by various organisms that are not directly involved in the growth, development, or reproduction of the organism but are essential in the ecological and other activities. Secondary metabolites are also termed specialized metabolites or natural products. Because secondary metabolites are not involved in the growth and development of the organism, the absence of these compounds causes little to no effect on the survivability of the organism. However, in the long run, some mild effects might be observed. Some secondary metabolites are specific to a species and are only found in them, but the horizontal transfer of these metabolites across species has seen to play an important role in the evolution of some organisms. Although they are not important for survival, secondary metabolites might be important for other activities like protection, competition, and species interaction. Secondary metabolites are classified into groups based on their biosynthetic origin. Some of the secondary metabolites are derived forms of primary metabolites.These are also formed during the stationary phase of growth in most organisms. This phase of growth is termed ‘idiophase’. Most secondary metabolites tend to act as a defense mechanism against various foreign invaders. These are produced in rather smaller quantities and are difficult to extract. Secondary metabolites are also not a part of the molecular organization of the organism. Some categories of secondary metabolites have been used in various biotechnological procedures for the formation of drugs and other compounds. Since secondary metabolites are specific to species, different secondary metabolites are involved in various procedures.Some examples of secondary metabolites include steroids, essential oils, phenolics, alkaloids, pigments, antibiotics, etc.

Enzymes

Enzymes are proteins that are produced in the body of different organisms as primary metabolites. Enzymes are important compounds that catalyze various metabolic pathways throughout the body. Enzymes are proteins formed of polypeptide chains of amino acids that are highly specific for the reaction they catalyze. In the absence of enzymes, the biological reactions would require a long period of time to complete. Enzymes are conservative and do not get spent during chemical reactions.

These are involved in almost all forms of metabolic pathways ranging from cellular respiration to internal digestion and absorption. The enzymes produced in various organisms are extracted for their use in industries for processes like fermenting of wine, the leavening of bread, curdling of cheese, and brewing of beer.Some examples of enzymes include lipases, amylases, proteases, etc.

Carbohydrates

Carbohydrate is a group of organic compounds that play an important role in the structural and physiological components of all living beings. Carbohydrates are among the most important primary metabolites that are common in all living organisms.

A carbohydrate is a biomolecule formed of carbon, hydrogen, and oxygen. These provide structure to organisms like cellulose in plants and peptidoglycan in bacteria. Besides, these are also compounds that are oxidized to obtain energy for the growth and functioning of the organism.

Pigments

Pigments are compounds of various colors that are produced by various organisms for various purposes. These are secondary metabolites and are produced by various organisms like plants and bacteria.

Flavonoids

Flavonoids are secondary metabolites in plants that are found in all fruits and vegetables. Flavonoids are phytonutrients, meaning plant chemical that also provides some coloration to many plants and animals. These are the largest groups of phytonutrients found in plants with more than 6000 types known. These compounds have important antioxidants with anti-inflammatory and immune system benefits.

Most people are not aware of the extent to which recombinant and nonrecombinant biotechnology goods produced by large-scale fermentation have impacted our everyday life. The general population may be familiar with traditional antibiotics or big recombinant therapeutic proteins like antibodies made by "industrial-scale fermentation." However, it is widely unknown what role fermentation plays in the production of small-molecule drugs, steroids, or cytotoxics, not to mention the products used in taste and fragrance, home appliances, chemical manufacture, and a variety of other industries.

Fermentation was an experimental process that was non-sterile before these two discoveries. Vinegar manufacturing from wine using a continuous "fill and draw" process was the first true commercial industrial fermentation use in France during the Renaissance. A floating mat of aerobic bacteria was allowed to oxidise wine in enormous barrels. As long as the oxidative biomass was still active, a significant portion of the acetic acid-containing liquid was then drained from the barrel and replaced with new wine.

Today, any submerged culture in a bioreactor—which are now mostly characterised by aerobic processes—is referred to as fermentation. Since the creation of sterile, large-scale culture technology for antibiotics in 1943 and the introduction of genetic engineering in the 1970s, it is amazing how far we have gone with the use of industrial-scale fermentation. The "living factories" of today are made up of wild-type, mutant, and recombinant microbial, fungal, plant, animal, mammalian, and stem cells. Recently, over 20 distinct genes were horizontally inserted, as in the instance of recombinant opioid synthesis in yeast. Commercial aims covered by "industrial-scale fermentation" range from biofuels to individualised medications. Industrial-Scale Fermentation, the phrase "industrial-scale" in relation to the liquid working quantities might signify multiple things. Every product category now has five. Platform chemicals, amino acids, and vitamins are manufactured in stirred-tank bioreactors or fermenters with operating capacities up to several hundred cubic metres for use in animal feed and other commodities.

Only maximum working volumes of several tens of cubic metres are needed for the commercial production of recombinant, parenteral (injectable), therapeutic proteins or monoclonal antibodies in stirred-tank reactors. Lastly, the term "industrial" refers to a working capacity of just a few hundred litres in the context of the industrial-scale production of adherent stem cells, which are currently cultured on microcarriers suspended in (disposable) stirred bioreactors. Thus, the nominal bioreactor operating capacity range covers at least two orders of magnitude, from low-cost goods to expensive pharmaceuticals. While the fundamental ideas behind suspension culture in bioreactors and the very basic layout of these bioreactors remain the same for all applications, they must be adjusted and changed in accordance with the specific needs of the targeted product and the cell type being cultivated in terms of parameters like the ones listed below:

A. Oxygen consumption
B. Necessary for heat transfer
C. Current Good Manufacturing Practice (cGMP) regulations; sensitivity to shear; sensitivity to process and culture fluctuations; sensitivity to local variables inside the bioreactor
D. Specific safety standards for very powerful active pharmacological components (containment levels are typically BLS1 and BLS2); (HPAPI).

There have been several descriptions of laboratory-scale fermentations. We walk the reader through the most crucial elements of large-scale industrial fermentation in this chapter. Which species are suited for suspension culture on a big scale?

Literature Review

In a study by Singh *et al.*, a vast array of natural compounds produced by microorganisms are a potential source and have significantly impacted almost every aspect of human, plant, and animal existence. Natural substances derived from microbes have shown their worth in food, farming, and medicine. Amino acids, enzymes, vitamins, organic acids, and alcohol are examples of primary metabolites that are employed both as nutritional supplements and in the biotransformation of raw materials to create industrial goods. While secondary metabolites are organic substances that are mostly extracted from tissues or plants. Due to their capacity to lower infectious illnesses in people and animals and so lengthen life expectancy, they are mostly employed in the biopharmaceutical business. Furthermore, the growth of sustainable agriculture is unavoidably greatly influenced by microorganisms and the goods they produce[5].

During their development, microorganisms create and release a large number of main and secondary metabolites into the environment. Extracellular metabolites therefore provide crucial information on the changes in microbial metabolism brought on by various environmental factors. Since there is no need to break cells, determining these metabolites is also simpler than extracting and analysing intracellular metabolites. Over the last 20 years, several analytical techniques have been used to the study of extracellular metabolites produced by bacteria. In a study by Pinu *et al.* they went through the uses and advantages of extracellular metabolite analysis. They also go through several sample preparation techniques that have been documented in the literature for extracellular microbial metabolites of both sorts (for example, metabolites in

solution and in gas). The validity of employing extracellular metabolomics data in the metabolic modelling of several industrially significant bacteria is evaluated last[6].

Bérdy *et al.* investigated a research on microbial metabolites, including antibiotics and other bioactive metabolites, has a brief history, distinct characteristics, and promising futures. On the basis of statistical data, it is primarily described how metabolites have a microbial origin, the variety of species that produce them, their diverse bioactivities, and special characteristics of their chemical structures. Future discoveries of potential metabolites, issues with dereplication of freshly isolated chemicals, and new directions and outlooks for the field of study are also highlighted[7].

The influence of the microbiome on host physiology is mediated through microbial metabolites. Here, Stinson *et al.* provided an early research suggesting that metabolites formed from maternally-derived microbial organisms are passed on to the newborn via human milk and highlight compounds of importance for early-life programming. We support the use of targeted and untargeted metabolomics as an addition to existing milk microbiome research.

A variety of microorganisms that create a diverse spectrum of bioactive chemicals make up the microbiome, which is a hidden treasure trove. There is evidence from recent research that the microbiome may have an effect on cancer treatments. Luu *et al.*provided a summary of how immunotherapy for cancer is enhanced by the molecular interaction of two classes of microbial metabolites with T cells.

Sanchez *et al.* investigated the microorganisms that thrive on cheap carbon and nitrogen sources undergo fermentation to generate important compounds including amino acids, nucleotides, organic acids, and vitamins that may be added to food to improve flavour or boost nutritional value. With the resurgence of interest in solvent fermentations, microbes play an increasingly important role in fields other than food and health. Many petroleum-derived products, as well as the ethanol required for liquid fuel, may be produced by microorganisms. The role that primary metabolites play as precursors in the synthesis of several medicinal molecules is one of their other uses[8].

Bacterial pigments are considered natural products, which are significant classes of highly active natural chemicals and also perform a variety of biological functions including antioxidant, antibacterial, and anticancer, etc. Bacterial pigments are classified as secondary metabolites; they have potential use in biomedicine and are commercially significant substances. Due to its requirements in treating many human health ailments, they are extensively employed in the biopharmaceutical industry. The generation of secondary metabolites in bacteria is influenced by physical, chemical, and biological variables. Scientifically based current instruments have a significant impact on signal processing, regulating the growth composition of bacteria, and bacterial metabolism impairment. Pradeepa *et al.* described the use of several contemporary approaches to detect the synthesis of secondary metabolites in microorganisms in order to understand the general processes involved in improving the production of bacterial colours[9].

Firáková *et al.* reported that scientists have recently concentrated their research on a class of relatively recent microorganisms known as endophytes. These microorganisms, mostly fungus

and bacteria, are defined as colonising the intercellular gaps of plant tissues. The taxonomy, ecology, and connection between endophytic microbes and their host plants are all being researched. A host-endophyte connection may be impacted by the bioactive secondary metabolites that some of these bacteria create. Numerous endophytic bioactive metabolites, both well-known and brand-new compounds, have recently been shown to possess a broad range of biological activities, including antibiotic, anticancer, anti-inflammatory, and antioxidant properties. For the biotechnological synthesis of bioactive compounds as therapeutically significant agents, microorganisms like endophytes may be particularly intriguing. So, the purpose of this review is to briefly describe endophytes, list the structurally various beneficial secondary metabolites generated by endophytic microbes, and describe the microbial sources of these compounds and their host plants[10].

Discussion

Secondary metabolites produced by microorganisms have a low molecular mass and unique structures.The structurally varied metabolites exhibit a wide range of biological actions, including antibacterial agents, enzyme inhibitors and anticancer agents, immune-suppressants and antiparasitic agents, plant growth stimulators, herbicides, insecticides, antihelmintics, etc. During the microorganisms' late development phase, they are created. Because the generation of secondary metabolites is often suppressed in the logarithmic phase and decreased in stationary growth phases, microorganisms have specific regulatory mechanisms that govern this process. About 40% of the secondary metabolites produced by microorganisms cannot be chemically manufactured and have unique molecular structures that are not present in chemical libraries.The stages of microorganism growth are connected to the overproduction of microbial metabolites. Various signal molecules, effectors, inhibitors, and inducers are involved in diverse forms of overproduction. In microbial cells, the biosynthesis of enzymes that catalyse metabolic events is regulated by well-known positive and negative mechanisms, including as induction, nutritional regulation (regulating the availability of carbon or nitrogen), feedback regulation, etc. The generation of primary metabolites by microorganisms has a big impact on how good life is. The synthesis of these chemicals by fermentation is still a key objective of contemporary biotechnology.

Characteristics of secondary microbial metabolites

To optimise its use in the fields of health, agriculture, food, and the environment, the natural fermentation product synthesis concept and technique may be scaled up and used. New analogue or templates in which secondary metabolite act as lead compounds will drive discovery and design of new medications, where the metabolite may serve as a beginning material for generating a product of interest, extended further by chemical or biological transformation.

Applications for microbial secondary metabolites

The use of microbes for the manufacture of secondary metabolites was made possible by the discovery of penicillin, which completely changed the area of microbiology. A number of -lactam-containing compounds and other classes of antibiotics have been discovered thanks to the development of novel screening and isolation procedures. 4000 of the approximately 6000

antibiotics identified so far are from actinobacteria. The prokaryotic group's most frequent manufacturers of antibiotics are the unicellular bacteria Bacillus and Pseudomonas. In eukaryotes, fungus are the second-largest producers of antibiotics after plants.Myxobacteria and cyanobacteria species have recently joined these notable organisms as productive species.Pharmaceutical products, particularly those with anti-infective properties, are made up of 62% antibacterials, 13% sera, immunoglobulins, and vaccines, 12% anti-HIV antivirals, 7% antifungals, and 6% nonHIV antivirals. Over 160 different antibiotics exist. The main producers of antibiotics on the market are Streptomyces hygroscopicus, which has over 200 antibiotics, Streptomyces griseus, which has over 40 antibiotics, and Bacillus subtilis, which has over 60 chemicals.

Pharmaceutical and dietary supplements

The discovery of the fungus-based statins, such as compactin, lovastatin, and pravastatin, which decrease cholesterol, was a great triumph. The ovastatin-producing bacteria A. terreus. Immunosuppressants like cyclosporin a, sirolimus (rapamycin), tacrolimus, and mycophenolate mofetil are very significant in human medicine. They established the field of organ transplantation and are utilised for heart, liver, and kidney transplants. Tolypocladium niveum is a fungus that produces cyclosporin A. The earliest known antibiotic, mycophenolic acid, is converted into the semi-synthetic drug mycophenolate mofetil by a fungus. Streptomycetes produce tacrolimus and sirolimus.

Probiotic bacteria's metabolic products are thought to be effective in treating and strengthening insulin sensitivity, minimising weight gain, avoiding obesity, extending satiety, decreasing food intake, and reducing fat deposition.

Secondary Metabolites – Sources and Applications. In a healthy human gastrointestinal tract, Firmicutes and Bacteroidetes predominate; the latter was found in lower concentrations in IBS patients who had constipation as their primary symptom. [38]. Utilized as food colouring, fish feed, nutraceuticals, cosmetics, and antioxidants are carotenoids of microbiological origin. Carotene from Blakeslea trispora, Dunaliella salina, and lycopene from B. trispora and Streptomyces chrestomyceticus, subsp. rubescens are two common food colouring sources. A recognised fish feed ingredient, astaxanthin is made from Xanthophyllomyces dendrorhous. Due to its superior antioxidant properties, astaxanthin, lutein, -carotene, zeaxanthin, and canthaxanthin are employed as nutraceuticals. Docosahexaenoic acid (DHA), a dietary supplement used in baby formula, is produced from the microalgae *Schizochytrium spp.*

Enzymes and inhibitors of enzymes

With yearly sales of $2.3 billion, microorganism-produced enzymes are used in detergents (34%), foods (27%), agriculture and feeds (16%), textiles and leather (10%), chemicals, pulp and paper (10%), and agriculture. 200 million dollars' worth of detergents are sold each year that contain the protease subtilisin. The other important enzymes are penicillin amidase (100,000 tonnes) and glucose isomerase (60,000 tons). Production of phytase and nitrilase (30,000 tonnes) totals $135 million. To produce 15 million tonnes of high fructose corn syrup annually, with a market value of $1 billion, Streptomyces glucose isomerase is utilised.

The most significant -lactamase inhibitor is clavulanic acid, which is produced by Streptomyces clavuligerus. Other inhibitors often target enzymes including glucosidases, amylases, lipases, proteases, and xanthine oxidase. Amylase inhibitors may be used to lose weight because they stop the body from absorbing dietary carbohydrates.

Products for animal and agricultural health

Applications for secondary metabolites in agriculture and animal health are numerous:As biopesticides, kasugamycin and polyoxins are employed; as bioinsecticides, nikkomycin and spinosyns; as bioherbicides, bialaphos; and as biopesticides, nikkomycin and polyoxins; The most widely used applications of plant hormones like gibberellins as growth regulators include ivermectin and doramectin as antihelmintics and endectocides against worms, lice, ticks, and mites.

Mutation and genetic recombination are the two processes by which microorganisms may produce new genetic traits. It is now feasible to alter the bacteria to create the desired colour thanks to the development of culture technology (genetic engineering). Nutrients, growth rate, feedback regulation, enzyme inactivation, and enzyme stimulation all influence the synthesis of secondary metabolites. When creating microbial pigments, the core biotechnological procedures are used, first looking for novel sources and then increasing the yield of sources that have previously been identified, either via optimization or strain enhancement.

The term "optimization" refers to the establishment of favourable circumstances that encourage the development of microorganisms and enhance the generation of secondary metabolites, such as those influenced by incubation time, moisture content, fermentation processes, carbon and nitrogen sources, pH, and UV. A variety of microbial physical processes, including symbiosis, competence, conjugation, sporulation, biofilm formation, pathogenicity, and motility, may alter the generation of secondary metabolites.

By releasing chemical signals when cell density exceeds a threshold concentration, quorum sensing technology enables communication to develop between cells.

When an inducer is added to biosynthesis, the growth rate lowers, or both, signals may be produced. These signals may have an impact on the series of regulatory processes that lead to chemical differentiation. The signal is often a low molecular weight inducer that functions by binding to or inactivating a regulatory protein, which inhibits the formation of secondary metabolites.

Chemical and physical sources, such as UV radiation, pH, nitrogen, oxygen, antibiotics, and inorganic ions, may be used to enhance strains. Chemical sources include nitrosoguanidine, 4-nitroquinolone-1-oxide, methyl methane sulfonate, ethylmethane sulfonate, and hydroxylamine. Additionally, it may result in changes to the chemical composition and colour of the pigment.

The synthesis of microbial products has long been influenced by genetics. Microorganisms that produce metabolites are subject to the following sorts of genetic regulation. The most important factors are structural genes that code for the creation of products, regulatory genes that control the initiation and expression of structural genes, resistance genes that control the producer's

resistance to its own antibiotic, permeability genes that control the entry, exclusion, and excretion of the products, and regulatory genes that regulate the pathways that produce precursors and cofactors.

The following paths are the key methods used in molecular genetics tools for identifying suitable vectors, efficient transformation procedures, and biosynthetic pathways.

The advantages of a strain that has been improved include increased production of desired metabolites, elimination of undesirable co-metabolites, improved utilisation of low-cost carbon and nitrogen sources, modification of cellular morphology for improved mycelium production, and improved oxygen transfer in the fermenter.For the selection of a specific trait of the desired genotype, which differs from the one of the ultimate interest but is simple to identify, basic knowledge of product metabolism and pathway regulation is required.

Conclusion

The goal of fermentation has always been to balance food preservation and availability. It is a less harmful way to produce and extract metabolites from natural sources. Through the metabolites created during fermentation, fermented foods have special nutritional qualities that improve health. Interaction and the creation of bioactive compounds provide fermented foods new flavours. Furthermore, given the growing consumer demand for nutritious meals, this industry has a lot of potential to grow in the near future. The sustained and potential advantages of fermentation have also been revealed in several scientific investigations. Numerous medicinal and food-related sectors may make use of microbial metabolites. However, only a relatively small number of GRAS bacteria are used in the synthesis of commercial metabolites, which are mostly produced by a variety of pathogenic microbes. Fermentation produces large amounts of many different metabolites, although not all of them have been well investigated or published. Finding metabolites produced during food fermentation that are safer to use is the actual difficulty. Therefore, the information on the positive effects of microbial metabolites generated during fermentation could be found in the current review.

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CHAPTER 4

A COMPREHENSIVE STUDY ON LARGE SCALE FERMENTATIONS

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Abstract:

This work discusses important facets of industrial-scale fermentation, an emerging field that now produces commercial values of over US$30 trillion yearly and is anticipated to fundamentally alter how we make chemicals in the long run. The most popular and flexible method of production, mass suspension culturing of cells in stirred bioreactors, is used to create anything from biofuels and bulk amino acids to monoclonal antibodies and stem cells. This method may now be used to produce a broad variety of cells, and for the majority of them, genetic editing tools are now accessible. Aspects of engineering and design, operational processes, economic factors, cost, and regulatory concerns are all included. There will also be a discussion of how we arrived to this point and the realities of industrial fermentation. The topic of large-scale manufacturing employed in industrial contexts is the exclusive focus of this chapter.

Keywords: Fermentation, Ethanol Fermentation, large-Scale Manufacturing,Protein.

Introduction

Fermentation includes the processes by which energy is extracted from the oxidation of organic compounds. The oxidation of organic compounds occurs by utilizing an endogenous electron acceptor to transfer electrons released from nutrients to molecules obtained from the breakdown of these same nutrients.There are various types of fermentation which occur at the industrial level such as ethanol fermentation and fermentation processes used to produce food and wine. The ability to utilize the fermentation process in anaerobic conditions is critical to organisms which demand ATP production by glycolysis. Fermentation can be carried out in aerobic conditions as well, as in the case of yeast cells which prefer fermentation to oxidative phosphorylation. The following is a brief overview of a few types of the large-scale fermentations utilized by industries in production creation [1], [2].

Ethanol fermentation is used to produce ethanol for use in food, alcoholic beverages, and both fuel and industry. The process of ethanol fermentation occurs when sugars are converted into cellular energy. The sugars which are most often used include glucose, fructose, and sucrose. These sugars are converted into cellular energy and produce both ethanol and carbon dioxide as waste products. Yeast is the most commonly used organism to produce ethanol via the fermentation process for beer, wine, and alcoholic drink production. As stated previously, despite abundant amounts of oxygen which may be present, yeast prefer to utilize fermentation. Hence, the use of yeast on a large-scale to produce ethanol and carbon dioxide occurs in an anaerobic environment [3], [4].

The ethanol which is produced can then be used in bread production. Yeast will convert the sugars present in the dough to cellular energy and produce both ethanol and carbon dioxide in the process. The ethanol will evaporate and the carbon dioxide will expand the dough. In regards to alcohol production, yeast will induce fermentation and produce ethanol. Specifically, in wine-making, the yeast will convert the sugars present in the grapes. In beer and additional alcohol such as vodka or whiskey, the yeast will convert the sugars produced as a result of the conversion of grain starches to sugar by amylase. Additionally, yeast fermentation is utilized to mass produce ethanol which is added to gasoline. The major source of sugar utilized for ethanol production in the US is currently corn; however, crops such as sugarcane or sugar beets can be used as well.

Ethanol fermentation is responsible for the rising of bread dough. Yeast organisms consume sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough, expanding it into something of a foam. Nearly all the ethanol evaporates from the dough when the bread is baked. The production of all alcoholic beverages employs ethanol fermentation by yeast. Wines and brandies are produced by fermentation of the natural sugars present in fruits, especially grapes. Beers, ales, and whiskeys employ fermentation of grain starches that have been converted to sugar by the application of the enzyme, amylase, which is present in grain kernels that have been germinated. Amylase-treated grain or amylase-treated potatos is fermented for the production of vodka. Fermentation of cane sugar is the first step in producing rum. In all cases, the fermentation must take place in a vessel that is arranged to allow carbon dioxide to escape, but that prevents outside air from coming in, as exposure to oxygen would prevent the formation of ethanol. Similar yeast fermentation of various carbohydrate products is used produce much of the ethanol used for fuel.

Fermentation is also utilized in the mass production of various recombinant products. These recombinant products include numerous pharmaceuticals such as insulin and hepatitis B vaccine. Insulin, produced by the pancreas, serves as a central regulator of carbohydrate and fat metabolism and is responsible for the regulation of glucose levels in the blood. Insulin is used medically to treat individuals diagnosed with diabetes mellitus. Specifically, individuals with type 1 diabetes are unable to produce insulin and those with type 2 diabetes often develop insulin resistance where the hormone is no longer effective.

Ethanol fermentation is the biological process by which sugars such as glucose, fructose, and sucrose, are converted into ethanol and carbon dioxide. Yeasts carry out ethanol fermentation on sugars in the absence of oxygen. Because the process does not require oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation is responsible for the rising of bread dough, the production of ethanol in alcoholic beverages, and for much of the production of ethanol for use as fuel.The increase in individuals diagnosed with diabetes mellitus has resulted in an increase in demand for external insulin. The mass production of insulin is performed by utilizing both recombinant DNA technology and fermentation processes. E. coli, which has been genetically altered to produce proinsulin, is grown to a large amount to produce sufficient amounts in a fermentation broth. The proinsulin is then isolated via disruption of the cell and

purified. There is further enzymatic reactions that occur to then convert the proinsulin to crude insulin which can be further altered for use as a medicinal compound.

An additional recombinant product that utilizes the fermentation process to be produced is the hepatitis B vaccine. The hepatitis B vaccine is developed to specifically target the hepatitis B virus infection. The creation of this vaccine utilizes both recombinant DNA technology and fermentation. A gene, HBV, which is specific for hepatitis B virus, is inserted into the genome of the organism yeast. The yeast is used to grow the HBV gene in large amounts and then harvested and purified. The process of fermentation is utilized to grow the yeast, thus promoting the production of large amounts of the HBV protein which was genetically added to the genome.

Fermentation was an experimental, non-sterile process before these two discoveries. Vinegar manufacture from wine using a continuous "fill and draw" technique during the French Renaissance was the first true commercial industrial fermentation use. Large barrels of wine were left open for an aerobic bacteria mat to float in to oxidise the wine. The procedure was then repeated as long as the oxidative biomass was still active, removing a significant portion of the liquid containing the acetic acid from the barrel and replacing it with new wine.

Today, any submerged cultivation in a bioreactor is referred to as fermentation. Aerobic processes now account for the majority of submerged cultivations in bioreactors. It is incredible how much progress has been made in the use of industrial-scale fermentation since the creation of sterile, large-scale culture technology for antibiotics in 1943 and the introduction of genetic engineering in the 1970s. Today's "living factories" include wild-type, mutant, and recombinant microbial, fungal, plant, animal, mammalian, and stem cells. More than 20 distinct genes have also recently been horizontally inserted, as in the example of recombinant opioid synthesis in yeast. "Industrial-scale fermentation" includes commercial goals like biofuels and individualised medications. When used in reference to liquid working quantities, the word "industrial-scale" may have many distinct meanings. Every product category has five now. In stirred-tank bioreactors or fermenters with working capacities of up to several hundred cubic metres, platform chemicals, amino acids, and vitamins are created for use in animal feed and other commodities.

In stirred-tank reactors, maximum operating volumes of a few tens of cubic metres are all that are needed for the commercial production of recombinant, parenteral (injectable), therapeutic proteins or monoclonal antibodies. In the context of the industrial-scale production of adherent stem cells, which are currently cultured on microcarriers suspended in (disposable) stirred bioreactors, "industrial" refers to a working capacity of only a few hundred litres. The nominal operating volume range of a bioreactor therefore covers at least two orders of magnitude, from low-cost goods to expensive pharmaceuticals. Although the fundamental concepts of suspension culture in bioreactors and the very basic layout of these bioreactors are the same for all applications, they must be adjusted and changed to meet the specific needs of the cultivated cell type and the intended product in terms of parameters like the following:

A. Heat transfer requirements; and oxygen needs
B. Current Good Manufacturing Practice (cGMP) regulations, sensitivity to shear, process and culture variations, local differences inside the bioreactor, and sensitivity to local variations.
C. Special safety standards for very powerful active pharmaceutical components;
D. Biosafety criteria (containment levels are typically BLS1 and BLS2) (HPAPI).

Literature Review

Tracy *et al.* Studied how MSG was produced at a large scale from the metabolic wastes or excretions of bacteria that were fed other agri-industrial waste products (e.g., sugar, soy, wheat). Since around 1960, the $8.4 billion (USD) Ajinomoto Company, Inc., which invented and is the leading producer of MSG (as well as other spices, processed foods, drinks, amino acids, medicines, and specialised chemicals), has been fermenting glutamate from altered bacterial strains. The expansion of the worldwide MSG market in the postwar era was driven by the fermentation method's financial viability, which had an effect on the aesthetic and health value of foodways all over the globe[5].

The fundamental concerns of industrial fermentations, process optimization, and scale up are targeted at maintaining optimal and uniform reaction conditions, limiting microbial stress exposure, and boosting metabolic accuracy in order to maximise product yields and to assure consistent product quality. With an emphasis on Escherichia coli as one of the most frequently fermented organisms, Schmidt *et al.*provides an overview of the problems typically associated with fermentation process optimization and scale up and presents currently used scale-up strategies while taking future technologies into consideration[6].

In a study by Di Paola, it was demonstrated that an essential component of comprehending the ecological and evolutionary dynamics influencing insects' adaptability to various niches is the interactions between yeasts and insects. Genetic variations between strains separated from gut and non-gut settings were revealed by studies on the evolutionary connections of *S. cerevisiae* populations (i.e., natural sources and fermentation). Recent research has shown that Saccharomyces is a reservoir and an evolutionary niche in insects' guts, helping it to spread, reproduce, and produce more interspecific hybrids while hibernating. This helps it survive and evolve. Here, we explore the possibility for using social insects to produce a variety of hybrid yeasts from ambient Saccharomyces isolates that are appropriate for commercial and biotechnological uses[7].

Sauer *et al.* reported that one of the first significant large-scale industrial fermentation processes was the microbial generation of acetone and butanol. It was, in fact, only surpassed in significance by the ethanol fermentation during the first half of the 20th century. Acetone-butanol-ethanol (ABE) fermentation, which saw a sharp drop in the 1950s, has lately attracted fresh attention in the context of biorefinery techniques for the generation of fuels and chemicals from renewable resources. Industrial microbiology has many new avenues to explore thanks to the availability of new techniques and expertise, and the renewed interest makes a thorough examination of this process valuable. The focus of this special issue of FEMS Microbiology

Letters is on the 100th anniversary of the first practical use of Chaim Weizmann's ABE fermentation method. It discusses both new and old advances and outlines a model path for biotechnology. This one procedure exemplifies all significant facets of industrial microbiology. This includes cutting-edge technical advancements, such the most recent advances in metabolic engineering, the use of biodiversity, the identification of novel regulatory systems, like those for microbial stress tolerance, and technological features, like bio- and down-stream processing[8].

Rodríguez *et al.* carried out an application of electrophoretic karyotyping was used to classify yeasts. This method was selected because it can show the substantial chromosomal rearrangements that produce mutations in the yeast genome. One of the primary drivers of the industrial yeasts' rapid development is thought to be this kind of mutation. Throughout the spontaneous fermentations over the course of two years, a diverse community of yeast strains was found. Four of the most prevalent strains were isolated, and they were examined for important industrial-scale microbiological traits. For the next seven years, starter yeasts were created using the chosen autochthonous strains. As a result of the majority of these experiments, we were able to produce homogenous yeast populations in which the karyotype of one of the injected strains—karyotype V—emerged as unmistakably dominant. Conclusions: Controlled fermentations were used to replace spontaneous fermentations, yielding a very acceptable end product. This was accomplished by inoculating the chosen strain with karyotype V and managing the inoculum scaling-up procedure properly. The study's significance and impact for nine years in all, we kept an eye on the wine yeast population in a commercial system. One of the first industrial-scale demonstrations of the viability of using molecular approaches to boost winemaking productivity is our study[9].

Formenti *et al.* provided a viewpoint that begins with a succinct description of key technical tools. The description of some of the most significant engineering challenges, however, is the primary focus. These include scaling up and scaling down fermentation processes, the impact of morphology on broth rheology and mass transfer, and developing new sensors to measure and control insightful process parameters. Due to their many uses as cell factories and consequent significance in a White Biotechnology environment, filamentous fungi's difficulties are highlighted the most. We offer computational fluid dynamics (CFD) as a potentially useful technique for evaluating mixing and possible gradient occurrence in a tank, as well as for supporting the scaling up and scaling down of bioreactors[10].

Discussion

Human history has always placed a high value on fermentation. Fermented foods have been produced by humans at least since the Neolithic era. After microorganisms were discovered in the 19th century, fermentation was discovered to be a practical method for creating synthetic compounds and medicines. Fermentation was the main method used to create alcohols and acetone between 1900 and 1930. But when oil prices dropped, chemical synthesis methods started to take precedence for making alcohols and other solvents. In the Pacific theatre of World War II, monosodium glutamate (MSG) made its way to America. Though the flavor-enhancing food additive was already well-known in the United States, the Japanese military's experience with rationing propelled American military and food sector interests to fully embrace the

technology and to invest in domestic manufacture. Industrial fermentation was a production technique for MSG that was unheard of in its efficiency and profitability when it was developed in 1957 by Japanese researchers. The term "industrial fermentation" describes the mass manufacture of products with high economic value using specialised microbial cultures and readily accessible basic ingredients.

To guarantee better fermentation control and a more stable product over time, yeast cultures are often chosen and standardised in the fermented food and beverage sector. It has been shown in several studies that the organoleptic properties of fermented goods reflect regional differences in the make-up of the microbial population. It is still unknown how and to what degree human involvement has altered the brewer's yeast population structure despite studies of the global distribution and genetic diversity of Saccharomyces cerevisiae. Industrial fermentation products may be greatly improved by the genotypic and phenotypic characterisation of ambient yeast populations and their possible use in the fermentative processes. The ecology of yeast has been shown to be closely related to social insects.

However, because fermentation facilities are typically not strictly designed in accordance with scale-up criteria, the process conditions in the culture vessels may differ significantly, and because any strategy or model can only inadequately consider and reflect the highly complex interdependence and mutual interaction of fermentation parameters, successful scale up in most cases is not the result of a clear-cut and linear experimental strategy but rather will be the result of a combination of factors. A significant technical tool for decreasing our reliance on chemicals and goods made from fossil fuels, industrial fermentation techniques are becoming more and more common. Although they are becoming more and more common, fermentation processes are still not as developed as conventional chemical ones, especially when it comes to applying engineering tools like mathematical models and optimization strategies.

Recently, there has been a focus on the utilisation of renewable resources, which has rekindled interest in microbial fermentations. The goal is to manufacture a wide range of required chemicals or biofuels using cheap and renewable raw materials such non-food crops, agricultural waste, and algae. While some biorefineries that utilise these feedstock materials will use naturally occurring microbes to manufacture the needed chemical products, the majority of them will depend on novel organisms that have been genetically altered to facilitate the creation of the target chemicals. Over the last several years, there has been an upsurge in demand for fine-flavour cocoa on a global scale. The finest chocolatiers in the world have a strong demand for fine-flavour cocoa because of its great quality and distinctive fruity and flowery flavour characteristics. The importance of cocoa fermentation to provide such properties has been underlined in several research. The microbial interactions and biochemistry that result in the production of these qualities on farms with industrial importance, where conventional fermentation techniques have been pre-standardized and scaled up, are, nevertheless, little understood.

Challenges

The difficulties in developing large-scale facilities for biochemical manufacturing are quite similar to those in constructing facilities for biopharmaceutical production. Because the genetically modified organisms (GMO) utilised in both kinds of facilities are sometimes weak by design, they frequently struggle to compete with microorganisms found in nature (in general, highly specialised breeding in a microorganism tends to reduce its viability overall). This is also a matter of safety. You don't want your innovative microbe to be able to outcompete other living things because you are producing it, simply in case it develops any unanticipated negative features. Therefore, it is crucial in both biochemical and biopharmaceutical facilities to avoid contamination of the bioreactor/fermentor systems.

This is done in biopharmaceutical facilities by implementing significant clean-in-place (CIP) and sterilize-in-place (SIP) systems, as well as by employing parts and equipment that can be cleaned and sterilised on-site. These criteria lead to extremely costly construction since all containers must be built for 25 psig or greater pressure and full vacuum to resist steam-sterilization conditions, and the majority of components must be constructed of highly polished stainless steel. The design of large-scale biochemical production may be based on the same ideas, but this must be balanced by the reality that other economic factors are at work. Biorefineries often generate goods that sell for, at best, a few dollars per kilogramme, in contrast to biopharmaceutical manufacturing facilities, which sometimes produce drugs that sell for thousands of dollars per gramme.

The latter use industrial-scale fermentation to produce bio-based chemicals and plastics on a scale that is orders-of-magnitude larger than the scale typically used for the production of biopharmaceuticals, which is another significant difference between industrial biorefineries and biopharmaceutical facilities. Because of this, it is anticipated that fermentors with a capacity of a few hundred thousand gallons or more will need to be developed in order to fulfil the prospective demand for chemicals produced by fermentation. The manufacturing of industrial biochemicals does not use many of the easily washable and sterilizable parts that have been developed for the food and pharmaceutical industries. Regarding scale, there are no clear definitions or cutoff points. The biggest bioreactors for cell-culture-based biopharmaceutical production currently available have a capacity of roughly 25,000 L. (6,500 gal). This page highlights several of the issues that may occur when using large-scale industrial fermentation vessels for industrial bioprocesses (i.e., those with a capacity up to 1 million gal). Additionally, it offers advice on how large-scale systems should be designed for proper CIP and sterilisation.

The utilisation of higher plants that have undergone genetic modification and create recombinant products in their leaves, fruits, roots, or other portions is a second option.

In order to produce items like insulin, lactoferrin, trypsin, secondary metabolites, and non-pharmaceutical goods like bioplastics, molecular farming—also known as plant-made pharmaceuticals—or transgenic plants are being seriously considered.

Therapeutic proteins may be produced in the milk, urine, blood, or other bodily fluids of genetically altered animals. In contrast to recombinant plants, transgenic production animals are

very rare. One example is the recombinant protein Ruconest against hereditary angiodema, which is obtained from transgenic rabbit milk and was authorised by the Food and Drug Administration (FDA) in 2014.

As it represents over 99% of biotechnological products made from individual or adherent cells of animals, mammals, plants, fungus, yeast, and bacteria, this chapter concentrates on the first approach, which is production in a sterile container (bioreactor or fermenter).

In "large-scale" suspension culture nowadays, other microorganisms are being grown. A separate book has extensive descriptions and discussions of each of the industrial production processes. The word "large-scale" or "industrial-scale" is obviously a relative one, as was previously established before.

There are four factors that can affect the success of a large-scale suspension culture, despite the fact that the cells and organisms vary greatly in taxonomy, form, size, and metabolism:

A. The cell's genotype, which is controlled and guided by the bioreactor's physicochemical environment; this environment can be monitored using a wide range of inline sterilizable sensors.
B. The optimal culture medium has a known chemical composition and is straightforward. Additionally, it is important to consider the medium's coalescence properties, which have an impact on kLa or foaming behaviour, early on when developing a culture medium composition.
C. The cultivation conditions (T, pH, pO2, pCO2, mixing duration, and shear), which are sustained by the bioreactor's capability for heat, gas, and momentum transfer.
D. Ad hoc hardware adjustments to industrial bioreactors are typically restricted to altering only the turbines and impellers.
E. A mode of operation like batch, fed-batch, continuous, or perfusion.

The Modern Large-Scale Fermentation

The easiest method of cell cultivation among the three fundamental operating choices is batch culture, however it has the drawback of having little control over factors like growth rate. As an alternative, a batch may be fed by carefully controlling the linear or nonlinear input of a carbon source and/or other nutrients.

Fed-batch culture with much greater volumetric and specific productivities because the cell phenotype is more tightly controlled. The most advanced and effective way to cultivate cells is by chemostat culture and its variation perfusion culture, which utilises cell recycling. The most popular method is fed-batch because it combines the great productivity of continuous fermentation with the operational safety of straightforward batch culture.

A bioreactor has to be built for efficient mass, heat, and gas transport as well as quick mixing times. However, despite the necessary transfer and mixing circumstances, the bioreactor's fluid's dynamic environment shouldn't kill shear-sensitive cells, such mammalian cells.

Operations involving open ponds or other open systems are not discussed in this chapter. There are around 20 distinct closed bioreactor design variations; there are two key considerations: the first is the state of the biomass, which refers to whether the cells are growing as individual single cells ("planktonic") suspended in a culture medium or whether they need to adhere to solid surfaces or to one another. The second is the method used to maintain the movement of the culture medium. The culture medium can be kept moving by a pump or by gravitational flow in tubular or flat-plate bioreactors, which are designs used for the phototrophic cultivation of microalgae, to achieve this I by mechanical mixing, (ii) by air injected at the bottom of a reactor, or (iii) by keeping the culture medium in motion by gravity flow.

Early manufacturing using animal cells used pneumatic mixing in airlift fermenters. Some cells in higher creatures, nevertheless, were even air bubble sensitive. There were significant constraints connected with attempts to preserve the fermenting medium bubble-free. Two variations of bubble-free aeration are available. The first, more effective method employs bubble-free aeration via a membrane that is gas permeable. The second method is simpler and works by just aerating the fermenting liquid's surface. On the other hand, when scale increases, mixing and mass-transfer operations with airlift, and particularly with bubble-free culture, quickly reach their limits. Therefore, for the industrial-scale culture of mammalian cells, the continuously stirred tank reactor (CSTR) has emerged as the preferred bioreactor design. A spinning shaft with attached impellers and/or propellers of various kinds makes for an efficient but simple design. Stirred tank and airlift are the two key concepts that are summarised. Even though CSTRs are the most popular form of fermentation vessel, airlift reactors are still employed for the largest-scale fermentations.

An airlift reactor's benefit is that aeration and mixing are accomplished via the gas phase, making it an energy-efficient process. The drawback is that very huge amounts of sterile air are needed, and it is very difficult to maintain long-term sterility. Therefore, this method works best when the process conditions—like a low pH—are favourable to the cultured organism. Air lift reactors were the subject of a thorough investigation that was published in 2010.

The synthesis of single-cell proteins (SCP) by Imperial Chemical Industries, ICI, in Billingham, United Kingdom, using methanol as a carbon source, required the construction of the biggest commercial reactor of its kind ever. With an operating capacity of 2000 m3, this airlift reactor was 70 m high. The system was put in place in the late 1970s, but as a result of rising oil prices following the 1973 first oil crisis and more dramatically during the 1979–1980 second, the plan to produce SCP-based food at a low cost using fossil carbon sources was quickly abandoned and the system was decommissioned in the early 1980s.

Billingham is the sole location where SCPs are still produced. Mycoprotein is produced by Fusarium venenatum and sold under the brand name Quorn in an airlift reactor that is 40 metres high.

In the sewage treatment facility Hoechst constructed in Leverkusen, Germany, there is a comparable design on an even bigger size. Aeration and mixing are ensured by radial air nozzles

and many internal guide sleeves. In Figure 1.5, the layout of this 8000 m3 "BIOHOCH reactor" is shown.

The CSTR design has become the industry standard for microbial and mammalian cell suspension culture, despite the fact that these unique application examples show the technology's scalability and despite the fact that airlift reactors have long been employed for shearsensitive cell-culture applications. The cultivations may be ramped up from laboratory to pilot size and up to 300 m3 in completely enclosed sterile fermenters thanks to its adaptable architecture.

The Armin Fiechter group investigated and evaluated many radically different bioreactor designs in the 1980s at the Swiss Federal Institute of Technology, Zürich (CSTR, Torus or horizontal loop bioreactor, jet loop bioreactor, and compact loop bioreactor). On a predetermined medium, a reference fermentation was conducted using the Trichosporon cutaneum yeast, which is completely aerobic, nonfermentative, and insensitive to glucose. The study's findings demonstrate why the conventional CSTR, which has many turbines, has long been and continues to be the most popular reactor design.

It can quickly mix very viscous fermentation broths and achieve high oxygen transfer and heat exchange rates. Impellers are used in lieu of turbines and flow breakers in the fermenter to reduce fluid dynamic stress in shearsensitive cells. Investors prefer a solution that offers flexibility and versatility, especially in the field of white biotechnology, where a variety of different products for different markets are produced using many different organisms. Designs, tanks, and equipment for the CSTR fermentation technology are widely standardised today, and this lowers the CAPEX and operational costs.

This is sufficient justification for focusing on suspension culture in closed containments or bioreactors in this chapter and going through their extensive design and operational requirements. We would like to call attention to three instances, nevertheless, that show there are fascinating niches where biomass is not always generated in conventional steel bioreactors.

The first application, created by Joule Unlimited, Inc, involves the suspension culture of genetically altered cyanobacteria in a 4000-l tube bioreactor that absorbs CO2 for phototrophic biofuel generation. The other two have to do with tissues or cells that need strong supports to develop.To proliferate, stem cells need a surface to attach to. These cells have typically been multiplied by letting them stick to and develop on the bottom of a flat plastic T-flask that is coated with a thin coating of the culture medium.Multiple interconnected flat-bottom stacks were created to accommodate the ever-increasing demand for such cells. However, as these stacks expanded in size, managing them proved increasingly difficult. Producers were compelled to convert to cells grown on carriers suspended in disposable stirred fermenters in order to satisfy the cell counts needed for therapeutic applications. Practically, a 10 stack system can grow and harvest 10 billion stem cells, while big hyper-stacks can yield 100 billion stem cells. To obtain a batch size of >100 billion cells, adherent culture in bioreactors is required.The stirred-tank bioreactor is still the safest method for manufacturing in large quantities almost all of the cells, including stem cells. Their variety of uses has been further expanded by the introduction of commercial, larger-scale, disposable stirred tanks.

Conclusion

To understand the technical aspects of a large-scale fermentation setup and to lower the financial risks, scale up studies must be carried out prior to building a large-scale fermentation unit. Scaledown operations are crucial because they replicate the difficulties of large-scale manufacturing at a smaller, more manageable size, helping to keep scaleup research within established cost bounds. However, carrying out such investigations used to be difficult on their own.

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CHAPTER 5

FERMENTATIVE PRODUCTION OF ANTIBIOTICS AND ORGANIC ACIDS

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Abstract:

Antibiotics are substances that may either kill or halt the development of a disease-causing microorganism, or a germ, at extremely low concentrations and are produced as a byproduct of the life process of one organism. The majority of antibiotics are produced by living things like bacteria. Antibiotics are derived from bacteria, fungi, and moulds in around 90% of cases. Others are entirely or partially created synthetically. It is detailed how several organic acids important to the industry are produced by microbes. A significant fraction of the global fermentation market is made up of organic acids, and for many of them, microbiological manufacturing provides a valuable economic option for chemical synthesis.

Keywords:

Antibiotics, Bacteria, Inoculum, Organic Acids.

Introduction

Antibiotics are chemicals that kill or inhibit the growth of bacteria and are used to treat bacterial infections. They are produced in nature by soil bacteria and fungi. Antibiotics are the most widely used chemotherapeutic agents. The most commonly used types of antibiotic are Aminoglycosides, Penicillins, Fluoroquinolones, Cephalosporins, Macrolides and Tetracyclines. Five basic mechanisms of antibiotic action against bacterial cells are inhibition of nucleic acid synthesis, inhibition of protein synthesis, inhibition of cell wall synthesis, alteration of cell membranes and antimetabolite activity [1], [2].

Antibiotic compounds are used either in their natural form or as semisynthetic derivatives; the latter arc is usually produced by isolating the antibiotic nucleus and subjecting it to chemical modification. Antibiotics are produced by both fungi and bacteria but over 50% of them are obtained from Streptomyces alone.

Use of Inoculum in the Production of Antibiotics

A high yielding strain is a prerequisite for antibiotic production. Therefore, constant strain improvement is an integral part of commercial production activities. The improved strain/strains are kept in long-term storage, from which the smallest quantity is taken to initiate inoculum development.

Inoculum development begins on solid media, and subsequently liquid media are used; the media used are specific for inoculum development. The inoculum is prepared usually in form of a spore suspension, which is transferred into the fermentor by placing it in a metal vessel that is attached to the fermenter [3], [4].

The spore suspension may be blown into the fermentor by using sterile air or it may be allowed to run in under gravity. As a rule, the number of stages between the preserved material and the final inoculum stage is kept to the minimum to minimise the risk of the organism losing its high yield potential.

Use of Fermentor in Production of Antibiotics

Antibiotics are generally produced in stainless steel fermentors (30,000-200,000 1 medium volume) used in the batch or fed-batch mode. Agitation is mostly by impellers, but air-lift system is also used. Water cooling is often used to maintain the temperature between 24-26°C for most antibiotic producers [5], [6].

Generally, the fermentor is maintained at above atmospheric pressure; this reduces contamination risk and enhances O_2 supply in the medium. Sterile air is supplied as per need, and for some processes, e.g., penicillin production, materials need to be added throughout the process. In most cases, it is critical to prevent contamination, and suitable cleaning procedures between fermentor runs must be adhered to.

The final stage fermentor is preferably used for antibiotic production for the longest possible period. But the initial stages of fermentation are designed for considerable microbial growth; typically these are carried out in seed-stage fermentors of smaller size.

One or more seed-stages may be used, depending on the process and the strain, to produce the maximum amount of biomass in the correct physiological state for high antibiotic production when introduced in the final stage fermentor.

Medium used for Antibiotic Production

Antibiotic production employs a variety of media, a different one for each stage of operation (Table 40.2). A considerable research effort is directed at developing seed-stage and production media to reduce costs and to enhance yields. A typical production medium has about 10% (w/v) solids.

Generally, yields are much higher on complex media. In some cases, a suitable precursor for the antibiotic is also provided, e.g., for penicillin G production, phenylacetic acid or phenoxyacetic acid is used as precursor. Since the antibiotics are secondary metabolites, the production medium is so designed that a key nutrient becomes limiting at a critical stage to initiate the secondary metabolism in the organism. The nutrient to be made limiting depends on the process, e.g., glucose for penicillin production and phosphate for several antibiotics produced by Streptomyces.

The initial step in antibiotic recovery is separation of cells from the broth; this is usually achieved by filtration or centrifugation. But in some cases, the whole, broth is used for extraction. Antibiotic isolation and purification employs solvent extraction, ion exchange, ultrafiltration, reverse osmosis, precipitation and crystallization.

Literature Review

In a study by Elander *et al.* over the last 50 years, significant advancements in fermentation technology and the productivity of the producer organisms Penicillium chrysogenum and Acremonium chrysogenum (syn. Cephalosporium acremonium) have led to increased production and significant cost savings. Today, it is anticipated that major fermentation manufacturers would achieve harvest titers of 20–25 g/l for cephalosporin C and 40–50 g/l for penicillin. Penicillin G or Penicillin V recovery yields are now >90%. For 6-aminopenicillanic acid or 7-aminocephalosporanic acid, chemical and enzymatic hydrolysis process technology is likewise very efficient (between 80 and 90%), with new enzyme technology contributing to significant cost reductions over the last ten years. Both cephalosporins and penicillins are still primarily produced in Europe. However, more bulk manufacturing is relocating to the Far East as a result of rising labour, energy, and raw material prices, with China, Korea, and India emerging as key production hubs while dosage form filling gains ground in Puerto Rico and Ireland[7].

The most successful agro-industrial waste, maize bran, impregnated with aminoglycoside production medium, yielded the highest paromomycin concentration (0.51 mg/g starting dry solids) after screening 6 different substrates. El-Housseiny *et al.* carried out an investiogation in which they used D-optimal design to optimise the environment led to a 4.3-fold increase in paromomycin concentration, reaching 2.21 mg/g starting dry solids at a pH of 8.5, inoculum size of 5% v/w, and temperature of 30 °C. Solid state fermentation, as compared to submerged liquid fermentation, produced equivalent paromomycin concentrations, lower raw material costs, less energy usage, and less waste water discharge. These results have significant ramifications for industrial fermentation. In order to produce paromomycin, solid state fermentation represents a possible alternative to submerged liquid fermentation. This is the first publication we are aware of on the enhanced solid state fermentation technique for paromomycin synthesis.

Sariola *et al.* provided an ethnographic emphasis is on a group of Finnish sourdough bakers, with a particular eye on a fermentation workshop in 2019. Emerging human-microbe relationships were shown in this session, drawing attention to a future without antibiotics. An approach that places microorganisms in the spotlight as the main players and situates it in the political context of rising populism and the Anthropocene is necessary to study bread manufacturing. In this essay, I discuss bakers who criticise capitalism by connecting ecological extraction, political oppression, and industrial food production. These bakers are known as "microbiohackers." I utilise the concept of diffraction to highlight the intricate relationships between the microbial/material and social/political, offering an alternative to the prevalent public health narrative that portrays bacteria as a danger and antimicrobial resistance. In the hands of bakers, sourdough, fermentation, and antimicrobial resistance provide a chance to challenge dualistic views of microorganisms and to forge emerging post-antibiotic futures.

Because there is a chance that remaining antibiotics may lead to the development of drug resistance, antibiotic fermentation residues (AFRs) must be disposed of safely. AFRs contain so much colloidal water that it is difficult to extract it using mechanical means, necessitating energy-intensive dewatering. The wet AFRs, in which colloidal water was transported into the hydrate of the DA and produced solid AFRs-DA combination at room temperature, may be

treated using dehydration agents (DAs). Guo *et al.* carried out an investigation of the AFRs' dehydration kinetics was done in this research. The findings demonstrate that the dehydration followed the first-order kinetic equation, with temperature, feed ratio, and DA particle size being the key determinants. Additionally, the feed ratio and temperature were used to determine the quantitative link between kinetic parameters, giving the fundamental information needed to further reduce the energy required for AFR disposal.

Bhargav *et al.* discussed the benefits of solid-state fermentation over submerged fermentation in the production of various value-added products are discussed in the study by Bhargav *et al.*along with significant aspects of various bioreactor designs, recent advancements in the use of various agro-industrial residues as substrates, and the significance of mathematical modelling. The use of SSF in manufacturing is beneficial and suited for the manufacture of numerous value-added goods, such as enzymes, antibiotics, and organic acids, thanks to advancements in modelling and optimization methods. This method lowers the cost of the production process while simultaneously lowering the price for customers[8].

Vo *et al.* discussed that Antibiotic synthesis and structure are the end products of multileveled regulation involving a network of several processes, intermediary steps, and molecules. The right combination of nutrients must be present for antibiotic synthesis. The most crucial sources that control secondary metabolism differently are carbon, nitrogen, and phosphorus. Through a mechanism known as carbon catabolite suppression, the carbon supply affects secondary metabolism by inhibiting the transcription of the biosynthetic genes, which prevents the creation of antibiotics. By using a mechanism known as nitrogen metabolite repression or nitrogen catabolite repression, nitrogen controls the production of antibiotics. This mechanism allows for the preferential use of easily assimilable nitrogen sources while only using secondary nitrogen sources when the primary substrate has been used up. It is nevertheless possible that phosphate supplies regulate the generation of antibiotics by influencing the activities of enzymes engaged in secondary metabolism, despite the fact that knowledge about the mechanism regulating phosphate is quite limited. The synthesis of antibiotics may be enhanced by the gene transfer between fungus and other microbes[9].

In this study, Xia *et al.*compiled the most current findings on the regulatory cascades that control the synthesis of antibiotics in Streptomyces at the four levels of signals that initiate biosynthesis, global regulators, pathway-specific regulators, and feedback regulation. By rewiring the regulatory networks, such as by the overexpression of positive regulators, inactivation of repressors, fine-tuning of the feedback, and ribosome engineering in Streptomyces, the production of antibiotics may be significantly increased. Because of the vast diversity and widespread distribution of biosynthetic gene clusters in Streptomyces genomes, the large quantity of genomic sequencing data suggests that the genus has the capacity to create far more antibiotics. The majority of these gene clusters have cryptic definitions for unidentified or undetectable natural products. By modifying the regulatory genes, it has been possible to effectively activate cryptic gene clusters in the age of synthetic biology. To unlock the potential of cryptic gene clusters, techniques including ribosome engineering, chemical elicitors, and regulatory gene rewiring have been used. These have been suggested as the most successful

method for finding novel antibiotics. We suggested that a deeper knowledge of the regulatory mechanism governing antibiotic synthesis will substantially aid in the identification of novel antibiotics and the optimization of industrial strains for the complex regulatory network in Streptomyces[10].

Discussion

Biotransformation is routinely used for commercial production of several useful antibiotics. Semisynthetic antibiotics, e.g., semisynthetic penicillin and cephalosporins, are produced by chemical modification of the penicillin nucleus, e.g., 6-aminopenicillanic acid. The penicillin nucleus is produced by microbial deacylation of naturally produced penicillin G and penicillin. One of the best instances of biotechnology is the industrial manufacture of -lactam antibiotics by fermentation during the last 50 years. The main biotechnology products in the world today are -lactam antibiotics, notably penicillins and cephalosporins, with global dosage form sales of US$ 15 billion, or 65% of the global antibiotics market. Chemicals called antibiotics are used to treat bacterial illnesses because they stop the development of germs or kill them. They are created in the natural world by fungus and soil bacteria. The most often used chemotherapy drugs are antibiotics. Aminoglycosides, Penicillins, Fluoroquinolones, Cephalosporins, Macrolides, and Tetracyclines are the antibiotic classes that are most often used. Antibiotics work against bacterial cells via five main mechanisms: suppression of protein synthesis, change of cell membranes, inhibition of cell wall production, inhibition of nucleic acid synthesis, and inhibition of antimetabolite activity.

The most plentiful antibiotics and other bio-active natural compounds, which are extensively employed in the pharmaceutical and agricultural industries, are produced by streptomyces. Typically, they are biosynthesized by secondary metabolic pathways that are encoded by cluster-located genes. And intertwined transcriptional regulatory cascades tightly control these gene groups. The understanding of the regulatory mechanisms behind antibiotic synthesis in Streptomyces has made significant strides in recent decades.The development of bacteria without a freely flowing aqueous phase is known as solid-state fermentation (SSF). For the production of value-added goods such antibiotics, single-cell proteins, polyunsaturated fatty acids, enzymes, organic acids, biopesticides, biofuel, and fragrance synthesis, the SSF is an alternative to submerged fermentation. However, it has been shown that SSF offers more benefits than submerged fermentation in a number of different procedures.

A class of antibacterial drugs known as penicillins are made by the fungi Penicillium notatum and Penicillium chrysogenum. Alexander Fleming made the first discovery of the penicillin-producing bacterium in 1926. The first significant commercially significant product created by an aerobic, submerged fermentation was penicillin. Penicillin, like other antibiotics, is created exclusively during the stationary phase since it is a secondary metabolite. Gram-positive bacteria that are growing aggressively are susceptible to penicillin. Pseudomonas aerugionsa is the only gram-negative bacterium that certain penicillins, such amoxicillin, are effective against.

Antibiotics may be made using industrial microbiology using the fermentation method, in which the source microbe is cultured in large containers (100,000–150,000 litres or more) with a liquid

growth medium. The right quantities of nutrients, oxygen concentration, temperature, and pH must be maintained; these variables are continuously watched and modified as required. Because antibiotics are secondary metabolites, population size has to be carefully managed to ensure that the highest yield is acquired before the cells are destroyed. The antibiotic must next be extracted and refined until it is a crystalline product, which is the last step in the procedure.

By cultivating the fungus Penicillium chrysogenum or Penicillium notatum, penicillin is commercially generated in the industry. Prior until now, the surface processes of surface liquid and solid state fermentation were used to produce penicillin. Currently, fed batch processing is used for commercial production.

To prepare the inoculum, strongly sporulated working stocks' spores are suspended in water or non-toxic wetting agents (Sodium sulfonate 1:10000). Following that, these spores are placed to a flask containing wheat bran and a nutritional solution for heavy sporulation. 5-7 days of incubation at 240C. After that, spores are moved to a seed tank and incubated there for 24 to 48 hours at 240°C with agitation and aeration to ensure adequate mycelial development. The fermentation process may employ these mycelia.Fermenters are used with stirred tanks or air lift tanks. PH is regulated to 5.5–6.0, which rises to 7–7.5 (optimal) owing to ammonia gas release and lactic acid consumption. CaCO3, MgCO3, or phosphate buffer is added if the pH is 8 or above. The temperature used to produce penicillin is 280C. The production of penicillin in a bioreactor is limited by the oxygen supply. The range of aeration speed is 3.0-1.5m3. Penicillin production is mostly dependent on biomass generation, hence a high biomass content in the vessel is ideal. By ratcheting up the agitation rate and intensity, it is accomplished.

Penicillin G is present in harvested culture broth along with a number of other metabolites. Mycelium is separated from the broth using a vacuum filter. Penicillin undergoes anionic form conversion at low pH levels (2.0-2.5). By adding phosphoric acid or sulfuric acid, the pH may be lowered. Active charcoal is used to treat a solvent that contains penicillin to remove colour and other impurities. By adding sodium hydroxide, the product is again extracted from the solvent and placed in water. After that, sodium or potassium penicillin is created by crystallising the penicillin product.The bacterium Streptomyces griseus produces the antibiotic streptomycin, which is effective against gram-negative bacteria as well as the TB bacterium, Mycobacterium tuberculosis. However, it has been shown to be effective in treating infections brought on by gram-positive bacteria that are particularly resistant to penicillin. As it affects plants systemically, it is also helpful in preventing bacterial plant diseases.

As a stock culture, S. griseus spores are kept as soil stocks or lyophilized in a carrier such sterile skimmed milk. In order to start liquid culture build-up of mycelial inoculum in flasks or inoculum tanks, the spores from the stock cultures are next transferred to a sporulation medium. This provides enough sporulated growth to launch liquid culture. When the mycelium has grown enough, it is fed to the production fermenter. Carbon source and nitrogen source are both present in a manufacturing medium. One of the finest carbon sources for increasing streptomycin production is glucose. Fructose, maltose, lactose, galactose, mannitol, xylose, and starch are other sugars that may be utilised as carbon sources in addition to glucose. As a source of nitrogen, you may utilise peptone, soy extracts, meat extracts, and leftovers from alcohol

distillation, ammonium salts, nitrates, and glycine. As growth-promoting substances, phenylacetic acid and L-naphthalene acetic acid may be added. It is also preferable to add proline to the medium, since this promotes the formation of high levels of streptomycin. Along with glucose, fats, oils, and fatty acids may also be employed.

The fermenter is fed a sterile liquid media. A suitable amount of inoculum is added to the fermenter. The ideal pH range for fermentation is between 7.0 and 8.0, while the ideal fermentation temperature is between 25 and 300C. Three steps are involved in the highly aerobic fermentation, which lasts between five and seven days.

It takes between 24 and 48 hours. There is a quick increase in size and mycelium production. Here, the ammonia released into the medium and the proteolytic activity of S. griseus cause the pH to increase to 8.0. Slow glucose uptake results in less streptomycin production.

For two days, it lasts. Rapid generation of streptomycin takes place in the absence of increased mycelial growth. Utilizing the ammonia generated during the first process, the pH is lowered to 7.6–8.0. Large amounts of glucose and oxygen are needed at this stage.

Here, cells lyse, release ammonia, and the pH rises before falling again over an extended time during which streptomycin is continuously produced. Oxygen needs decrease together with the medium's sugar content's exhaustion. Production of streptomycin finally comes to an end. Streptomycin is produced at 1200 micrograms per millilitre. After fermentation is finished, filtering is used to remove the mycelium from the broth. Several processes are used to recover streptomycin. The fermentation broth is often acidified, filtered, and neutralised. The streptomycin in the soup is subsequently removed by passing it through a column containing cation exchange resin. The antibiotic is then eluted from the column using hydrochloric acid, cyclohexanol, or phosphoric acid after the column has been cleaned with water. Then, it is vacuum-concentrated at 600C. The streptomycin is then dissolved in methanol, filtered, and precipitated with acetone using the filtrate. The precipitate is vacuum dried after being rinsed with acetone once more. It is further refined by methanol solution. The calcium chloride complex is used to extract streptomycin in its purest form. The production of vitamin B12, which is a by-product, has no negative effects on the streptomycin output.

The first vinegar was spoiled wine. Glacial acetic acid is the purest form of acetic acid (99.98%). Vinegar is a product of acetic acid. It is a colourless liquid organic complex with a strong scent. A certain set of individuals is responsible for the commercial manufacture of acetic acid. Two genera of acetic acid bacteria are recognised. Gluconobacter that only converts ethanol to acetic acid during oxidation. Acetobacter that turns ethanol into acetic acid, CO2, and water before oxidising it further. Gram-negative bacteria are those that produce acetic acid. For instance, A. aceti, A. peroxidans, and A. pasteurianus. The result of insufficient ethanol oxidation is acetic acid. Alcohol dehydrogenase first converts ethanol to acetaldehyde, which is subsequently hydrated to generate acetaldehyde hydrate. Acetaldehyde dehydrogenase then reacts with the latter to produce acetic acid.

Acetic acid is created for every ethanol molecule that is oxidised. Therefore, high yielding strains may convert 12% alcohol to 11–12% acetic acid. A sufficient supply of oxygen is crucial for

optimum manufacturing. Microorganisms perish as a consequence of a lack of oxygen and a high concentration of alcohol and acetic acid. Acetic acid may be made by the surface fermentation or submerged fermentation processes.

Vinegar Vinegar is an aqueous solution with trace amounts of alcohol, salts, sugars, and esters as well as acetic acid, which makes up around 4% of its total volume. It is often used as an additive to flavour processed liquid foods like sauces and ketchup. Wine, whey, and malt are the primary ingredients used to make vinegar. Surface technique (using a trickling generator) or submerged process are also options for producing vinegar.Spraying the fermentation material over the surface allows it to seep through the shavings that are home to the acetic acid-producing bacteria. On the top section, the temperature is around 300°C, while on the bottom part, it is about 350°C. It takes roughly 3 days to manufacture vinegar.

Stainless steel is used to construct the fermentation bioreactors. A suction pump at the top provides aeration. About ten times as much is produced in the submerged process as there is in the surface process.Acetobacter aceti, a bacterial species, is employed as a bioreactor. Vinegar is really produced by two fermentation processes: the first uses yeast to create alcohol from sugar, and the second uses acetic acid bacteria to convert ethanol to acetic acid through acetaldehyde. The aerobic fermentation required for the microbial conversion of ethanol to acetic acid is high. For the purpose of producing vinegar commercially, acetobacter bacteria are used. Gluconobacter and Acetobacter are the two types of bacteria that make up the genus Acetobacter. Gluconobacter converts ethanol to acetic acid, while Acetobacter first converts ethanol to acetic acid, then to CO2 and water. The Acetobacter aceti and A. pasteurianum species are those that are utilised economically. Similar to this, Gluconobacter oxydans and its subspecies are used to make commercial vinegar. The process of turning ethanol into acetic acid involves two oxidation stages. In the first stage, ethanol is oxidised to acetaldehyde in the presence of NAD or NADP, and under the catalytic activity of the enzyme alcohol dehydrogenase, acetaldehyde is converted to acetic acid in the second step.

Two processes, surface fermentation and submerged fermentation, are used to manufacture acetic acid for commercial use. The amount of nutrients utilised in submerged fermentation is typically five times more than those used in surface fermentation.It is constructed of wood, has a maximum internal capacity of 60 m3, and contains birch wood shavings lining the inside surface. The generator's starting ingredient, ethanol, enters from the top and trickles through birch wood shavings containingbacteria into the bottom basin, which is where the partly transformed solution is cooled and pushed back up to the top of the generator to be re-passed through. Up until 88–90% of the alcohol is converted to acetic acid, this procedure is repeatedly repeated. To promote the best possible development of Acetobacter, the starting material must include both acetic acid and ethanol. Currently, better yielding strains are used in the fermentation of vinegar that may produce 13–14% acetic acid.

Mashes were first used in the early phases of submerged fermentation, which often do not need aeration. But at the moment, high yielding materials that can produce 13% acetic acid are used. Nevertheless, the process with such high yielding material needs a lot of aeration, up to 50m3 oxygen. Employed are stainless steel fermenters, which are agitated from the bottom. A suction

rotor is used to produce aeration, and air is drawn in by a pipe that descends from the top of the vessel. Along with foam removers, a heat exchanger is offered to regulate the temperature. Up to 35 hours of fermentation time are spent at a temperature of 400C. In a completely continuous operation, the acetic acid production is over 98%.

Because there are bacteria present, submerged fermentation produces murky vinegar. Filtration is used to make it clear. Typically, plate filters and filter aids are used. If necessary, the finished product is decolored using K4 [Fe (CN) 6] after filtering.The most prevalent weak organic acid, or citric acid, is a naturally occurring substance found in lemon fruits. Citric acid manufacturing is an industrial process that uses raw materials including substrates, microbes and enzymes that promote citric acid development, among other things, to produce citric acid for commercial use. In most cases, the fermentation process is used to produce them commercially. Citric acid is highly demanded in the food, pharmaceutical, and cosmetic industries as well as other sectors including hygiene and cosmetics, giving commercial production of the substance a great deal of significance. The John and Edmund Sturage Company, in the United Kingdom, was the first to produce citric acid for use in commerce in the year 1826. Both natural and artificial processes are used to produce citrus acids. Citrus trees like the lemon, orange, and others are used to naturally manufacture citric acid. Enzymes and biological fermentation by microorganisms are used in the chemical synthesis of citric acid.

As a main metabolite, bacteria make citric acid. Due to faulty citric acid or krebs cycle, citric acid is produced during tropophase cell development. When the Krebs cycle is faulty, a large quantity of sugar is carried by the EMP route, forming acetyl-CoA. Citrate synthase, an enzyme, aids in the condensing of acetyl-CoA with oxaloacetic acid to produce "citric acid." Therefore Deactivating Krebs cycle enzymes like the enzyme Aconitase/Isocitrate Dehydrogenase, which may further degrade citric acid, is necessary for the synthesis of citric acid.The Koji method is often referred to as "Solid- State Fermentation." Japan is where this method was initially made available. It has to do with producing citric acid from agricultural and industrial waste. You may utilise raw materials like apple pomace, sugar cane, beet molasses, and more. These sources of food were used by *Aspergillus niger*. The raw material is modified to have a pH of 4-5 and a moisture content of 70%. Following cooling at 30-600C, A. niger is injected into the raw materials. After inoculation, the medium is put into big trays, which are 3-5 cm deep, and incubated for three to seven days at 25 to 300 C. Finally, the citric acid was removed from the fermenting tank. The *Aspergillus niger* amylase enzyme converts the input material's starch content into citric acid. The Koji method does not need pre-treatment of the substrate since the synthesis of citric acid is unaffected by trace elements.

The "Submerged Culture Fermentation" method is another name for submerged culture. Approximately 80% of citric acid is produced utilising this submerged fermentation process. Black Aspergillus japonicus is used in submerged fermentation. The procedure is carried out in a stainless steel bioreactor that includes a cooling system, impellers, enough aeration, etc. Carbon sources include materials like maize starch and beet molasses. Ammonia is utilised as the source of nitrogen. The substrate that is employed must undergo pre-treatment, such as nutrition addition or sterilising, among other things. A. japonicus was added to the culture medium, and

the temperature was maintained at 300C. The majority of submerged fermentation occurs in a batch bioreactor, which can convert 2500 kg of glucose and 860 kg of oxygen into 1500 kg of citric acid and 500 kg of biomass.The term "Liquid Surface Fermentation" is another name for it. The earliest technique for producing citric acid was surface culture fermentation, which was established in 1919. When doing liquid surface fermentation, a culture medium with a pH of 5–6 is introduced to aluminium shallow trays to a depth of 5–20 cm. This procedure is carried out in a fermentation chamber, which preserves relative temperature and humidity while providing consistent air circulation. A. niger spores are first blasted over the surface of the culture medium for approximately 5 to 6 days, and then dry air is blown over the surface. The medium's pH is adjusted to be between 1.5 and 2.0. The spores began to germinate after 24 hours, and the development of white mycelium can be seen on the surface of the culture media. The residual liquid is removed from the mycelial mat after the mould has used all the sugar content. A. niger produces citric acid as one of its main metabolites throughout the surface culture phase.

The result of fermentation is fermented liquor, which has a hazy appearance becausedue to the presence of mycelia, antifoaming chemicals, etc. Calcium hydroxide, or Ca (OH) 2, is employed as a slurry to separate these components, resulting in a calcium citrate precipitate. The precipitated calcium citrate is filtered and cleaned. Following filtering, it is treated with H2SO4 to cause calcium to precipitate as CaSo4. After successively leaving the ion exchange bed, calcium sulphate is subsequently treated with activated carbon, which causes it to become demineralized. The resulting solution is put through crystallizers that circulate. Centrifugation is used to separate the crystals that have formed as a consequence of crystallisation. After that, the leftover solvent is dried, sieved, and finally packaged.

Conclusion

Antibiotics are necessary for the successful completion of complex surgical operations including organ and prosthesis transplants as well as for the treatment of infectious infections. Antibiotic-resistance mechanisms place a significant clinical and financial burden on healthcare systems across the globe. Despite the issue of antibiotic resistance in pathogenic bacteria, little is known about the variety, distribution, and sources of resistance genes, particularly for the uncultivable bulk of environmental bacteria. Both antibiotics newly licenced for use in humans and those that have long been considered the gold standard of care are subject to significant levels of resistance.

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CHAPTER 6

MICROBIAL PRODUCTION OF VITAMINS AND AMINO ACIDS

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Abstract:

There are several steps in "Microbial production of vitamins and amino acids". In this regard, "Microbial production of vitamins and amino acids" are crucial for the production process in food, water, pharmaceuticals industry as well as in industrial processes. Amino acids are considered as building blocks in a protein structure. In this regard, if this protein is being hydrolyzed the requisite amino acids can be isolated. Amino acid production is important in the food industry as well as in other industrial production processes.

Keywords:

Amino Acids, Microbial production, Vitamins, Vitamin B12.

Introduction

Vitamins are important for the production of medicines as well as other products with industrial importance. Vitamins that have been produced by using organic synthesis methods frequently require several reactions in an Industrial environment. The steps for "Microbial production of vitamins and amino acids" include the following.Vitamin B12 (cobalamin), one of the most intriguing and fascinating molecules in science and medicine, was first identified as the agent that prevented pernicious anaemia in the early 1920s when two American doctors, Minot and Murphy, showed it could treat the condition with a diet that included raw liver. Pernicious anaemia was a disorder that was first described in 1835. Only two enzymes, methionine synthase and (R)-methylmalonylCoA mutase, need the vitamin in humans; it is only needed in trace amounts (roughly 1 mg/day). Nevertheless, more than 10 t of vitamin B12 are commercially produced each year from a variety of bacterial species. For substances of the cobalamin group, the phrase "vitamin B12" is frequently used. There are three natural types of cobalamin: adenosylcobalamin, methylcobalamin, and hydroxocobalamin.Only fermentation is a source of vitamin B12 in nature. To fulfil yearly requests across the globe, it is made by a variety of pharmaceutical businesses. In 1952, Merck started using Pseudomonas denitrificans to produce vitamin B12. Since then, genetic engineering and microbial screening have increased the culture's effectiveness more than 30 times compared to the results of the original soil isolates. As a byproduct of the fermentation of the antibiotics (neomycin, chlortetracycline) Streptomyces, vitamin B12 was initially obtained for use in human therapy and as a food or feed supplement. From sewage sludge and manure, beneficial strains were also isolated[1], [2].

Improved activity has been shown after mutagenic treatments, however in each instance cobalt ions and 5, 6-dimethylbenzimidazole (5, 6-DMBI) must be added in addition to the precursors

like glycine, threonine, and aminopropanol. A variety of microorganisms have been used over the past two to three decades to produce vitamin B12 in an effective manner[3], [4].

Amino acid production

Microbial amino acid production involves direct fermentation method, conversion of intermediates of metabolic process to amino acids, using different microbial enzymes as well as immobilized cells. Using direct fermentation methods, amino acid production can be performed by using the amino acids produced by microorganisms from several carbon sources like alkenes, glucose, fructose, glycerol, and propionate. In this regard, byproducts like molasses as well as scratch hydrolysates can be used. By using different fermentation methods these amino acids can be isolated. On the other hand, these microorganisms can be used to carry out certain metabolic processes for amino acids productions, like the production of serine by using glycine. Furthermore, immobilized cells, crude cell extracts as well as reactors of enzyme-membrane can be used for amino acid production. In this regard, certain dehydrogenase enzymes isolated from Bacillus megaterium and other organisms are used to produce several L-amino acids. Industrial amino acid production is maintained by pH, nitrogen, carbon sources, and temperature. “Microbial production of vitamins and amino acids” is the crucial area that deals with the development of knowledge regarding different types of microorganisms.

L-Glutamic acid

L-Glutamic acid can be produced from Corynebacterium glutamicum traditionally. On the other hand, another organism that is employed is Brevibacteriam. In this regard, glucose is generally broken down to phosphoenolpyruvate and then to pyruvate in glycolysis. Phosphoenol pyruvate can be converted to oxaloacetate using biotin as a cofactor. Furthermore, using Krebs cycle α-ketoglutarate dehydrogenase is produced and converted to glutamic acid using glutamate dehydrogenase[5]–[8].

L-Lysine

Glucose is converted to phosphoenolpyruvate and pyruvate using glycolysis and PEP is further converted to Oxaloacetate by reacting in the Krebs cycle. Oxaloacetate can form aspartate by transamination. Aspartate semialdehyde can be converted to L-lysine using several enzymes. In order to produce L-lysine industrially several strains of B. flavum or C. glutamicum can be used.

Production of Vitamins

There are several types of vitamins as well as amino acids that are4 produced by microorganisms such as L-glutamic acid, L-Lysine, L-Threonine, L-Phenylalanine, L-aspartic acids, Vitamin E, Vitamin B12, Vitamin K.

Vitamin B12

It is generally produced by fermenting Propionibacterium and Pseudomonas. On the other hand, Propionibacterium shermanii can be used to produce vitamin B12 in two steps. In step one growth and production, intermediates are produced and in step two vitamins B12 are produced from corn steep liquor, CoCl2, and glucose.

Vitamin C

There are several metabolic and genetic engineering processes that have been developed widely to produce 2-Kolagen from different microorganisms like Erwinia herbicola, G. Oxydans, Pseudomonas putda

Vitamin D

Vitamin D or ergosterol can be produced by using yeasts such as Saccharomyces cerevisiae, S. uvarum. In this regard, for the production of concentrates of vitamins fish oils are employed in order to extract vitamin D3 directly. Furthermore, *Saccharomyces cerevisiae* can be bioengineered in order to enhance the accumulation of the vitamin through increasing the overexpression of several enzymes that are involved in the biosynthetic pathway. In this case, molasses is used as a carbon source.

Vitamin A

Several engineering approaches have been developed to produce carotenoids or vitamin A in different organisms, In this regard, *Saccharomyces cerevisiae* and Candida utilis are modified in order to express genes for producing carotenoids On the other hand, E. coli. Can also be bioengineered in order to produce carotenoids.

The biological production of vitamins has been developed by determining microbes that are capable of producing these industrially important enzymes. On the other hand, microbial amino acid production involves three types of methods that include extraction of amino acids and chemical synthesis of amino acids.

Literature Review

Revuelta *et al.* discussed aboutMicronutrients known as folates (vitamin B9) serve as cofactors in one-carbon transfer processes that are important in the production of nucleotides and amino acids. Important disorders including cancer, anaemia, cardiovascular problems, or neural tube anomalies are linked to folate deficiency. According to epidemiological statistics, folate insufficiency is still quite common in many communities. Folic acid supplementation, or the fortification of food with synthetic folic acid, is now required in many industrialised nations. However, potential alternatives to folic acid supplementation include the biofortification of folate in dairy products and staple crops, as well as the synthesis of folate utilising metabolically engineered microbes. In order to establish an economically viable technique for the biotechnological synthesis of the vitamin, we will now analyse the existing approaches to increasing the production of folate in microorganisms[9].

Bioactive substances are a group of substances that, when consumed by humans or other animals, have specific effects. Since many bioactive substances have positive effects on human health, they are becoming more and more popular in modern society. This increased interest encourages the creation of novel production techniques that are more effective than the conventional methods, which rely on chemical synthesis or extraction from natural tissues. One of the newest methods of production is biotechnology-based generation, which uses fermentation

of genetically modified microbes as an alternative to the existing production processes. This method has already been used to offer several bioactive substances to industry. In a study by Wu *et al.* they focused on some common bioactive substances produced by microbes, such as polyphenols, polysaccharides, amino acids, and vitamins. We also go through several microbial systems' inherent drawbacks and highlight potential future possibilities for system improvement[10].

Jannathulla *et al.* discussed on about Algae, bacteria, fungus, and yeast are the primary sources of many microbial products in terms of isolates, whole-cell biomass, and living organisms via the fermentation process. They include a variety of minerals and vitamins and have a balanced amino acid profile. However, compared to the other three microorganisms, the essential amino acid index (EAAI) of the fungal-based microbial meal was significantly lower (0.57–0.67). (0.77–0.90). Based on the nucleic acid composition of the microbial products produced, bacteria were used more often than algae, fungi, or yeast. In 2017, the market for microbial products was estimated to be worth US$5.3 billion. This market is expected to grow by 8.6%. They might replace fishmeal in aquatic species' meals by 25–50%. Despite this, they may manage infectious illnesses in a variety of aquatic species and serve as a strong immunostimulant, growth booster, and disease controller. The current assessment reiterates the use of microbial products in addressing problems faced by the worldwide aquafeed sector, particularly the need for fishmeal. However, in order to overcome the current constraints and make genetic engineering and fermentation technology economically viable, innovative ways must be devised.

Discussion

The creation of strains of microbes for better amino acid synthesis is briefly detailed, along with some general thoughts on production techniques. One or more of the three following procedures are used to produce amino acids in commercial quantities:The components of proteins' structure are amino acids. The necessary amino acids, such as cysteine, tyrosine, and leucine, may be extracted by hydrolyzing the proteins.A combination of D- and L-amino acids is produced during chemical synthesis. The majority of the amino acids used for industrial applications belong to the L group. However, chemical processes are utilised to synthesise glycine (optically inactive) and a few other amino acids that may be used in L- or D-form for specific applications (D, L-alanine, D, L-methionine).Microbiological techniques are used for the large-scale manufacture of amino acids. There are three methods to choose from. Amino acids may be generated by microbes directly using a variety of carbon sources, such as glucose, fructose, alkanes, ethanol, glycerol, and propionate. Molasses and starch hydrolysate, two industrial byproducts, may also be used. With little effectiveness, methanol is used as a cheap carbon source in the manufacture of amino acids.

(a) Conversion of metabolic intermediates into amino acids: In this method, specific processes for the synthesis of amino acids, such as the conversion of glycine to serine, are carried out by microorganisms.

(b) Direct use of microbial enzymes or immobilised cells: Amino acid synthesis may sometimes be carried out using immobilised cells, crude cell extracts, immobilised cells, resting cells, or

enzyme-membrane reactors. Below are a few examples. For the amination of -keto acids to form L-amino acids such as alanine (from pyruvate), leucine (from -ketoisocaproic acid), and phenylalanine, some bacteria, such as Bacillus megaterium, have amino acid dehydrogenases (from phenyl pyruvate). You may utilise enzyme-membrane reactors or immobilised cells. For the synthesis of a number of additional amino acids, including tryptophan, tyrosine, lysine, and valine, enzymes or immobilised cells are also used.

Microorganisms use metabolic pathways that are tightly regulated and run efficiently to synthesise amino acids. As a result, an overproduction of amino acids in nature is unusual. It has been possible to identify several strains that excrete specific amino acids, such as glutamic acid, alanine, and valine.A way must be found to get rid of the metabolic regulatory/control mechanisms in order for a microorganism to produce an excess of any amino acid. In reality, mutagenesis and screening programmes have produced a number of amino acid-producing microbes.The main methods of strain formation are as follows. In fact, a novel strain for the production of amino acids is effectively developed by combining numerous techniques.A lack of the production of regulatory end products distinguishes these mutants as being auxotrophic (i.e. repressor or regulatory effector).

The metabolic pathway intermediates build up and are eventually eliminated.Genetic recombination: Mutants with excessive amino acid synthesis may be created by genetic recombination. Certain bacteria, such as Corynebacterium glutamicum and Bacillus flavum, employ protoplast fusion to create hybrids.For the generation of new strains, traditional genetic engineering approaches may be used. There have been generated strains with increasing activity of rate-limiting enzymes. In one method, the genes for the manufacture of amino acids such glutamic acid, lysine, phenylalanine, and valine were increased using E. coli and the cloning vector pBR322.Improved strains of microorganisms may be produced by analysing the genomes of both wild and mutant strains. When the whole chromosomal sequence of an organism (such as C. glutamicum or E. coli) is known, attempts may be made to carry out genetic alterations to effectively overproduce the necessary amino acids.

In turn, the fermentation activities may be detected using chip technology.The first amino acid to be generated by microbes was glutamic acid, or L-glutamine. Even now, *Corynebacterium glutamicum*, the initial bacterium used to produce glutamic acid on a big scale, is still being utilised effectively. The other significant organisms utilised to produce glutamic acid, despite their lower output, are from the genera Microbacterium, Brevibacterium, and Arthrobacter.These species are all similar to C. glutamicum in terms of morphology and physiology. Glutamate dehydrogenase activity is high in bacteria that produce glutamic acid, but -ketoglutarate dehydrogenase activity is low. The vitamin biotin is also necessary for them.

For the strains to manufacture and excrete glutamic acid in increasing amounts, many changes have been achieved, especially in *C. glutamicum*. These include lysozyme-sensitive mutants with excellent yields as well as strains that can withstand high biotin doses.Phosphoenol pyruvate and pyruvate are the results of the breakdown of glucose. Acetyl CoA is made from pyruvate. Oxaloacetate may be produced on its own when phosphoenol pyruvate is converted to it by the

enzyme phosphoenol pyruvate carboxylase. Both of these very important carboxylation processes need the cofactor biotin.

The classic citric acid (Krebs) cycle events, which come next, are responsible for producing the important metabolite -ketoglutarate. In the regular citric acid cycle, the enzyme ketoglutarate dehydrogenase reacts with -ketoglutarate to produce succinyl CoA. Glutamate dehydrogenase converts -ketoglutarate to L-glutamic acid for the purpose of producing glutamic acid (GDH). With a molecular weight of 49,000, this enzyme is a multimer. The reducing equivalents are needed by GDH in the form of NADPH + H+. They are produced in the Krebs cycle reaction that comes before it, which is catalysed by the enzyme isocitrate dehydrogenase, which changes isocitrate into -ketoglutarate. The involvement of the two enzymes, glutamate dehydrogenase and isocitrate dehydrogenase, results in the supply and consumption of NADPH + H+ in a cyclic manner (Fig. 26.2).

Theoretically, one glucose molecule may be converted into one glutamic acid molecule. In actual use, it was discovered that 70% of glucose could be converted into glutamic acid.The high capacity for the delivery of the citric acid cycle metabolites is a crucial need for glutamic acid synthesis. This is accomplished by the effective conversion of phosphoenol pyruvate and pyruvate to oxaloacetate. In order to effectively make oxaloacetate, two enzymes are required: phosphoenol pyruvate carboxylase and pyruvate carboxylase, whereas pyruvate dehydrogenase is the sole enzyme required to produce acetyl CoA.Insignificant amounts of glutamic acid cannot be produced by certain bacteria that contain either phosphoenol pyruvate carboxylase (e.g., E. coli) or pyruvate carboxylase (e.g., B. subtilis). Since C. glutamicum contains both of the necessary enzymes, it may replace intermediates in the citric acid cycle (via oxaloacetate) while glutamic acid is being synthesised.Ketoglutarate dehydrogenase of the citric acid cycle is another important enzyme that may promote the best possible synthesis of glutamic acid. For effective glutamic acid production, as shown in C. glutamicum, it must have very low activity.

Additionally, the activity of -ketoglutarate dehydrogenase is decreased when cells are exposed to antibiotics (penicillin) and surfactants, although glutamate dehydrogenase activity is unaffected. By doing this, the synthesis of glutamic acid is maximised while the oxidation of -ketoglutarate through the citric acid cycle is minimised.

Due to the fact that glutamic acid is made inside of cells, its release or export is crucial. The export of glutamic acid currently seems to include an active, carrier-mediated, energy-dependent mechanism.Use of oleic acid auxotrophs, addition of penicillin, and iii. Use the VI of glycerol auxotrophs. Include local anaesthetics. It has been determined how a biotin shortage affects the ability of intracellular glutamic acid to be released. The cofactor biotin is crucial for the production of fatty acids and is needed by the enzyme acetyl CoA carboxylase. The production of fatty acids and, as a result, phospholipids is significantly decreased when there is a shortage or insufficient availability of biotin. Defective membrane formation (protein-phospholipid complex) leads to altered permeability and increased glutamic acid export from intracellular sources.

Mutants of the oleic acid and glycerol auxotrophs are discovered to have altered membrane phospholipid composition. This makes intracellular glutamic acid release easier. For enhanced industrial production of glutamic acid, the information about the membrane permeability of glutamic acid has been effectively used.

Growth factors, pH, oxygen availability, and carbon and nitrogen sources all have an impact on the industrial production of glutamic acid. It briefly describes the important elements. Carbon sources may be refined (such as glucose, sucrose, fructose, and maltose) or unprocessed (such as sugar cane or sugar beet molasses). Acetate, which is cheap, is used in nations like Japan. Alkanes, ethanol, and methanol are among other less common substrates.

The amount of ammonia present is essential for turning the carbon source into glutamic acid. A high ammonia concentration, however, prevents organism development. Ammonium salts and ammonia with a low concentration are supplied at the start of the fermentation process.Ammonia in aqueous solution is continually added during the fermentation process. In addition to a constant supply of nitrogen source, pH may be adjusted in this manner. Given that bacteria that produce glutamic acid have urease, which can break down urea and release ammonia, urea is sometimes employed as a nitrogen source.

The concentration of biotin in the medium is affected by the carbon source and is a crucial growth component. In the case of 10% glucose as the carbon source, a supply of 5 g of biotin per litre medium is advised; however, the biotin need is substantially lower (0.1–1.0 g/l) using acetate as the carbon source. For certain strains, it is advised to add L-cysteine to the medium.O2 supply should be kept up to par and without interruption. According to observations, high oxygen levels prevent organisms from growing, whereas low oxygen levels cause the creation of lactic and succinic acids. The production of glutamic acid is minimal in both cases. Following the completion of the fermentation, the cells are removed, and the culture broth is put through an anion exchanger. The ammonia that is released may be utilised again while the glutamic acid attached to the resins is eluted in NaOH. Monosodium glutamate, or MSG, is created when glutamic acid reacts with NaOH and may be purified by running it through an anion exchanger. Both evaporation and crystallisation are possible processes for MSG.

Phosphoenol pyruvate and pyruvate are produced as a result of the glycolysis of the glucose. The citric acid cycle's essential compound, oxaloacetate, may be produced from any of these metabolites. Oxaloacetate transforms into aspartate upon transamination. Aspartate is changed by the enzyme aspartate kinase into aspartyl phosphate, which then develops into aspartate semi-aldehyde.

The end products' feedback inhibition regulates this enzyme. Aspartate kinase has three isoenzymes, the first of which is inhibited and repressed by L-methionine, the second of which is inhibited and repressed by L-threonine and L-isoleucine, and the third of which is inhibited and repressed by L-lysine. Aspartate kinase's structure and amino acid composition are now known. Additionally, mutants of aspartate kinase that are resistant to L-feedback lysine's control have been developed by genetic engineering.

Improved Production Strains:

Based on the biosynthetic pathway and the regulatory steps certain improvements have been made in the strains of C. glutamicum and B. flavum for overproduction of lysine.

- Mutant organisms resistant to lysine antimetabolites (e.g. b-amino ethyl-L-lysine).
- A mutant strain with an altered enzyme aspartokinase, so that it is not regulated by end product inhibition.
- A strain with a decreased homoserine dehydrogenase activity (so that diversion for the synthesis of methionine, threonine and isoleucine is minimised).
- A strain with reduced citrate synthase activity (to lower the occurrence of citric acid cycle).

Release of L-Lysine

The export or release of L-lysine from the cells into the surrounding medium occurs through a lysine-export (LysE) carrier protein. It is a Transmembrane protein (mol. wt-25,400) with six segments that participate in lysine transport. The exporter system is very efficient active process to export large quantities of intracellular lysine.

Production Process of L-lysine

The most commonly used carbon sources for lysine manufacture is molasses (cane or sugar beet), starch hydro lysates or sucrose. The other sources like acetate, ethanol or alkanes are used to a lesser extent. The nitrogen sources are ammonium salts, gaseous ammonia. Protein hydro lysates are added to supply certain amino acids (L-methionine, L-homoserine, and L-threonine). The protein hydro lysates also supply growth factors such as biotin.

A time-course graphic representation for the formation of lysine is depicted in Fig. 26.5. As is evident, a continuous supply of glucose (or other sugar) is required for sustained production of lysine. Under optimal fermentation conditions, the yield of lysine (in the form of L-lysine HCI) is 40-50 g per 100 g carbon source.

Production of L-lysine

There are different recovery processes for lysine depending on its application.

- An alkaline solution containing about 50% L-lysine can be obtained after biomass separation, evaporation and filtration.
- A crystalline preparation with 98-99% L-lysine (as L-lysine HCI) can be obtained by subjecting the culture broth to ion-exchange chromatography, evaporation and crystallization.

Both the above grades of lysine are suitable for supplementation of feeds.

L-Threonine

L-Threonine is manufactured industrially by employing either E. coli or C. glutamicum. With the mutant strains of E. coli, the product yield is better.The metabolic pathway for the synthesis of L-threonine is depicted in Fig. 26.6. Some of the reactions of this pathway are common for the biosynthesis of L-lysine and methionine, besides isoleucine .Starting with aspartic acid, in a sequence of five steps, threonine is produced.The regulatory reactions in E. coli for L-threonine biosynthesis have been elucidated. Three isoenzymes of aspartate kinase, separately inhibited by

the end products have been identified- one by L-threonine, one by L-methionine and one by L-lysine.Further, two isoenzymes of homoserine dehydrogenase-one inhibited by L-threonine and other by L-methionine are also known. A gene thrABC that encodes three polypeptides (one polypeptide possesses the activity of kinase and homoserine dehydrogenase, the second homoserine kinase and the third threonine synthase) in E. coli has been identified.

Improved Production Strains

The efficiency of the producer strains can be increased by creating E. coli mutants with high-level expression of the gene thrABC. Further, mutants with minimal production of L-isoleucine also result is high yield of L-threonine.The culture medium containing glucose or sucrose, yeast extract and ammonium salts is adequate for L-threonine production. The sugar feeding has to be continued for good yield (about 60% of the carbon source). The downstream processing for the isolation of L-threonine consists of coagulation of the cell mass (by heat), filtration, and concentration by evaporation, and crystallization.

L-Phenylalanine

Both E. coli and C. glutamicum can be used for the production of L-phenylalanine. The biosynthetic pathway is quite complex and an outline is shown in Fig. 26.7. An interesting feature is that the same pathway is responsible for the synthesis of all the three aromatic amino acids-tyrosine and tryptophan, besides phenylalanine.

Pathway for the Synthesis of L-phenylalanine, L-tyrosine and L-tryptophan

The synthetic pathway commences with the condensation of erythrose 4-phosphate with phosphoenol pyruvate to form deoxyarabinoheptulosonate phosphate (DAHP). DAHP in the next series of reactions is converted to chorismate which can form L-tryptophan. Chorismate mutase converts chorismate to prephenate which forms L-phenylalanine through the participation of prephenate dehydrogenase. Prephenate also serves as a precursor for the synthesis of tyrosine.The genes responsible for the formation of the regulatory enzymes of L-phenylalanine have been identified. By employing genetic manipulations, strains for improved production of L-phenylalanine have been developed.

L-Tryptophan

There are different ways of synthesizing L-tryptophan-chemical, enzymatic and fermentation methods. At present, large scale manufacture of tryptophan is carried out by using the enzyme tryptophan synthase of E. coli. Tryptophan synthase combines indole with L-serine to form tryptophan.

Indole is available from petrochemical industries while L-serine can be recovered from molasses during sugar refinement. Mutant strains of E. coli with high activity of tryptophan synthase have been developed for large scale manufacture of tryptophan.Tryptophan can also be produced by fermentation employing C. glutamicum, or E. coli. Mutant strains of both these organisms have been developed for increased yield of tryptophan.The production of tryptophan by C. glutamicum was increased by introducing a second gene encoding anthranilate synthase, a key

enzyme in its biosynthesis. Further, genes encoding other important enzymes (deoxyarabinoheptulosonate phosphate synthase, anthranilate phosphoribosyltransferase) were also be modified. The result is that the pathway becomes insensitive to feedback inhibition by end products, leading to an overproduction of L-tryptophan. There is a growing demand for aspartate, as it is a component of aspartame (an artificial sweetener), besides its use as a food additive, and in pharmaceutical preparations. The preferred method for aspartate production is enzymatic in nature. The enzyme aspartase converts fumarate and ammonia to aspartate. Although this reaction is reversible, aspartate formation is favoured.

Conclusion

The identification of bacteria capable of manufacturing these crucial for industry enzymes has led to the development of the biological manufacture of vitamins. On the other hand, the generation of amino acids by microorganisms uses three different techniques, including amino acid extraction and chemical synthesis.

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CHAPTER 7

A REVIEW STUDY ON STEROIDS

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Abstract:

Major areas of research include the discovery and engineering of microorganisms capable of producing value-added products from phytosterol and other renewable raw materials, whole-cell, and enzyme biocatalysts performing different reactions of steroid modifications with special attention to the oxyfunctionalization of inactive carbons in steroid molecules and rare steroid production.

Keywords:

Biotransformation, Steroids, Hormones, Engineering.

Introduction

Generation of microbial strains with improved biocatalytic features using genetic and metabolic engineering, as well as synthetic biology approaches, cascade bioconversions, new steroid bioproduction schemes, new insights on steroid bioproduction (upstream and downstream processing), the development of new approaches for steroid bioconversion enhancement, and method development for environmental protection from endocrine disruptors are of great importance for the development of steroid biotechnologies.The production of steroid drugs and hormones is one of the best examples of the applications that biotransformations have on an industrial scale. Microbiological transformations are an effective tool for the preparation of various compounds, which can be difficult to obtain by conventional chemical methods and have been widely used in the bioconversion of steroids. In 1950, the pharmacological effects of cortisol and progesterone were reported, in addition to the hydroxylation of the latter in C-11α using Rhizopus species. This began a very important stage in the development of the synthesis of steroids with biological activity [1], [2].

Currently, a great versatility of microbial systems in the pharmaceutical industry for the commercial production of steroids and other drugs is recognized. Several hundreds of microbiological transformations of steroids have been reported in the literature; also, many bioconversions have been incorporated into numerous partial syntheses of new compounds for their evaluation such as hormones or drugs [3], [4]. Chemical derivatives of some steroids are reported to have better therapeutic advantages than the starting materials.Steroids are a broad class of terpenoid lipids that typically a contain gonane core of the four fused rings (A–D) composed of 17 carbon atoms and vary mainly by the presence, type and position of functional groups and the side chain at C17. Being essential molecules in all living organisms, steroids play vital functions in vertebrates acting as signal molecules that regulate signal transduction

pathways by the binding to the respective intracellular receptors. Another principal function of steroids (such as cholesterol, sitosterol, or ergosterol) is regulating cell membrane fluidity and proper operating of the lipid bilayer. It is believed that steroids originated hundreds of millions of years ago, and in the course of evolution, microorganisms acquired the ability to completely degrade or detoxify steroids through their structural modification, i.e., carry out the biotransformation of steroids[5], [6].

Steroids are ubiquitous in nature and serve as carbon and energy sources for many bacteria. Recent studies showed global distribution of the aerobic microbial degradation pathway in wastewater treatment plants, soil, plant rhizosphere, and the marine environment. Various representatives of Actinobacteria and Proteobacteria are capable of degrading different natural plant and the vertebrate steroids excreted into the environment mainly via the 9(10)-seco-pathway. The wastes of synthetic hormone production mills may contain residual amounts of steroids that can also be partially oxidized or completely degraded by microorganisms. Microbiological transformation of plant sterols is currently the technological basis for the production of so-called synthons, from which various pharmaceutical steroids are produced by chemical or combined chemical-enzymatic syntheses. The steroids that can be produced from the synthons are the adrenal cortex hormones, sex hormones, progestins, mineralocorticoids and the non-hormonal steroids for the use in various sectors, including medicine, veterinary medicine, aquaculture, agriculture, food industry[7], [8].

The global needs of the pharmaceutical industry alone for steroid substances exceed 1500 tons annually, and the market for steroid drugs produced from them in 2015 exceeded USD 100 billion, second only to antibiotics. Biotechnologies of microbiological transformation of phytosterols into key synthons of androstane series, such as androstenedione (AD), androstadienedione (ADD), 9-hydroxyandrostenedione (9-OH-AD) have already been implemented on an industrial scale in several countries (USA, Germany, China, India and others). The discovery of new promising steroid molecules with rare bioactivity, including those with antiviral and neuroprotective effects, the growth of the vitamin D market, re-direction of the production schemes to the non-animal raw materials, allows us to predict a significant increase in the demand of the global market for steroid synthons produced by microbiological methods from phytosterols.The biotransformation processes of different steroid compounds described in this review, although not exhaustive, aim to highlight the importance of biotransformation through different microorganisms, as a useful chemical-biological tool for obtaining novel derivatives for research purpose and as industrial applications. An example includes obtaining steroid compounds for the pharmaceutical industry.

Biotransformation of steroids has been implemented in an important way in the partial synthesis of new steroids, for their evaluation as hormones and drugs. Currently, there is a wide variety of steroids used as diuretics, anabolic, anti-inflammatory, antiandrogenic, anticontraceptive, and antitumor, among other applications. Chemical functionalization in different carbon atoms of the sternum skeleton is related to the biological activity of the molecule. The interest in the biotransformation of steroid compounds has been increasing in recent years, due to the obtaining of new and useful pharmacologically active compounds. In addition to the development of new

genetically modified strains, there is an increase in the availability of immobilized enzymes and the manipulation of culture media. Biotransformation of steroids proceeds with low to moderate yields in general. One of the main causes is their low solubility in water. Currently, methodologies are developed that allow the incorporation of chemicals—surfactants, ionic liquids, cyclodextrins, liposomes, among others—that contribute to improve the yields of each biotransformation process and the processes friendly to the environment.

Literature Review

Schiffer *et al.* carried out a study in which they discussed about the technology advancements that have made it possible to quantitatively profile steroid precursors, bioactive steroids, and inactive metabolites with sensitivity, specificity, and simultaneity, enabling thorough analysis of the serum and urine steroid metabolomes. Thus, the quantification of steroid panels is becoming more popular in clinical and research facilities than the measurement of individual marker molecules. Despite the fact that the biochemical processes involved in the manufacture and metabolism of steroid hormones have been extensively described, there is still a gap between what is known and how it is applied to the observed steroid profiles. With a focus on the routes connecting and distinguishing the serum and urine steroid metabolomes, we provide an overview of steroid hormone manufacture and metabolism by the liver and peripheral tissues in this study. There is also a short summary of the steroid profiling process[1].

Ojoghoro *et al.* investigated a study in which recent studies have shown that combinations of several steroid hormones may prevent pregnancy even when each hormone is present at a level at which it would not have a detectable impact on its own. The findings of the laboratory investigations indicating steroid hormones may be substantial environmental pollutants have been validated by a small number of field studies. More investigation is needed to pinpoint the principal sources of steroid hormones entering the aquatic environment, describe the intricate combinations of steroid hormones that are now understood to be present everywhere, and ascertain the effects of environmentally plausible combinations of steroid hormones on aquatic vertebrates, particularly fish. A thorough evaluation of the risks of steroid hormones in aquatic environments cannot be made until that study is finished.

Buchholz *et al.* reported that by examining genes and their relationships throughout evolution, basic research in biochemistry and molecular biology has both significantly broadened and united the field of life sciences. The range of available goods and services greatly increased. Understanding life at the molecular level paves the path for the development of unique goods and effective, environmentally friendly manufacturing processes[9].

The synthesis of very valuable steroidal pharmaceuticals and their precursors for the pharmaceutical sector became possible via the steroid alterations carried out by chosen wild-type and modified strains of microorganisms. Some microbes are proficient at carrying out redox reactions at various locations on the steroid molecule as well as the oxyfunctionalization of the steroid core and the degradation of the side chains on sterols. Donova *et al.* demonstrated that several steroid-converting microbial strain-based bioprocesses have a strong industrial foundation. The development of improved bioprocesses and production plans for obtaining

known and new metabolites with strong biological activity depends greatly on the choice of suitable microorganisms as well as the development of new engineered strains, even though a variety of biocatalytic methods have been developed. The development of steroid-transforming strains via genetic and metabolic engineering, together with fresh ideas in enzymatic and whole-cell biocatalysis, has created a platform for extremely efficient and selective biotransformations[10].

The discipline of synthetic chemistry was first exposed to microbial transformation technologies in the middle of the 1950s. The issues with steroid hormone production by microbial transformation suddenly attracted study attention. The Institute of Applied Microbiology (IAM), University of Tokyo, developed the first project at that time, known as the "Tsuda Project," in the spring of 1956. I participated in it. It was named "The Study for Microbial Transformation of Steroids." In addition to synthesising steroidal chemicals, our microbial transformation processes have also been used more extensively to synthesise other organic molecules, such as pravastatin, etc. Naito *et al.* presented a number of the findings from these reactions. Five kinds of outcomes are presented: Fortunately, Streptomyces carbophilus SANK 62585 was ultimately chosen as a strong microbial converter with a reduced level of by-products. A lot of research was done and the culturing conditions were improved with the goal of producing pravastatin on an industrial scale[11].

Discussion

The placenta, the gonads, and the adrenal cortex all produce steroid hormones that are all produced from cholesterol and have a variety of clinically significant functions. The smooth endoplasmic reticulum and mitochondria are where steroid hormones are made. They must be generated as precursors when required since they are lipophilic and cannot be kept in vesicles where they would quickly disperse. Steroid hormone precursors are transformed into active hormones upon stimulation of the parent cell, and when their intracellular concentration increases, they simply diffuse outside of the parent cell. All steroid hormones are insoluble in plasma and other bodily fluids because they are produced from cholesterol. Steroids are thus linked to transport proteins, which lengthens their half-life and ensures widespread dispersion. A minor portion of free steroids that are "active" are in balance with the protein-bound steroids. Steroids may trigger gene transcription either fast by attaching to cell surface receptors or gradually by attaching to cytoplasmic or nucleic receptors. All vertebrates naturally produce steroid hormones, which are very significant hormones. They regulate a variety of physiological functions, including as osmoregulation, sexual development, reproduction, and stress reactions. Additionally, several synthetic steroid hormones are in common usage as medications for both humans and animals. The amounts of steroid hormones in rivers have been measured thanks to recent developments in environmental analytical chemistry. The identification and quantification of several steroid hormones, both natural and synthetic, as well as transformation products, show that they are pervasive aquatic pollutants. Some steroid hormones, both natural and synthetic, may negatively affect reproduction when present in the water at very low quantities, even sub-ng/L, according to laboratory ecotoxicology investigations, which were mostly done with fish but also amphibians.

Early biotechnology (BT) was inspired by exciting findings like the discovery that yeast, a living organism, is what causes beer and wine to ferment. Significant disagreements between vitalists and chemists led to the reversal of beliefs and paradigms, but also to further inquiry and advancement. By creating pure monoculture in sterile media, Pasteur's work contributed to the development of the science of microbiology and, together with Robert Koch's work, to the understanding that a single pathogenic organism is the causal agent for a certain illness. Pasteur also made advancements in the production of beer, wine, and alcohol, three industrial processes with significant economic significance. By the middle of the 20th century, biotechnology was a recognised field of study, and courses in it had been created in the biological sciences divisions of several institutions. Beginning in the 1970s and 1980s, governmental organisations in Germany, the UK, Japan, the USA, and other countries began to pay attention to BT as a subject with inventive potential and economic development, which spurred the industry's expansion.

The adrenal medulla and the adrenal cortex make up the adrenal glands. The zona glomerulosa, which generates aldosterone, and the zonae fasciculata and reticularis, which together create cortisol and adrenal androgens, are the three principal anatomic zones that make up the adrenal cortex. In the medulla, catecholamines are produced. The adrenal cortex produces more than 30 different steroids, which fall into three different functional groups: mineralocorticoids, glucocorticoids, and androgens.

The steroid hormones cortisol, 11-deoxycortisol, aldosterone, corticosterone, and 11-deoxycorticosterone are all primarily produced in the adrenal glands. The adrenal glands and the gonads produce the majority of other steroid hormones, including the oestrogens.

The zona glomerulosa is where the mineralocorticoids are created. At the collecting tubule, distal tubule, and collecting ducts, the mineralocorticoids' primary job is to encourage tubular reabsorption of sodium and the release of potassium and hydrogen ions. Water is also reabsorbed at the same time as salt. Fluid volume and artery pressure are both increased by salt and water absorption.

The most powerful mineralocorticoid, aldosterone, is responsible for nearly 90% of all mineralocorticoid activity. Aldosterone, 11-deoxycorticosterone, 18-oxocortisol, corticosterone, and cortisol are the mineralocorticoids with the highest potency. Cortisol contains some mineralocorticoid action in addition to its major glucocorticoid activity. Although cortisol is 80 times more concentrated than aldosterone, it has a potency that is 1/400 that of aldosterone. Aldosterone is produced at a rate of 100 g per day and cortisol at a rate of around 25 mg per day by the adrenal glands. In addition to modest mineralocorticoid action, corticosterone mostly exhibits glucocorticoid activity.Renin-angiotensin system is principally responsible for controlling aldosterone production; elevated serum potassium concentrations also promote it. Aldosterone levels rise in response to hyperkalemia and angiotensin II. Elevated sodium concentration restricts aldosterone secretion to a lesser extent, while corticotropin promotes aldosterone production.

Most of the glucocorticoids are produced in the zona fasciculata. The glucocorticoids have a variety of metabolic effects. Cells consume less glucose when exposed to glucocorticoids, which

increase gluconeogenesis. With the exception of the liver, cortisol decreases protein reserves in every cell in the body while increasing protein synthesis in the liver. Additionally, cortisol raises blood levels of amino acids, reduces the transport of amino acids into extrahepatic cells, and raises the transport of amino acids into liver cells. The hormone cortisol raises plasma levels of free fatty acids and enhances the utilisation of free fatty acids for energy by mobilising fatty acids from adipose tissue. About 95% of total glucocorticoid action is accounted for by cortisol, the most clinically significant glucocorticoid.

A minor but considerable portion of the overall glucocorticoid action is accounted for by corticosterone. Corticotropin, which is released by the anterior pituitary gland in response to corticotropin-releasing hormone (CRH) from the brain, regulates cortisol output virtually exclusively. Serum cortisol suppresses the release of CRH and corticotropin, which stops the adrenal glands from secreting too much cortisol. In combination with growth hormones like insulin-like growth factor (IGF)-1 and IGF-2, corticotropin increases the release of cortisol and supports the development of the adrenal cortex. The peak cortisol levels occur an hour or so before waking, and cortisol release has a diurnal cycle. Cortisol production is heightened by stress, discomfort, and inflammation.

Any steroid hormone that exhibits masculinizing effects is referred to as an "androgen." Secondary sexual traits in males arise as a result of androgens. The androgens are less significant in women, but the adrenal androgens are mostly responsible for pubic and axillary hair development. The main androgen is testosterone. The gonads and the adrenal glands both create androgens. The testes produce roughly 7000 g of testosterone each day, whereas the adrenals produce about 100 g per day. In women, 30% of testosterone is directly generated by the adrenals, 50% to 60% is obtained via androstenedione conversion in peripheral tissues, and 20% is produced by the ovary.

The zona reticularis is where the adrenal androgens are largely produced. The main steroid that is generated by the adrenal glands is dehydroepiandrosterone (DHEA). DHEA sulphate is created when DHEA is sulfated (DHEA-S). The male sex hormones adrenal androgens are fairly active. A portion of the androgens in the adrenal glands are transformed to testosterone. It is not entirely clear how the adrenals stimulate the release of androgen. Between the ages of 5 and 20 is known as the adrenalrhea, which results in an increase in these androgens. Therefore, adrenarche starts well before puberty. Corticotropin and other unidentified substances both have a role in the regulation of adrenal androgen secretion.Dihydrotestosterone (DHT), androstenedione, and testosterone are all secreted by the testes. GnRH secretion from the hypothalamus regulates the gonadal synthesis of androgens by causing the anterior pituitary to produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In response to stimulation by LH, the testes' Leydig cells produce testosterone. In the target tissues, the majority of the testosterone is transformed into the more active DHT.

The primary role of oestrogens in females is to encourage the growth and proliferation of certain cells in the body that give rise to the majority of secondary sexual traits. The uterus and breasts are prepared for pregnancy and lactation, respectively, by the progestins. Estrogens and progestins often have no clinically meaningful impact on how males acquire their sexual

characteristics. Estrogen and progestin are produced by the adrenal gland or the gonad in women. The adrenal gland's contribution to circulating oestrogens in females with intact ovaries is minimal. Variable phases of the female menstrual cycle include different rates of estradiol and progesterone secretion. The predominant ovarian oestrogen is estradiol; the other two are estrone and estriol. Estriol and estrone are both 80 and 12 times more powerful than estradiol, respectively. Estrone is produced by the ovaries in very minute levels, although it is mostly created by peripheral androgen conversion. In non-pregnant women, estriol is primarily a metabolite of estrone and estradiol. Estriol, however, serves as the placenta's main oestrogen throughout pregnancy. The placenta transforms DHEA-S from the foetal adrenal glands into estriol.

Progesterone is a primary progestin, whereas 17-hydroxy-progesterone is a minor progestin. Small levels of progesterone are generated throughout the first half of the menstrual cycle, with the adrenal cortex and ovaries producing about equal amounts of the hormone. The corpus luteum secretes more progesterone in the second part of the menstrual cycle. About 1/5 as much oestrogen is produced by men as it is by non-pregnant women. A little bit of testosterone is converted to oestrogen in the Sertoli cells. Additionally, oestrogens are produced peripherally in the liver from androstenediol and testosterone.

Albumin and cortisol-binding globulin (CBG) interact to form cortisol. Between 3 and 10 percent of cortisol is free, 80 to 90 percent is bound to CBG, and between 5 and 10 percent is linked to albumin [6, 7]. All that is functional in cortisol is the free form. The CBG may rise or fall in certain clinical circumstances. For instance, a rise in thyroid hormone or oestrogen might result in a rise in CBG. Alternately, a reduced CBG may be brought on by hypothyroidism, elevated androgens, acute stress, and nephrotic syndrome. The total cortisol level is impacted by changes in CBG concentration, but not the free cortisol level. Additionally, progesterone, 17-hydroxyprogesterone, 11-deoxycorticosterone, cortisone, and corticosterone all bind to CBG.

When compared to CBG, albumin, and red blood cells, aldosterone is only weakly bound, leaving around 50% of it free. DHEA-S is firmly attached to albumin, while DHEA and androstenedione are loosely bound. Sex hormone-binding globulin (SHBG), also known as testosterone binding globulin, is where testosterone and estradiol are bound. 20% to 40% of serum testosterone is linked to albumin, 60% to 75% to SHBG, and just 1% to 2% is free [4]. DHT, testosterone, androstenediol, estradiol, and estrone are listed in decreasing affinity for SHBG. DHEA and androstenedione have a tenuous bond with albumin. Age and testosterone may lower SHBG, whereas oestrogen, diabetes mellitus, hyperthyroidism, and cirrhosis can raise it. Aldosterone and cortisol may fix in the target tissue or break down in the liver. An estimated 75% of the decomposed steroid is eliminated in the urine, with the remaining 25% passing via the bile and faeces. Cortisol has a half-life of 60 to 100 minutes. Aldosterone, DHEA, androstenedione, testosterone, and estradiol all have half-lives that are under 20 minutes. Aldosterone has a half-life of under 15 minutes. About 0.1% of the total urine cortisol metabolites are unmetabolized cortisol.

Approximately 2% of hypertension patients also have primary hyperaldosteronism. Primary hyperaldosteronism is most often brought on by an adenoma that produces aldosterone and is

also referred to as Conn's syndrome. Dexamethasone-suppressible hyperaldosteronism, primary adrenal hyperplasia, idiopathic hyperaldosteronism, and adrenal cortical cancer are further reasons. High plasma aldosterone (PA) levels and low plasma renin activity differentiate primary hyperaldosteronism from other types of hyperaldosteronism (PRA). A genuine or apparent mineralocorticoid excess that is not due to aldosterone (induced by an 11-hydroxylase deficit, 17-hydroxylase deficiency, Liddle syndrome, an 11-hydroxysteroid dehydrogenase deficiency, or licorice consumption) is characterised by a low PRA and a low PA in the patient. High PRA and PA are indicators of secondary hyper-aldosteronism. Renal artery stenosis, a tumour that secretes renin, malignant hypertension, or chronic renal illness are all potential causes of secondary hyperaldosteronism with hypertension. Cirrhosis, the use of diuretics or laxatives, renal diseases (such as renal tubular acidosis or Barters syndrome), congestive heart failure, vomiting, and familial chloride diarrhoea may all result in secondary hyperaldosteronism with nor-motension.

Because primary hyperaldosteronism, secondary hyperaldosteronism, and essential hypertension overlap, measuring aldosterone concentration alone is not an effective screening test. Using the PA: PRA ratio, primary hyperaldosteronism is assessed. The best time to measure the PA:PRA ratio is after 2 hours of standing and when the patient has not taken any drugs for 2 to 4 weeks before to the test (particularly diuretics, ace inhibitors, and -blockers). A ratio of more than 25 between PA (ng/dL) and PRA (ng/mL/hour) is considered indicative of primary hyperaldosteronism, and one of more than 50 is considered diagnostic.

The typical next step after a diagnosis of primary hyperaldosteronism is suspected based on the PA: PRA ratio is confirmation testing to show the independence of aldosterone secretion. Giving two litres of saline over four hours and checking for potential aldosterone suppression may do this. Aldosterone suppression to less than 5 ng/dL is regarded as normal. The diagnosis of hyperaldosteronism is confirmed if the aldosterone level continues to be higher than 10 ng/dL.Lack of CRH or corticotropin as well as primary adrenal insufficiency, which prevents the adrenal glands from producing cortisol, may both result in low levels of cortisol (secondary or tertiary adrenal insufficiency). Aldosterone and cortisol depletion are symptoms of primary adrenal insufficiency (Addison's disease). Due to intact angiotensin II activation of the adrenal glands, the adrenal glands may produce aldosterone but not cortisol in secondary and tertiary adrenal insufficiency. Primary adrenal insufficiency may be brought on by autoimmune disorders, adrenal haemorrhages, HIV, TB, sarcoidosis, or amyloidosis, among other conditions. Infiltration of the anterior pituitary or hypothalamus by craniopharyngioma, pituitary adenoma, metastasis, sarcoidosis, or TB, or suppression of corticotropin by long-term steroid usage are causes of secondary or tertiary adrenal insufficiency.

Adrenal insufficiency may cause weakness, exhaustion, anorexia, nausea, stomach discomfort, and diarrhoea over a prolonged period of time. Any kind of adrenal insufficiency may cause hyponatremia. Due to the absence of aldosterone, primary adrenal insufficiency may result in hyperkalemia. Patients with acute adrenal insufficiency may have hypotension due to reduced cardiac output, decreased vascular tone, and relative hypovolemia. Adrenal insufficiency may cause a coma and perhaps death if left untreated. Measurement of serum cortisol is often used to

assess adrenal insufficiency. The value of random cortisol levels is low due to the diurnal rhythm of cortisol production. A blood cortisol level more than 18 g/dL is often regarded as appropriate in critically sick patients. The tests that are favoured in an outpatient situation are either a morning cortisol test or a corticotropin-stimulation test. A morning cortisol level of less than 3 g/dL may be used to diagnose adrenal insufficiency, whereas a reading of more than 18 g/dL can rule it out. A corticotropin-stimulation test may assess morning serum cortisol levels that are between 3 g/dL to 18 g/dL.

A corticotropin-stimulation test involves administering 250 g of synthetic cosyntropin intravenously (or intramuscularly), and measuring the levels of cortisol at baseline, 30 minutes, and 60 minutes. To rule out adrenal insufficiency, a peak cortisol measurement of 18 to 20 g/dL or more is often employed. Another kind of stimulation test, such a CRH-stimulation test, must be conducted to rule out acute secondary adrenal insufficiency if it is suspected. Because albumin and CBG levels may drop under acute stress, this complicates calculations of blood cortisol levels. As a result, even if the amount of free (active) cortisol remains same, the total serum cortisol may drop. Although there are techniques for measuring serum free cortisol, they are costly, difficult to use, and not widely accessible. To calculate a free cortisol index that is comparable to the serum free cortisol, a cortisol:CBG ratio has been suggested.

"Cushing's syndrome" is the term used to describe the condition of chronic and excessive cortisol. Exogenous glucocorticoids, excessive adrenal cortisol production (due to an adrenal adenoma, carcinoma, or nodular hyperplasia), or excessive corticotropin or CRH production are all potential causes of excessive cortisol. The anterior pituitary gland or an ectopic source, such as small cell lung cancer or bronchial carcinoid, may both generate corticotropin. Similar to bronchial carcinoid, medullary thyroid cancer, or metastatic prostate cancer, CRH may also be generated ectopically. Hypertension, diabetes mellitus, androgen-type hirsutism, irregular menstruation, weight gain, ecchymoses, myopathy, osteopenia, truncal obesity, and purple striae are only a few clinical signs of excessive cortisol.

A screening test is often the first step in the diagnostic process for Cushing's syndrome. A high false-positive rate is one challenge with Cushing's syndrome screening tests. The only people who should be tested are those who have suspected Cushing's syndrome (ie, patients who have central obesity, facial plethora, proximal muscle weakness, purple striae, and so forth). A screening test that measures free cortisol in 24-hour urine is both specific (98%) and sensitive (95–100%). A 1-mg overnight dexamethasone suppression test is another option that is often used for screening. This test involves taking 1 mg of dexamethasone orally at 10 or 11 p.m., then measuring the cortisol level at 8 a.m. the following morning.

Conclusion

Although there is some debate in the literature, Cushing's syndrome cannot exist if the blood cortisol level is less than 5 g/dL. Following screening tests, further testing should be done if the diagnosis of Cushing's syndrome is uncertain or suggests it. The measurement of salivary cortisol levels is a more recent method for diagnosing Cushing's syndrome. Salivary cortisol accurately measures the amount of serum free cortisol because it readily diffuses into saliva. As

an outpatient, saliva may be collected at several times of the day, enabling serial cortisol assessments without requiring serial blood draws.

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CHAPTER 8

AN EXPLORATIVE STUDY ON INDUSTRIAL ENZYMES

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Abstract:

Enzymes are considered as a potential biocatalyst for a large number of reactions. Particularly, the microbial enzymes have widespread uses in industries and medicine. The microbial enzymes are also more active and stable than plant and animal enzymes. In addition, the microorganisms represent an alternative source of enzymes because they can be cultured in large quantities in a short time by fermentation and owing to their biochemical diversity and susceptibility to gene manipulation. Industries are looking for new microbial strains in order to produce different enzymes to fulfil the current enzyme requirements. This special issue covers ten articles including three review articles, mainly highlighting the importance and applications of biotechnologically and industrially valuable microbial enzymes.

Keywords:

Biocatalyst, Enzymes, Protease, Technology.

Introduction

Enzymes are used in the chemical industry and other industrial applications when extremely specific catalysts are required. However, enzymes in general are limited in the number of reactions they have evolved to catalyze, and by their lack of stability in organic solvents and at high temperatures. As a consequence, protein engineering is an active area of research and involves attempts to create new enzymes with novel properties, either through rational design or in vitro evolution. These efforts have begun to be successful, and a few enzymes have now been designed “from scratch” to catalyze reactions that do not occur in nature.

Microorganisms are favored sources for industrial enzymes due to easy availability, and fast growth rate. Genetic changes using recombinant DNA technology can easily be done on microbial cells for elevated enzyme production and scientific development. Production of microbial enzymes is a necessary event in the industrial sectors, due to the high and superior performances of enzymes from different microbes, which work well under a wide range of varied physical and chemical conditions. Further, microbial enzymes are used in the treatment of health disorders associated with deficiency of human enzymes caused by genetic problems. For instance, patients with inherited congenital sucrase-isomaltase deficiency are unable to digest sucrose, and therefore, sacrosidase enzyme is given orally to facilitate digestion of sucrose. In

addition, phenylalanine ammonia lyase is used to degrade phenylalanine in genetic phenylketonuria disorder

Food processing

In food processing, the enzymes used include amylases from fungi and plants. These enzymes are used in the production of sugars from starch, such as in making high-fructose corn syrup. In baking, they catalyze the breakdown of starch in the flour to sugar. Yeast fermentation of sugar produces the carbon dioxide that raises the dough. Proteases are used by biscuit manufacturers to lower the protein level of flour. Trypsin is used to predigest baby foods. For the processing of fruit juices, cellulases and pectinases are used to clarify fruit juices. Papain is used to tenderize meat for cooking.

Dairy industry

In the dairy industry, rennin, derived from the stomachs of young ruminant animals (like calves and lambs) is used to manufacture of cheese, used to hydrolyze protein. Lipases are implemented during the production of Roquefort cheese to enhance the ripening of the blue-mold cheese. Lactases are used to break down lactose to glucose and galactose.

Brewing industry

In the brewing industry, enzymes from barley are released during the mashing stage of beer production. They degrade starch and proteins to produce simple sugar, amino acids, and peptides that are used by yeast for fermentation. Industrially-produced barley enzymes are widely used in the brewing process to substitute for the natural enzymes found in barley. Amylase, glucanases, and proteases are used to split polysaccharides and proteins in the malt. Betaglucanases and arabinoxylanases are used to improve the wort and beer filtration characteristics. Amyloglucosidase and pullulanases are used for low-calorie beer and adjustment of fermentability. Proteases are used to remove cloudiness produced during storage of beers.

Starch industry

In the starch industry, amylases, amyloglucosideases, and glucoamylases convert starch into glucose and various syrups. Glucose isomerase converts glucose into fructose in production of high-fructose syrups from starchy materials.

Paper industry

In the paper industry, amylases, xylanases, cellulases, and ligninases are used to degrade starch to lower viscosity, aiding sizing and coating paper.

Biofuel industry

In the biofuel industry, cellulases used to break down cellulose into sugars that can be fermented (see cellulosic ethanol).

In the production of biological detergents, proteases, produced in an extracellular form from bacteria, are used in pre-soak conditions and direct liquid applications, helping with the removal

of protein stains from clothes. In molecular biology, restriction enzymes, DNA ligase, and polymerases are used to manipulate DNA in genetic engineering, important in pharmacology, agriculture and medicine, and are essential for restriction digestion and the polymerase chain reaction. Molecular biology is also important in forensic science.

Enzymes have developed over millions of years to effectively function in the cellular milieu and catalyse tens of thousands of distinct reactions. The living world undergoes diversity, adaptation, optimization, and invention thanks to the basic yet very effective mutation and selection system known as evolution (3). Over thousands of years, evolution has given enzymes their many properties and functions.

Literature Review

For many biotechnological applications, industrial enzymes are crucial. Currently, little is known about the variety of marine bacteria from Malaysia that produce industrial enzymes. Cheng *et al.*examined the variety of marine bacteria that produce industrial enzymes in culture collections at the Institute of Marine Biotechnology, University of Malaysia Terengganu. Of the 200 bacterial isolates that were successfully resurrected, 163 of them were able to grow. Using 16S rDNA, marine bacteria that generated enzymes with total scores greater than four were chosen for molecular identification. 161 bacterium isolates produced amylase (68.7%), lipase (88.3%), and protease (68.7%) in secretions. Three main phyla—Proteobacteria, Firmicutes, and Bacteroidetes—were discovered by phylogenetic research. These nine genera—Bacillus, Chryseomicrobium, Photobacterium, Pseudoalteromonas, Ruegeria, Shewanella, Solibacillus, Tenacibaculum, and Vibrio—were formed from the phyla[1].

Zhang *et al.* discussed on the Industrial enzymes as they are the "chip" of contemporary bio-sectors, promoting the growth of downstream industries by tens or even hundreds of times. For industrial applications, it is crucial to clarify how enzyme structures and activities relate to one another. The structure-function interactions have recently undergone substantial study thanks to enhanced breakthroughs in protein crystallisation and computer modelling technologies, opening the door to rational design and de novo design. This paper examines current advancements in the structure-function connections of commercial enzymes and applications[2].

Duman-Özdamar *et al.*highlighted the successes in producing biocatalysts to highlight P. pastoris' function as a cell factory. Additionally, the advantages and difficulties of the most important expression systems, including P. pastoris, Saccharomyces cerevisiae, and Escherichia coli, as well as recent developments and future prospects, were extensively discussed. The newest developments to improve the manufacturing of recombinant proteins and offers for possibilities were then explored.

Industrial biotechnology is biotechnology used to streamline industrial operations and the creation of goods. Many believe that the next phase of the biotechnology revolution will be industrial biotechnology. The extensive use of enzymes in numerous industrial processes and products, including those in the pulp and paper, chemical, detergent, textile, food, and animal feed industries, is one of the key aspects of industrial biotechnology. This practise enables the creation of sustainable products and processes. In the recent years, advances in recombinant

DNA technology and sophisticated bioprocesses have made it feasible to mass manufacture enzymes to satisfy the ever-increasing demand. Recent advances in site-directed evolution and protein engineering have made it possible for us to design novel enzymes with unique functions. This results in an industry that is rapidly expanding and presently influences every aspect of our everyday lives. The main industrial enzyme types and the numerous uses for them are covered in this chapter. These applications will continue to benefit society by enhancing our quality of life, conserving resources, and preserving the environment[3].

In industrial processes, enzymes may often replace the use of high temperatures, organic solvents, and pH extremes while also improving reaction specificity, product purity, and environmental effect. The increasing usage of industrial enzymes depends on ongoing innovation to boost efficiency and save costs. Recombinant DNA and fermentation technologies, which make it possible to manufacture this variety at a cheap cost, as well as protein modification tools, which allow enzymes to be modified so that they may be tailored to the needs of the industrial market, are the driving forces behind this breakthrough[4].

Engineers are using evolution to improve enzymes for use in chemical plants because it can produce such a broad, strong, and specialised arsenal of enzymes. Training is necessary for high-performance athletes, and the evolutionary algorithm serves as the training programme for enzymes. The strongest evidence of the evolutionary principles of natural selection—variation, inheritance, and selection is how quickly they have permeated both academic research and commercial applications.

Headon *et al.* demonstrated the current biotechnology sector's primary objective is the manufacturing of enzymes. While the need for new biocatalysts is always rising due to the continuing focus on biotechnological endeavours, markets for conventional industrial enzymes are nevertheless expanding. Despite being one of the oldest divisions of the biological sciences, enzymology is nevertheless a subject of active, continuing study. Numerous innovative uses for these catalytic activities are likely given the ongoing discovery of new enzymes and our growing knowledge of already known enzymes and their functional importance. The synthesis and use of enzymes in the intestines will always be crucial to the biotechnology sector[5].

Littlechild *et al.* carried out a study in which the rate at which microbial genomes and metagenomes are being sequenced makes it possible to identify novel, robust biocatalysts with commercial uses for a variety of industrial biotechnology applications. A never-ending supply of novel, reliable commercial biocatalysts may be obtained via the use of better bioinformatic methods and the creation of new, quick enzyme activity screening methodologies. Several recent case studies where industrial enzymes of "high priority" have been found and characterised will be included in this mini-review. It will feature certain hydrolase enzymes as well as current case studies that have been completed by our team in Exeter[6].

Chapman *et al.* demonstrated that in terms of sustainability and process effectiveness, using enzymes as industrial biocatalysts has several benefits over using conventional chemical processes. Enzyme catalysis has been scaled up for commercial processes in the pharmaceutical, food, and beverage industries; however, additional improvements in stability and biocatalyst

functionality are needed for the best biocatalytic processes in the energy sector for producing biofuels and converting natural gas. Immobilized biocatalysts for use in such industrial-scale processes must be developed using a multidisciplinary approach in order to overcome the technological challenges connected with the use of immobilised enzymes. The next generation of immobilised biocatalysts and the effective scaling up of their induced processes will be defined, in particular, by the convergence of technological competence in protein, enzyme, and process engineering. This review covers the successful applications of biocatalysis, how enzyme immobilisation can enhance industrial processes, and the analysis tools necessary for the multi-scale implementation of enzyme immobilisation for increased product yield at maximum market profitability and minimal logistical burden on the environment and user[7].

Kirk *et al.* carried out a study in which they discussed in the Enzymes that have previously been encouraged to be used in a number of industrial goods and processes due to their efficient catalytic characteristics. The effective creation of novel enzymes has been made possible by recent advances in biotechnology, notably in fields like protein engineering and guided evolution. This has led to the creation of new enzymes especially suited for wholly novel areas of application where enzymes have never before been utilised, as well as the improvement of existing enzymes with better features for well-established technological applications[8].

Binod *et al.* evaluated the state of industrial enzyme research globally as well as in the context of India, including its use in various industries, its application, and the current status of R&D and commercialization. A special section on enzymes engaged in biotransformation is also discussed in length since they are now often utilised in biotransformation and because of their significance in the production of chiral compounds of medicinal interest. The scientific databases SciVerse Scopus, Google, and other online resources were searched for this research[9].

Kant Bhatia *et al.* discussed on the hunt for new enzymes from harsh environments, as well as the refinement of existing enzymes and tweaking them towards certain desirable features, have all been revolutionised by metagenomics and directed evolution technologies. Researchers may now design enzymes for increased activity, stability, and substrate specificity to fulfil commercial demand using new molecular biology technologies, such as next generation sequencing, site guided mutagenesis, fusion proteins, surface display, etc. Although many enzymatic processes have been improved to an industrial level, difficulties in sustaining activity throughout the catalytic process still need to be resolved. This article reviews current improvements in metabolic engineering techniques to increase enzyme effectiveness and production as well as new discoveries in industrial uses of enzymes[10], [11].

Discussion

Beginning in the 1960s, researchers attempted to speed up evolution by analysing the structure of the enzymes, on the theory that they could foretell which amino acids would need to be altered to adjust the function of the enzymes. By introducing specific amino acid swaps into the encoded enzyme via DNA modifications, biochemists have been able to better understand the architectures of enzymes. However, they discovered through this research that enzymes are not straightforward to comprehend and that the majority of the polypeptide chain around the active

site contributes to enzyme performance. Due to the intricacy of enzymes, it is still practically difficult to anticipate how mutations would affect them. However, the use of computers and bioinformatics in data analysis and the development of novel ideas is becoming more and more significant.Since the development of genetic engineering, enzymes and other proteins that are created on a small scale naturally may now be manufactured on a huge scale. Depending on the enzyme's intended use, different levels of downstream processing are used. Industrial enzymes are often prepared in a very basic manner and need minimal downstream processing. Enzymes intended for medical use undergo much more downstream processing, sometimes including three or four chromatographic stages.

The evolutionary algorithm was brought into the lab in the early 1990s, which led to a breakthrough in enzyme engineering. In this technique, known as directed evolution, enzymes are randomly mutated rather than being carefully studied and transformed into clever mutants. Although the fundamental reasons behind many of the changes are challenging for scientists to explain, random mutagenesis proved essential in assisting researchers in identifying advantageous mutations. Optimizing enzymes for a desired reaction may be done extremely well via directed evolution. Enzyme engineers make decisions on how many mutations should be introduced, how many variants should be screened, how to generate libraries, etc. rather than making mutation predictions. Due to recent improvements, industrial enzymes are now extensively used in a variety of sectors, including chemical manufacturing, food and beverage production, pharmaceuticals, textiles, cosmetics, etc. Compared to employing complete cells, they are thought to be more cost-effective and environmentally beneficial. The growth in demand for industrial enzymes has accelerated the development of manufacturing methods. Due to its remarkable potential for in-depth research, Pichia pastoris (P. pastoris) has shown its effectiveness as a host for heterologous protein production. A well-researched and effective technique for fermenting P. pastoris is specifically high-cell density fermentation. Additionally, the options for P. pastoris strain enhancement have been expanded because to advancements in state-of-the-art gene-editing techniques.

A well-established area of biochemical research called enzyme technology is in the process of maturing and evolving. In this period of global industrialisation, the maturation is shown by the growth of the theory, their function, and the construction and configuration of their three-dimensional structure. A deeper knowledge of enzymes and the relevance of their functional roles proposes a wide range of innovative uses for their catalytic activities as well as for ongoing R&D discovery of novel features. For the wide range of reactions catalysed by environmental factors, they are employed on an industrial and scientific scale. This is just the beginning of the industrial enzyme age, which is getting ready to employ already-known enzymes in unique ways and newly-discovered or specially-designed enzymes to catalyse unexplored processes. The use of enzymes that can replace hazardous chemical processes is vitally important since there is a desire for cleaner, greener technology to protect our mother planet for future generations. Most of the present R&D on enzymes is focused on this problem. Similar to this, it is becoming increasingly common to utilise enzymes under very hard environments such extremely high and low temperatures and pH levels. As a result, it seems important to evaluate current R&D directions on industrial enzymes.

A sustainable method for the chemical synthesis of fine chemicals, general chemicals like surfactants, and new consumer-based goods like biodegradable plastics is to use "nature's catalysts." This offers a sustainable and "green chemistry" approach to chemical synthesis that produces no harmful waste and is safe for the environment. Enzymes may also be used for a variety of other purposes, such as the collection of carbon dioxide, the breakdown of food, and the treatment of various waste streams, opening the door to the idea of a "circular economy" in which nothing is wasted.

You get what you screen for, according to Frances Arnold, a Nobel winner who created the first rule of directed evolution. The implementation of screening methods to produce useful measurements of the hundreds of enzyme variations is therefore the key problem in directed evolution. Chromogenic substrates were employed in the early screening devices to track enzyme activity and offer a visual or photometric output. These technologies made it simple to get hold of enzymes with better qualities. The enzymes, however, were solely enhanced for the specific chromogenic substrate. The intended reaction, which did not always include chromophores, was often not affected by the laboratory results. Other screening methods including growth selection, agar-plate screens, and coupled processes that look for byproducts have also been studied. These techniques either have poor accuracy, are too complicated, or are too specialised for a certain response. High-performance liquid chromatography (HPLC) analysis, which allows desired reactions to be monitored in a microtiter plate screening format, is now the preferred approach for commercial enzyme development. This approach may be used for a variety of responses. The difficulty, however, is in accurately simulating the required reaction's process conditions in an analytical system that is compatible with microtiter plates.

An enzyme called phytose, which is introduced to feed, breaks down phytic acid to produce phosphate. It enhances calcium, zinc, and iron absorption in animals by increasing the bioavailability of the phosphorous that is bonded to phytate and reducing the antinutritional action of phytate. Although wild-type enzymes sufficiently catalyse the reaction, the demands placed on the enzyme in this application are extremely high (for more details, see Chapter 3.3 in Ref. 1). The animal's upper digestive system must efficiently hydrolyze phosphorus that is bound to phytate. The creatures' body temperatures are about 37°C, and the surrounding atmosphere is acidic, which is generally beyond the range of what the enzymes can tolerate. Additionally, the enzyme has to endure the 65–80°C feed pelleting process. Since phytase was developed to resist these demanding conditions, it has dominated the worldwide feed enzyme market.

Pharmaceutically active substances (APIs). In the preceding instances, molecules were broken down by enzymes. In the chemical and pharmaceutical sectors, using enzymes to manufacture compounds is a burgeoning application area. Currently, enzymes are the preferred method for creating APIs such chiral alcohols and amines. Despite the fact that enzymes are naturally stereoselective, they cannot always be employed to make synthetic compounds that don't exist in nature. A wide variety of chiral alcohols and amines that cannot be generated by wild-type enzymes may be synthesised by enzyme engineers. For these compounds, enzymes often function better than conventional catalysts.

The method used by Merck and Codexis to produce sitagliptin, a popular medication for the treatment of Type 2 diabetes, is an amazing illustration of enzymatic chiral amine synthesis. An enzymatic method took the role of a chemical procedure that used a Rh-t-Bu-Josiphos catalyst for asymmetric hydrogenation. The researchers' planned wild-type enzyme, nevertheless, was unable to produce sitagliptin. A first variation with very low activity, or 0.2% conversion of 2 g/L substrate using 10 g/L enzyme, was created by preliminary enzyme engineering. Following multiple iterations of directed evolution, the final enzyme variant was able to convert 200 g/L of ketone to sitagliptin at 92% yield and good selectivity (99.5% enantiomeric excess [ee]) using 6 g/L of enzyme in 50% DMSO (4). The transition metal catalyst, the chiral purification step, and high-pressure hydrogenation are no longer required by the new, simplified method. The enzymatic technique minimises waste formation by 19% while increasing the total yield of the synthesis process by 13%.

Ingredients in food. For synthetic chemists, linking sugar molecules may be a headache. Due to the many connection possibilities, functional groups must be sequentially protected and deprotected, which leads to complicated synthetic paths and poor end product yields. On the other hand, enzymes excel in spotting minute variations in molecules. They have the ability to bind two sugar molecules together in very precise ways. This feature has been employed to produce complex human milk oligosaccharides (HMO) with up to five sugar building blocks and to scale up the production of the disaccharide cellobiose.

Exclusive to humans, HMOs are a complex and structurally varied family of glycans. They are attributed with several of breastfeeding's health advantages. Demand for HMO synthesis on an industrial scale has surged among manufacturers of infant formula. With no protecting group chemistry or activation, Glycom and c-LEcta created enzymes that selectively attach fucose or sialic acid to a tetrasaccharide in a single step. This kind of reaction cannot be catalysed using conventional chemical techniques. The secret to achieving regioselective catalysis with a high yield of desired products was enzyme engineering.

Since their original discovery, inventive engineers have employed enzymes in industrial applications. The spectrum of industrial enzyme uses has increased with the use of evolutionary engineering techniques. Enzyme engineering has developed into a discipline that can reliably tune a catalyst for the production of a desired product that functions well in an industrial process.

Conclusion

The importance of bioinformatics in providing advanced design techniques for variant libraries has increased recently. Even while it is currently required to create enzymes in the lab, bioinformatics may one day be so advanced that computers will be able to anticipate the ideal enzyme, doing away with the need to screen huge libraries of enzyme variants. We have to put in the effort to comprehend the specifics of these intricate high-performance athletes in order to get at this future situation. We merely need to use this ability to unlock the latent potential of enzymes for even more applications using the techniques of today's enzyme engineering.

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CHAPTER 9

AN OVERVIEW ON WASTEWATER TREATMENT

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Abstract:

Water is an inevitable part of the daily routine of almost all living beings. During recent years, most parts of the earth have begun to face severe water scarcity and there is a need to adapt the methods for reuse of wastewater. Freshwater availability is not sufficient to fulfill the consumption needs of the entire planet, and most of the freshwater is in the form of ice and snow in Polar Regions. Besides emerging, chemical pollutants and pharmaceuticals contamination in the aquatic environment raises the concern.

Keywords:

Activated Sludge, Microbes, Water Technology, Wastewater Treatment.

Introduction

The population increase requires a constant supply of clean water for drinking, sanitation, irrigation, and various other uses. The presence of pathogens, microbial toxins, and spores in natural water bodies also affects the day-to-day water requirements.Wastewater can be detrimental to the environment if left untreated. That's because waste from humans and pets are a source of several types of waterborne diseases and bacterial contamination.Thanks in part to microorganisms, treating wastewater and sewage is possible. The role of microorganisms in wastewater treatment helps to treat and purify wastewater and make it less harmful to the environment[1], [2].

Microrganisms can also have important impacts on the environment. All healthy ecosystems have their own communities of bacteria that decompose biological matter. However, contamination by sewage and human waste can disrupt the natural balance of bacteria and affect aquatic ecosystems. An influx of human pathogens can cause problems for ecosystems in several ways. First, sewage bacteria can cause hypoxic "dead zones" in aquatic ecosystems. The foreign bacteria rapidly reproduce and consume debris and nutrients in the sewage, but use up all the oxygen in the water in doing so. The de-oxygenated water is harmful to fish and other aquatic life. Coral reefs are also affected by sewage contaminated water. Coral can become infected by human gut bacteria, and this can cause "coral bleaching disease" where coral lose their normal bacterial and algae communities and die[3], [4]. Water quality is not just important for human health, it is important for the human communities that depend on aquatic and marine ecosystems.While there are many different microbes used in sewage treatment, there are three well-known microbes that play an instrumental role in keeping sewage clean. Each of these types

of bacteria help the treatment process in a unique way to ensure there is little to no impact on the surrounding environment[2], [5], [6].

Common microorganisms used in wastewater treatment

Here is a list of bacteria used in sewage treatment you can reference.

Aerobic bacteria

Aerobic bacteria are mostly used in new treatment plants in what is known as an aerated environment. This bacterium uses the free oxygen within the water to degrade the pollutants in the wastewater and then converts it into energy that it can use to grow and reproduce.For this type of bacteria to be used correctly, it must have oxygen added mechanically. This will ensure the bacteria are able to do their job correctly and continue to grow and reproduce on its food source[7]–[9].

Anaerobic bacteria

Anaerobic bacteria are used in wastewater treatment on a normal basis. The main role of these bacteria in sewage treatment is to reduce the volume of sludge and produce methane gas from it.The great thing about this type of bacteria and why it’s used more frequently than aerobic bacteria is that the methane gas, if cleaned and handled properly, can be used as an alternative energy source. This is a huge benefit considering the already high wastewater treatment energy consumption levels. Unlike aerobic bacteria, this type of bacteria is able to get more than enough oxygen from its food source and will not require adding oxygen to help do its job. Phosphorus removal from wastewater is another benefit of anaerobic microbes used in sewage treatment.

Facultative

Facultative microorganisms in sewage treatment are bacteria that can change between aerobic and anaerobic depending on the environment they are in. Note that these bacteria normally prefer to be in an aerobic condition.

Final thoughts on the role of microorganisms in water treatment

Many industrial and municipal wastewater treatment plants use bacteria and other microorganisms to help with the process of cleaning sewage. Picking the right bacteria can be tricky since your selection depends on the condition of your area for effective use. Wastewater treatment can also provide a great source for alternative energy if the anaerobic bacteria are handled correctly. Learning the names of microbes used in sewage treatment and the role bacteria in sewage treatment plays doesn’t have to be a solo job. Consider the water treatment solutions available from AOS to learn more about the role of microorganisms in water treatment and how microorganisms in the wastewater treatment process can help keep your water healthy.

Literature Review

Mohsenpour *et al.* carried out a study in which they discussed about themany industrialized and developing countries are putting an increasing amount of effort into improving the ecological state of their water sources, particularly by lowering the levels of nitrogen and phosphorus in

wastewater discharge. Mixotrophic microalgae have attracted more attention recently because they can be used to clean wastewater. This is predicated on the fact that they may use the organic and inorganic carbon, as well as the inorganic nitrogen (N) and phosphorous (P) in wastewater for their development, leading to the desired decrease in the concentration of these components in the water. The study provided a critical account of microalgae as a crucial stage in the treatment of wastewater to improve the reduction of N, P, and COD while using a small portion of the energy required by traditional biological treatment systems. Here, we start with a summary of the numerous treatment phases, then cover the cellular and metabolic processes that microalgae employ to decrease N, P, and COD of wastewater with identification of the circumstances under which the process may be most likely to be successful. Along with a discussion of bioreactor structure and design, we also discuss the numerous abiotic and biotic elements that affect the treatment of microalgal wastewater.

Abdel-Raouf *et al.* demonstrated that the organic and inorganic pollution is caused by pollutants that were released into the environment as a consequence of home, agricultural, and industrial water activities. In an increasing number of locations, the standard primary and secondary treatment methods for these wastewaters have been implemented in order to remove the readily settled debris and oxidise the organic material that is present in wastewater. The end result is an effluent that is dumped into natural water bodies that is transparent and seems to be clean. However, due to the release of refractory organics and heavy metals, this secondary effluent is highly concentrated in inorganic nitrogen and phosphorus and causes eutrophication as well as further long-term issues. Microalgae cultivation is an intriguing stage in the treatment of wastewater since it enables a tertiary biotreatment together with the creation of potentially valuable biomass that may be utilised for a variety of applications. Because microalgae can utilise inorganic nitrogen and phosphorus for growth, microalgae cultures provide an elegant answer to tertiary and quaternary treatments[5].

Crini *et al.* in their study highlighted the environmental concerns with the chemical and biological pollution of water have gained significant attention from society, government agencies, and business during the last 30 years. The majority of home and industrial processes result in wastewaters with unwanted hazardous pollutants. Water resources must be continuously protected in this situation. The removal of insoluble particles and soluble pollutants from effluents is done using a mix of physical, chemical, and biological processes in current wastewater treatment techniques. This article gives a general review of wastewater treatment techniques and discusses the benefits and drawbacks of the current technology[1].

Jasim *et al.*designed a system for the Al-Hay wastewater treatment plant (WWTP) takes the requirements into account. Also mentioned are the features of wastewater that is physical, chemical, and biological. The project is launched to build a wastewater treatment facility based on the population of Al-Hay city. Following the design of the girt chamber, equalisation basin, oil and grease removal system, aeration tank, and secondary settling tank, the mean cell residence time, aeration tank volume, hydraulic retention time, F/M ratio, return sludge flow rate, sludge production, and oxygen requirement values were calculated. These data have also been subjected to GPS X modelling. A typical schematic of a wastewater treatment plant is shown,

beginning with the influent flow, aeration tank, and settling (clarifier) tank. Additionally, the simulation time is shown. The metrics, such as TSS and solids, are often improved with longer times. This is a measure to enhance the model's fit to the real data for the secondary effluent TSS. The study illustrates the design of the Al-Hay wastewater treatment plant's treatment procedure (WWTP). The mathematical process design for WWTP was also included in the article. The estimated sludge age (c) and measured yield have been linked. The mixed liquor suspended solid and sludge age are correlated. The measured yield's value has been noted; values range from 0.2 to 0.6 kgVSS/kg. The production of sludge is 3339.18 Kg/day, and the sludge retention duration is 27.7 days. These results show how well the biological tank of the Al-Hay WWTP was working[10].

Wollmann *et al.* discussed on theusage of natural resources is faced with inescapable obstacles as a result of current global environmental concerns. The provision of clean water for the human population is becoming into a worldwide issue. Our nutrition and health are threatened by a variety of organic and inorganic contaminants in urban, industrial, and agricultural streams, including microplastics, excessive nutrient loads, and heavy metals. Thus, it is becoming more and more crucial to create effective technology for wastewater treatment as well as circular economic theories. The prospect of recycling industrial waste to produce new sources of raw materials for energy and material usage is shown by the biomass production of microalgae utilising industrial wastewater. The potential of unconventional extremophilic (thermophilic, acidophilic, and psychrophilic) microalgae as well as industrial algae-wastewater treatment ideas that have actually been implemented are all highlighted in this study of algae-based wastewater treatment technologies[2].

Jain *et al.* highlighted that a thriving economy and the multifaceted growth of society depend on access to clean, safe water. Rapid population growth, growing industrialization, urbanization, and extensive agricultural practises have led to the production of wastewater that has made the water not only contaminated or deadly, but also dirty or polluted. Every year, millions of people pass away from diseases spread by drinking water tainted with harmful pathogens. This promising technology has accomplished amazing feats in a number of industries, including wastewater treatment. Nanomaterials are well suited for use in wastewater treatment because of their high surface to volume ratio, high sensitivity and reactivity, high adsorption capacity, and ease of functionalization. The methods being developed for wastewater treatment using nanotechnology have been discussed in this article and are based on adsorption and biosorption, nanofiltration, photocatalysis, disinfection, and sensing technology .

Discussion

Waste water disposal is a major concern due to inadequate sewage treatment capacity and rising sewage production. The Water and Sewerage Board now bypasses a significant percentage of waste water in STPs and sells it to surrounding farmers on a fee basis, or the majority of the untreated waste water ends up in river basins and is indirectly utilised for irrigation. Selling wastewater and renting pumps to raise it are two of the lowest socioeconomic strata's most profitable sources of revenue in places like Vadodara, Gujarat, where there are no other water sources (Bhamoriya, 2004). According to reports, irrigation using sewage or sewage combined

with industrial effluents results in savings of 25 to 50 percent on N and P fertiliser and increases crop output by 15 to 27% compared to irrigation using normal waters. According to Strauss and Blumenthal (1990), about 73,000 acres of per-urban agriculture in India are irrigated using effluent. Farmers in peri-urban locations often use intense year-round vegetable production methods (300–400% cropping intensity) or other perishable commodities like fodder, earning up to four times as much from a unit of land area as freshwater farmers. Major crops that get irrigation from waste water include:

Cereals: 2100 acres of land are irrigated with waste water to grow paddy along a 10 km section of the Musi River (Hyderabad, Andhra Pradesh) where wastewater from Hyderabad is disposed-off. In Kanpur and Ahmedabad, waste water is used to irrigate wheat.

Vegetables: Near the Keshopur and Okhla STPs in New Delhi, 1700 acres of land are used to grow a variety of vegetables. These farms cultivate a variety of vegetables, including cucumbers, eggplant, okra, and coriander in the summer and spinach, mustard, cauliflower, and cabbage in the winter. Spinach, amaranth, mint, coriander, and other crops are cultivated all year round in Hyderabad's Musi River Basin.Kanpur farmers use wastewater to produce marigolds and roses. Farmers in Hyderabad are growing jasmine using wastewater.Avenue trees and parks: In Hyderabad, public parks and avenue trees are irrigated with secondary processed effluent.

Fodder crops: 10,000 acres of land are irrigated with wastewater to grow paragrass, a kind of fodder grass, near Hyderabad beside the Musi River.The biggest single wastewater usage system in aquaculture in the world is the East Kolkata sewage fishery.Agroforestry: In the Karnataka villages close to Hubli-Dharwad, waste water is used to irrigate plantations of trees such sapota, guava, coconut, mango, arecanut, teak, neem, banana, ramphal, curry leaf, pomegranate, lemon, galimara, and mulberry.

Fields that are irrigated with wastewater provide both male and female agricultural employees with excellent job opportunities to grow fruits, vegetables, flowers, and fodder that may be sold in surrounding markets or used by their animals. Wastewater helps a 266 million rupee yearly agricultural output in the rural districts of Gujarat's Vadodara. According to estimates, sewage fluids in India have the capacity to yearly irrigate between 1 Mha and 1.5 Mha of land, as well as provide up to 1 Mt of nutrients and 130 Mt of labour. However, there are some restrictions regarding waste water treatment and reuse in agriculture, including the production of waste water when crops do not require irrigation water, the location of the plants in relation to the land needing irrigation, the match between the waste water fertiliser content and the crop requirements, the risk of over-application, and a high incidence of weeds and insect pests due to, generally speaking, low pesticide use in agro-forestry systems. In fact, salt buildup in the soil, odour issues, salt and colour leaching impacting groundwater and downstream water quality, etc., have all been associated with intensive land application.

Institutional structures and policies for wastewater management:

For the time being, there are no specific laws or rules governing the safe management, transportation, and disposal of wastewater in the nation. The Constitutional Provisions on sanitation and water pollution, the National Environment Policy of 2006, the National Sanitation

Policy of 2008, the Hazardous Waste 6 (Management and Handling) Rules of 1989, the Municipalities Act, the District Municipalities Act, and other legal provisions serve as the foundation for the current policies governing wastewater management.

State governments and urban local bodies are in charge of building sewerage infrastructure for sewage disposal, though central programmes like the Jawaharlal Nehru National Urban Renewal Mission, the Urban Infrastructure Scheme for Small and Medium Towns, the National River Conservation Plan, and the National Lake Conservation Plan supplement their efforts. However, State governments/urban local authorities and their agencies are in charge of operating and maintaining sewage infrastructure, including treatment facilities. State Pollution Control Boards have the legal authority to take action against any noncompliant agency under the Water Act of 1974. The Water Act of 1974 also places a strong emphasis on using treated sewage for irrigation, but state governments have chosen to overlook this problem.

The government of India's Ministry of Environment and Forests (MoEF) launched a technical and financial assistance programme to encourage the development of shared facilities for the treatment of effluents produced by SSI units that are grouped together. A 50% project capital cost subsidy was given under the Common Effluent Treatment Plant (CETP) financial aid plan, with 25% of the cost split by the Central and State Governments. As a consequence, 88 CETPs encompassing more than 10,000 polluting enterprises have been established across India, with a combined capacity of 560 MLD.

In addition to building treatment facilities, the federal, state, and board governments have offered financial incentives to businesses and investors to encourage them to make investments in pollution management. The rewards/concessions that are available to them include:

Devices and systems implemented to reduce pollution or conserve natural resources are eligible for a greater depreciation allowance.

Systems and equipment classified under depreciation allowance are eligible for higher rates of investment allowance.Industries are urged to relocate from urban regions in order to lessen pollution and clear congestion in metropolitan areas. If the proceeds are utilised to purchase land or build a building in order to move the company to a new location, the capital gains resulting from the transfer of the buildings or lands used for the business are free from tax.The Central Government provides customs tax exemptions for goods imported to enhance chemical industry safety and pollution management.Large quantities of seriously contaminated effluents are produced by bio-refineries that make fuel ethanol. For such heavily laden wastewaters, anaerobic digestion is often used as the initial stage in the treatment process. Currently, 90% of the Chemical Oxygen Demand (COD) in the effluent stream is effectively removed by anaerobic biological treatment of biorefinery effluents. 80–90% of the BOD is removed at this step, and 85–90% of the biological energy is recovered as biogas. The effluent from an anaerobic digestion phase needs further aerobic treatment to lower the BOD to acceptable levels. However, biological treatment methods by themselves are insufficient to comply with increasingly stringent environmental laws. A wise tertiary therapy selection might lessen colour and lingering COD.

A further strategy is to use algae. By sequentially using heterotrophic and autotrophic algal species, wastewater can be treated with algae, which has the benefit of reducing organic and inorganic loads, raising dissolved oxygen levels, reducing CO2 pollution, and producing valuable biomass, which can be used to make "organic" fertilisers. Algae are known to flourish at very high concentrations of inorganic nitrates and phosphates, which are harmful to other species, as shown in research on eutrophication. This specific feature of algae may aid in the cleanup of heavily contaminated wastewater.

Numerous research on the effectiveness of constructed wetlands (CWs) in the treatment of municipal water have been done. CWs are a practical solution for treating municipal wastewater. Given that wetland hydraulics determine how well it treats runoff, a well-designed built wetland should be able to sustain its hydraulic loading rates (HLR) and hydraulic retention time (HRT). Indian experience treating various types of wastewater using engineered wetland systems has mostly been on an experimental scale. Field-scale artificial wetland systems in developing nations like India are severely hampered by the need for a sizeable amount of land that is not always accessible. Subsurface (horizontal/vertical) flow systems are therefore being viewed as the more advantageous choice for developing nations because they typically have a 100 times smaller size range and three times shorter HRTs (generally 2.9 days) than surface flow systems. HRTs that are shorter often mean less land is needed. Batch flow systems with shorter detention times have reportedly been linked to smaller treatment areas and more effective pollutant removal (Kaur *et al.*, 2012a, b). So it would appear that batch-fed vertical subsurface flow wetlands would be more acceptable under Indian settings

The most vulnerable groups are farm labourers and their families that use furrow or flood waste water irrigation methods. Spray and sprinkler irrigation increases the likelihood that salts, pathogens, and other pollutants may be deposited on crop surfaces and have an impact on the adjacent populations. Although drip irrigation is the safest watering technique, it might block emitters depending on the amounts of total suspended solids in the effluent. It has been shown that using drip systems in conjunction with the proper filters, such as gravel, screen, and disc filters, significantly lowers the frequency of clogging and coliform.Post-harvest treatments are a crucial feature of wastewater-irrigated crops' health risk reduction strategies and are especially crucial for addressing contamination that may occur both on the farm and after the crops have been harvested. Adopting some of the low-cost techniques, such as frequent washings, exposing the product to sunshine, growing the crops on beds, removing the two outermost leaves of cabbage, and also cutting beyond certain height from ground level, might significantly reduce the health risks.

Situation and need for knowledge and expertise in wastewater safety

Wastewater is more salty because it contains dissolved particles from urban areas, which are then concentrated even more by high levels of evaporation in dry and tropical regions. Heavy wastewater usage in agriculture may result in salinity issues and reduce the productivity of the land. When harmful contaminants are released into the environment in excess, they may accumulate and worsen groundwater and downstream water quality by promoting the development of weeds, algae, and cyanobacteria.Water quality and the local climate have an

impact on the kinds of crops that farmers can grow. High evaporation rates in arid and semiarid areas make wastewater more salty, necessitating the production of salt-tolerant crops and variations. Since many fodder crops can tolerate salt, it may be encouraged to utilise wastewater for fodder cultivation in urban and peri-urban regions, especially where there is a high demand for dairy products. However, the quality of milk may be impacted, posing a risk to people as a result, and the health of the cattle fed on wastewater-irrigated fodder may be substantially compromised (as is the case today in Hyderabad).

Wastewater is a rich source of plant nutrients, therefore soils that get irrigation from it are fertilised. As a result, the amounts of fertilisers to be applied should be modified based on the nutritional content of the wastewater, the quantity to be applied, and the crop's need for nutrients. Additionally, routine soil testing should be done to look for signs of soil disease or unbalanced nutrients.Crop contamination may be efficiently reduced by ceasing watering 1-2 weeks before to harvest.This is difficult to put into practise, however, since many plants (particularly green vegetables) need irrigation up until harvest in order to maximise their market value. Some fodder crops that don't need to be picked at the height of freshness may be suitable for this strategy.

Alternative land uses such as the establishment of manmade forests with high economic value and having high rate transpiring trees like sisal, mahogany, Eucalyptus, poplar, bamboo, neem (Azadirachta indica), shisham (Dalbergia sissoo), etc. for nonedible products like fuel and timber and developing green belts around cities can be another approach to overcome health hazards in situations where land has already been contaminated and food crops The quality of groundwater has been shown to be unaffected by wastewater discharges under such systems, and soil heavy metal concentrations have also been found to be low. According to Thawale *et al.* (2006), the biochemical oxygen demand reduction effectiveness of tree plantings ranges from 80.0 to 94.3%. Therefore, areas to be covered by high rate transpiration systems may develop depending on changing water demand in various seasons.The tolerance of different crops to the presence of heavy metals in the soil varies. They vary as well in terms of the accumulation of absorbed heavy metals in various plant sections and metal affinities. Therefore, crops should be chosen such that they can withstand the poisonous components of wastewater and accumulate in plant parts that are of the utmost value or are not eaten.

The right mixture of timber trees, fruit trees, fodder, industrial crops, and cereals should be developed depending on the amount and quality of the wastewater that is available for usage. It is important to encourage the use of wastewater in public parks, golf courses, green spaces, and tree plantations.Farmers should be encouraged to use contemporary irrigation techniques like drip irrigation.

For wastewater of various grades, combinations of emitter size, locations, and filter units must be devised for optimum management.In the field, effective microbial strains for wastewater cleanup should be sought out and used.Research to develop effective, affordable, and sustainable natural wastewater treatment technologies that preserve nutrients while eliminating pathogens and other contaminants may get more financing.Similar to this, further study is required to identify profitable crops with an economic non-edible component in order to prevent food chain contamination and improve phyto-remediation of contaminated locations.Caste, class, ethnicity,

gender, and land tenure are socioeconomic factors that affect the kind of livelihood activities that rely on wastewater. If the study findings are to be used in a sustainable manner, participatory research that takes into consideration farmers' concerns, views, and behaviours is required. For the creation of realistic policies, both socioeconomic and bio-chemo-physical data must be gathered via field surveys, water, soil, and 10 plant sample and analysis, group discussions, and in-depth interviews with users, researchers, and policy makers.

Farmers should be reminded to wash their product in fresh water before bringing it to market. To lower the pathogen burden, consumers should cook and wash their dishes often.Farmers, consumers, and policymakers should be informed about the problems and effects of wastewater via regular health checks, the delivery of antihelmintic medications, and awareness campaigns.For the use of wastewater that is safe and sustainable, local knowledge, indigenous technical knowledge (ITK), and traditional knowledge should also be carefully recorded.A group of research institutions and businesses will work together to discover effective methods for using and treating wastewater in this situation. This co-creation approach will encourage the growth of businesses in the bio-treatment, wastewater re-use, and agricultural water-saving technology sectors. Additionally, it would incorporate the importance of co-learning, establish connections between conventional and industrial agri-production systems, better take advantage of market opportunities, and help researchers and project partners conduct science-based research on wastewater treatment and management, opening up a variety of opportunities for low-cost, long-term sustainable up-scaling procedures.

Conclusion

Because wastewater isn't treated in impoverished nations like India, reusing it might cause issues. Finding low-cost, low-tech, user-friendly ways that both preserve the deterioration of our priceless natural resources and do not jeopardise the lives of those who rely heavily on wastewater is therefore a problem. It is widely acknowledged that the utilisation of built wetlands is an effective method for treating wastewater. Constructed wetlands use less material and energy than traditional treatment systems, are simple to run, don't have any issues with sludge disposal, and can be maintained by unskilled workers. As they are powered by the natural energy of the sun, wind, soil, microbes, plants, and animals, these systems also have cheaper building, maintenance, and operating costs.

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CHAPTER 10

ROLE OF MICROORGANISMS IN FOOD INDUSTRY

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Abstract:

The microbiota in the human microbiome plays an important, critical role in our health. Diet is a contributing factor to the optimal function of this biological system and techniques for processing food contribute toward this. Overly processed foods can have a detrimental effect on the proper function of the human microbiome. Techniques to improve the health of this system are therefore critical in food design.

Keywords:

Diet, Food, Nutrition, Microbiome, Microorganisms, Probiotics, Yeast.

Introduction

Microbes have been used in the production of various important foods and beverages which are part of the average human diet for thousands of years. Even though we have utilized micro-organisms for the production, preservation, and fermentation of foods for a long time, food microbiology does not have a precise beginning. The development of the discipline has been a gradual process built upon observation of natural processes and experimentation. There are several specific uses of microbes in the food industry today. Yeast, including Saccharomyces cerevisiae, is used to leaven bread as well as in the fermentation of alcoholic beverages. Certain bacteria, including lactic acid bacteria, are used to produce yogurt, cheese, and pickles. Some cheeses (blue cheese, Stilton, Gorgonzola, and so forth) use molds to aid in ripening and provide characteristic flavoring. Other uses include citric acid production by fungi, the addition of probiotic supplements in yogurt and drinks, and the production of vinegar[1], [2].

Currently, there are around 3500 traditionally fermented foods available worldwide. In addition to alcoholic beverages, fermentation is used to enhance taste in coffee, grains, and tea leaves after harvest. Market availability for non-alcoholic beverages like kombucha is also quite widespread. The eating of fermented foods varies geographically in a number of ways. Foods fermented from manioc are popular in Africa along with cheese and bread, while fermented fish and soy items are often eaten in Asia.Microorganisms are crucial to the food business. They are utilised in the manufacturing of a variety of food items and are also in charge of food spoiling, which results in infections and intoxication, as was previously covered in the preceding article Contributions of Microbiology in the Food Industry[3], [4].

The majority of the time, food items get contaminated by microbes while being processed, stored, transported, or distributed, or even just before consumption.The growing conditions offered by various food sources vary for microorganisms. Inherent variables including nutrients,

pH, moisture content, and the physical makeup of the food, as well as external elements like temperature, relative humidity, and gases, regulate microbial development (CO_2, O_2).

Thus, given the ideal environments created by the internal and external elements, microorganisms flourish and cause the food product to deteriorate and degrade, resulting in a sour, rancid-smelling, or fungus-covered mass that is inedible.Additionally, microbial development in food may result in outward modifications including colour changes, the deposit of powdery growth, effervescences on the food surface, etc. Food may get contaminated by microbes at any stage of the manufacturing process, including growing, harvesting, transit, storage, and final preparation.Foods that are not kept correctly may also get spoiled. Protein- and fat-rich meat and dairy products provide a perfect habitat for microbial deterioration, which leads to proteolysis and putrefaction of the food items. Fruits and vegetables deteriorate in a different way than meat and dairy products because they contain significantly less protein and fat.

Uses of Microorganisms

There are already around 3500 traditionally fermented foods available worldwide. They are a staple of our everyday lives and may be either animal or vegetable based. Not only are alcoholic beverages fermented; following harvest, cocoa beans, coffee grains, and tea leaves are also fermented to produce their distinctive flavour characteristics.The biggest class of unicellular creatures is the bacteria. Cocci, or spherical cells, bacilli, or cylindrical or rod-shaped cells, and spiral or curved forms are the three categories into which the morphologies of medically significant bacteria are categorised. The bacteria that cause sickness are mostly gram-negative pathogens, although three gram-positive rods have been linked to food intoxications*: Clostridium perfringens, Bacillus cereus,* and *Clostridium botulinum*

Other common bacteria that cause food to spoil, infect people, and cause disease include Acinetobacter, Aeromonas, Escherichia coli, Proteus, Alcaligenes, Flavobacterium, Pseudomonas, Arcobacter, Salmonella, Lactococcus, Serratia, Campylobacter, Shigella, Citrobacter, Listeria, Staphylococcus, Micrococcus, Corynebacterium. Additionally, diverse food and dairy products are produced using various bacterial strains. The manufacturing of fermented foods and dairy products uses strains of Streptococcus, Lactobacillus, Bifidobacterium, Erwinia, etc. Yogurt is made using Streptococcus thermophilus and Lactobacillus bulgaricus.Microorganisms are studied, applied to, and used in analytical microbiology as reagents for the quantitative analysis of specific chemical substances. These processes are dependent on how a certain microbe responds to its surroundings.This analytical technique for the quantitative assessment of the substance may be developed to meet the needs of food culture, fermentation, or preservation if a microbe responds to a specific chemical entity with a measurable reaction and produces an acceptable result.

Molds

Molds are multicellular filamentous fungus that may be easily identified by their fuzzy or cottony appearance when they grow on food. They have a low pH, a minimal moisture requirement, and are mostly responsible for food spoiling at room temperature (25–30 oC).Grain and maize may quickly develop mould if they are kept in wet circumstances. Because moulds

need free air to proliferate, they develop on the surface of tainted food. Molds are also used in the production of various meals and food items. They are used in the ripening of several food items, including cheese (e.g. Roquefort,Camembert).Molds are also raised for food and as animal feed, and they are used to make components like citric acid, which is used in soft drinks, and enzymes like amylase, which is used to make bread. aNumerous oriental foods ripen in large part due to moulds. Wine is made by allowing grapes to decay using a species of Bothrytiscinerea. The distinctive Finnish fermented milk known as viili is produced through lactic fermentation utilising mould.

Yeasts

Yeasts are widely used in the food business because they can ferment glucose into ethanol and carbon dioxide. The baker's yeast, which is the most used kind, is produced industrially. The majority of beers are fermented using Saccharomyces carlsbergensis most often. Brettanomyces, Schizosaccharomyce, Candida, Cryptococcus, Debaryomyces, Zygosaccharomyces, Hanseniaspora, and Saccharomyces are the other yeast species of relevance.

Factors Affecting Microorganism Growth

Understanding how to employ the microorganisms is essential to understanding their usage in the food business since they behave differently depending on the environment and circumstances in which they are used.

A. By cutting, washing, boiling, or pickling, removing or destroying them.
B. By introducing substances like acid or alcohol or by fostering competition, organisms may develop.
C. Reducing contamination from people, objects, the environment, and uncooked or undercooked food.
D. Reducing microbial growth by thoroughly cleaning and sterilising the apparatus (container etc).
E. Modifying the temperature, light exposure, pH of the storage area, and other environmental variables.
F. Although each of these influences on development may happen independently, they may also happen concurrently in nature. The impacts of many factors that are slightly detrimental to microbial development are additive.

The most significant microorganisms responsible for food spoiling are bacteria, moulds, and yeast, which are also used to the greatest extent in the preparation of food and food products.A broad range of cultured milk products are produced by fermenting dairy products using various strains of bacteria and fungus. These cheese-making procedures use both fungus and bacteria.Milk that may be processed to produce a broad range of cheeses, including soft unripened, soft ripened, semisoft, hard, and extremely hard kinds, is coagulated using lactic acid bacteria.In the food and health industries, microorganisms like Lactobacillus and Bifidobacterium are employed.Another well-liked food source is spirulina, a cyanobacterium that is offered in specialised shops.Molds are employed to cause grapes to rot so that various types of wines may be produced.One of the most significant fungi utilised as a food source is the

mushroom (Agaricus bisporus).Alcoholic drinks like beer are made by fermenting grains and cereals with various yeast strains.

Literature Review

Alizadeh-Sani *et al.* demonstrated that Emulsifiers are a broad class of substances referred to as surface active agents or surfactants. A chemical reaction's speed is slowed down and its stability is increased by an emulsifier. Since they vary from chemical surfactants in that they are non-toxic, biodegradable, foamable, biocompatible, effective at low concentrations, and highly selective in a range of pH, temperatures, and salinities, bioemulsifiers are sometimes referred to as surface active biomolecule compounds. Emulsifiers may be made by bacteria, fungi, and yeast and are present in many different natural resources. The molecular weight of biosurfactants is lower than that of bioemulsifiers. The way that emulsions work is intimately correlated with their chemical composition. In order to better understand the numerous bioemulsifiers utilised in the pharmaceutical and food industries, this report looked at how they are made from microorganisms. We investigated species that can produce biosurfactants in this study. Both the petroleum sector and environmental remediation might make use of these low-cost substrates[5].

Rather *et al.* highlighted that in order to breach human defences and counteract the effects of powerful antibiotics, pathogenic bacteria have evolved a variety of tactics throughout time. One such tactic used by bacteria to resist and live even in the presence of antibiotics and other unfavourable environmental circumstances is the creation of biofilm on both biotic and abiotic surfaces. Microorganisms may live safely within biofilms, which are self-produced extracellular polymeric materials made of polysaccharides, extracellular proteins, nucleic acids, and water. Pathogenic bacteria are severely affecting creatures' health and ability to live as a result of this adaption method. In this overview, we talk about how harmful bacteria may colonise human tissues and medical implants. We also concentrate on food deterioration, disease outbreaks, mortality linked to biofilms, economic costs, and other significant issues related to pathogenic bacteria that create biofilms in the dairy, poultry, ready-to-eat food, meat, and aquaculture sectors of the food industry.

Quinto *et al.* carried out a study in which they demonstrated that after consuming tainted food, microbial infections are the cause of several foodborne illnesses. Numerous preservation techniques have been created to guarantee the microbiological food safety as well as the nutritional benefits and sensory qualities of food. However, because of consumer concern about health risks, the need for natural antibacterial agents is rising. Furthermore, the development of multidrug resistant microbes as a result of antibiotic usage has caused researchers and the food sector to place an even greater emphasis on natural antimicrobials. Natural antimicrobial substances derived from flora, fauna, bacteria, viruses, algae, and mushrooms are discussed. The food industry's interest in innovations is evaluated, along with fresh viewpoints from relevant academics. The shelf-life of food would also be increased using these novel methods, which should help reduce microorganisms that cause foodborne illness[6].

Faridah *et al.* reported that up to this point, biotechnology has advanced quickly. The use of biotechnology for human needs, particularly the food business, was fairly widespread. Some

traditional and contemporary biotechnological procedures need the employment of microbes in order to function. Through fermentation, certain microbes were added to traditional foods including yoghurt, tape, cheese, and salted vegetables. Additionally, microbes are also employed in the DNA recombination process to create premium food items like GMOs (Genetically Modified Organism). Indonesia is a nation where Muslims make up the majority of the population, hence halal considerations were important while providing meals. Foods that use microorganisms and fall under the biotechnology category must pay close attention to the halal requirement. In this situation, consideration must be given in order to guarantee that non-halal ingredients are not contaminated throughout any stage of the manufacturing process[7].

Kumar Verma highlighted the primary elements of food that enhance the organoleptic properties of food and increase consumer acceptance of food are aroma and flavour. Although the industries microbiological source for commercially manufactured aromatic and flavouring chemicals, its idea has been a driving force for human actions from the dawn of humanity. The sustainable method that microbial flavour compounds may offer natural additives for the food processing industry has increased interest in them over the last several decades. Microbial bioprocess products, which range from antibiotics to fermented functional meals, also have a number of positive health effects.

In the food sector, microbial contamination is a growing problem. Studying microorganisms' traits and behaviours over a range of platforms is crucial for comprehending their impact. Rapid, on-site, and sensitive approaches for the identification of microorganisms in food matrices are required due to growing concerns about foodborne outbreaks and the obsolescence of labor-intensive, time-consuming culture-based enumeration techniques for real-time applications. A short overview of microorganism biomarkers and frequently utilised bio-recognition ligands for detection assays are included in the current article. In order to analyse microorganism activity in complicated food matrices, current advancements in bio-recognition based detection approaches are discussed, and the molecular interaction between biomarkers and ligands is critically analysed. Important findings and judgements. Jayan *et al.* carried out the study by examining certain biomarkers of microorganisms as nucleic acids, proteins, antigens, and metabolic products, it is possible to identify the presence of microbes in food. Recent bio-recognition ligands may enhance the selectivity of cell detection from complicated food matrices using detection methods as biosensors, lateral flow assay, and microfluidic devices. The new bio-recognition-based techniques might be used in the industry to assure food safety since they bridge the gap between culture-dependent enumeration and molecular techniques. To evaluate the effectiveness of all developing approaches in actual samples, a validation process must be developed[8].

Koubaa *et al.* demonstrated that the food sector is becoming more and more interested in finding cheaper, more environmentally friendly alternatives to traditional methods of plant extraction and microbial inactivation. Chemical extraction procedures are often hampered by a number of challenges, such as the use of chemical solvents, which is connected to several health and environmental problems. Furthermore, it is often necessary to employ high temperatures to enhance and/or expedite the processes that might harm and destroy the thermolabile substances.

Ultrasound aided supercritical fluid extraction is one of the most intriguing ways that could be competitive to the present methods of extracting compounds from plant matrices and microbial inactivation of food goods. This non-exhaustive study, which spans the previous 20 years, provides a critical analysis of the most significant published findings using this cutting-edge technique [9].

Due to the high percentage of spoilage bacteria, consumers are becoming more concerned about the sustainability of the food supply. The food industry has to invest in cutting-edge technology that can preserve food's nutritional value, improve bioactive chemical bioavailability, ensure economic and environmental sustainability, and satisfy sensory preferences of customers. Because bioactives are susceptible to high-temperature processing, heat treatment has a detrimental impact on the nutritional and sensory characteristics of food samples. Non-thermal techniques are required to decrease food losses, and sustainable advancements in food safety, nutrition security, and preservation are key factors for the age to come. Non-thermal procedures have been authorised because they enhance food quality, use less water, produce fewer emissions, are more energy efficient, guarantee clean labelling, and make use of leftover food byproducts. These procedures include cold plasma, sonication, high-pressure processing, pulsed light, and pulsed electric field (PEF).

Nabi *et al.*addressed how this method may affect the microbiological, physicochemical, and nutritional qualities of foods for a sustainable food supply. This strategy also highlights the limits of this new method. The analysis of HPP to satisfy the worldwide requirements was successful. The raw material, water, energy, and nutritional contents must all be balanced in a restricted global food supply. HPP has shown success in preventing microbial deterioration while maintaining nutritional content. The fundamental prerequisites for producing clean-label, sustainable food are met by HPP technology. Producing meals that are nutritionally adequate for customers' health demands a limited amount of resources.

Peng *et al.* highlighted that lactic acid bacteria (LAB) have been used for fermentation and preservation for a very long time. Their metabolic products, which may enhance the nutritional and sensory qualities of foods, as well as their antibacterial substances, which help to increase food items' shelf lives, are responsible for this property. Pulsed electric fields (PEF), power ultrasound (US), high-pressure processing (HPP), ultraviolet (UV), and microwave (MW) are some developing technologies that have received a lot of interest for their use as mild processing methods in the food business. They benefit from effectively inactivating the bacteria and preserving the food goods' fresh qualities. These technologies have the potential to improve a number of processes when used at a sub-lethal level, including better microbial growth and fermentation conditions as well as altered LAB metabolic characteristics. The characteristics of LAB and their uses in the food business are covered in this review. With a particular emphasis on microbial inactivation, growth stimulation, and enhancement of the advantageous characteristics of LAB by new technologies, it addresses the effects of developing technologies on these bacteria[10].

Discussion

Our food supply serves as a source of nutrients and energy for microorganisms. They take advantage of nutrition to multiply. The food may deteriorate as a consequence of this. They decompose a nutrient or create new chemicals to cause enzymatic changes and off flavours in meals. They "spoil" our food by doing this, rendering it unsuitable for ingestion. To avoid this, we restrict the interaction between microorganisms and our foods (prevent contamination), remove them from our foods, or modify storage conditions so that their development is inhibited (preservation), preventing food spoiling.

If the microorganisms in question are harmful, their presence in our diet will also cause an epidemic of illnesses that are transmitted via food. Many of our meals either act as a source of harmful germs or promote their development. Once again, we make an effort to stop them from entering and growing in our food or to get rid of them via processing.The interactions of microbes with our diet are also advantageous. Numerous popular cultured foods, such as cultured buttermilk, yoghurt, sauerkraut, pickles, and tofu, are made possible by the helpful actions of microbes.Food serves as the substrate for the development of microbes, hence its properties are crucial. Understanding the properties of the food or substrate is important since they will dictate which microbes can or cannot thrive there. The microbial flora that may eventually emerge and thrive in it can only be predicted by one person at that point. Due to their actions, these microflora will cause biochemical alterations in food. Depending on the biochemical alterations, they will either be advantageous or detrimental.

It is crucial to understand the elements that encourage or impede the development of microbes. It will aid in our comprehension of the principles governing food preservation and deterioration. Hydrogen-ion concentration, moisture, oxidation-reduction (O-R) potential, nutrients, biological structure, and the presence of inhibitory chemicals are the primary food compositional elements that affect microbial activity.

The activity and stability of macromolecules like enzymes are significantly influenced by the environment's acidity and alkalinity (pH). These enzymes are crucial for the development and metabolism of microorganisms. Thus, pH has an impact on the development and metabolism of bacteria.

For growth, every microbe has a minimum, maximum, and ideal pH. The pH ranges for filamentous fungi are 3.5–4.0, 6.0–8.0 for yeasts, and 4.5–6.0 for bacteria in general. In contrast to most yeasts and bacteria, moulds can develop across a larger pH range, and many moulds can tolerate acidities that are too high for yeasts and bacteria to survive. The majority of fermentative yeasts, such as fruit juices, grow well in the pH range of roughly 4.0 to 4.5, whereas film yeasts thrive on foods that are acidic, such sauerkraut and pickles. However, most yeast do not thrive in alkaline environments and do not contribute much to the rotting of foods with high pH levels. However, many yeasts thrive very close to neutral pH levels. There are certain exceptions, such as the fact that some bacteria, such as lactobacilli and acetic acid bacteria, may thrive at mild acidity. This is especially true of bacteria that make significant acids as a consequence of their

activity. These thrive in a pH range of 5.0 to 6.0, but other organisms, such as proteolytic bacteria, may grow in meals with a high (alkaline) pH like that of preserved egg white.

Molds and yeasts are less sensitive to pH than bacteria, with pathogenic bacteria being the most sensitive. Table 2.1 lists the pH values of several popular meals as well as the pH range required for certain groups of microorganisms to flourish and a few dangerous bacteria that are connected with food.

Microorganisms' pH minima and maxima differ as a result of other significant parameters including temperature, moisture content, salt concentration, redox potential, and so on. Alcaligenes faecalis, for instance, may grow across a larger pH range in the presence of 0.2 M sodium citrate than it can in the absence of NaCI. The pH minimum of certain lactobacilli also relies on the kind of acid being employed; for instance, growth may occur at lower pHs in the presence of citric, hydrochloric, phosphoric, and tartaric acids than in the presence of acetic or lactic acids. In general, bacteria are less acid-tolerant than yeast and mould.

The lag phase of the microbe increases when it is cultivated at a pH that is either higher or lower than its optimal pH. If the meal has a strong buffering capacity as opposed to one that has a low buffering capacity, the greater lag would last for a longer period of time. Food with a good buffering capacity would have a delayed microbial-induced shift in pH. The pH has a negative impact on a respiring microbial cell because it has an impact on how enzymes work and how nutrients are transported into the cell. Along with influencing the rate of development of microbes, pH also has an impact on how long they survive through storage, heating, drying, and other types of processing. Many times, the pH may be favourable at first, but as the organism grows, it may change and become unfavourable. In contrast, a pH that is initially limiting may be changed by a small number of bacteria to one that is more conducive to the development of several more microbes.

Foods' natural pH vary, although the majority are neutral or acidic. With certain exceptions, such as egg white, where the pH rises to around 9.2 when CO_2 is expelled from the egg after laying, materials with an alkaline pH often have a somewhat disagreeable taste. With the use of a pH metre, it is simple to determine the product's pH. This number, however, is insufficient on its own to foretell microbial spoilages. Knowing the acid responsible for a certain pH is also advantageous since some acids, especially organic acids, are more inhibitive than others.

The kind of microflora present in food, as well as the pace and kind of food decomposition, are significantly influenced by the acidity of a foodstuff. For instance, the majority of meats and seafoods have a final pH of 5.6 or higher. As a result, these items may get spoiled by bacteria, mould, or yeast. Similar to fruits, most vegetables have higher pH values than those of fruits, making them more susceptible to bacterial deterioration than fungal decomposition. Their rotting is mostly caused by bacteria that cause soft rot, such Erwinia carotovora and pseudomonads. However, a lower pH (below 4.5) in fruits hinders bacterial development, and yeasts and moulds predominate in sourness.

Meat spoils more quickly than fish when it is cold. This is because post-rigor mammalian muscle has a pH of around 5.6, which adds to the meat's extended storage life. Fish, on the other hand,

have a pH of 6.2 to 6.5. Under cool temperatures, Shewanella (formerly Alteromonas) mostly causes spoilage. Because it is a pH-sensitive bacterium, it is important for fish rotting but not for normal meat (pH 6.0).

Halibut (pH 5.6) and other fish with naturally low pH levels have superior keeping properties than other fish. A diet with a naturally low pH would thus be more likely to have a stable microbiome than a food with a neutral pH.

A well-rested meat animal's normal 1% glycogen is converted to lactic acid upon death, which immediately lowers pH levels from roughly 7.4 to about 5.6. Meat has a longer shelf life because most germs cannot survive in environments with a lower pH. Compared to meat from rested animals, meat from exhausted animals degrades more quickly. This is because the majority of the glycogen in the body had already been used up throughout its existence; as a result, the ultimate pH reached when rigour mortis was complete was not as low as that of an animal that had had enough rest. Bacteria may develop and ruin it as a result.

Certain items, such as fruits, soft beverages, fermented milks, sauerkraut, and pickles, which have an acidic pH, are ideal keepers because of their limiting pH. The pH of fruits, soft drinks, vinegar, and wines is well below the range where bacteria often thrive, which contributes to their outstanding keeping quality. Because yeast and mould can develop at pH levels below 3.5, which is far lower than the minimum for most food deterioration and all food poisoning bacteria, fruits often experience mould and yeast spoiling.

Some foods have a low pH because they are naturally acidic, while others, including fermented foods like sauerkraut, pickles, and fermented milks, have a low pH because of the acidity that microorganisms create during the fermentation process.

This acidity, which is sometimes referred to as biological acidity, is often brought on by lactic acid buildup during fermentation. Whatever the source of acidity, it seems to have the same impact on preserving quality. Since the earliest times, the capacity of low pH to inhibit microbial development has been used to preserve foods using acetic acid and lactic acids.

With the exception of soft drinks that contain phosphoric acid, the majority of other acidic foods' acidity is caused by the presence of weak organic acids, according to 26 Factors influencing Growth and Inhibition of Microorganisms in Food. In solution, they do not entirely separate into protons and conjugate bases but instead reach equilibrium:

Weak acids, like acetic acid, partially dissociate, which is crucial to their capacity to prevent microbial development. Strong acids have a more significant impact on pH when added, although at the same pH, they are less inhibitive than weak lipophilic acids. This is due to the fact that the content of undissociated acid is closely correlated with the suppression of microbes by weak acids. These undissociated lipophilic acid molecules may readily move across the membrane, transitioning from the cytoplasm's high pH to the external environment's low pH, where the equilibrium favours the undissociated molecule. The balance swings in favour of the dissociated molecule at this higher pH, causing the acid to ionise and produce protons. These protons make the cytoplasm more acidic. By ejecting protons that have entered the cell, the cell

typically maintains its internal pH. The microorganism uses energy from growth-related processes to remove protons from the cell, which slows the development of the cell. The cell can no longer handle the load. When growth is no longer viable due to a reduction in cytoplasmic pH, the cell finally perishes. On the other hand, strong acids in solution totally dissociate into protons and conjugate bases. The cell membrane is unable to readily accommodate these fragmented acid molecules. As a result, the pH of the cytoplasm hardly changes. Therefore, at the same pH, they are less inhibitory than weak acids.

Some foods are more resistant to pH fluctuations than others. Since they are buffered, they have a tendency to resist pH fluctuations, and the capability of a substance to do so is referred to as its buffering capacity. The substances in food known as buffers are crucial because they withstand pH shifts. Within a certain pH range, they are very effective. Buffers make it feasible for an acid (or alkaline) fermentation to last longer and produce more products and organisms than would be possible without them. Vegetables are often less buffered than meats. Meats' different proteins help to increase their capacity as buffers. Since vegetables typically contain little protein, they lack the buffering power needed to withstand pH fluctuations brought on by the development of bacteria. Therefore, during the early stages of the fermentations for pickles and sauerkraut, they allow a noticeable pH reduction with the generation of tiny quantities of acid by the lactic acid bacteria. This is advantageous because it gives the lactic acid bacteria a chance to control the spoilage-causing pectin-hydrolyzing and proteolytic organisms. In contrast to strong buffering power, low buffering power causes a succession of microorganisms to arise more quickly during fermentation. Since milk has a significant amount of protein (a good buffer), lactic acid bacteria may thrive and produce a lot of acid when producing fermented milks before their development is inhibited.

Conclusion

Since ancient times, people have employed yeasts, moulds, and bacteria to create foods like bread, beer, wine, vinegar, yoghurt, and cheese, as well as fermented fish, meat, and vegetables. In nature, microorganisms carry out the fermentation processes. One of the earliest methods of transforming and preserving food is fermentation. In addition to preserving food, this biological process also enhances its nutritive and gustatory characteristics (relating to the senses; taste, sight, smell, touch). An effective fermentation will favour beneficial flora over unfavourable flora in order to delay deterioration and enhance flavour and texture.

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CHAPTER 11

A REVIEW ON MICROBIAL FOOD PRODUCTS

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Abstract:

Currently, the food sector uses microbial products on a regular basis. Products made from microbes are highly sought after across several sectors. Microbiological components and entire microorganisms are presently used in a variety of food compositions by the expanding food business. Foods that have undergone fermentation have become highly popular. The processing or production of various food items involves the employment of microbial enzymes, pigments, flavouring agents, acids, whole cell proteins, polysaccharides, sweeteners, and other products. In contrast to their chemical equivalents, microbial metabolites and products are seen as being natural, safe for consumption, and environment-friendly.

Keywords:

Alcohol, Beer, Fermented Food, Microbes, Organic Acids, Zymase

Introduction

The production of alcohol beverages is a process that involves the active participation of microorganisms, most often yeasts.Beer is the most consumed alcoholic beverage in the world. It is made most often of malted barley and malted wheat. Sometimes a mixture of starch sources can be used, such as rice. Unmalted maize can be added to the barley or wheat to lower cost. Potatoes, millet and other foods high in starch are used in different places in the world as the primary carbohydrate source. The process of making beer is called brewing. It includes breaking the starch in the grains into a sugary liquid, called wort, and fermenting the sugars in the wort into alcohol and carbon dioxide by yeasts. Two main species are used in the fermentation process: *Saccharomyces cerevisiae* (top-fermenting, since it forms foam on top of the wort) and Saccharomyces uvarum (bottom-fermenting). Top-fermenting yeasts are used to produce ale, while bottom-fermenting produce lagers. The temperature used for top-fermenting (15-24ºC) leads to the production of a lot of esters and flavor products that give beer a fruity taste. Hops are added to introduce a bitter taste and to serve as a preservative [1], [2].

Brewer's yeasts are very rich in essential minerals and B vitamins, with the exception of vitamin B12. Beer brewing in modern days is performed by added pure cultures of the desired yeast species to the wort. Additional yeasts species that are used in making beer are Dekkera/Brettanomyces. After the fermentation is finished, the beer is cleared of the yeasts by precipitation or with the use of clearing additives. Other types of alcohol beverages are made by

the fermentation activity of microorganisms as well. A few examples are sake (uses the fungus Aspergillus oryzae to facilitate starch fermentation from rice), brandy, whiskey (both are distilled alcohol), and other alcohol beverages with higher percentage of alcohol compared to wine and beer[3], [4].

Wine production

Wine is made from grapes or other fruit. The grapes are first cleaned of leaves and stems and the fruit is crushed into must that is ready for fermentation. The yeasts used for the fermentation grow a film on the fruit or in the environment. These wild strains play an important role in the final properties of the drink. However, cultivated strains of *Saccharomyces cerevisiae* are often added to improve the consistency of the final product. There are hundreds of commercially available yeast strains for wine fermentation.

Vinegar

Vinegar has been used for cooking and in the household and different industries due to its mildly acidic nature for many centuries. It is one of the foods together with beer, wine, bread and fermented dairy products that is the result of fermentation by microorganisms and has been around for thousands of years. It is a mixture of acetic acid (most often 5%) and water. The fermentation is performed usually by acetic acid bacteria, from the genus Acetobacter, from the alcohol in variety of sources (e.g., apple cider, wine, potatoes, and fermented grain). Acetobacter bacteria are Gram negative aerobic rods. They are naturally present in environments where alcohol is being produced and can be isolated from damaged fruit, apple cider, etc. In these liquids, the bacteria form a film on the surface, since they are aerobic and need good oxygen supply. This film, called mother of vinegar, can be used as a starter culture of acetic fermentation in fresh alcohol liquids. Mother of vinegar can also be found in unpasteurized store brand vinegar. Acetic acid bacteria are transmitted in nature by vectors like fruit flies and Vinegar eels.

Citric acid (citrate)

Citric acid (citrate) is an important substance in the Krebs cycle. It is produced from acetyl coenzyme A and oxaloacetate in the presence of the enzyme citrate synthase. The Krebs cycle is key in the oxidation of sugars, proteins and fats to carbon dioxide and water. Many of the cycle compounds are also needed for the synthesis of the cells' own proteins, carbohydrates, and fats.Citrate has been used for centuries in different industries and in the households. It is used as a food additive to give a sour taste to foods or to preserve certain qualities of food products (e.g., prevents separating of the fats in ice cream). It has natural antibacterial properties and is used as a preservative as well. Its buffering property is used in cosmetics and pharmaceuticals to adjust the pH of products.

Literature Review

Bokulich *et al.* carried out a study in which they investigated about Wine grapes offer a distinctive biogeography model in which patterns of microbial biodiversity across viticultural zones not only address issues of dispersal and community maintenance, but are also an essential element of the quality, consumer acceptance, and economic value of a food product with

significant cultural significance. Wine quality measures unquestionably suffer as a result of the microbial activity that occurs throughout the transformation of grapes into wine from the vineyard to the wine bottle. Many of the bacteria found in wine grapes are known to contribute to the health of the grapevine and the quality of the wine. Wine grapes naturally contain a broad variety of germs from the environment. Regional wine traits' determinants, however, have not yet been discovered, despite common belief that they are only influenced by geological or viticultural variables. In order to show how regional, site-specific, and grape variety characteristics influence the fungal and bacterial consortia that live on wine-grape surfaces, this research used a high-throughput, short-amplicon sequencing technique. Additionally, the correlation between these microbial assemblages and certain climatic characteristics raises the possibility of a connection between the environmental circumstances in vineyards and the patterns of microbial inhabitation. When these elements are considered together, they form the distinctive microbial inputs to local wine fermentations, suggesting the possibility of nonrandom "microbial terroir" as a deciding factor in regional diversity among wine grapes[5].

Liu *et al.* talked about how local circumstances are impacted by a changing climate, and how this may change microbial diversity and, in turn, wine style. Wine production may be optimised by strengthening the expression of regional characteristics by identifying and regulating the microorganisms present, thanks to advances in our knowledge of microbial diversity and its impact on wine fermentation[6].

Filamentous fungi, yeasts, and bacteria with a variety of physiological traits and influences on wine production are part of the complex microbial ecology of grapes. Most of the time, due to insufficient grape sample, the effect of damaged grapes on yeast ecology has been understated. When full bunches are harvested, a greater percentage of species recover, which likely the result of damaged berries is being buried in seemingly healthy bunches. The primary factor enhancing both microbial populations and species diversity in grapes is grape health state. Consequently, the impact of biotic (such as insects, birds, phytopathogenic and saprophytic moulds), abiotic (such as weather, rain, and hail), and viticultural (such as fungicides) variables depends on their principal destructing effect[7].

Long believed to provide wine its distinctive regional characteristics, soil composition has been studied. Here, we demonstrate that the key factor influencing wine fragrances in vineyards in southern Australia is the microbial populations in the soil. We suggest a method through which fungus may pass through the vine from the earth. In addition to microorganisms, which create the tastes of fermented plant meals and drinks, the location and environmental characteristics of the vineyard site also impart unique scents and flavours to the wine in the case of wine. By affecting the health of the grapevine, wine fermentation, and the taste, fragrance, and quality of finished wines, microbial development and metabolism are essential to the process of making wine. It is unknown how microbial dispersion patterns affect wine metabolites, and although taste has been linked to both fungal and bacterial composition in wine, bacterial activity in wine fermentation results in fewer sensorially active biochemical transformations than fungi. Here, we gathered samples from six different wine-growing regions in southern Australia in order to look at the geographical distribution patterns of fungus and bacteria as well as their relationships to

the chemical makeup of wine. According to the findings, wine-growing areas may be identified by their soil and must microbiota. Under various environmental settings, including measurements of soil characteristics and weather, we discovered a link between microbial and wine metabolic profiles. The individuality of a wine area is linked to fungus communities. As a result of our discovery that the soil microbiome is a source of fungus related to grapes and must, we hypothesise that weather and soil might affect wine qualities via the soil microbial population. Our study outlines a thorough scenario of wine microbial biogeography in which microbial diversity reacts to the environment and connects with the composition of the wine and geographical features. Through a knowledge of fungal activity and abundance, these results provide possibilities for considerate human activities to improve the nutritional content of food. IMPORTANCE long believed to provide wine its distinctive regional characteristics, soil composition has been studied. Here, we demonstrate that the key factor influencing wine fragrances in vineyards in southern Australia is the microbial populations in the soil. We suggest a method through which fungus may pass through the vine from the earth[8].

Microorganisms may change wine that has been matured in barrels or bottles, which can lower the quality of the final product. Our understanding of the ageing microbiota and the mechanisms that affect the microbial populations is still fairly restricted. The current study deals with the meta-taxonomic characterisation of microbial consortia present in red wines along with 12 months of maturing. This is done using high-throughput sequencing (HTS) methods. The wines made from two separate grape types were stored in two different cellars and compared based on the amount of time the wines spent in the barrels, how long they had previously been used, and whether they were aged in oak barrels or glass bottles. The microbial diversity was not considerably impacted by barrel ageing, while fungal and bacterial communities underwent changes in structure and makeup. Acetobacter, Oenococcus, Lactobacillus, Gluconobacter, Lactococcus, and Komagataeibacter, as well as the fungi Malassezia, Hanseniaspora, and Torulaspora, were the principal microbes responsible for these modifications. Our findings demonstrated that the oak barrels had a greater impact on microbial diversity than glass bottles, whose microbial communities were quite comparable to those of the wine put into the barrels at the start of the ageing process. Additionally, the wine had a larger amount of Lactobacillus but a lower proportion of Acetobacter in the bottles. Finally, it seems that ageing the barrels for only one year did not significantly alter the variety or composition of the microbiota compared to brand-new barrels. This is the first meta-taxonomic investigation on microbial communities during wine ageing, and it demonstrates that both cellars' barrel-aged wines have a comparable microorganism makeup. These findings raise the idea of an age-related common and stable microbiota in the absence of external modifications. The comparison and identification of microbial changes throughout age that might possibly stop financial losses in the wine business would benefit from further confirmations of the present result[9].

Reid *et al.* discussed about Microbial alcohol oxidoreductases which come in an astounding variety. They exhibit a broad range of substrate preferences and carry out a number of significant but quite distinct physiological tasks. Many of these enzymes play crucial roles in the creation of vinegar, alcoholic drinks, and industrial solvents. Still others take part in the breakdown of xenobiotic and naturally occurring aromatic compounds as well as the development of bacteria

and yeasts on methanol. Three main groups may be formed from them. The NAD- or NADP-dependent dehydrogenases are the first. These can be further divided into group I "iron-activated" enzymes, which typically contain approximately 350 amino acid residues, group II "short-chain" enzymes, which have approximately 250 residues, and group IE "zinc-independent" enzymes, which include ribitol dehydrogenase of Klebsiella aerogenes, and group IE "long-chain" enzymes, which have approximately 350 amino acid residues. The methanol dehydrogenases of Amycolatopsis methanolica and Mycobacterium gasti are 4-nitroso-N, N-dimethylaniline-dependent nicotinoproteins, as are the aldehyde/alcohol oxidoreductase of this organism. (2) Enzymes that are NAD (P)-independent and employ the cofactors pyrroloquinoline quinone, haem, or cofactor F420, such as the alcohol dehydrogenases found in certain archaebacteria and methanol dehydrogenase of Paracoccus denitrificans. (3) Alcohol oxidases that catalyse an almost irreversible oxidation of alcohols, such as Hansenula polymorpha's methanol oxidase and likely the veratryl alcohol oxidases of several fungi involved in the breakdown of lignin. The majority of the enzymes included in this study are those for which entire amino acid sequences are known[10].

Lamsen *et al.* focused on improved biological production processes for liquid fuels from renewable sources are of interest due to the rising need to solve current energy and environmental issues. Although the generation of ethanol by microorganisms is widely known, higher-chain alcohols have more chemical characteristics in common with gasoline. Unfortunately, these alcohols (with the exception of 1-butanol) cannot be generated effectively by natural microbes, making it difficult to make them economically in large quantities. To assist boost the titers and production of these advanced biofuels, synthetic biology, on the other hand, provides further tools for engineering synthetic pathways in approachable hosts. This study focuses on current advances in synthetic biology that may create higher-chain alcohols as potential sustainable alternatives to fossil fuels[11].

Hu *et al.* identified five main bacteria in the fermentation by metagenomic and metatranscriptomic analyses: Saccharomyces cerevisiae, Rhizopus delemar, Pichia kudriavzevii, Lactobacillus helveticus, and Rhizopus oryzae. Although P. kudriavzevii was more active than S. cerevisiae, which was previously thought to be the most prevalent yeast in Baijiu fermentation, our metatranscriptomic findings contradicted this notion. These two analyses showed that S. cerevisiae and P. kudriavzevii had higher initiation abundances in traditional technology than in new technology, whereas R. delemar and R. oryzae had lower initiation abundances. They also showed that Lactobacillus showed apparent advantages in traditional technology, whereas Lactobacillus and yeast showed obvious advantages in new technology at the end of fermentation. Other microorganisms, such as non-saccharomyces yeasts, moulds, and bacteria, were also implicated in greater alcohol production in addition to S. cerevisiae. This paper offers understanding of the microbial dynamics and greater alcohol generation, as well as an effective method for streamlining the fermentation process for baijiu.

Discussion

For centuries, the source of citric acid were citrus fruits. After World War I, the ability of some microorganisms to produce citric acid was further explored and the technology for industrial

production was developed. Penicillium mold was the first described organism to produce citric acid but industrially another mold, *Aspergillus niger*, became the microorganism of choice. The mold is grown in a medium with sucrose or glucose as the main carbon source. The sugar source is usually an inexpensive solution like molasses or corn steep liquor. The microorganism makes more citric acid in the Krebs cycle than needed for the cell's metabolism and exports it outside the cell. The citric acid is then precipitated out of solution and regenerated. The process of making wine, from the vineyard to the winery, is intricate.

Microbes are critical to this trip. The bacteria present might possibly alter the composition of wine by affecting the soil, geography, weather, and climate where the vines grow as well as vineyard management techniques. Microbial populations are further altered by the addition of grapes to the winery and the commencement of winemaking procedures. Recent developments in next-generation sequencing (NGS) technology have advanced our knowledge of the microbial populations related to fermentations and grapes. Now that we have a deeper understanding of the microbial diversity found in different wine-producing locations, we may start to speculate on how diversity can influence the qualities of wine quality and style. The literature on wine-related microorganisms and how it interacts with, shapes, and affects microbial populations and wine quality is highlighted in this review. We assert that microbial biogeography is a novel approach that may affect wine quality and regionality by considering the geography, climate, and soil of habitats as well as viticulture and winemaking procedures. The microbial community reacts to local circumstances based on geographic scales, habitats, and taxa.Microorganisms are crucial to the development of the taste of Chinese baijiu. Baijiu may be developed by mechanisation. Therefore, it is important to research how automation affects the microbial ecology and taste in the manufacturing of baijiu. The microbial communities of the two technologies showed changes throughout fermentation, and at the height of fermentation, there were much more yeasts and bacteria in the new mechanical technique than in the conventional method.

There are certain species, including parasitic fungus and environmental bacteria that can only be found in grapes, while there are others that can live and flourish in wine, making up the wine microbial consortium. The acetic acid bacteria, lactic acid bacteria, and yeast species are all included in this consortium. Based on the grape's state of ripening and the availability of nutrients, these bacteria' percentage changes. The microbiota of truly intact grape berries is similar to that of plant leaves after véraison, when it is dominated by basidiomycetous yeasts (e.g., Cryptococcus spp., Rhodotorula spp., Sporobolomyces spp.) and the yeast-like fungus Aureobasidium pullulans. Until véraison, grape berries are susceptible to fungal parasites. The cuticle of berries that are visually intact may have microfissures and soften as they ripen, increasing the availability of nutrients and possibly explaining the predominance of oxidative or weakly fermentative ascomycetous populations (such as Candida spp., Hanseniaspora spp., Metschnikowia spp., and Pichia spp.) as harvest time approaches. When grape skin is obviously damaged, the presence of high sugar concentrations on the berry surface encourages the growth of acetic acid bacteria and ascomycetes with higher fermentative activity, such as Pichia spp. and Zygoascus hellenicus, as well as potentially harmful wine-spoiling yeasts (such as Zygosaccharomyces spp., Torulaspora spp). (E.g. Gluconobacter spp., Acetobacter spp.). Saccharomyces cerevisiae, a microorganism that ferments sugar, is often found on berries with

damage from grapes and is uncommon on immaculate fruit. Oenococcus oeni is the usual agent of malolactic fermentation, although it has only sometimes been identified from grapes in the vineyard. Lactic acid bacteria are minor members of the grape microbiota. Environmentally common bacteria, including those in the genera Enterobacter and Enterococcus, Bacillus and Bacillus, Burkholderia and Serratia, and Staphylococcus and Staphylococcus, have been identified from grapes but cannot thrive in wine. Saprophytic moulds, such as *Botrytis cinerea*, which causes grey rot, or *Aspergillus spp*., which may produce ochratoxin, are only present in the vineyard, yet their metabolites may have an impact on wine quality after grapes are processed.Microorganisms replaced the industrial chemical production of many different organic compounds, like enzymes and amino acids. Enzymes, such as glucoamylase (used to make high-fructose corn syrup) and pectinase (clearing agent for apple cider and wines) are produced industrially by Aspergillus. The food additivemonosodium glutamate (MSG) is produced in the form of glutamic acid by *Corynebacterium glutamicum*.

Microbes are everywhere, which means that they may be found in the soil, the air, the water, as well as in the bodies of people, other living things, and plants. In areas without any other forms of life, they may also be found. Microorganism examples include bacteria, fungus, protozoa, and viroids. Due to the fact that certain bacteria may cause illness in both plants and animals, including humans, the conventional assumption is that these organisms are detrimental to us. Microorganisms are valuable to humans in a variety of ways, albeit there are many of them. The industrial applications of microorganisms will be covered in this article.

Fermentation is the name for the anaerobic respiration process in which the activity of microorganisms causes complex compounds to break down partially into simpler ones. The byproducts produced when pyruvate or its derivatives are fermented may discriminate among a variety of fermentation processes. Ethanol fermentation (used in beer and bread) and lactic acid fermentation are the two processes that humans employ most often to generate commercial foods (used to flavour and preserve dairy and vegetables). Malted grains and fruit juices are used to manufacture alcoholic beverages such as wine, beer, whisky, brandy, and rum. Without distillation, beer and wine are made. Whereas after distillation, rum, brandy, and whisky are created. When wine, beer, and other alcoholic beverages are made, yeasts serve as the primary fermenter and source of alcohol. By turning the alcohol in alcoholic beverages into acetic acid, acetic acid bacteria may produce vinegar, a food product.

In order to produce great wine, the first stage in the process is harvesting. The only fruit that continuously produces natural wine that is stable and free from artificial flavours, colours, or esters is grapes. In order to turn the grapes into what is known as must, mechanical presses are next employed to stomp or tread them. Must is just freshly squeezed grape juice that has the skins, seeds, and solids in it. The must is allowed to organically ferment for 6–12 hours after being crushed and pressed. In the presence of airborne wild yeasts. *Saccharomyces cerevisiae* variety ellipsodeus is the microorganism used to make wine. (Brewing yeast) By using several flavours, various flavours may be produced. Following the completion of the fermentation phase, the process is clarified, removing any remaining solids such proteins, tannins, and dead yeast cells. The wine is then moved, or "racked," into a separate container, either an oak barrel or a

stainless steel tank. However, ageing wine in oak barrels will result in a wine that is rounder, more vanilla-flavored, and smoother. Wine may be distributed for consumption right away. Additionally, it increases the amount of oxygen that wine is exposed to while it matures, reducing tannin and assisting the wine in achieving its ideal fruitiness.

Grain, typically barley, is fermented to produce beer. It takes around two days to rinse and soak the barley grains. Once the extra water has been removed, the barley is incubated for 4-5 days to facilitate germination. Malting is the term for the procedure. The seeds are then slowly heated to an ambient temperature of 80° to destroy them. Kilning is the term for this procedure. A greater kilning temperature will result in darker beer, thus it is important to keep an eye on the temperature. In order to create grist, a coarse powder, the dry barley grains are next crushed between rollers. Warm water is combined with the grit, and the mixture is then kept at 65°C for nearly an hour. Mash is the term for the procedure. Following that, the filtrate is cooked for two to three hours while being stirred. And after that, fermentation takes place. *Saccharomyces cerevisiae* is a kind of microbe used to make beer. It takes a few days for the fermenting solution to stand. After that, it is crystallised, carboxylated, and then bottled.

A mixture of grains, including maize, wheat, barley, etc., are fermented to produce whisky. Distillation is a fermentation byproduct. Simply purifying and removing any diluting ingredients, such as water, requires further processing of the fermented beverage during distillation. To differentiate distilled drinks from undistilled ones, this raises the percentage of their alcohol content, which is why they are also sometimes referred to as Hard Liquor. Utilizing bacteria for fermentation, several organic acids are produced. Fungi are used in the production of organic acids. Acetic acid, citric acid, gluconic acid, fumaric acid, and lactic acid are a few examples of acids that may be produced in huge quantities utilising fungus. The following list includes a few organic acids that were derived from microbial sources. Acetic acid or vinegar (Acetobacter aceti), butyric acid (Clostridium butylicum), lactic acid, and citric acid are all produced by the bacteria *Aspergillus niger*, Gluconic acid, Fumaric acid, and Rhizopus arrhizus. (Lactobacillus). For a person to grow and develop normally, vitamins, which are complex chemical molecules, are needed in very little amounts. The vitamins B and C are water-soluble, whereas the other kind of vitamin is fat-soluble (vitamins A, D, E, and K). Since the body cannot generate all vitamins, we must consume food, supplements, and pills to get our daily dose. Here are a few essential vitamins (from microorganisms). Vitamins B2, B12, and C are all found in *Neurospora gossypii* and Eremothecium ashbyi (*Aspergillus niger*).

Some secondary metabolites, or fermentation byproducts, are used therapeutically. Extracting antigens and enzymes from isolated bacteria and viruses. In the creation of antibiotics and antivirals, these antigens are helpful. Alexander Fleming developed penicillin while dealing with Staphylococcus aureus from Penicillium notatum. The discovery of penicillin as a powerful antibiotic earned Earnest Chain and Howard Plorey the 1945 Nobel Prize. Leprosy, diphtheria, whooping cough, and other illnesses are all treated with penicillin.The following list includes a few significant antibiotics (microbial sources). Erythromycin (*Streptomyces erythreus*), Penicillin (*Penicillium chrysogenum*), Streptomycin, Chlormycetin (*Streptomyces venezuelae*), and (*Streptomyces griseus*).

Detergents with lipases are used to clean laundry of oil stains. To make bottled juices clearer, use pectinases and proteases. In the treatment of myocardial infarction, streptokinase (from Streptococcus) acts as a clot-buster (heart attack). Organ transplant recipients are given the immunosuppressant Cyclosporin A (Trichoderma polysporum). As a blood, cholesterol-lowering agent, statins made by the yeast Monascus purpureus are employed. In order to create steroids that may be administered through injection into a human body for various reasons, certain bacterial and fungal species are employed.

Conclusion

Today, microorganisms play a significant role in the human food chain. In the modern human food system, several microorganisms and microbial products are used. The demand for microbial goods is steadily rising since they have certain clear benefits over their synthetic chemical counterparts. The food business today often uses microbial pigments, enzymes, alcohol, acids, polysaccharides, and other metabolites. For the synthesis of microbial metabolites on a large scale and at a low cost, new strategies, innovations, and improvements in current technology are needed.

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CHAPTER 12

APPLICATION OF MICROBIAL TECHNOLOGY IN DAIRY INDUSTRY

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Abstract:

Since ancient times, dairy products have been part of human diet. These serve as good source of calcium, vitamin D, proteins and other essential nutrients. These products also provide phosphorus, potassium, magnesium, and various vitamins viz. vitamin A (retinols), vitamin B_{12} (Cyanocobalamin), and riboflavin. Various fermented dairy products are prepared using different microbial strains. Microbes ferment the carbohydrates present in milk, which is mainly lactose to lactic acid and some other products. The acid precipitates the proteins in the milk; therefore fermented products are usually of thicker consistency than milk.

Keywords:

Curd, Cheese, Dairy products, Fermentation, Microbial Technology

Introduction

The high acidity and low pH hinders the growth of other bacteria including pathogens. The fermentation of milk provided a simple way to increase its shelf-life while improving its safety. Humans learned to control fermentation processes from the initial accidental events in fermentation. This learning of controlled fermentation of milk in domestic practices gave rise to a diverse dairy products influenced by habits of different ethnicities, geographical environments and type of dairy farming.Now, a huge variety of fermented dairy products are available for consumers. Although a small proportion of these products are homemade, most of them are produced industrially[1], [2]. The production of fermented products is economically important in many countries. As the requirement of fermented products is increasing day by day, and in many countries dairy industries are contributing in economic growth. The first example of fermented milk was presumably produced accidentally by nomads. This milk turned sour and coagulated under the influence of certain microorganisms. By luck it was having harmless, acidifying type and non toxinproducing bacteria. Various types of fermented milks and derived products have been developed in all parts of the world each with its own characteristic history. Their nature depends very much on the type of milk used, on the pre-treatment of the milk, on the temperature (climate), conditions of fermentation and on the subsequent technological treatments. Most commonly used dairy products include curd, yogurt, cheese, kefir and kumis.

Curd

Curd is made by curdling or coagulating the milk. This can be done by mixing edible acidic substances in to the milk, such as lemon juice or vinegar. By adding these substances to the milk,

it will curdle the milk and separate into two parts. The liquid part is the whey and the solid milk is the curd. The whey contains whey proteins of the milk, whereas the curds are the milk proteins or casein. Sometimes old milk might get soured and is separated without any added acidic substance. This happens because raw milk contains Lactobacillus. Lactobacillus is a genus of bacteria that converts sugars into lactic acid by means of fermentation[3]–[5].

Cheese

Cheese is a fermented milk product and historically serving as a mean of preserving milk. Cheese making occurs in three main stages: In the first stage, milk is moulded into solid curd and liquid whey by the coagulation of the milk protein, casein. The coagulation of casein is done through two complementary methods: acidification and proteolysis. Acidification occurs when lactic acid bacteria ferment the disaccharide lactose to produce lactic acid. Originally, it can be done by naturally occurring lactic acid bacteria in the milk but today, dairy industries usually standardize the process by the addition of domesticated bacterial cultures, including strains of *Lactococcus lactis, Streptococcus thermophilus* and *Lactobacillus sp.*

Yogurt

Yogurt is most commonly used dairy product. It is prepared by heating the milk up to nearly 80°C in order to kill any additional bacteria that may be present and to denature milk proteins. The milk is then allowed to cool slowly to around 45°C, and thereafter, it is inoculated with a bacteria, and is allowed to ferment at room temperature. The bacteria used are Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus. If bacteria is not available, then a spoonful of yogurt can also be used as it contains bacteria. Probiotic bacteria like Streptococcus thermophilus, Lactobacillus acidophilus and bifidobacteria can also be used for the production of yogurt and it is commonly referred as bioyogurt.

Kefir

Kefir is a fermented milk beverage which has its ancient origin in Eastern Europe. This light alcoholic beverage is prepared by inoculation of raw milk with irregularly shaped, gelatinous white/yellow grain called kefir grains. These Kefir grains have varying and complex microbial composition that includes species of yeasts, lactic acid bacteria, acetic acid bacteria and mycelial fungi.

Kumis

Kumis and kefir are similar dairy products but kumis is produced from a liquid starter culture as compared to solid kefir "grains". It has mild alcohol content as compared to kefir because mare's milk contains more sugars than other milks. It is very popular in Kirgizstan, Mongolia, Kazakhstan and some regions of Russia and Bulgaria. It is usually made from, area’s milk by spontaneous fermentation of lactose to lactic acid and alcohol. Depending on the lactic acid contents, kumis is of three types- strong, moderate and light. A. Strong kumis: It is generated by lactic acid bacteria like Lactobacillus bulgaricus, Lactobacillus rhamnosus. There is acidification of milk to pH 3.6–3.3 and conversion ratio of lactose into lactic acid is about 80–90%. B. Moderate kumis: It involves Lactobacillus bacteria viz. L. Acidophilus, L. Plantarum, L. Casei,

L. fermentum with restricted acidification properties that lower the pH to 4.5–3.9 at the end of the process and the conversion ratio averages 50%. C. Light kumis: It is a slightly acidified product (pH 4.5– 5.0) and is produced using *Streptococcus thermophilus* and *Streptococcus cremoris*.

Literature Review

Feil *et al.*demonstrate that the dairy industry's sustainability indicators are still in their infancy and that further study is needed. Seven publications were identified that highlight 12 environmental, 11 social, and 8 economic indicators that may be regarded as preliminary and fragile. The benefits on the theme are related to solutions to the challenges, such as electricity reduction, sustainable practises, and wastewater treatment methods, among other things. The studied problems are related to these and other issues, such as electric power consumption, industrial plant efficiency, and wastewater treatment methods. Among other things, it is determined that the dairy sectors have been addressing the sustainability issue since 2011, with a hazy tendency, with evidence of the fragility of the sustainability indicators being identified, primarily in the early stages of their formation, when examining holistic approach (triple bottom line)[6].

Akansha *et al.* in their research, effluent from the dairy sector was treated using aerated electrocoagulation and phytoremediation. With aluminium and iron electrodes, electrocoagulation was carried out, and the effects of different operational parameters such electrode combination, pH, and voltage were studied. At neutral pH, electrocoagulation was shown to be successful, and when applied voltage was raised, so did this effectiveness. At 120 min of reaction time, starting pH 7, voltage 5 V, the Al-Fe electrode combination with aeration achieved the highest COD elimination efficiency of 86.4%. When compared to raw dairy effluent, a significant increase of Canna indica was seen in wastewater that had undergone electrocoagulation treatment. When electrocoagulation and phytoremediation were applied together, 97% of COD was removed. As a result, it shows to be an effective solution for treating effluent from the dairy sector. In addition to the aforementioned, studies on the toxicity of bacteria were carried out to determine the toxicity of wastewater. The findings revealed that bacteria thrived in both treated and untreated wastewater[7].

Aziz *et al.* highlighted that due to its natural resources that are suited for cows, New Zealand may have the greatest dairy industry in the whole globe. It now has fresh potential since a new industrial generation known as Industry 4.0 is on the horizon. How can the New Zealand conventional dairy sector improve and evolve in the face of new technology by using the golden prospects presented by Industry 4.0? This essay examines the state of the dairy business in New Zealand today and presents some research findings. The dairy sector, which is thinking about using Industry 4.0 enabled solutions, might benefit from some key insights and lessons from this research[8].

Krampe *et al.* described that the actions Oatly has made up to this point in order to become the biggest producer of "dairy and milk" worldwide. With the overarching goal of promoting structural changes in the dairy business, Oatly develops, manufactures, and distributes dairy and

milk analogues. The strategy used by Oatly is based on cutting-edge technology that can produce dairy analogues and milk-like fluids. The resultant goods are sold under their own brands and supported by controversial and avant-garde advertising tactics including storytelling, activities pertaining to public policy, social media campaigns, and more conventional sales ideas. While using a variety of marketing techniques, the corporation is always creating new products and constructing factories that allow them to enter international markets. Other stores and food businesses followed Oatly's lead and indicated interest in dairy and milk substitute goods, intensifying competition in the industry.

Dairy processing with ultrasound is becoming more popular, particularly for fermented dairy products that serve as the foundation for functional meals. Power ultrasonography has been proven in experiments to improve the lactic acid bacteria's ability to ferment by altering their metabolic activity, shortening the fermentation period, and increasing the qualitative features of fermented milk products. The fermentation process is one of the crucial steps in the manufacturing of dairy products, but it also demands a lot of time and resources. Therefore, the advantages of ultrasonography for fermentation owing to microbial activation become more significant. Actually, bacteria are impacted by ultrasonic applications in two different ways. Depending on the ultrasonic intensity and frequency, sonication duration, kind of microbe, pH, and temperature, it may be utilised for microbial activation in the dairy sector as well as for the inactivation of microorganisms. Akdeniz *et al.* provided a theoretical basis on ultrasound, research results, and an overview of the impact of power ultrasound on microbial inactivation and growth based on the fermentation profile of dairy products. Details on the power ultrasound's activation and deactivation processes for microbes are also provided.

In the dairy business, food safety and quality control are crucial. A meal that is both very nutrient-dense and a great environment for the development of harmful bacteria is milk. As a result, the dairy sector spends the majority of its resources and procedures on minimising contamination. Although thermal methods for microbial decontamination may be efficient, they cannot concurrently provide foods with outstanding organoleptic, nutritional, and decontamination qualities. Due to its inherent antibacterial characteristics devoid of any temperature impact, microbial inactivation by exposure to blue light in this setting is a viable alternative strategy in the food sector.

In order to ascertain the inactivation kinetics of blue light against bacteria suspended in whole milk or saline solution, dos Anjos *et al.*examined the effects of the light on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhimurium, and Mycobacterium fortuitum. In order to look into potential milk component deterioration, we also carried out a number of optic spectroscopies. All species were photoinactivation sensitive when suspended in milk or saline solution. Depending on the suspension media, inactivation kinetics varies greatly, and each species is impacted differently. Within less than two hours of exposure to radiation (720 J/cm2), every studied bacterial species shown more than 5 log10 inactivation. No substantial changes in any of the components of milk were found using infrared spectroscopy (e.g., sugars, proteins, and lipids). The only highly damaged component identified was riboflavin

(vitamin B2). As a result, we draw the conclusion that blue light-based microbial inactivation has tremendous promise for dairy sector operations[9].

It is essential to concentrate on policies that safeguard the public's health and lower the prevalence of foodborne pathogens and spoilage microorganisms in light of the ongoing outbreaks of foodborne illness in recent years. Ibrahim *et al.* investigated the usage of chemical preservatives and heat treatments are two examples of restrictions currently present in traditional microbial control approaches. For instance, such traditional procedures have a negative influence on the sensory qualities of food, leading to poor organoleptic traits. Additionally, the rising consumer advocacy for safe and healthy food items and the ensuing paradigm shift toward clean labelling have raised interest in natural and efficient antibacterial alternatives. For instance, lactic acid bacteria (LAB) produce natural antibacterial components that are typically antagonistic to pathogens and severely restrict the activity of organisms that cause food rotting. Bacteriocins and other LAB metabolites have been commercially exploited for their antibacterial characteristics and utilised in several applications in the dairy industry to inhibit the development of unwanted microbes. With an emphasis on the mechanisms of action and applications for microbial food spoilage prevention and disease management, we outlined the natural antimicrobial chemicals produced by LAB in this study. In addition, we provide evidence in the review to back up our suggestion that LAB be used as a possible alternative antimicrobial approach to deal with the problems caused by pathogen antibiotic resistance.

Niaz *et al.* investigated that the balanced amino acid content of milk proteins suggests the development of new functional products. These improve the consistency and sensory qualities of certain dairy products. Lactoferrin has both bacteriostatic and bactericidal activity against a number of bacteria. It binds iron, depriving organisms including L. monocytogenes, Salmonella spp., Escherichia coli, Bacillus stearothermophilus, Shigella dysenteriae, and Bacillus subtilis of the nutrients they need to flourish. As an alternative to antibiotics, lactoferrin works. It functions as a natural antimicrobial for bio preservation, extending shelf life, ensuring safety, and improving health by acting against life-threatening diseases like cancer, hepatitis, respiratory infections, and foodborne diseases in infants, children, and adults, among other products, such as dairy, meat, seafood, beverages, bakery products, acne care, infant formulas, etc. In a summary, the present study examines lactoferrin's significance and safety considerations in the food business and healthcare fields[10].

Discussion

The presence of bacteria, yeast, mould, and viruses has a significant impact on the final dairy product's quality. The ultimate desired Product was defined by the regulated development of these microorganisms. Milk is regarded as a complete food since it contains a healthy mix of protein, fat, and sugar. However, these nutritional benefits have the potential to promote rapid population increase, which might sometimes result in unfavourable alterations to milk and its byproducts. Numerous elements throughout the processes of production, processing, and distribution to consumers have an impact on the hygienic properties of milk.

Examples of dairy products that rely on microbial activity include:

1. Cheese: This dairy products sector is more reliant on the advantageous enzymatic modifications brought on by microbes. The way different species transform the components of milk into cheese affects the flavour and texture of the cheese in significant ways. During cheese production, the right amount of bacterial and mould species are introduced, and a large portion of the microbial activity that occurs during ripening is caused by microbial species that accidentally enter the milk at various points.
2. Butter production: Starter cultures, which are chosen mixed bacterial cultures responsible for producing acid and flavour, are what give butter its flavour and scent. The circumstances in which the best flavour develops and the interactions with microorganisms are well established, and it is clear that the changes starters bring about are desired.
3. Other examples of fermented milks formed by microorganisms added in various compositions and proportions to milk, cream, and skim milk, respectively, are dahi, yoghurt, sour cream, and buttermilk. These products' taste, texture, and flavour vary and are contributed by various microorganism groups.
4. Different microorganism interventions may now be carried out on a commercial scale to provide key economically valued goods including vitamins, solvents, and food additives. Before being released into the environment, certain milk ingredients that are generally discarded and have little economic value must be changed into stable oxidising and non-toxic compounds. The required changes in the organic components of dairy wastes during sewage treatment are caused here as well by microbial action.
5. Milk microbes are important: Knowing the microbial load in milk and milk products allows us to evaluate their quality. Each product is allowed a particular amount of microbial load before it is deemed unsafe for ingestion. If the natural flora found in milk is allowed to grow, it may ruin the milk. Since milk is a complete food, it may be contaminated with pathogens; as a result, it must be preserved carefully to prevent their uncontrolled development and measures must be taken to eradicate them.

When making cheese, yoghurt, and other fermented milk products, certain bacteria cause chemical changes that are advantageous.Untreated dairy products may contain various dangerous germs that might have an impact on human health. Studies have shown that bacteria carried by milk may sometimes cause infections in both humans and animals. A dairy product maker must understand the origins of pathogens in dairy products, the environments in which they thrive, and the best ways to avoid or eliminate them. Although the manufacturer and supervisor are responsible for ensuring the safety of the consumer, it is crucial that the customer be aware of whether a certain dairy product is suitable for eating. Even at the farm level, the farmer should be aware of the likelihood that a diseased animal may infect their herds and spread the disease.

Milk-borne illnesses including TB, brucellosis, and typhoid fever are less likely to spread thanks to sanitary milk production procedures, correct handling and storage of milk and milk products, and adequate pasteurisation. However, consuming raw milk or dairy products manufactured with milk that was improperly pasteurised or handled carelessly, resulting in postprocessing contamination, has been linked to a variety of milk-borne disorders. Major concerns exist over the presence of the following bacterial pathogens in raw milk and other dairy products:

It should be mentioned that several types of mould, namely Aspergillus, Fusarium, and Penicillium species, may develop in milk and can create mycotoxins, which can pose a major risk to the health of consumers.Microorganisms are to blame for milk spoiling because of the unwanted alterations they bring about via growth or metabolic processes. The source of microorganisms that cause quick changes, the environments that support their development, and the strategies for stopping their activity should all be known to the milk producer. As butter, cheeses, etc. are commonly held for extended periods of time, which may further degrade quality, the maker of milk products must deal with issues that are comparable to those of the producer of milk as well as new ones. The issue of spoiling is particularly significant when it comes to cheeses that need to mature since the right circumstances must exist for the growth of certain beneficial microbes while simultaneously posing a risk for the development of harmful ones.

For the creation of high-quality dairy products, the initial microbiological quality of raw milk is very important. The word "spoilage" is used to describe food that has lost its texture, colour, flavour, or smell to the point that it is no longer fit for human consumption. Protein, carbohydrates, and lipids are often degraded by bacteria or their enzymes during microbial food deterioration. Psychrotrophs are the bacteria that are primarily responsible for milk deterioration. Pasteurization kills the majority of psychrotrophs, but some, such Pseudomonas fluorescens and Pseudomonas fragi, may create proteolytic and lipolytic extracellular enzymes that are heat-stable and can ruin food. Some Bacillus, Clostridium, Corynebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus, and Streptococcus species and strains may withstand pasteurisation and thrive under refrigerated conditions, which might result in spoiling issues in milk and milk-derived products.

When it became necessary to milk bigger cows, the cows were transported to a shed or barn that had been outfitted with stalls (milking stalls), where they could be kept for the whole of their lives while being milked. This allowed for the milking of more cows by one person, up to 20 for a trained worker. However, having cows stand about in the yard and shed as they wait to be milked is bad for the cow since she needs as much time as possible to graze in the paddock. The two daily milking sessions should typically last no more than an hour and a half each. No matter how many cows are milked—whether there are 10 or 1000—the total daily milking time for each cow should not exceed three hours. This is because cows should spend as much time as possible in their stalls and on their backs, which will boost their comfort and help them produce more milk. Depending on the quantity and frequency of milkings, a cow is only physically milked for 10 minutes daily.

As herd numbers grew, it became increasingly important to have effective milking equipment, sheds, milk storage tanks, bulk milk conveyance, shed cleaning equipment, and ways to move cows from paddock to shed and back. Animal health issues grew in severity as herd sizes grew. Two methods have been used in New Zealand to solve this issue. The first was the development of better veterinary drugs that the farmer could use (as well as government control of the drugs). The second was the establishment of veterinary clubs, wherein groups of farmers would hire a veterinarian (vet) full-time and share their services all year long. It was in the veterinarian's best

interests to keep the animals healthy and decrease the frequency of calls from farmers rather than to make sure that the farmer required to call for treatment and pay on a frequent basis.

For the 300 to 320 days that the cow is in milk each year, this daily milking cycle is continued. The last 20 days of the production cycle may see some small herds being milked once daily, although this is uncommon for big herds. If a cow is left unmilked even once, she will likely start to produce less milk nearly right away, and the remainder of the season, she may dry out (provide no milk) while still ingesting feed. To make more money and to improve lifestyle, once-daily milking is currently more regularly used in New Zealand. The decrease in milk output is at least partly compensated by labour and expense savings from just milking once each day, making this strategy beneficial. Compared to certain intensive farm systems in the US, which milk three or more times daily owing to greater milk yields per cow and lower marginal labour costs, this one produces milk more often from each cow.When a farmer is contracted to provide liquid milk for human consumption (as opposed to milk for making butter, cheese, and other products; see milk), they frequently have to manage their herd to keep the contracted number of cows in milk throughout the year or to maintain the necessary minimum milk output. By mating cows outside of their usual breeding cycle, this is accomplished such that the herd's peak production times are rotated throughout the year.

Farmers in the northern hemisphere who keep cows in barns virtually the whole year often manage their herds to provide consistent milk output so they may be paid all year round. Due to the cooperative dairying systems' design to maximise grass and milk production in the spring and the milk processing plants' payment of bonuses during the dry (winter) season to help farmers get through the mid-winter break from milking, the southern hemisphere cooperative dairying systems allow for two months of no productivity. Additionally, it implies that during their most prolonged pregnancies, cows stop producing milk. Some dairy farms that operate all year round suffer financial consequences when they produce too much milk at any one period of the year because they can't sell it at the going rate. In order to enhance the genetics of the female progeny who will be bred as replacements, artificial insemination, or AI, is a typical practise in all high-production herds. Additionally, the necessity to maintain potentially aggressive bulls on the farm is reduced by AI. Due to a lack of profit, male calves are either sold to be grown for meat or veal, or they are killed. Until she is killed due to decreased output, sterility, or other health issues, a cow will freshen or calve roughly once each year. The cow will then be sold and usually end up being butchered.

Since the beginning of domestication, milk-producing animals have been used for food. They began as a component of the nomads' subsistence farming. Animals followed the group as they travelled across the countryside. The symbiotic connection between the animals and the herders depended heavily on feeding and defending the animals. People who lived in agricultural civilizations in more recent times kept dairy cows, which they milked for home and regional (village) consumption, a classic example of a cottage enterprise. Multiple uses for the animals might exist (for example, as a draught animal for pulling a plough as a youngster, and at the end of its useful life as meat). The herd size in this example was fairly modest, and as the animals were often milked by hand, the whole herd could be milked in under an hour—roughly 10 per

milker. A dairyman or dairymaid would do these responsibilities. The origins of the term "dairy" may be traced to Old English daege and Middle English dayerie, deyerie, from the noun deye (female servant or dairymaid) (kneader of bread).

As society got more industrialised and urban, the production of milk turned into a lucrative business, and dairy cattle evolved into a separate breed from beef or draught cattle. When machines were developed to do the milking, more individuals were hired as milkers than at first.

In the past, a dairy farm was the location where the milking and processing were done side by side in both space and time. The animals were milked by hand; hand-milking may still be done today on farms with a limited number of animals retained. Hand-milking is done by holding the teats in the hand and squeezing the teat between the thumb and index finger, or by squeezing the teat between the thumb and index finger, then sliding the hand downward from the udder towards the end of the teat, to express milk. In order to release the trapped milk, the milk duct is intended to be closed up at the udder (upper) end and gradually closed by the movement of the fingers as they proceed down the duct. Emptying one milk-duct capacity at a time, each half or quarter of the udder is removed. Repeatedly, the process of stripping is done quickly with both hands. Both techniques result in the milk that was clogged in the milk duct being squirted out the end into a bucket that is held between the knees (or rests on the ground) of the milker, who often sits on a low stool, and is supported by the bucket. When milking a cow or cows, it was customary for them to stand in a field or paddock. Heifers in the early stages of production would need to be educated to hold still while being milked. The cows were milked while attached to a post in several nations.

Conclusion

For medical purposes, lactic acid bacteria have been studied. Elie Metchnikoff, who won the Nobel Prize in medicine in the early 20th century, thought that the large consumption of milk-fermented goods among Bulgarian and Russian peasants was responsible for their extended life expectancy. He postulated that following intake, lactic acid bacteria would populate the gut, provide an acidic environment as they proliferate, and therefore inhibit the growth of protease. After learning that Lactobacillus bulgaricus cannot survive in the human stomach, the concept was dropped. Research was restarted once it was discovered that some strains of Lactobacillus acidophilus thrived in the intestine after implantation years later. When given in sufficient concentration, living microorganisms have been characterised as "probiotics," which have positive benefits on their host. Most of the species that have been studied have been isolated from various fermented dairy products. Numerous disorders including diarrhoea, intestinal inflammations, urogenital infections, allergies, etc. have been the subject of research aimed at healing or avoiding them. Certain species have been processed and offered as dietary supplements. However, there is currently insufficient data to demonstrate a clear cause and effect link for any of the marketed products.

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CHAPTER 13

CLASSIFICATION OF MAJOR MICROBIAL PRODUCTS

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Abstract:

Industrial microbiology has produced goods that directly and often unappreciatedly touched our life. These devices have significantly affected how we live and how long we live. They consist of biofuels, food additives, medicinal supplies for both human and animal health, and industrial and agricultural items. Nonantibiotic substances employed in medicine and health have significantly enhanced the wellbeing of animal and human populations, especially in recent years. We'll just talk about the top items in each category. Antibiotics Microorganisms, primarily filamentous fungus and actinomycetes of the genus Streptomyces, generate a large number of antibiotics.

Keywords:

Antibiotics, Fermentation, Microorganisms, Medium, Penicillin.

Introduction

To highlight the crucial significance of medium formulation and environmental control in the manufacture of these significant compounds, this chapter will examine the synthesis of a few of the most important antibiotics.*Penicillium chrysogenum*, which produces penicillin, is a great example of a fermentation for which meticulous medium composition manipulation is employed to attain maximum yields. Maximum antibiotic yields are not produced as quickly as they may be when large quantities of glucose are employed as the carbon source. When lactose, a slowly hydrolyzed disaccharide, is present and nitrogen is scarce, this encourages a higher buildup of penicillin after growth has ended.A steady, continuous glucose feed may provide the same outcome. The appropriate precursor is added to the medium if a specific penicillin is required. To increase the synthesis of penicillin G, which contains a benzyl side chain, for instance, phenylacetic acid is added. To optimise the synthesis of desired chemicals, this "steering" method is used. In order to ensure optimal stability of the freshly manufactured penicillin, the fermentation pH is kept around neutrality by the addition of sterile alkali. The broth is isolated from the fungal mycelium and processed by absorption, precipitation, and crystallisation to obtain the finished product when the fermentation is finished, which typically takes 6 to 7 days.Then, several semisynthetic penicillins may be produced by chemically altering this fundamental product. Streptomycin Streptomyces griseus produces the secondary metabolite streptomycin, whose accumulation is influenced by variations in substrate availability and environmental circumstances. Glucose is employed as a carbon source in this fermentation, which takes place on a medium made of soybeans. As a result, the nitrogen supply (soybean meal) is mixed, which restricts growth. After growth, the culture's antibiotic levels start to rise under carefully regulated nitrogen constraint. The area of developing antibiotics is continuously

growing. 6,000 antibiotics have been described as of this writing, 4,000 of which were from actinomycetes[1], [2].

Each year, over 300 new antibiotics are discovered. Acids Amino In the food business, amino acids like lysine and glutamic acid are utilised as nutritional supplements in bread goods and as flavor-improving ingredients like monosodium glutamate (MSG). Regulatory mutants, which have a decreased capacity to restrict the synthesis of an end product, are commonly used to produce amino acids. The typical microorganism carefully controls cellular metabolism to prevent the overproduction of biochemical intermediates. As shown in figure 42.12, *Corynebacterium glutamicum* mutants that lack or have a restricted capacity to convert the TCA cycle intermediate -ketoglutarate to succinyl-CoA are currently used to produce glutamic acid and numerous other amino acids in significant amounts. Increased membrane permeability and the excretion of high levels of glutamic acid are caused by a regulated low biotin level and the addition of fatty acid derivatives. The glyoxylate pathway is used by the damaged bacteria to get the vital metabolic intermediates they need, particularly while they are growing[3]–[5].

Isocitrate undergoes a nearly full molar conversion to glutamate (or an 81.7% weight conversion) once development is restricted due to altered food supply. An initial two-step microbial procedure was utilised to create lysine, an important amino acid added to breads and cereals. This has been replaced by a single-step fermentation in which the *Corynebacterium glutamicum bacterium* accumulates lysine while being prevented from synthesising homoserine. In a three day fermentation, more than 44 g/liter may be generated.

The series of metabolic events that result in Corynebacterium glutamicum accumulating glutamate from glucose. Bold arrows indicate significant carbon fluxes.

a) Development using the glyoxylate bypass to provide essential TCA cycle intermediates.
b) The majority of the carbon in the substrate is converted to glutamate after development is complete (note shifted bold arrows). Less frequently utilised reactions are shown by the dashed lines. Microorganisms with comparable regulatory alterations have been exploited to create a number of 5′ purine nucleotides that are used as flavour enhancers for soups and meat dishes, while this practise is not very common in the United States. Organizing Acids It is crucial to understand how trace metal levels and balances affect the synthesis and excretion of organic acids by bacteria in industrial microbiology. Major products include citric, acetic, lactic, fumaric, and gluconic acids. Citrusfruit from Italy was the main source of citric acid prior to the development of microbial methods. Nowadays, bacteria create the majority of the citric acid; 70% of it is utilised in the food and beverage business, 20% in medicines, and the remaining portion in various industrial uses.

The essence of the fermentation of citric acid is restricting the concentrations of trace metals like manganese and iron to halt *Aspergillus niger* development at a certain stage of the fermentation. To achieve low and regulated quantities of accessible metals, the medium is often treated using ion exchange resins. Formerly carried out via static surface growth, the fermentation of citric acid currently takes place in aerobic agitated fermenters. It has been discovered that copper

counteracts the suppression of citric acid synthesis by iron above 0.2 ppm, therefore high sugar concentrations (15 to 18%) are often employed.

The control and efficiency of the tricarboxylic acid cycle and the glycolytic pathway are necessary for this fermentation to be successful. When the substrate level is high after the active growth phase, citrate synthase activity rises while aconitase and isocitrate dehydrogenase activity falls. The stressed bacteria then accumulates and excretes citric acid as a consequence. In contrast, just one microbial enzyme, glucose oxidase, which is present in *Aspergillus niger*, is involved in the synthesis of gluconic acid. A. niger is optimally cultivated in a medium of corn-steep liquor. When nitrogen starts to restrict growth, the remaining glucose is converted to gluconic acid in a single step by the resting cells. Gluconic acid is a component of detergents as well as a calcium and iron transporter.

Specialty Substances for Medical and Health Use Microorganisms are employed to manufacture nonantibiotic speciality chemicals in addition to the bulk goods that have been produced during the last 30 to 40 years, such as antibiotics, amino acids, and organic acids. Sex hormones, cancer-fighting drugs, ionophores, and unique substances that affect bacteria, fungus, amoebae, insects, and plants are among them. To ensure that these medically significant substances reach the user in a stable, functional state, it is always required to create and recover the goods under carefully regulated circumstances. Biopolymers Biopolymers are produced by microorganisms and are used as gelling agents and to change the way liquids flow. These are used widely in the food and pharmaceutical sectors. Utilizing microbial biopolymers has the benefit of allowing manufacturing to be independent of the weather, political events that might restrict the availability of raw materials, and the depletion of natural resources. Additionally, production facilities might be situated close to sources of cheap substrates (e.g., near agricultural areas).

Exopolysaccharides of bacteria At least 75% of all polysaccharides are used in a variety of goods as stabilisers, for the dispersion of particles, as agents that produce films, or to encourage water retention. Many frozen foods, like ice cream, that are prone to sharp temperature fluctuations benefit from the texture maintenance provided by polysaccharides. These polysaccharides must be compatible with other polysaccharides and preserve their qualities under the pH values present in the specific meal. If heated, they shouldn't lose their physical properties. Dextrans, which is used to expand and absorb blood, and Erwinia polysaccharides, which are in biosurfactants, are examples of biopolymers. All surfactants that have been utilised commercially are the result of synthetic chemistry. The usage of biosurfactants is now gaining more and more attention. In environmental applications where biodegradability is a key need, they are particularly crucial. B-iosurfactants are employed for solubiliz ation, emulsification, enhancing detergency, wetting, and phase dispersion. These characteristics are crucial for bioremediation, oil spill cleanup, and improved oil recovery.

Glycolipids are the most widely utilised biosurfactants generated by microorganisms. These compounds have unique hydrophilic and hydrophobic ion pairs, and the final compound structure and properties are determined by the specific growth circumstances and the carbon source used. Good yields are often produced using insoluble substrates. These biosurfactants have been employed with the Exxon Valdez oil spill and are good dispersion agents. The

bioconversion P rocess B ioconv ersions, also known as microscopic transform ations or b iotransform ations, are small modifications that non-grow ing microorganisms carry out on molecules, such as the insertion of a hydroxyl or keto function or the saturation/desaturation of a complicated cyclic structure. Thus, the microorganisms serve as catalysts. There are various advantages over chemical processes in biological transformations. Stereochemical production of a product's biologically active form is a significant disadvantage. In contrast, the majority of chemical syntheses result in racemic mixes in which the organism can effectively use only one of the two isomers. Additionally, under moderate circumstances, enzymatic processes allow for the transformation of larger water-insoluble compounds. In several bioconversions, unicellular bacteria, actinomycetes, yeasts, and moulds have been employed. These transformations may be caused by internal or extracellular enzymes. To carry out required bioconversions, cells may be manufactured in more specialised ways or grown in batch or continuous culture and dried for immediate use. The hydroxylation of a steroid is a common bioconversion.

The water-insoluble steroid is dissolved in acetone in this case, and it is then introduced to the reaction system containing the pregnancies of microbial cells. The process of alteration is watched, and the finished product is removed from the medium and purified. B-iotransformations carried out by unhampered non-dividing cells or free enzymes do have restrictions. The majority of processes that take place without active metabolism—without constant access to reducing power or ATP—are exergonic reactions. An energy source like glucose must be given under strictly regulated non-random circumstances if ATP or reductants are required. When using vegetative cells or spores that are freely floating, the microbial biomass is often only utilised once. The cells are thrown away after the procedure is finished. After being immobilised in a polymeric matrix or attached to ion-exchange resins through ionic interactions, cells may often be reused again. Cells, spores, and enzymes from microorganisms may be imprisoned using ionic, capacitative, and physical entrapment techniques. On the inside walls of fine tubes, microorganisms may also be immobilised. This method is used in several industrial and environmental operations. The solution to be changed is then simply fed through the microorganism-lined tube. These include the biotransformation of steroids, the phenol degrading process, and the creation of a wide variety of antibiotics, enzymes, organic acids, and metabolic intermediates. The recovery of valuable metals from diluted process streams is one use of cells as biocatalysts.

Literature Review

In a study carried out by Schink *et al.* they as a prominent end product during growth on pectin but not on glucose or polygalacturonic acid, a number of pectinolytic bacteria of the Clostridium, Erwinia, and Pseudomonas species generated methanol. Clostridium butyricum strain 4P1's pectin metabolism was connected with a final product concentration of 16 mM at the conclusion of growth, a 1:1 stoichiometry for methanol synthesis, and a percent starting substrate methoxylation. High pectin methylesterase activity and a lack of methanol consumption were both related with pectin-based growth. Discussions are held on the ecological importance of pectin metabolism and the emergence of microbial methylotrophic metabolism in the natural world[6].

Atanasov e*t al.*highlighted that in the past, natural compounds and their structural analogues have significantly influenced pharmacology, particularly for the treatment of cancer and infectious disorders. However, natural compounds also pose difficulties for drug development, such as technological obstacles to screening, isolation, characterisation, and optimization, which led the pharmaceutical industry to stop looking for them in the 1990s. Recent advancements in technology and science, including as enhanced analytical tools, genome mining and engineering techniques, and improvements in microbial culture, are tackling these issues and creating new possibilities. In order to combat antimicrobial resistance, this has revived interest in natural compounds as drug leads. Here, they listed recent technical advancements that have made natural product-based drug discovery possible, highlight particular applications, and go over important prospects.

Papadochristopoulos *et al.* discussed that Consumers and the food industry are very concerned about food contamination caused by microbes. Scientists and the food industry are working to address consumer desires for wholesome, risk-free, and "clean label" goods that are devoid of synthetic preservatives. Utilizing natural anti-microbial compounds in food items or packaging is one option for replacing synthetic preservatives. Natural anti-microbials may be used to increase the safety and shelf life of meat and processed meat products since they are typical instances of extremely perishable items. Despite several instances of beneficial usage of natural anti-microbial agents in meat products documented in scientific studies, their commercial use is still in its infancy. This review's main goal is to provide a thorough summary of current work on natural anti-microbials, including essential oils, plant extracts, and flavonoids, chemicals originating from animals, organic acids, bacteriocins, and nanoparticles. Future prospects, legislation, use restrictions, in situ meat product research, and the agents' anti-microbial mechanism of action are all outlined. According to the study, organically produced anti-microbials may help the meat industry provide "clean label," wholesome, and secure meat products for customers.

Bradáčová *et al.* suggested that as a method to improve crop stress tolerance and nutrient usage efficiency, the use of biostimulants with plant growth-promoting qualities but without major nutritional. A significant difficulty still exists with regard to low repeatability in actual production settings. As a potential solution to this issue, the use of combination products based on microbial and non-microbial biostimulants or microbial consortia is discussed. The variety of bacterial communities at the root surface (rhizoplane) was decreased by phosphorus restriction, but this impact was reversed by MCP inoculation, demonstrating the increased P status of the plants. The findings provide credence to the idea that, especially under difficult environmental situations, the introduction of microbial consortia might boost the effectiveness and repeatability of BS-assisted agricultural production systems[7].

In a study by Fusco *et al.* it was discussed that Humans have been consuming milk and milk products for tens of thousands of years. The development of metagenomic investigations has expanded our understanding of the microbiota of milk and milk products, particularly as it relates to environmental, manufacturing, and storage factors. The price of milk is influenced by the quality of the milk, which is determined by chemical characteristics (fat and protein content, lack

of inhibitory compounds, microbial and somatic cell counts). In this situation, re-emerging and new viruses pose a significant threat to food safety. It is probable that as a consequence of global warming, not only will there be a rise in microbiological dangers, but also an increase in chemical risks related to the presence of mycotoxins or plant toxins in milk. Here, they provided a general review of the main microbiological risks that are present in the twenty-first century[8].

Li *et al.* demonstrated that one of the main by-products of the chicken business is feathers. They mostly consist of keratins, which are widely used in a variety of industries. Due to their resistance to protease breakdown, untreated feathers might become pollutants as a result of the rising output of feathers from the chicken industry. Because feathers are so full of amino acids, they are a desirable ingredient in fertilisers and animal diets. A growing body of research indicates that waste materials containing feathers may be turned into products with additional value. A variety of bacteria and fungi have shown the ability to breakdown chicken feathers by secreting enzymes like keratinases. This paper discusses current developments in feather breakdown by microbes, keratinase structure, feather application, and microbes that may produce keratinase. The discussion also covers the enzymes essential for the breakdown of keratin as well as their mode of operation. Additionally, we put up a technique for feather deterioration. According to the research that have been conducted, the microbial decomposition of feathers has a tremendous potential to transform them into a variety of products, including biofertilizer and animal feeds[9].

Since ancient times, nature has provided numerous valuable medications, with many of them being derived from plant sources. After the penicillins were discovered, drugs from microbiological sources were discovered, and diving methods in the 1970s opened up the oceans. Late in the 1980s, combinatorial chemistry changed the emphasis of drug discovery efforts from Nature to the lab bench. Cragg *et al.* examined the development of natural goods medications, highlighting significant pharmaceuticals derived from natural sources that transformed the management of critical disorders. It is obvious that novel structural leads from Nature will continue to be a primary source, and successful medication development rely on interdisciplinary partnerships. Principal Conclusions In addition to creating new screening methods, the explosion of genetic data also made it possible to use genome mining and combinatorial biosynthetic technologies. Unknown compounds have been identified thanks to the information obtained. Combinatorial chemistry may be used to optimise these unique bioactive structures, creating new therapeutic candidates for a variety of illnesses. Overall Importance The development of genetic tools that made it possible to isolate and express biosynthetic cassettes from bacteria may represent the next big step in the search for natural products lead. It is now clear that such creatures may have a significantly higher biodiversity. In comparison to plants and multicellular animals, the number of possible species in the microbial world is several orders of magnitude bigger. Combining these figures with the number of previously undetected biosynthetic clusters (> 10 per species) shows that the promise of microbial diversity is yet largely unrealized[10].

Discussion

Proteolytic and lipolytic bacteria, such as Pseudomonas or Acinetobacter spp., predominate the spoilage bacterial populations, according to research on the impact of hygiene and efficient

chilling on the spoilage microbiota. These bacteria have the ability to create heat-stable proteases and lipases, which are still active after pasteurisation and may contaminate milk when kept for an extended period of time. After pasteurisation, milk may still get contaminated, therefore there is still a strong need for improved cleaning and sanitation practises, tools, and test methods to (quantitatively) identify important pathogenic or spoilage bacteria. Worldwide usage of raw milk and raw milk cheese is rising along with the desire for locally grown, minimally processed, healthful foods. The goal is to exploit complementary or synergistic interactions and increase the flexibility of responses under various environmental conditions. This research compared the efficacy of single-strain microbial inoculants with known plant growth-promoting capabilities to consortium products in actual production circumstances in massive tomato culture systems subjected to various environmental obstacles. Different fungal and bacterial single-strain inoculants, as well as microbial consortium products, exhibited very comparable positive responses in a protected greenhouse production system in Timisoara, Romania, with composted cow dung, guano, hair, and feather meals as key nutrients. Over the course of two growing seasons, there was a considerable improvement in the nursery's performance, fruit setting, fruit size distribution, seasonal yield share, and cumulative yield (39–84% compared to the control). An open-field drip-fertigated tomato production system in the Negev desert of Israel with mineral fertilisation on a high pH (7.9), low fertility, and sandy soil, on the other hand, recorded superior performance of the microbial consortia products (MCPs) under more difficult environmental conditions. When there was a restricted supply of phosphate, this was shown by better phosphate (P) acquisition, an increase in the ultimate fruit yield, and stimulation of vegetative shoot biomass production. Furthermore, MCP inoculation was linked to specific alterations in the bacterial community composition of the rhizosphere, especially with regard to Sphingobacteria and Flavobacteria, which have been described as salt indicators and drought stress protectors.

The well-known traditional technique of generating folic acid by chemical synthesis has a high cost of raw ingredients and a poor yield of the finished product. Some bacteria and yeast that produce folic acid may grow in whey or milk plasma and produce large levels of folic acid in the culture medium. It has been shown that a number of bacteria, including Lactococcus lactis sub sp. cremoris, L. lactis sub sp. lactis, Bifidobacterium adolescentis, and B. pseudocatenulatum, as well as yeasts such as Candida famata, C. guilliermondii, C. glabrata, Yarrowia lipolytica, Saccharomy Pseudomonas denitrificans and Propionibacterium shermanii are the microorganisms responsible for generating vitamin B12.

Therefore, megaloblastic anaemia may be avoided by using probiotic microorganisms. It includes eating meals that have been fermented with lactic acid, which improves the pH in the digestive system to promote iron absorption, activate the enzyme phytases, and create organic acids and other digestive enzymes. A type of probiotic bacteria created by the Swedish company Probi increased the absorption of iron from diet in women, according to a research. Lp299v, commonly known as Lactobacillus plantarum V, strengthens the immune system in addition to the digestive system. Additionally beneficial to the heart, it helps to lessen unforeseen gas and bloating situations. By making it more normal and consistent, it improves bowel movements. The probiotic strain Lactobacillus plantarum 299v lessens an antibiotic's detrimental effects on

colonic fermentation. 34 In the recent past, numerous medications have been created utilising genetically modified bacteria or fungus that generate the medication in enormous bioreactors. Erythropoietin is used to treat anaemia and may be produced artificially in bioreactors by microorganisms. However, this therapy involves frequent, even daily injections. Specialized cells from human blood that typically restore the lining of blood arteries have been isolated by researchers. These cells were genetically altered to express erythropoietin. Mesenchymal stem cells, which may develop into blood arteries, were then added to the mixture of cells. Then, mice that had become anaemic due to radiation (as sometimes happens in chemotherapy patients) or the loss of kidney tissue were injected with this combination under the skin. Under the skin, networks of blood vessels spontaneously developed from the cell mixture. Both kinds of mice's anaemia was treated by the vessel lining's secretion of erythropoietin.

Major illnesses include Type II diabetes, coronary heart disease, hypertension, and several types of cancer are all significantly increased by obesity. When energy intake—which is mostly stored as triglycerides—exceeds energy expenditure, obesity results. Diet, stage of development, age, level of physical activity, and genes all have a role in the complex condition known as obesity. The production of a natural cure using probiotic bacteria helps with weight management, avoiding obesity, extending feelings of satiety, decreasing food intake, lowering fat deposition, boosting energy metabolism, treating & strengthening insulin sensitivity, and treating obesity. Recent research indicated that altering the microbial ecosystem's makeup in the gut could be a unique strategy for treating obesity. Such a course of therapy may include changing the make-up of an obese person's microbial communities by giving those probiotics, or helpful microbes. Studies on animals have shown that certain LAB strains are effective at reducing serum cholesterol levels. This is likely because they break down bile in the stomach, which prevents the re-absorption of cholesterol (which enters the blood as cholesterol). A meta-analysis that included five double-blind studies that looked at the short-term (2–8 weeks) effects of yoghurt with probiotic strains on serum cholesterol levels found only a small change in total cholesterol concentration of 8.5mg/dL (0.22mmol/L) (4% decrease) and a decrease in serum LDL concentration of 7.7mg/dL (0.2mmol/L) (5% decrease).

A somewhat lengthier investigation on the impact of probiotic-enriched yoghurt on 29 patients over the course of six months showed no statistically significant variations in total blood cholesterol or LDL levels. However, the research did find that therapy led to a considerable rise in serum HDL from 50mg/dL (1.28mmol/L) to 62mg/dL (1.6mmol/L). This relates to a potential increase in the LDL/HDL ratio. Additionally, it has been reported that, depending on the strain, members of the Lactobacillus (Lb. sporogenes and Lb. acidophilus NCFB 1748) and Bifidobacterium genus may play a significant role in the regulation of weight as an anti-obesity effect in experimental models and humans or as a growth-promoter effect in agriculture.

The most prevalent functional gastrointestinal condition that causes abdominal discomfort, changed bowel habits, and abnormal stool features is irritable bowel syndrome (IBS). With the administration of probiotics, Lactobacillus salivarius or Bifidobacterium infantis discovered that the usual IBS symptoms significantly improved. Reductions in bloating, gas, colonic transit time, and abdominal discomfort were often noted benefits. Multiple functions of intestinal

microorganisms in maintaining human health include complicated food digesting, medication metabolism, toxic chemical detoxification, production of vital vitamins, and avoiding the colonisation of infections. The majority of the microbes in the GI tract are anaerobic bacteria, which are impossible to grow in a laboratory setting. Age, gender, geographic origin, and environmental variables including nutrition and dietary supplements all affect the kind and quantity of bacteria in the GI tract. In a healthy human GI tract, Firmicutes and Bacteroidetes predominate; the latter was found in lower concentrations in IBS patients who had constipation as their primary symptom.

With a frequency of 10% to 20%, atopic dermatitis (AD) is the most prevalent chronic skin disorder in newborns and young children. The prevalence of this condition is influenced by geography, with the United States and Europe having the greatest frequency. Genetics account for 82% of the vulnerability to AD, while environmental variables account for 18% of the susceptibility. Additionally, dietary sensitivity, particularly to milk and egg proteins, has been associated to AD. Recently, the name eczema has been suggested; nonetheless, for the purposes of this article, both AD and eczema shall be used.

An imbalance in the TH1/TH2 cytokine activation of TH2 cells and the stimulation of IgE and IgA production, which results in allergic responses, are related with allergic disorders. 45 Probiotics may suppress the TH2 response while increasing the TH1 and TH1 cytokine production, including interferon. The usage of probiotics was linked to an increase in interferon production and a suppression of allergen-induced tumour necrosis factor, IgE, and numerous allergy-induced cytokines in children with atopic illness.

Atopic dermatitis, which affects 17.2% of Americans, has been dramatically improved by two probiotic strains, according to a research. A combination of Lactobacillus acidophilus DDS-1 and Bifidobacterium lactis UABLA-12 (from UAS Labs) was tested in a clinical trial on 90 preschoolers with moderate to severe atopic dermatitis (ages 1 to 3 years) (AD). In children with atopic dermatitis, probiotic therapy reduces gastrointestinal symptoms and stabilises the intestinal barrier function. Probiotics may thus be appealing options given the minimal likelihood of side effects developing.

The prevalent chronic condition Crohn's disease, which affects the gastrointestinal system, is thought to arise from an abnormal immune response to intestinal bacteria in a genetically vulnerable host. Depending on whatever region of the digestive system is damaged, there are many forms of Crohn's disease. The small intestine, the large intestine, the rectum, or the mouth may all be affected by Crohn's disease.

Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, and Mycobacterium avium subsp. paratuberculosis are the primary pathogenic pathogens linked to Crohn's disease. A secure probiotic bacteria called Lactobacillus GG is known to momentarily invade the human gut. It has been discovered to be effective in treating a number of digestive diseases marked by increased gut permeability. A research found that Lactobacillus GG may help children with slightly to moderately active, stable Crohn's disease by improving gut barrier function and clinical status. 48

Free radicals are now more clearly implicated in the genesis of a number of illnesses, including cancer, diabetes, cardiovascular disease, autoimmune disorders, neurodegenerative diseases, and ageing. Although several studies have revealed a link between antioxidants and microbes, the isolation of microbial antioxidants became the focus of study in the early 1980s.

In animal trials, the probiotic bacterium Streptococcus thermophilus has been found to lessen the intestinal atrophy that results from short-term fasting and to assist in the recovery from malnutrition. Additionally, S. thermophilus is recognised for its potent antioxidant properties, which defend the body against harmful free radicals that develop in the body as a result of ageing, stress, sugar, antibiotics, and other chemicals and pollutants. The anti-oxidant activity (AOA) of six Penicillium and Aspergillus species, including Rhizopus oryzae, was assessed in a research, and it was discovered that the extracts of two Penicillium and four Aspergillus species protected linoleic acid better than the control.

In one species, Aspergillus candidus CCRC 31543, the oil and BHA were both preserved. In a follow-up investigation, it was discovered that the presence of ammonium sulphate, sucrose, or lactose in the culture medium improved the antioxidant production in A. candidus CCRC 31543. Both the mycelium and the broth generated extracts with comparable activity after being extracted with ethyl acetate. A phenolic acid called gallic acid is present in a wide range of natural sources, including microbial products. From Penicillium and Aspergillus cultures, gallic acid has been isolated. One of the three antioxidants produced by Streptomyces sp. USF-319 inhibits 5-lipoxygenase. Mycotrienin II, trienomycins A and B, which are ansamycin antibiotics, are among the antioxidants. The most active of the three compounds, mycotrienin II, was rated as a moderate antioxidant when compared to BHT (butylated hydroxytoluene). However, it was discovered that mycotrienin II inhibits 5-lipoxygenase.

The last class of antioxidants that microbes can produce are carotenoids. According to research, the colourants -carotene from Blakeslea trispora and Duniella salina and lycopene from Blakeslea trispora and Streptomyces chrestomyceticus, subsp. rubescens, are acceptable for use in human meals. For use in fish meals, astaxanthin from microbiological sources, such as Xanthophyllomyces dendrorhous, has been permitted. It was discovered that lycopene and astaxanthin have high singlet oxygen quenching ability. According to a research, lutein, beta-carotene, zeazanthin, and canthaxanthan all have AOAs that are 10 times lower than astaxanthin.

An emerging area is the extraction of colours from microbial sources. Numerous microorganisms, including bacteria, fungus, yeasts, and algae, have different colours. These sources may provide natural colours when simple and efficient processes are followed. Microorganisms may produce pigments without being affected by the weather, grow quickly and easily, and produce colours in a variety of tints when grown on inexpensive substrates. The ability to mass produce microorganisms due to their rapid development rate is one of the main benefits of employing them as a source of natural colours. Red, yellow, and blue pigments make up the majority of what microorganisms create. The majority of study has been concentrated on the generation of yellow and red pigments, such as xanthomonadin from Xanthomonas campestris pv. And carotenoid from Phaffia rhodozyma, Micrococcus roseus, Brevibacterium linens, and Monascus sp.

However, since many bacteria are unable to produce blue pigment, research into blue bacterial pigments is restricted. Streptomyces coelicolor A3 (2) produces actinohodine-related blue pigments, as do Chromobacterium violaceum and Janthinobacterium lividum, which combine violacein and deoxybiolacein. 4 In addition to its use in colouring textiles, violacein also has anti-leishmanial, anti-ulcerogenic, antiviral, antibiotic, anti-tumoral, and anti-Trypanosoma cruzi properties. It also has cytotoxic effect against human colon cancer cells.

When the body cannot effectively break down sugar due to a lack of insulin, diabetes, a common and sometimes deadly condition, develops. Most of the insulin that individuals use to control their diabetes is made utilising biotechnology. Large amounts of human insulin are produced by genetically altered bacteria and purified for medicinal application. Humuline, a well-known brand name for 'human' insulin made using GM bacteria, is being used by millions of people all over the globe. Recently, friendly gut bacteria have been trained to produce a particular protein that may assist diabetic mice control their blood sugar. The microorganisms can be cultured in yoghurt and may provide an alternative therapy for patients with diabetes, however the study is currently in its very early stages.

A strain of non-pathogenic E. coli bacterium that produces the protein GLP-1 was developed by the researchers. This protein causes the pancreatic cells to start producing insulin in healthy individuals. Recent research has shown that human intestinal cells in a dish may be stimulated by modified bacterial cells secreting the protein to generate insulin in response to glucose. In a recent study, animals with diabetes were given the modified bacteria. The mice progressed from having diabetes to having normal blood glucose levels after 80 days. Despite not receiving the modified microorganisms, diabetic mice still exhibited elevated blood sugar levels. An alternative to insulin injections for diabetics is the consumption of yoghurt or smoothies as glucose-responsive insulin treatment. The production of the protein by bacteria offers a number of benefits over utilising the protein itself as a therapeutic. In reaction to circumstances in the host, the bacteria can release precisely the exact quantity of the protein, which might eventually reduce the need for self-monitoring and enable the patient's own cells (or the cells of the commensal E. coli) to give the proper amount of insulin when required. Additionally, by creating the protein where it is required, some of the drawbacks of protein-based medications—which may be costly to produce and often breakdown during digestion—are eliminated.

Asthma, allergic rhinitis, atopic dermatitis, and other atopic illnesses are becoming more common, according to reports. Probiotic strains may reduce some local and systemic immunological markers, which is evidence that they have an impact on inflammatory processes. These effects may be mediated via the GALT, one of the three intestinal lines of defence. Probiotics may reduce the number of cells that cause inflammation and the accessibility of allergens, normalise the intestinal microbiota, improve the function of the intestinal barrier, and control the release of inflammatory mediators.

Conclusion

The quantity of novel microbial metabolites is continuously increasing, according to our current understanding and future projections, but the quality improvement is much more significant. This

offers important practical outcomes for both agricultural and human treatment. The majority of the biodiversity on earth has yet to be discovered, and the new, quick techniques enable its effective utilisation. Alternative strategies and the opportunity to incorporate adequate biosynthetic pathways from non-cultivable strains into compatible hosts are provided through cloning and genetic engineering. It is reasonable to anticipate that natural compounds derived from microorganisms will be crucial in the current shift from empirical drug screening to really logical drug design.

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CHAPTER 14

AN OVERVIEW ON MICROBIAL GROWTH

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Abstract:

Microorganisms have historically been excellent models for the study of fundamental unicellular cellular processes. However, these ostensibly monocellular creatures spontaneously organise into multicellular communities, develop into specialised cells that serve the needs of the whole population, and, under certain circumstances, coordinate their behaviour. In particular, wild microorganisms may adapt to nutrient scarcity by changing their characteristics in response to the availability of resources (for instance, phase variation in bacteria).

Keywords:

Antibiotics, Cultivation Medium, Fermentation, Microorganisms, Penicillin.

Introduction

Using uncommon nutrition sources, including their own waste products, microbes may lower their rate of metabolism under this circumstance, which occurs often in nature (for example, ammonia signalling in yeast colonies). When microorganisms are transported to pleasant laboratory circumstances, some of the processes that seem to be significant in nature, such as the creation of the extracellular matrix, are turned off, and others that seem to be more universal survive. This study examines the characteristics of multicellular microorganisms, including as differentiation, interaction, and long-range signalling, and investigates how these characteristics vary in the microorganisms that are often investigated in the lab.Microorganisms are often handled as individuals in the laboratory under ideal nutrition supply, temperature, humidity, and other circumstances, and are mostly researched during the relatively brief window of time when they develop as exponential pure, liquid cultures. However, microbes seldom find circumstances in the natural environment that enable them to thrive and reproduce at the fastest possible pace. Microorganisms in the wild must be able to live for lengthy periods of time without reproducing because they must contend with conditions including nutrition scarcity, temperature variations, and a lack of moisture. Furthermore, since one of their methods for survival is the capacity to group together into multicellular communities and to develop into specialised cell types, microbes seldom survive in nature as individuals.Motile amoebas and bacteria create the fruiting bodies of myxobacteria and slime moulds, which have been the subject of traditional studies. These structures are created as a consequence of cell differentiation, which creates very hardy spores (germinal cells) and fruiting body stalks that eventually lyse (terminal phenotype cells). The environment of the fruiting body seems to influence the growth of mature spores that can endure harsh conditions. The substance produced by lysed stalk cells is integrated into the spore cell wall during this time, giving the spore’s complete resistance to adverse circumstances. These results show that differentiation programmes do exist, but they are restricted to certain cells and

regions of the fruiting body and cannot be carried out by individual free-living cells.The growth and division of bacteria, yeast, and moulds are prerequisites for the development of colonies and biofilms, as opposed to the active aggregation and subsequent differentiation of non-diverging motile microorganisms that results in the production of fruiting bodies. However, much like the cells in fruiting bodies, the cells in colonies and biofilms may differentiate and act in various parts of these multicellular communities in a variety of diverse ways.

Numerous data suggest that the environment has a significant impact on the characteristics of the multicellular communities that are created by microorganisms. This is especially apparent in bacterial and yeast biofilms and colonies. These highly structured biofilms may cling to solid surfaces in liquid settings or form a pellicle on the surface of water. Complex social communities of various, coexisting microorganisms are prevalent in nature. In fact, they build so quickly that it is difficult to maintain surfaces clear of an encroaching biofilm. Contrarily, it is challenging to build artificial experimental environments in the lab that mimic natural ones; in other words, to produce artificial biofilms with characteristics like fully grown natural biofilms. As a result, despite the abundance of knowledge about the existence and ultrastructure of biofilms in situ, very little is known regarding the ecology and biology of cells inside biofilms as well as concerning routes specific to biofilms.Another kind of multicellular community of microorganisms that develops in nature are colonies. They seem to be less sophisticated and environment-resistant than biofilms, serving as an "intermediary" between naturally occurring biofilms and unprotected individual cells. This makes colonies of bacteria, yeast, and other microorganisms an intriguing subject for studies of, for example, the interactions and signalling between microorganisms within multicellular communities[1], [2].

This is in addition to the fact that colonies of bacteria, yeast, and other microorganisms are frequently grown in laboratories. One fascinating topic is whether (and if so, how) natural colonies vary from those that are grown in a lab. Some hints have been offered by recent research on the differences between colonies created by laboratory strains of *Saccharomyces cerevisiae* and those formed by strains recently obtained from wild. In contrast to colonies of wild S. cerevisiae strains, which generate "fluffy"-structured colonies even in the laboratory, S. cerevisiae laboratory strains often create smooth colonies with no discernibly organised pattern. Cells inside fluffy colonies are joined by an extensive, highly glycosylated extracellular matrix that serves as a framework for the colony, according to biochemical studies and ultrastructural native electron microscopy. The smooth colony included no such material. The colony has to be protected from desiccation and hazardous environmental elements by this extracellular matrix. It can also create tiny pathways for the passage of nutrients and water, as well as chambers and microenvironments for the emergence of new cell generations, much like biofilms may. This is corroborated by the finding that fluffier colonies spread and occupy new land more quickly than smoother colonies[3]–[5].

Literature Review

Modernization of the use of microbes in food and beverage manufacturing over countless years may be envisaged. The fast use of biotechnology methods, which enable the quick discovery of novel chemicals and microbes or even the genetic enhancement of well-known species, is what

has sped up the manufacture of new food at the present time. Microorganisms had never before in history been so prevalent in fields like agriculture and medicine, unless they were known bad guys. However, right now, a variety of agricultural crops need helpful microorganisms like plant development promoters and phytopathogen controllers, and several species are utilised as biofactories of significant pharmaceutical compounds. The use of biofactories does not stop there; microorganisms have also been investigated for the synthesis of various chemicals, fuel molecules, and industrial polymers, and strains that are important for the environment because of their capacity for biodecomposition or biosorption have drawn attention in both research labs and industrial settings.

Vitorino *et al.*to this emerging field of microbiology as technological microbiology, and we think that sophisticated methods, including metabolic engineering and heterologous expression, may be progressively integrated into this applied science to provide new and better goods and services[6].

Stavropoulou *et al.* discussed on the food contamination by microorganisms may result in food degradation and health hazards when ingested. Foods contain a native flora and a transitory flora that reflects their surroundings; they are not sterile. We must eliminate these germs or stop their development in order to guarantee the safety of food. It is impossible to eliminate recurring risks brought on by errors in the handling, processing, and distribution of food using antiquated techniques and insufficient solutions. They need to be approached positively, and problems need to be solved by combining all available information. Mathematical models that have been built to analyse microbial reactions must be verified for the particular situation. Predictive microbiological modelling may thus be used as a valuable technique for quantitative risk assessment. Here, they examined the predictive models that have been modified to enhance the food industry chain using a procedure that simulates real-world circumstances or a virtual replica of the finished product. Predictive models are therefore anticipated to be a helpful and important tool in research as well as in commercial food preservation operations today[7].Worldwide, there is a significant amount of agro-food industrial residue (AFIR) production. These wastes are mostly lignocellulosic wastes, which may be used to create goods with additional value. Technologies like solid-state fermentation (SSF) for the bioconversion of lignocellulosic waste, which are based on the creation of a broad variety of bioproducts, have both financial and environmental advantages. SSF is one of the value-adding methods for AFIRs that has the potential to significantly alter the environment of the larger community due to its adaptability and interest in implementing the circular bioeconomy's principles. The choice of the right substrate and microbe, as well as the choice of the best process parameters for the microorganism's growth and the generation of the necessary metabolites, are crucial factors in SSF.

Šelo *et al.* provided a general overview of the management of AFIRs by SSF, including information on their current uses, classifications, and chemical makeup; the catalytic function and potential applications of enzymes produced by various microorganisms during SSF cultivation on AFIRs; the production of phenolic compounds by SSF; and a brief explanation of the role of SSF treatment of AFIRs for feed improvement and biofuel production.

Ma *et al.* carried out a study in which they highlighted that Plants and bacteria cohabit or compete for existence, and their harmonious interactions play a crucial role in adapting to settings rich in metalliferous materials. These interactions may thus be investigated to enhance microbe-assisted phytoremediation. Soil microbes, with whom they develop complex communication networks, may utilise plant root exudates as sources of nutrients and energy. Some helpful bacteria and fungi, known as plant growth promoting microorganisms (PGPMs), can reduce metal phytotoxicity and stimulate plant growth either indirectly by triggering defence mechanisms against phytopathogens or directly by solubilizing mineral nutrients (such as nitrogen, phosphate, potassium, iron, etc.), producing plant growth promoting substances (such as phytohormones), and secreting particular enzymes (e.g., 1-aminocyclopropane- 1-carboxylate deaminase). Through a variety of processes, including acidification, precipitation, chelation, complexation, and redox reactions, PGPM may also alter the metal bioavailability in soil. They highlighted recent developments and applications in our understanding of the biochemical and molecular mechanisms underlying plant-microbe interactions and how they contribute to the key steps in phytoremediation, including heavy metal detoxification, mobilisation, immobilisation, transformation, transport, and distribution[8].

Koskey *et al.* reported that In order to promote soil nutrient availability, crop nutrient quality, plant tolerance to biotic and abiotic stressors, biocontrol of pests and diseases, and water absorption, PGPMs play crucial roles in agroecological cycles. In order to promote the long-term maintenance of plant and soil health and to improve agroecosystem resilience against unpredictably changing climatic conditions, this review examines various research strategies involving the use of helpful microorganisms in the particular context of smallholder agroecosystems.

Numerous bacteria have the potential to increase plant production in cropping systems, according to a growing body of research, albeit their practical field use may be hampered by a number of biotic and abiotic restrictions. The goal of the current effort was to create multifunctional synthetic microbial consortia that could be combined with the right bioactive chemicals to increase crop production and quality. A bottom-up strategy was used to find plant growth-promoting microorganisms (PGPMs) with various functional characteristics. By looking at peer-reviewed scientific papers and outcomes from pertinent European initiatives, a thorough literature assessment on PGPMs connected to maize, wheat, potato, and tomato, as well as on commercial formulations, was carried out. To find plant growth-promoting (PGP) strains, metagenome fragment recruitments on the genomes of prospective PGPMs represented in databases were also carried out. Isolated PGPMs were artificially combined into three separate microbial consortiums once evidence of their capacity to cohabit emerged. Additionally, starving circumstances were used to evaluate how bioactive substances affected the proliferation of particular PGPMs. To choose those that may be used in sustainable agriculture, several combination products based on microbial and non-microbial bio stimulants (BS) are worth investigating for greenhouse and open field studies.

Gallagher *et al.* investigated that in closed industrial settings, genetically modified organisms (GMOs) are often exploited to create valuable substances. Their newer uses in public clinical or

environmental contexts, nevertheless, need more stringent safety and security procedures. A significant unresolved biosafety issue is intrinsic biocontainment, or the development of bacterial hosts incapable of surviving in natural conditions. In order to limit the viability of Escherichia coli cells to media containing exogenously supplied synthetic small molecules, we developed a new biocontainment strategy with overlapping "safeguards"—engineered riboregulators that tightly control expression of essential genes and an engineered addiction module based on nucleases that cleaves the host genome. These several layers of protection prevent persistence, sustain strong growth under permissive circumstances, and keep escape frequencie. The gradual adoption of safety measures exposed escape mechanisms and made it possible to develop methods to circumvent them. Our safeguarding approach is modular and makes use of conserved processes that might be expanded to include undomesticated animals and creatures important to clinical or industrial settings[9].An essential strategy for boosting agricultural output is the employment of microorganisms that encourage plant development. Additionally, there is a growing need to reduce the use of artificial fertilisers and to establish sustainable agriculture.

Silva *et al.*determined how well the growth-promoting microbes Bradyrhizobium elkanii and Trichoderma harzianum treated soybean seedlings. The Regional Institute of Rural Development (IRDeR) in Augusto Pestana, RS, Brazil, is where this experiment was carried out. Trichoderma harzianum, Bradyrhizobium elkanii, and co-inoculated Trichoderma harzianum + Bradyrhizobium were the four treatments, each of which had four replications. The experimental design was randomised blocks. The components of soybean yield were examined using five rows that were each 5 m long, separated at 0.5 m, and three centre lines. Two of these lines had their fourth metre of growth harvested, and one of these lines had its incidence of soil illnesses assessed. Tukey test was used to compare treatment means. Treatments had an impact on grain yield: B. elkanii and treatment with co-inoculation of B. elkanii and T. harzianum did so significantly. The biologically inactive chemical treatment resulted in a greater frequency of illnesses and a decreased grain yield[10].

Discussion

Because the mortality of these microbes balances out their development, the number of microorganisms in the biosphere is essentially constant. Any microbial group's ability to survive within an environmental niche is ultimately impacted by its ability to compete successfully for resources and by maintaining a pool of all live cells, which is often made up of human cells and a variety of other bacteria (referred to as the microbiome or microbiota). Understanding resource competition in a certain microenvironment is crucial to comprehending the development, survival, and demise of bacterial species (also known as physiology).Predictive modelling may provide the food sector a significant competitive edge since the industrial domain is evolving quickly and there is demand to constantly enhance both goods and procedures. Investigating the wide range of elements that may have an impact on the finished product has helped the industry maintain their worries about research and development capital. Meal quality depends heavily on the presence of microbes in the food. However, the characteristics of food itself, such as water activity, pH, storage circumstances, temperature, and relative humidity, are intimately connected to microbial behaviour. Mathematical modelling derived from quantitative research on microbial

populations may be used to anticipate the influence of these elements together facilitating development of bacteria in foods. Utilizing predictive models enables us to assess changes in microbial populations in food from harvest through production, providing a permanent and impartial assessment of the relevant parameters. Predictive microbiology, which ensures the quality and safety of food, is the study of microbial activity in response to certain environmental variables.Over 50% of the world's food production is generated by smallholder agroecosystems, which are essential to maintaining food security. Although these distinctive agroecosystems confront several difficulties and get little assistance, they are believed to be an essential resource for feeding the world's growing population in the years to come. Due to the new challenge of increasing food production through agricultural intensification in the face of declining per capita arable lands, faltering global economies, and unpredictable climate change, there is now an excessive reliance on agrochemical inputs, which are frequently expensive and harmful to both human and animal health as well as the environment. Alternative eco-friendly tactics that are most compatible with smallholder systems have been sought for to maintain healthy crop production methods. Biological agents, particularly plant growth-promoting microorganisms (PGPMs), which provide crucial agroecosystem services within a holistic vision of enhancing farm productivity and environmental protection, are the most common and widely accepted solution that has drawn a lot of interest from researchers and smallholder farmers.

We have learned a lot about the physiology of microorganisms by studying isolated cultures that were cultivated in labs under ideal circumstances (nutrient excess). But the majority of microbes face nutritional stress when they compete in the natural environment. Also, a new microbiota composition will soon occupy an empty environmental microbial niche. Understanding the intricate relationships that guarantee the survival of a particular microbiome is ultimately a balance between nutritional availability and physiological effectiveness.Growth is the systematic expansion of an organism's total number of parts. True growth does not occur when a cell absorbs water, deposits lipid or polysaccharides, or deposits both. Binary fission has the side effect of increasing the number of single bacteria in a population, or "culture," by cell multiplication.

Monitoring Microbial Concentrations

Cell concentration (the number of viable cells per unit volume of culture) or biomass concentration are two ways to quantify microbial concentrations (dry weight of cells per unit volume of culture). Because the average dry weight of the cell fluctuates at various phases of a culture, these two criteria are not always equal. Neither are them equally important: For instance, cell concentration is the relevant amount in studies of microbial genetics and the inactivation of microorganisms, but biomass concentration is the significant quantity in studies of microbial biochemistry or feeding.

Usually, cell concentration is determined by the viable cell count. For this, a 1-mL volume from a bacterial suspension is taken, serially diluted ten times, and then 0.1-mL aliquots are plated on an agar medium. Every single invisible bacterium (or clump of invisible germs) develops into a countable visible colony. Plates with between 30 and 300 colonies provide the best reliable data for statistical analysis. The number of colony forming units (CFU)/mL in the undiluted bacterial

solution will be determined by the plate count, the dilution, and a factor of 10. By using this technique, the final bacterial count is not affected by dead bacteria in the suspension.

For the majority of applications, a standard curve may be used to connect a culture's photoelectrically determined turbidity to the viable count. An approximate visual estimate is often an alternative: Escherichia coli, for instance, has around 107 cells per millilitre in a scarcely turbid solution and about 108 cells in a very turbid suspension. The relationship between turbidity and viable count may change as a culture grows and dies; cells can lose viability without the culture becoming less turbid. C. Biomass Density

A microbial culture's dry weight after being cleaned with distilled water may theoretically be used to directly estimate biomass. This process is laborious in reality, therefore the researcher usually creates a standard curve that links dry weight to viable cell count. Alternately, the volume filled by cells that have settled out of suspension or by measuring an essential cellular component like protein may be used to indirectly determine the concentration of biomass.A curve is often produced when microbial cells from a culture that has already reached saturation are added to a set amount of liquid medium, and the number of viable cells per millilitre is regularly measured and plotted. The stages of the bacterial growth curve are not representations of the occurrences in a single cell, but rather of a community of cells. A batch culture is the name for this kind of culture. There are four stages that may be used to describe the normal growth curve. In contrast to the environment of the human host, where nutrients are digested by bacteria and human cells, batch culture is a closed system with restricted resources. The genetics and physiology of bacterial replication, including the lag, exponential, stationary, and death phases that make up this process, may nonetheless be fundamentally understood by studying growth in batch culture.

The lag phase is the time when cells adjust to their new environment after being depleted of metabolites and enzymes as a consequence of the adverse circumstances prevalent at the conclusion of their previous culture cycle. It takes time for intermediates and enzymes to build up to quantities that allow growth to restart.It often occurs that cells transferred from a completely different media are genetically unable to grow in the new medium. When this happens, there may be a significant growth delay because it takes time for a few variations in the inoculum to reproduce enough for a net increase in cell number to become visible.

The cells expand while in a steady state during the exponential phase, which is depicted by equations 5-7. A steady amount of new cell material is being created, but since it is catalytic in nature, the bulk grows exponentially. This continues until one of two things occurs: either all of the available nutrients in the medium are used up, or toxic metabolic waste builds up and prevents development. The nutrient that becomes scarce for aerobic organisms is often oxygen. Unless oxygen is introduced into the medium by agitation or by bubbling in air, the growth rate declines when the cell concentration surpasses around 1 107/mL. When the bacterial concentration exceeds 4-5 109/mL, even in an aerated medium, the rate of oxygen diffusion cannot keep up with the demand, and growth gradually slows.

Growth eventually comes to an end due to the depletion of nutrients or the buildup of harmful substances. The majority of the time, however, cell turnover occurs during the stationary phase, when the gradual death of cells is balanced by the development and division of new cells. When this happens, the total number of cells gradually rises but the number of viable cells remains constant.

After some time in the stationary phase, a certain rate of cell viability loss starts to occur. The mortality rate rises until it reaches a constant level, depending on the organism and the culture circumstances. The following is a discussion of steady-state death's mathematics. Cell death often occurs at a pace that is significantly slower than exponential expansion. The mortality rate often drops sharply after the majority of cells have perished, thus a small number of survivors may endure for months or even years. This persistence may sometimes be the result of cell turnover, with a few cells thriving while absorbing nutrients from nearby cells that lyse and die.

It is believed that a genetic response induced in hungry, stationary phase cells is the cause of the bacterial culture phenomena known as viable but not culturable (VBNC) cells. Some bacteria may survive by producing spores, whereas others can go dormant without altering their shape. When the right circumstances arise (such passing through a mammal), VNBC microorganisms begin to multiply again.

Microbes, commonly referred to as microorganisms, may be found inside of us as well as on the surfaces we touch and breathe. All microorganisms are too tiny to be seen without a microscope, as the name would imply. Microbes are very varied, regardless of size. Protists, fungus, and bacteria are examples of microbes. We use a variety of techniques to manage their development so that we may coexist peacefully with all of these bacteria.

On the one hand, we attempt to avoid a lot of hazardous bacteria. Think about the dangerous microorganisms Listeria monocytogenes and Salmonella enterica, which are responsible for foodborne diseases. To stop the development of microorganisms like these and make food safe to consume, we employ refrigeration. On the other hand, we purposefully grow certain bacteria because they serve valuable purposes. To make bread dough rise, for instance, bakers utilise warm temperatures to encourage the development of the yeast Saccharomyces cerevisiae. Some bacteria may be both harmful and good depending on the situation. For instance, Escherichia coli may make synthetic insulin that can save lives in an industrial environment but can also induce gastrointestinal disease when consumed. Understanding how germs grow is intriguing, and it helps humans create procedures for maintaining harmonious connections with them.Diverse organisms make up microbes. While many have distinctive qualities and skills, they all have a few things in common. The majority of microorganisms only have one or a few cells. A cell membrane encloses every microbiological cell. The membrane regulates the flow of substances into and out of the cell. This enables the cell to take in vital substances like nutrition while removing waste. Some microorganisms also have a cell wall protecting them. The cell's interior parts are contained by the wall, which serves as a structure. Each cell has the DNA that codes for its genome inside of it. The cell's other components carry out vital metabolic tasks that keep life alive.

Features of a Microbial Cell, Figure 2. This representation of a bacterial cell highlights the DNA, cell membrane, and other critical internal components of a microbial cell. This cell has flagella and a cell wall (an appendage some bacteria use for movement).Instead of a rise in cell size, microbial growth refers to an increase in the number of cells. Numerous microorganisms are unicellular, or made out of only one cell, such as Escherichia coli, Salmonella enterica, and Listeria monocytogenes. Any unicellular microbe's size is limited by the ability of the cell's vital components to ensure its survival. For instance, when cells grow too big, the integrity of the cell wall is compromised. Cells may split or create new cells from the parent cell to allow growth despite the limitations on cell size. As a result, even if the population as a whole is steady in size, the population is growing.

Most often, during one growth cycle, a single-cellular microorganism splits into two identical new cells. In addition to producing enough material to create the membrane, wall, and molecular machinery for two cells, the original cell, also known as the parent cell, copies its DNA. To fit these extra ingredients, the parent cell enlarges only a little. The parent cell then starts to constrict in the centre, and a fresh section of cell wall is assembled there. This procedure is repeated until the parent cell has divided into two fully developed cells. The cells that arise are known as the daughter cells. Cell replication, also known as cell division, is the process of creating two identical daughter cells. This kind of replication quickly multiplies the number of cells since each daughter cell starts the cycle again by taking on the role of the parent cell. For bacteria with varying geometries, cell division might appear somewhat differently (Figure 3b), but the fundamental ideas are the same.

One cell continuously generates two brand-new cells as long as the circumstances are right. Every cycle, the population's cell count doubles. We call this exponential growth. E. coli has a short division cycle that may last as little as 20 minutes, depending on the circumstances. This quick division causes the population to grow quickly (Figure 3c). The population eventually reaches a size where we can see the effects, like the development of a physical building. This might be a "colony" of bacteria on solid growth medium in a research facility. Plaque on your teeth may be a sign of this. When circumstances are ideal for E. coli, a visible structure may be formed in only eight hours, when it normally takes over a million germs to do so.

Conclusion

The rise in research that explicitly examined quorum sensing and biofilms over the last several years is a strong indication of microbiologists' growing interest in natural microbial life. According to Shapiro, bacteria should be thought of as "sensitive, communicative, and decisive animals assimilating information from their environment and from their neighbours in order to carry out the complicated tasks of reproduction and survival in organised multicellular communities". The implications of "multicellular microorganisms" for microbiological study have not yet been fully understood, and much more work is required in order to at least somewhat comprehend this viewpoint.

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CHAPTER 15

ROLE OF FUNGI IN INDUSTRIAL MICROBIOLOGY

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Abstract:

In order to solve pressing global concerns, fungi are crucial. Through more effective use of natural resources, the utilisation of fungal processes and products may promote sustainability. Numerous commercial fermentation processes including the manufacture of polysaccharides, vitamins, pigments, lipids, glycolipids, and polyhydric alcohols all include the employment of fungi. They are employed as biofertilizers, a food source due to their high protein content, and they have antibacterial properties. In addition to helping plants develop faster and preventing illness, fungi are quite important for producing mycoproteins. In the study, the significance of fungus in the petroleum, agro-allied, agricultural, and pharmaceutical sectors is examined.

Keywords:

Agro-allied, Biofertilizers,Cosmetics, Fungi, Mushroom, Mycoproteins.

Introduction

Fungi are eukaryotic creatures that typically reproduce sexually and asexually and have a distinctive stiff cell wall made of chitin, cellulose, or both. They use a variety of substrates, from basic sugars to complex polysaccharides, since they are osmotrophic chemoheterotrophs. The majority of fungi are aerobic, although some are facultative or obligate anaerobes. They create extracellular enzymes, such as cellulases, xylanases, and pectinases that allow insoluble materials to liberate their soluble components. There are hundreds of types of fungi, and they are very important to man's economy. In actuality, fungus and humans have a close relationship. There is seldom a day that goes by when we are not affected negatively or positively by these creatures. The Latin term fungus is immediately translated into the English word "fungus" (mushroom). The term "mycology" is used to describe the scientific study of fungus. It is derived from the Greek words "mykes" (mushroom) and "logos" (discourse). Fungi are found all throughout the planet and may flourish in a variety of ecosystems, even the harshest ones. The term "mycobiota" refers to a collection of all the fungi found in a certain location or geographic area. Taxonomists have identified over 120,000 different species of fungus[1], [2].

They contribute significantly to agriculture by preserving soil fertility and causing crop and fruit illnesses, to medicine by producing antibiotics, too many other sectors, and as a significant source of food. The study of basic biological processes often makes use of fungus as research tools. Some of these fungi, especially mould and yeasts, have a detrimental impact on stored items including foodstuffs, textiles, leather, and rubber, plastic, lumber, and even glass by causing deterioration. They are also employed as supplies of antibiotics and crucial compounds for industry (such as alcohols, acetone, and enzymes), in addition to their function in

fermentation processes (e.g., the production of alcoholic beverages, vinegar, cheese and bread dough). Fungi are the foundation of several significant enterprises. The use of fungal enzymes in place of chemical processes has allowed industries including textile, leather, paper, and pulp to switch from chemical to biological processing, greatly reducing their environmental effect. Using enzymes[3], [4].

What we can produce from biological raw materials has substantially improved in the food and feed industries, including animal feed, baking, brewing, wine, and juice. By washing clothes thoroughly even at low temperatures, microbial enzymes added to detergents have dramatically decreased CO_2 emissions. The biochemical abilities of certain fungi have been successfully used in a variety of industrial applications. Major worldwide concerns are addressed in part thanks to fungi. Utilizing fungal processes and products may promote sustainability by making better use of available resources. Applications vary from converting biowaste for value-added goods to using biomass from renewable plant sources as a replacement for products dependent on oil, such as biochemicals, polymers, fertiliser, and gasoline.

When combined with seed, a fungus called an inoculum may help plants develop more robustly by improving their intake of nutrients and water. This robustness is crucial for preserving food production in the face of climate change. The synthesis of dietary components with prebiotic effects for a healthy human gut flora and thus increased resistance to lifestyle illnesses may be facilitated by fungi. Similar to this, using fungus may be a quick way to produce better animal feed and use less antibiotics, such as in the production of meat, which is now one of the main sources of bacteria that are multidrug resistant. One of nature's most fruitful areas for discovering novel medication candidates and antimicrobials is fungi. Last but not least, fungi offer intriguing promise as a fresh approach to producing biological treatments and a variety of novel bio-based goods with added value. Therefore, the research looks at how fungi may be used in the pharmaceutical, agro-allied, and petroleum sectors.

Literature Review

Wang *et al.* highlighted that the incorporation of the CAS9 gene into the genome, transient production of Cas9/sgRNA, the AMA1-based plasmid strategy, and the Cas9 RNP method are only a few of the CRISPR/Cas9 tools and approaches that have been created for various filamentous fungi and are discussed in this study. The many CRISPR/Cas9 applications that have been used in filamentous fungus are examined, with a focus on precise genome modification by gene tagging and change in gene regulation, as well as gene disruption/deletion and genome modification. A CRISPR/Cas9 system for filamentous fungus may face difficulties that are highlighted, including the nuclear localization sequence for the CAS9 gene, possible off-target effects, and very effective transformation techniques. Overcoming these challenges could help this technology be used more widely. CRISPR/Cas9 systems have the potential to be used in the future to several molecular features of filamentous fungus since they are an easy-to-use, affordable, and effective technique[5].

Devi *et al.* investigated that PGP fungi aid in promoting plant development and relieving various abiotic stressors in difficult settings in addition to inducing a plant defence response against

infections. Different genera of the phyla Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota have reported PGP fungus. Fungi also have a lot of potential uses in the food and medicinal sectors, among others. Fungi and fungal compounds are utilised in medicinal applications to treat human and animal illnesses. Fungi are employed in the agricultural sectors to make plants healthy and resistant to infections, as well as in the fermentation industries to produce alcoholic beverages, cheeses, bread, kefir, yoghurt, and a variety of other culinary preparations. The variety of helpful fungi from various ecosystems and their biotechnological uses in promoting plant development and soil health are the topics of the current review[6].

Selim *et al.* studied the Growing interest has been shown in endophytic fungi, which are present asymptomatically in the interior tissues of all higher plants and represent potential sources of physiologically active substances. The biology of endophytic fungi, including their isolation, variety, and biological activity in maintaining the environment and agriculture, are the main topics of this paper. Additionally, it takes into account their medical uses, particularly in the creation of anticancer, antibacterial, antioxidant, and antiviral substances. One of the most inventive families of secondary metabolite makers that play significant biological functions in supporting human existence are endophytic fungus. They might be sources of innovative natural products that could be used in the pharmaceutical sector, in agriculture, or in environmental applications[7].China has established a position in the global market while the World Trade Organization membership process drags on. China now holds the top spot in the global market for the amount of agricultural and ancillary goods traded, particularly when it comes to the growth of international commerce in Southeast Asia, Europe, and Africa. China is becoming a global leader in the developing agricultural sector as the growth velocity of the edible fungus business increases.

Zhang *et al.* carried out a study on a distinct, specialised trophic subgroup of fungus known as entomopathogens is made up mostly of members of the order Hypocreales (class Sordariomycetes, phylum Ascomycota). The genomes of these Hypocrealean Entomopathogenic Fungi (HEF), which generate a wide range of secondary metabolites (SMs), score highly in terms of the number of expected, distinctive SM biosynthetic gene clusters. By modifying different interactions between the producer fungus and its insect host, SMs from HEF play a variety of functions in insect pathogenicity as virulence factors. Additionally, these SMs facilitate intra- and interspecies communication, protect the corpse of the prey from opportunistic microbial invaders, and lessen abiotic and biotic stressors. These SMs offer lead compounds for the development of chemical pesticides for crop protection and support the function of HEF as commercial biopesticides in the context of integrated pest management systems. By enabling the contemporary pharmaceutical industry to repurpose some of these compounds as life-saving human pharmaceuticals, these bioactive SMs also support the widespread use of certain HEF as nutraceuticals and conventional treatments. Here, we review recent data on the functions of these metabolites in fungal virulence while also surveying the structures and biological activities of SMs reported from HEF[8].

Numerous fungus communities, sometimes referred to as manglicolous fungi, live in mangroves. They may be divided into saprophytic, parasitic, and symbiotic fungi and consist mostly of marine fungus and a small group of terrestrial fungi. By producing a range of extracellular degradative enzymes as cellulase, xylanase, pectinase, and amylase, fungi in mangrove environments play a significant ecological role in the breakdown of organic materials. These enzymes may be extracted from mangrove fungus and used in a variety of biotechnological processes.

Thatoi *et al.* investigated that in particular, mangrove endophytic fungi generate a number of bioactive compounds that are employed in the pharmaceutical and nutraceutical sectors to make antibacterial, anticancer, antioxidant, antidiabetic, and other therapeutic agents. Additionally, certain mangrove fungus contribute to the creation of biopesticides that are helpful in the management of plant diseases, while others create microbial lipids that have the potential to be utilised as a feedstock for the generation of biodiesel. Mangrove fungus are not well explored despite having a significant ecological significance and offering several biotechnology opportunities. The material in the current study covers the ecological significance of mangrove fungus, their variety, and their biotechnological potential as sources of new medications, enzymes, biodiesel, biopesticides, and other products[9].

Wakai *et al.* studied on the medical and food sectors may use filamentous fungus because they have a variety of uses, such as the synthesis of protein, organic acid fermentation, and secondary metabolism, among others. Their further potential as host microorganisms for bioproduction was discovered by previous genomic investigations of a number of filamentous fungus. Based on optimum design combined with computer science, modifications to transformation and metabolism may be made using recent developments in molecular genetics, marker recycling, and genome editing. In this review, we summarise the existing uses of filamentous fungus and discuss contemporary molecular genetic technologies that may be used to increase the contribution of these microorganisms to bioproduction. The current analysis clarified whether filamentous fungus may someday serve as hosts for other microorganisms in the bioproduction industry[10].

Discussion

Filamentous fungus are abundant in many different environmental niches and are crucial for industry, medicinal research, and plant and animal health. The function of genes and their control must be understood by manipulation of the genome and coding sequences, however in certain filamentous fungus, conventional genetic techniques are either ineffective or nonfunctional. The initial framework for adapting this gene editing technology for filamentous fungi has been made possible by the quick development and widespread use of CRISPR/Cas9 technology for a variety of model and non-model organisms. Both beneficial and harmful fungi play a part in our everyday lives. They are both our adversary and our buddy.Indirectly or directly, fungi are helpful to people. Fungi are employed in agriculture, food production, the pharmaceutical sector, other industries, and food as well as medicine. Among the beneficial activities are:

Making Medicine: Several species of fungus are utilised in the manufacture of numerous medications. The most significant species are Aspergillus proliferous, Claviceps purpurea, Saccharomyces cerevisiae, and Penicillium notatum.

Antibiotics are certain microorganisms' metabolic byproducts that are active against other microorganisms. Penicillin, a miracle medicine from Penicillium notatum with the Fusidium coccineum medication Fusidin (Fusidic acid).

Vitamins are the micronutrients necessary for the development of all living things. Saccharomyces cerevisiae, Eremothemium ashbyii, and Vitamin B-12, respectively, are present in these organisms.

Steroid: Steroid is used to treat rheumatoid arthritis, allergies, and a few other conditions. Numerous fungi are capable of producing a variety of steroids. *Aspergillus niger* ferments plant glycosides to create steroid-like cortisol.

Alkaloid: The sclerotium of Claviceps purpurea, which causes rye's ergot illness, produces and accumulates a number of alkaloids. Ergo-metrine and its semi-synthetic analogue, among other alkaloids, logues such as methyl ergometrine and methyl ergometrine maleate show noticeable uterine activity; they prevent maternal haemorrhage during childbirth while having an adverse effect of raising blood pressure, sure and reduced milk production

Foods

Humans have been eating fungi for a very long time. Some fungi are employed in food processing while others have been consumed directly as food:Due to their high protein content (21-30% on dry weight), excellent quantity of lysine, an amino acid; minerals like Na, Ca, K and P; vitamins like B, C, D and K; and very small amount of fat, the fruit bodies of various fungi, such mushrooms and truffles, are eaten as food.

These are suggested as the best meals for diabetics and those with heart disease. Commercially, the aforementioned fungus may be artificially cultivated. Recent years have seen a significant increase in mushroom production, which has benefited various East Asian nations' economies.

A variety of fungi are utilised in the manufacturing of organic acids, bread, cheese, alcohol, and enzymes.

(a) Alcohol Production: The brewing business is built on the alcoholic fermentation of fungus. Alcohol is produced by microorganisms like yeast using the enzyme zymase.Saccharomyces ellipsoideus produces wines from grapes or other fruits with an alcohol content of around 14%. *Saccharomyces cerevisiae* uses barley malt to produce beer that has 3-8% alcohol.

(b) Bread and Cake Production: CO2, which is generated as bubbles during alcoholic fermentation by yeast, is employed in the baking industry to give breads and cakes a spongy look.

(c) Making Cheese: By hydrolyzing lipids, some Penicillium species (P. roquiforti and P. camemberti) are employed to make Roquefort and Camembert cheese as well as to give cheese a distinct flavour.

(d) Production of enzymes and organic acids:

The commercial manufacturing of enzymes and other organic acids uses a variety of fungus.Here is a list of various fungus, their generated enzymes and/or acids, and their applications:

Fertility of Soil

The combined activity of several types of fungus results in the decomposition of trash and wood, which occurs mostly in forests. The structural polymers like cellulose, hemicellulose, lipid, protein, starch, etc. may be broken down by fungi like Fusarium, Chaetomium, Chitridium, Penicillium, Aspergillus, etc.

Fungi aid to increase minerals and other substances by decomposing organic matter, which increases the soil's fertility.

Plant Nutrition

A number of fungi, including Rhizoctonia, Tricholoma, Boletus, Phallus, Amanita, and others, create a mycorrhizal association with the roots of higher plants. The fungus partner provides the water and minerals, and in exchange, they consume the plant's nutrients.As Insecticide: Various sorts of insects are controlled by using fungi like Cordyceps melonthae as insecticides.In biological research, fungi such as yeast, neurospora, and others have been utilised in cytological and genetic investigations. Researchers have employed Physarum polysephalum to explore DNA synthesis.Test Organism: Even when the compounds are present in very small amounts in the substrate, several strains of *Aspergillus niger* have been utilised to detect trace metals including Zn, Cu, and Mo. These substances give the conidia a specific colour when ingested by the fungus. In a similar manner, Vitamin B complex has been found using Neurospora crassa.

Hormone production in plants Soil fungus Gibberella fujikuroi uses certain fungi to make plant hormones like gibberellin.

Biological prevention

Trichoderma sp., for example, shown that it is parasitic on several soil-borne and foliar diseases by its antagonistic action. In sustainable disease management systems, Trichoderma sp. is utilised to control plant illnesse a naturally occurring fungus called Beauveria bassiana has been studied for its ability to manage soil-borne insects, such as the European beetle.

Fungi's Negative Activities: Fungi may negatively impact humans in a number of ways, either directly or indirectly. They might result in food deterioration, infections in humans, animals, and plants, among other things.

1. Fungi are the primary cause of a number of minor and serious plant diseases. Some of these also contribute to starvation in various regions of the globe. Phytophthora infestans, which causes late blight of potato diseases, Pythium debaryanum, which causes damping of seed

diseases, White rust caused by the family Albuginaceae, the family Peronosporaceae, which causes downey mildew, etc.

2. Food and fruit decay as a result of storage fungus.

Poor crop and fruit storage promotes the development of fungi that cause substantial economic losses, such as green rot on fruit caused by Penicillium sp., black rot in fruit caused by Aspergillus sp., and green rot in grains caused by Aspergillus flavus, etc.

3. Diseases Caused by Fungi in Humans and Animals:

Some fungi, such as the Malassezia species, and dermatophytes, which may utilise keratin as a food source and have a special enzymatic capability [keratinase] by Trichophyton rubrum, etc., parasitize people and animals and cause diseases of the skin, hair, or nails.

Fungi, such as the aquatic fungus Saprolegnia parasitica, exist as parasites on fish eggs and gills in mammals. Additionally, Achlya species seriously harm fish.

4. Creation of fungi-produced poisons

Mycotoxins are harmful secondary metabolites that certain fungi may create, and they play a part in the spread of some illnesses to both people and other animals.

Mycotoxins, such as patulin, aflatoxin, ergot alkaloids, and ochratoxin, may cause short-term immunological deficiencies, liver and kidney fibrosis, and cancer, as well as long-term impacts like these.

5. Hallucinogenic Substance: The well-known hallucinogenic substance, LSD (d-lysergic acid diethylamide), is made from the sclerotia of Claviceps purpurea, the rye ergot disease causative agent. Psilocin and Psilocybin, which have psychedelic characteristics, are produced by other fungi, such as Psilocybe mexicana. Hallucinogenic drugs have the potential to kill brain cells and alter human experience.

6. Clothes damage:

Fungi may develop on damp clothing and footwear, harming them. Natural fibre clothing, including that made of cotton, linen, rayon, wool, and silk, is more prone to microbial deterioration than clothing made of synthetic materials. Enzymes produced by mould on clothing break down cellulose or protein into chemicals that the mould, such *Aspergillus niger*, uses as food.

6. Wood and paper damage:

The primary microbes degrading paper-based collections globally are filamentous fungus from the Ascomycota phylum responsible for the formation of various colour patches on paper that have a biological origin, such as the genera Aspergillus, Penicillium, and Chaetomium.

Damage to building materials

A black mould called Stachybotrys chartarum produces conidia in the form of slime heads. Although it may sometimes be discovered in grain and soil, the mould is more often identified in cellulose-rich construction materials taken from wet or water-damaged structures. It is linked to wet gypsum material and wallpaper and needs a high moisture level to thrive.

White Biotechnology is a group of scientific methods and tools used to increase the effectiveness and environmental impact of contemporary industrial production. The foundation of industrial biotechnology is microbial technology. The term "microbial technology" describes the process of using bacteria to produce a useful product or service.

The fungus have been studied over the last 20 years to examine different biological processes. The fungi are the most suitable for use as test organisms since they have a rapid rate of growth and a brief life cycle. For genetic studies and other biological processes, fungi provide excellent study material. In contrast to Physarum polycephalum, which is utilised to research the mitotic cycle, morphogenesis, and DNA synthesis processes, the genus Neurospora has developed into highly useful material for genetic studies. Neurospora crassa is widely used to determine the amount and presence of vitamin B in a given sample. Similar to this, *Aspergillus niger* is used to identify trace metals like zinc, nickel, and copper even when they are present in very small amounts. There are several reasons why fungi are used in many sectors.

The bioremediation of oil-polluted settings prefers fungal species due to their ease of transportation, genetic engineering, and scaling-up to create necessary amounts, as is common in yeast and Penicillium strains in the fermentation sector. The surface area accessible for petroleum hydrocarbon biodegradation is increased when fungal extensive mycelia penetrate insoluble materials like oil. Given their propensity for aerobic development, fungi are able to flourish in environments where bacteria could be constrained, such as those with low pH and inadequate food levels. When a combination of bacteria, moulds, and yeasts is used, the amount of synthetic petroleum mixture that is decomposed increases by twofold. In order to breakdown hydrocarbons, fungi have developed unique features throughout time.

These unique characteristics include I Petroleum-degrading microorganisms have special traits of efficient hydrocarbon up-take, which entails special receptor sites for binding hydrocarbons and/or the production of distinct chemical substances that aid in the emulsification and transport of hydrocarbons into the cell; They have group-specific oxygenases, which are enzymes that introduce molecular oxygen into the hydrocarbon and with relatively few side effects. Two orders, the Mucorales and the Monilales, were primarily home to fungi with the capacity to metabolise hydrocarbons.

The world has to be made aware of the vital role that fungus and mycology play in sustainable development, which makes better and more sustainable use of resources like water, land, and biological materials. This is a chance to describe how fungi contribute to the bio-economy while profiling mycology. Support for mycology may be increased globally by raising understanding of and appreciation for the function of fungus.

Support will enable mycologists worldwide to raise more money for important fundamental research, draw more talent to our area of study, and improve the international mycology network.

Such advancements are necessary to utilise fungi to their full potential in the bio-economy. Even more ideas may be drawn from the kingdom of fungi. To prevent microbiological contamination of goods and equipment, the appropriate antimicrobial compounds are often utilised in a variety of sectors. The moment is ripe to spread awareness of the enormous significance of fungus and mycology for sustainable.

By describing the function performed by fungi in the different sectors and the bio-economy, we may profile mycology in this context. It is possible to increase support for mycology globally through raising understanding of and appreciation for the function of fungus. Additional talent will be drawn to the area of study as a result of support, which will also enable mycologists worldwide to raise more money for crucial fundamental research and fortify the network's international reach. Such advancements are necessary to fully use fungus in the pharmaceutical, agro-allied, and petroleum sectors. To address the global concerns of climate change, growing demand on natural resources, and the rising burden of lifestyle illnesses, the potential of fungal organisms for a more sustainable world must be unlocked.

To preserve this variety and realise the full potential of the fungal kingdom for use in the future around the globe, mycology has to be stimulated on a global scale and collaborate more effectively. The field of mycology must advance to the point where it can draw talent for the next generation of mycological researchers and for developing the expertise required for the transition to a new, more sustainable bioeconomy. Expanding the exploitation of fungal biodiversity for additional value-added applications requires a better knowledge of the fungal kingdom, phylogeny, and phylogenomics as a foundation. It is important to develop mycological platforms and mycological know-how and capabilities throughout the globe.

The distinguishing traits that set fungi apart from other living forms include the following. They develop by lateral branching, apical development, and heterotrophic feeding in a filamentous branching pattern [see also - Mushroom Production]. The germination of their dormant structure or spore starts their life cycle. The next stage is vegetative development, during which the biomass rises as the substrate is used and new cells (hyphae) emerge. Mycelium is the term for the porous three-dimensional network that the fungus hyphae create. When resources are scarce at a certain point in the life cycle, sporulation may take place, producing morphologically different structures that may separate from the mycelium. Not all filamentous fungus generate spores, but when they do, it's typically because the nutritional requirements aren't right. When those conditions are present, spores will be formed. The majority of filamentous fungi are discovered developing in an unsatisfactory stage where no sexual cycle is known, whereas some are found in a perfect stage (a sexual cycle), which is similarly reliant on the proper nutritional circumstances. The filamentous fungus are crucial to the biosphere because they break down organic matter, which replenishes the biosphere with nutrients including carbon, nitrogen, phosphorus, and minerals. The filamentous fungi are used in a range of operations, including the production of beneficial metabolites, the food industry, and other industries.

Conclusion

The world has to be made aware of the vital role that fungus and mycology play in sustainable development, which makes better and more sustainable use of resources like water, land, and biological materials. This is a chance to describe how fungi contribute to the bio-economy while profiling mycology. Support for mycology may be increased globally by raising understanding of and appreciation for the function of fungus. Support will enable mycologists worldwide to raise more money for important fundamental research, draw more talent to our area of study, and improve the international mycology network. Such advancements are necessary to utilise fungi to their full potential in the bio-economy. Even more ideas may be drawn from the kingdom of fungi.

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CHAPTER 16

ANALYSIS ON THE FUNGI IN PETROLEUM

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Abstract:

The petroleum industry, also known as the oil industry or the oil patch, includes the global processes of exploration, extraction, refining, transporting (often by oil tankers and pipelines) and marketing of petroleum products. In the future, when complex organic substrates are to be broken down to complex products to solve challenging problems, fungal and microbial consortia could provide a short-cut to a solution. Onygena species, Non-pathogenic species of Onygenales, are specialized in breaking down the keratin found in feather, hooves, and horn. The keratin is composed of proteins, bound in a non-bio-accessible form.

Keywords:

Biodegradation, Crude Oil, Fossil fuel, *Onygena corvine,* Petroleum.

Introduction

Among the large number of different proteases produced by *Onygena corvina*, we discovered that just three enzymes, belonging to two types of protease families, are sufficient to breakdown both feather and pig bristles. The decomposition of substances by biologicalsystems is knownas biodegradation. The conversion of original substances into new products which, in most cases, do not have the same properties as the first product is known as primary biodegradation while the complete conversion of original substance into carbon (IV) oxide, water, and new microbial biomass through simple mineralization is called ultimate biodegradation. Bioremediation is the environmental application of biotechnology and is defined as the use of biological agents or organisms to breakdown pollutants in the environment by the process of biodegradation[1], [2].

Oil waste causes a considerable and accelerating biological damage of the earth's active surface. There are several waste pits around Grabownica Plant that date from 1925 to 1950 and are used to store drill debris. The wastes are the result of percussive drilling operations and are polluted with petroleum hydrocarbons.One of the most popular study topics nowadays is the biotechnological acceleration of petroleum hydrocarbon biodegradation using efficient bacteria cultures (derived from highly polluted locations). Technically speaking, biological approaches have been used on a scale of 1 to 8 due to their great efficacy and comparatively cheap cost[3]–[5].

However, the weathered drill wastes kept in pits provide a challenge to bioremediation efforts since they contain a significant quantity of petroleum pollutants (up to 200,000 mg TPH/kg dry

mass). Because of this, it has been said that the technical idea of waste purification, which is based on stage application of subsequent purification procedures, would permit a progressive decline in petroleum contamination levels. As a result, consecutive procedures will be used to purify a contaminated region more thoroughly.The following procedures are used in laboratory research on the ex-situ biodegradation of petroleum pollutants from weathered drill waste:

- basic/initial bioremediation stimulated by bioventilation (providing oxygen through aeration) and waste environment enrichment with biogenic ingredients in doses determined on the basis of laboratory tests, which promoted the growth of indigenous microflora in conditions of temperature and humidity similar to those found in the field.

- bioaugmentation with inoculation of originally cleaned waste carried out by biopreparation using identified, chosen, and multiplied native microorganisms.

The aforementioned studies allowed for the optimization of the various steps of a sophisticated waste-purification technology, as well as the efficiency of the utilised biopreparations and the discovery of a function for fungus in the modification of a biopreparation based on native bacteria.

The multi-constituent structure of kerosene is intricate. In order to degrade it, a variety of microbe cultures with a sophisticated enzymatic system must be used. To prevent the hostile impact of the native soil microflora on foreign microorganism cultures that are not suited to a particular environment, it is advised to utilise bacterial consortia in the form of a biopreparation based on these microorganisms.

Numerous publications on the study of biodegradation using fungus may be found in international literature. Most filamentous fungi are unable to completely mineralize aromatic hydrocarbons; instead, they can only change them into indirectly harmful by-products that are more bacterially degradable.

Cladosporium and *Aspergillus* are filamentous fungus involved in aliphatic hydrocarbon biodegradation, while *Cunninghamella, Penicillinum, Fusarium,* and *Aspergillus* are involved in aromatic hydrocarbon breakdown. It is a means of cleaning-up contaminated environments by exploiting the diverse biodegradation abilities of microorganisms to convert contaminant compound of concern to harmless products by mineralization which in turns lead to the generation of carbon (IV) oxide and water or by conversion into microbial biomass (cell materials). Different strains of soil fungi including *Graphium, Fusarium, Penicillium, Paecilomyces, Acremoniun, Mortierella, Giiocladium, Trichoderma* and *Sphaeropsidales* are found to be important groups capable of utilizing petroleum hydrocarbons.

Petroleum hydrocarbons degradation by different species of filamentous fungi Fungi Compound *Trichoderma harzianum Naphthalene Aspergillus spp. Crude oil Cunninghamella elegans Phenanthrene Aspergillus niger n-hexadecane Cunninghamella elegans Pyrene Aspergillus ochraceus Benzo [a] pyrene Penicillium spp.*

Biodegradation of pesticides/ toxic chemicals and petroleum: White Rot fungi have the potential role in degradation of toxic pesticides like DDT, PCB and Lindane. In addition

to this, it can degrade certain toxic chemicals like dioxin, benzopyrene, cyanides, azides, CCl4 and Pentachlorophenol (PCP). Aspergillus, Penicillium, Paecilomyces and Fusarium has found to be involved in petroleum degradation at 30 oC in contaminated soil environment.

Chemical dispersants simply divide up oil into tiny pieces in an effort to make it easier for natural microbes to break it down. They do precisely what their name says. However, this approach has two drawbacks:

First, research done in collaboration by Dr. Sara Kleindienst and her colleagues in 2015 revealed that chemical dispersants may actually inhibit natural bacteria's capacity to break down oil, possibly causing an increase in the amount of oil present in the marine environment.

Second, the dispersants themselves may be more harmful than beneficial. These dispersants include toxic substances that may be exceedingly hazardous to marine species, notably disrupting fish growth, according to an assessment of sustainable ways for managing oil spills.

The ideal option would be to use a naturally existing animal that can degrade the oil in place of these chemicals. What animal, however, could survive on very poisonous oil? Not really animals, but new study has uncovered marine fungal organisms that can efficiently degrade environmental oil pollutants. Fungi are a wholly distinct category of creatures that are neither plants nor animals. When we think about fungus, we often picture mushrooms and scenes right out of Alice in Wonderland in our thoughts. Marine fungus are quite unique. These yeast-like creatures are seldom visible to the unaided eye, and when they are, they often resemble the unappealing fluffy material that could be growing on an old casserole in the back of your refrigerator (ew).

Many marine fungus species are natural decomposers, which means they can chemically break down organic materials like dead creatures or wood into their constituent parts, from which they may extract energy, carbon, and nutrients. Polycyclic Aromatic Hydrocarbons, or PAHs for short, are one of the most hazardous components of oil and have been successfully degraded by three coastal species of marine fungus from the Gulf of Mexico, according to research lead by Dr. Rachel Simister in the Department of Chemistry at Haverford College.

By using certain enzymes to break down the oil and absorb and utilise the carbon from it, these fungi are able to "feed" off of this deadly chemical. This method of hydrocarbon elimination provides a natural remedy that may be able to restore oil-contaminated ecosystems to their former splendour while also greatly reducing the toxicity of oil.

It's improbable that we will ever completely get rid of oil since it remains one of the most valuable commodities in the planet. This implies that there is always a chance of spillage. A fungus could be able to eliminate every rainbow-colored greasy sheen seen on the ocean's surface in the future, thanks to breakthroughs in science and cleaner, more natural techniques of cleanup.

Literature Review

Benguenab *et al.* attempted to identify telluric filamentous fungus that are effective in reducing pollution caused by petroleum hydrocarbons. Used engine (UE) oil-contaminated soil yielded six different fungi strains. The use of 2.6-dichlorophenol indophenol allowed for the screening of fungi for the degradation of crude oil, diesel, and UE oil (DCPIP). Aspergillus ustus HM3.aaa and Purpureocillium lilacinum HM4.aaa were the two isolates that were chosen, recognised, and registered at NCBI. The radial growth diameter assay was used to determine if fungi were tolerant to various petroleum oil concentrations. Gravimetric analysis was used to determine the hydrocarbon elimination %. 10 days were used as the study period to examine the kinetics of crude oil deterioration. In solid medium with a high concentration of petroleum oils, A.ustus was the fungus that could tolerate it best. Based on quantitative research, it was determined that crude oil was the kind of oil that both isolates degraded the most, *P. lilacinium* and *A. ustus* eliminated 44.55% and 30.43%, respectively, of the crude oil. The two fungi were able to break down UE oil and diesel by a respective margin of 14.39 and 16.00% and 27.66 and 21.27%. These fungi produced much more biomass in liquid media containing all petroleum oils than the controls did. As well, for *P. lilacinium* and *A. ustus*, the crude oil removal rate constant (K) and half-lives (t1/2) were 0.02 day-1, 34.66 day, and 0.015 day-1, 46.21 day, respectively. Scale-up investigations are needed before using the chosen fungus in soil bioremediation, even if they seem promising for degrading petroleum oils.

Mohammadian *et al.* identified the species variety of fungus that live in the soils of oil fields in southern Iran that have been polluted with petroleum. On atmospheres of phenolic hydrocarbons and crude oil as substrate, enrichment was one of two separate approaches employed for fungal isolation. For taxonomic identification, a phylogenetic analysis of the ribosomal DNA's internal transcribed spacer was employed, along with supplementary data from the -tubulin gene for certain species. Aspergillus, Alternaria, and Exophiala were the taxa from which isolated strains were most commonly found. The toluene substrate produced the most strains from the four hydrocarbon enrichments, and the crude oil substrate was the technique of isolation that was most effective. Herpotrichiellaceous fungi as well as other filamentous fungi were produced by enrichment on xylene and benzene[6].

In this study by Liu et al, a novel model of a bioremediation system for petroleum-contaminated soil was established. After 30 days of remediation, the system had a TPH degradation rate of 57.72 5.55%. The rehabilitation of petroleum-contaminated soil in this system used a combination of white-rot fungus and bacteria that break down petroleum. White-rot fungus generated a remediation material that could constantly release important enzymes under particular stimulation via solid-state fermentation (SSF), which could then be employed in the remediation after achieving the stable structure of a white-rot fungi carrier (after SSF). The white-rot fungal remediation substance was made to be applied within or on top of the soil, and it simultaneously broke down the bacteria and petroleum hydrocarbons in the soil. The white-rot fungi's enzyme secretions produced during the SSF and the fungi's contribution to the restoration of petroleum-contaminated soil were studied. Sand, straw, and biosurfactants were also added to help the bioremediation process and improve the collaborative remediation system for bacteria-

white-rot fungus. Additionally, orthogonal experiments were run to investigate the impact of other remediation-related parameters[7].

Sari *et al.* carried out a study looked at locally adapted fungus isolates from petroleum hydrocarbon-contaminated soil in Siak that have the ability to break down hydrocarbons. A crude oil-contaminated soil sample that was taken from an oil field near Siak, Riau, was used to identify native fungus. By cultivating the isolates in Bushnell-Haas broth containing crude oil (5% v/v) for 16 days, the efficiency of isolates on the breakdown of crude oil was examined. Indirect signs of crude oil breakdown by the fungus included a drop in pH, alteration in optical density, and quantity of CO2 produced. Gravimetric analysis was used to calculate the crude oil biodegradation percentage. The two colonies were chosen and designated as Penicillium sp. LBKURCC153 and Aspergillus sp. LBKURCC151, respectively. According to the findings, Aspergillus sp. LBKURCC151 obtained a greater degree of biodegradation (61%), after 16 days under the ideal circumstances, than Penicillium sp. LBKURCC153 (46%)[8].

Due to its effects on ecosystems and human health, pollution discharged by crude oil refineries is regarded as one of the most serious environmental issues. Because it is a low-cost, safe technique to improve a healthy environment, biological control is now an effective process for removing dangerous materials from the environment. Ibrahim *et al.*demonstrated that every kind of fungus was capable of decomposing crude oil to different degrees. From the severely petroleum oil-contaminated soil, a total of three genera and seven species have been found. Only three isolates—*Aspergillus flavus, Aspergillus niger,* and *Absidia corymbifera*—were capable of biodegrading oil, which changed the colour of Czapek's broth from deep blue to colourless as it was reduced from a higher concentration to below the detectable limit. However, fungal species isolated from contaminated soil may be used in the bioremediation of crude oil to remove petroleum hydrocarbon from polluted settings. Absidia corymbifera demonstrated the better crude oil biodegradation efficiency compared to other species[9].

Mohsenzade *et al.*, In order to identify petroleum-resistant plant species and rhizospheral fungi for use at bioremediation, a field research was carried out in a petroleum-contaminated site at the Kermanshah refinery (Iran). According to the findings, 2.2% of vegetated regions and 6.8% of non-vegetated areas have petroleum contamination respectively. Plant samples were obtained from petroleum-contaminated locations, and morphological traits were used to identify them. The findings showed that there were 21 species present in the rhizosphere of the plants growing in the contaminated locations; three of these species were shared by all of the plants, while the rest had distributions peculiar to their respective species within the plants. P. aviculare had the maximum number of rhizospheral fungus (11 species) in regions without pollution and nine species in areas with pollution. The plant seems to be the ideal option for phytoremediation. However, fungus in petroleum-polluted environments varied more than in unpolluted areas. A few species, particularly Fusarium species, showed higher resistance to petroleum pollution (10% v/v), and as a result they may be suitable for mycoremediation in highly polluted areas[10].

Discussion

Peroxidase enzyme of Penicillium crysosporium and Streptomyces spp. have potential biodegradable activities that degrade Amaranth dye, Orange G, heterocyclic dyes like, Azure B and Lip dye. The filamentous fungi are also having role in degradation of toxic hydrocarbons (Figure 1).

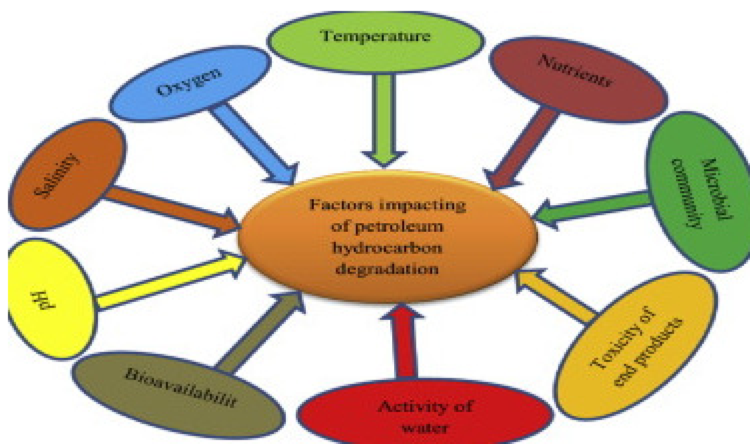

Figure 1: Illustrating the factors influencing the petroleum hydrocarbon degradation.

Fungi in hazardous waste remediation: Fungi help in remediation of explosive contaminated soil by its lignin degrading Enzymes. TNT, RDX, HMX are some of the potential explosives that contaminates soil and water. Other degradable nitro explosives by Pleurotus ostreatus are Nitrobenzene, 4-Nitrophenol, 4-Nitroaniline, 1-Methoxy 4 nitrobenzene, 2-Methoxy 4-nitro phenol and 1, 2, di Methoxy 4 nitrobenzene.

The worldwide catastrophe of petroleum pollution may be cleaned up using a variety of techniques, including bioremediation. Methods: In a field study, fugal strains were isolated from oil-contaminated areas of the Arak refinery in Iran, and their ability to grow in potato dextrose agar (PDA) media containing 0-10% v/v crude oil was assessed. The activity of three enzymes (Catalase, Peroxidase, and Phenol Oxidase) was also assessed in the fungal colonies. Finally, the ability of the fungi to bioremedi.Four fungi were chosen as the most resilient ones: Acromonium sp., Alternaria sp., Aspergillus terreus, and Penicillium sp. They were capable of expanding in the tested concentrations, with Alternaria sp. exhibiting the best growth potential in the medium containing petroleum. The results of the enzyme experiment indicated that the oil-contaminated medium had higher levels of enzymatic activity. Results of bioremediation revealed that the investigated fungus might lessen petroleum contamination. Aspergillus terreus, Penicillium sp., Alternaria sp., and Acromonium sp. all had the best effectiveness for removing petroleum in the ranges of 10%, 8%, 8%, and 2%, respectively. Conclusions: Fungi have a significant role in reducing petroleum pollution.

Biomineralization of Heavy Metals: The fungi have eminent role in the removal and recovery of heavy metals from wastewater and industrial effluents. Hg, Cu, Ni, Pb, Cd are extracted at pH 2-5 by myceliar beads of Penicillium. The fungi also play the following harmful roles: Destruction of timber: Several fungi such as Polyporus, Serpula lacrymans,

Fusarium negundi, Coniophora cerebella, Lentinus lapidens and Penicillium divaricatum cause destruction of valuable timbers by reducing the mechanical strength of the wood.

Destruction of textiles: Several fungi are able to grow on cotton and woolen textiles causing their destruction. These include species of Alternaria, Penicillum, Aspergillus, Mucor and Fusarium. spp. of Stachybotrys which causes destruction of cotton in storage houses. Chaetomium globosum is reported to cause greatest damage to textiles.

Destruction of Paper: Paper pulp wood is destroyed by the growth of Polyporus adustus, Polystictus hirsutus etc. several fungi such as species of Chaetomium, Aspergillus, Stachybotrys, Alternaria, Fusarium, Dematium, Mucor and Cladosporium cause extensive damage to industrial materials including papers, books, newspapers and wood industry. Cellulose degradation by fungi: Heap of agricultural residues, forest residues deposited ample of celluloses in the soil. Only fungal cellulases are involved in degradation of deposited cellulose. Fusarium, Trichoderma, Penicillium derived cellulases are involved in degradation of celluloses. Degradation ofthese leads maximum bioenergy production. Other fungal enzymes are gluconase and glucosidase (cellobiase).

Bioconversion of lignin: White Rot fungi such as Coriolus versicolor, Polyporus ance and Brown Rot fungi like Poria monticola, Lenzitis trabea are used in depolymerization and degradation of lignin to low molecular weight Petroleum products. These fungi are also used in softening of wood in paper making industries.

One of the most important environmental issues today is soil degradation from petroleum. This kind of pollution reduces or completely eliminates soil fertility, alters the chemical makeup of soil and water cycles, reduces the aesthetic value of ecosystems, pollutes secondary groundwater and air sources, and hinders or completely eradicates soil organisms. The breakdown of soil organic matter, the production of humus, the cycling of nutrients, and the stimulation of plant development are all significantly influenced by soil microorganisms, mostly bacteria and fungus. Hydrocarbons have an indirect or direct impact on soil microorganisms after entering the soil. Petroleum use indirectly raises soil surface temperatures, alters the amount of organic matter in the soil, disrupts the oxygen and water cycles, and reduces the availability of nutrients. The composition and operation of soil microbial communities are therefore altered by these changes. Petroleum hydrocarbons have the potential to negatively impact soil community members directly by resulting in cell lysis, growth suppression, and non-specific membrane disruptions. The growth stimulation of microbial communities that can breakdown or tolerate hydrocarbons is another direct impact of petroleum injected into the soil. Although these organisms are common in soils, it has been shown that the physicochemical properties of the soil and the soil's genesis affect both their quantity and patterns of breakdown.

Numerous hydrocarbons with various C chain lengths (from 1 to higher than 30), structures, and saturation levels may be found in crude oil. These hydrocarbons are often divided into four categories: aliphatics, aromatics, asphaltenes, and resins. Petroleum hydrocarbons are less biodegradable in the following order: n-alkanes are followed by branched alkanes, low-weight aromatics, cyclic alkanes, high-weight aromatics, and polycyclic aromatic compounds.

Asphaltenes and resins are thought to be weakly biodegradable hydrocarbons. Hydrocarbons are the only carbon source that microorganisms consume, or they modify them to make them less harmful. Although bacteria may use hydrocarbons anaerobically, aerobic conditions are where the major routes of microbial hydrocarbon breakdown take place. Fascinatingly, it has been shown that bacteria can break down aliphatic and aromatic chemicals, and that fungi can likewise break down polycyclic aromatics. Furthermore, although fungi degrade lignin and cellulose using non-specific enzyme complexes such cytochrome P450, lignin peroxidase, manganese peroxidase, and laccase, bacteria employ specialised metabolic pathways like those of alkane monooxygenase and dioxygenase to break down hydrocarbons.

Microbial community successions in polluted soils are influenced by the original soil microbial composition, as well as by the soil's physicochemical qualities and oil content. This selection of populations that can degrade hydrocarbons happens following petroleum contamination. The soil's organic carbon concentration, pH, moisture and oxygen content, climate, geographic location, soil genesis, and plants all have a significant impact on the original microbial makeup. The key variables influencing the sorption and desorption of hydrocarbons in soil particles, which in turn affects their toxicity and bioavailability, are particle size, clay concentration, and organic matter content.

At relatively low initial petroleum concentration (up to 5%), efficient microbial breakdown of petroleum hydrocarbons occurs in the soil; thus, the dynamics of microbial communities, notably those of bacteria, have been well researched in such situations. However, since they are thought to be destroyed or severely hindered owing to their low activity, the microbial communities of soils with high petroleum concentrations are less well understood. To comprehend the possibility of rebuilding soil ecosystems, for example, after a lengthy period of time or with further remediation techniques, knowledge of such communities is crucial. The dynamics of bacterial and fungal populations in petroleum-polluted soils are poorly understood, with an emphasis on the individual genes that enable the various metabolic pathways of hydrocarbon degradation. The similarities and differences of microbial community successions in various soils after oil disruption of the soil ecosystems are little documented. In the case of both self-restoration and bioremediation, the missing knowledge has both theoretical and practical relevance since bacteria and fungus are the primary agents that allow improvement of soil quality following petroleum contamination.

The quantity of bacterial catabolic genes, the architecture of fungal and bacterial communities, and the amount of hydrocarbons in soils were also examined. Our hypothesis was that the principal soil physicochemical properties would be less important than hydrocarbons in the successions taking place in the soil microbial communities at high petroleum concentrations. Furthermore, it was proposed that the existence of particular metabolic pathways for hydrocarbon breakdown and the lack of general metabolic pathways affect bacterial populations more profoundly than fungal communities.

Conclusion

Fungi are more efficient and effective in removing hydrocarbon pollutants from the environment, such as water and soil, according to our research, which analysed and discussed the biodegradation potentials of these organisms. The promise of fungus hasn't, however, been completely realised. Similar to this, there have been numerous improvements made in the bioremediation of hydrocarbons, such as the use of fungus-derived enzymes and genetically altered fungi to speed up and lower the cost of bioremediation. Nevertheless, these improvements are insufficient, and more work in this area is required. The use of omic technology, which combines genomics, transcriptomics, and proteomics, has increased the biodegradation of fungi. While transcriptomics can provide details about active pathways during hydrocarbon degradation, genomics provides the fungal organism with a blueprint and biochemical pathways. As a result, by using omics technology, it is possible to find novel pathways and functional genes that may code for the synthesis of additional biodegradative enzymes, hence increasing the capacity of fungi for the biodegradation of hydrocarbons.

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CHAPTER 17

STUDY ON FUNGI IN AGRO-BASED INDUSTRY

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Abstract:

Fungi are a class of eukaryotic organisms that are a source of food, organic acids, alcohol, and antibiotics, compounds that promote growth, enzymes, and amino acids. They consist of microscopic creatures like moulds, yeasts, and mushrooms. They eat plant or animal tissue that is either dead or alive. Because they are the main decomposers of materials in the ecological system, fungi vary greatly from other living species.

Keywords:

Agro-products, Fungi, Mycoprotein, Plants,VAM.

Introduction

Fungi are excellent decomposers of organic waste and most rapidly target cellulose, lignins, gums, and other organic complex materials. From acidic to alkaline soil responses, fungi may also operate in a variety of other soil reactions. Together, fungi and other organisms play a fundamental part in a variety of physiological processes, including mineral and water uptake, chemical alterations, stomatal movement, and the biosynthesis of auxins, lignans, and ethylene—compounds known as "biostimulants" that help plants recognise and adapt to environmental stresses like drought, salinity, heat, cold, and heavy metals.Even before their presence was widely understood, the microbe was employed in agricultural and industrial activities from the very dawn of civilization.

Traditional processors have been in use since the dawn of civilization for the production of fermented drinks, bread, and vinegar. Recent improvements in our knowledge of the genetics, physiology, and biochemistry of fungus have prompted the use of fungi in the creation of several agricultural and commercial goods that are significant economically. The distribution of the soil's fungi is influenced by all environmental conditions [1], [2].

In the soil, filamentous fungus mostly break down organic materials and aid in soil aggregation. In addition to this characteristic, bound species of Alternaria, Aspergillus, Cladosporium, Dematium, Gliocladium, Humicola, and Metarhizium produce substances that resemble organic molecules in soil and may thus be required for the preservation of soil organic matter. Chemical fertilisers and plant growth regulators have both been employed to boost agricultural output. The use of chemical fertilisers on agricultural plants has a severe impact on the environment and human health. Recent research has concentrated on finding alternate strategies to improve plant

yield and safeguard the soil. Although many plant-associated fungi are widely recognised for their ability to support plant development, the interaction between these microorganisms and plants is still unknown. Soil-borne bacteria may penetrate roots and establish their population in plants as endophytes[3], [4].

Microorganisms may synthesise phytohormones, dissolve insoluble phosphate, and simplify complicated chemical compounds. Biostimulants in Plant Science. It has also been shown that endophytic fungi provide plants a resistance to salt, drought, heat, and diseases. The capacity of the four endophytic fungi (GM-1, GM-2, GM-3, and GM-4) to enhance soybean plant development under salt stress conditions was examined. In comparison to controls, seeds prepared with endophytic fungal cultures had increased seed germination and plant growth rates. Gibberellins study of culture filtrate (CF), which revealed a vast variety and varied amounts of Gibberellic acids, confirmed the beneficial impact of fungi on plant development.

Rhizospheric fungus application is an efficient and sustainable way to improve plant development and manage a variety of plant diseases. For their ability to stimulate growth in sesame seedlings, three dominant fungi (PNF1, PNF2, and PNF3) isolated from the rhizospheric soil of peanut plants were tested. PNF2 substantially boosted the shoot length and fresh weight of seedlings across these isolates as compared to controls. PNF2 had a larger quantity of indole acetic acid than the other isolates, according to an analysis of the fungal culture filtrate.

At all developmental stages, fungal interactions with plants have an impact on their main and secondary metabolism. The principal process of photosynthesis and the source of energy for plants. Chlorophyll and carotenoids are examples of photosynthetic pigments that are connected to this efficiency. More so than in the controls, plants treated with fungus showed a rise in leaf chlorophyll.In low pH or slightly acidic soils, where soils often aren't disturbed, fungi predominate. Numerous different types of bacteria will start to degrade and transform the organic wastes into useable products as a result of fungi breaking down the organic leftovers. Approximately 90% of all plants have hyphae networks that symbiotically connect them to mycorrhizal fungus. The plant mostly receives phosphate and other minerals from the soil via mycorrhizae, including zinc and copper. The plant root serves as a source of nutrition for the fungus, including sugars. A mycorrhizae network is the term used to describe this cooperative interaction.

Although soil fungus may thrive in a broad variety of pH levels, they are more prevalent in acidic circumstances due to intense competition with bacteria at neutral pH levels. Most fungi like to thrive at the ideal soil moisture level and are aerobic in nature. These organisms have a very little impact on metabolic transformation when there is an excessive amount of moisture.The area near to the ground where various chemicals and organic chemistry processes take place is known as the rhizosphere. It is dominated by soil bacteria. Up to 10–30% of the soil rhizosphere is made up of soil fungus. Due to their capacity to create a broad range of extracellular enzymes, fungus can decompose many types of organic materials, consequently controlling the balance of nutrients and carbon to maintain healthy soil. This enables fungus to traverse soil gaps and provide nutrients to plants over relatively large distances. Fungal growth mostly occurs in soil, which is a component of all plant species' roots. Numerous bioactive

metabolites produced by fungi may enhance plant development. Fungi are also utilised as biofertilizers and provide plants with inorganic nutrients including ammonium, nitrate, and phosphate.

Rhizosphere microorganisms are capable of surviving in a variety of environmental circumstances and competing with other soil elements.Microorganisms have been used for agricultural and industrial purposes since the beginning of civilisation, even before their presence was fully recognised. Since the start of civilization, traditional processors including fermented beverages, bread, and vinegar have existed. Fungi are now used to produce a wide range of agricultural and industrial goods because to recent developments in our knowledge of their genetics, physiology, and biochemistry. All environmental factors have an impact on how the soil's fungi are distributed.

In the soil, filamentous fungi perform two key tasks: they break down organic materials and promote soil aggregation. In addition to this characteristic, bound species of Alternaria, Aspergillus, Cladosporium, Dematium, Gliocladium, Humicola, and Metarhizium create substances that are comparable to the organic compounds in soil and may thus be necessary for the preservation of soil organic matter. The introduction of artificial fertilisers and plant growth regulators has increased crop yield. The environment and human health are severely impacted by the use of chemical fertilisers on agricultural plants. The focus of recent research has been on developing other methods for boosting plant production while yet safeguarding the soil. Although soil-borne microorganisms may penetrate roots and establish their population in plants as endophytes and several plant-associated fungi are well recognised for their capacity to promote plant development, it is still unclear how these bacteria and plants interact.

Literature Review

Rajesh *et al.* studied the recent discoveries from structural and functional genomics methods point to the potential use of these bacteria as new research models for the processes underlying multi-player interactions, namely interactions between microbes, plants, and the environment. The historical development of Trichoderma spp., its mode of action against various biological agents, potential applications, and recent mass production techniques are summarised and thoroughly discussed in this work, along with the most recent developments and their use in agriculture and the sustainable environment[5].

Interest in finding more environmentally friendly approaches to fertilisation and pest management has grown in light of the negative health impacts associated with pollution caused by chemical fertilisers and pesticides. The term "fungal endophytes" (endobionts) refers to tiny fungi that live within plant tissues without likely creating disease symptoms. The growth promotion and plant tolerance to diverse biotic and abiotic stressors and diseases are significantly influenced by endophytic fungus. They also create several agrochemical bioactive metabolites, phytohormones, and antibacterial chemicals. Given their extensive range of activities that support plant growth, these endophytes have a great deal of potential to be used as a secure and affordable substitute for chemical pesticides and fertilisers[6].

De Lucca *et al.*Most fungus are saprophytic, which means they are not harmful to people, animals, or plants. Nevertheless, only a small number of fungal species are phytopathogenic, create toxins that are harmful to plants, animals, and people, and inflict sickness (such as infections and allergies) on humans. Members of the Aspergillus and Fusarium genera as well as other genera (such as Alternaria and Mucor) that make up the "emerging pathogen" category in humans are among these fungi. Both agricultural productivity and the health of healthy and immunocompromised people are often threatened by these fungus. When these very few fungi are combined, they may result in significant financial losses for agriculture, a loss of food for human consumption, and severe, often deadly infections in both people and animals. Given that they have to create substances to fend against fungus in their environment, plants may be a source of antifungal chemicals[7].

Rangel *et al.* investigated and highlighted that Increasing global food security, reversing rising cancer rates, defending environmental health, and reducing climate change are all urgently needed right now. In order to achieve these goals, it is crucial to increase agricultural yield and soil health, decrease food spoilage, employ more biological control instead of pesticides, maximise bioremediation of contaminated locations, and produce energy from renewable sources like biofuels. In this study, fungi that may provide assistance in finding answers to such issues are highlighted. In the context of the studies included in this Special Issue of Current Genetics, we go through important facets of fungal stress biology. This field of biology is relevant to both fundamental and applied research on fungal (and other) systems, including biological control of insect pests, roles of saprotrophic fungi in agriculture and forestry, mycotoxin contamination of the food supply chain, optimization of microbial fermentations, including those used to produce bioethanol, plant pathology, the limits of life on Earth, and astrobiology[8].

Selim *et al.* demonstrated the Growing attention is being shown in endophytic fungi as potential sources of physiologically active substances since they are found asymptomatically in the interior tissues of all higher plants. The biology of endophytic fungus, their discovery, isolation, identification, and variety, as well as their biological functions in agricultural and environmental sustainability, are the main topics of this review. It also takes into account their medical uses, particularly in the creation of antiviral, anticancer, and antibacterial chemicals. One of the most inventive types of secondary metabolite makers that are crucial to human survival are endophytic fungi. They are prospective sources of new natural agents that might be used for environmental, agricultural, and medicinal purposes[9].

Chitin, a common polymer made from the cuticles of insects, the shells of crustaceans, and the cell walls of fungus, is degraded by chitinases. In the fields of medical, agriculture, the food and pharmaceuticals industries, and environmental management, they are receiving more and more attention. They are desirable for these uses because of their functions in the breakdown of chitin for the creation of industrially valuable chemicals and in the management of fungal diseases and insect pests. Chitinases, however, have a variety of origins, traits, and modes of action that seem to limit optimization processes and make standardising strategies for improved practical applications difficult. Consequently, the outcomes of laboratory experiments often do not translate to practical applications. These difficulties may be resolved with the expanding science

of protein engineering by changing or redesigning chitinases to improve certain properties necessary for particular applications. Oyeleye *et al.* compiled the differences in chitinases' properties and processes that prevent their widespread use in biotechnological applications. Also mentioned are recent efforts to enhance chitinase efficiency[10].

Discussion

All aspects of agriculture have benefited from new technology, although some contemporary methods have an adverse effect on the environment. The most current difficulty for sophisticated farming is to increase yields while protecting the environment. Finding environmentally acceptable alternatives, such as expanding the use of biocontrol agents, is therefore urgently needed. Fungi and bacteria are two of the many species that are utilised as biocontrol agents. The fungus genus Trichoderma provides a variety of enzymes that are important for biocontrol processes such cell wall disintegration, resistance to biotic or abiotic stimuli, hyphal development, and others. Since the past two decades, knowledge of filamentous fungus belonging to the genus Trichoderma has steadily advanced, from the basic ideas of biocontrol agents to their newly proven position as symbionts with a variety of beneficial impacts on the plants.

Complex organic molecules may be converted into simpler forms using phosphate. It has also been shown that endophytic fungi provide plants resilience to heat, salt, drought, and disease. Under salt stress circumstances, the ability of four endophytic fungi (GM-1, GM-2, GM-3, and GM-4) to promote soybean plant development was examined. Compared to controls, endophytic fungal cultures greatly increased seed germination and plant development. Culture filtrate (CF) was the subject of a research. Gibberellic acids, which come in different concentrations, supported the beneficial effect of fungus on plant development. The use of rhizospheric fungus to enhance plant growth and prevent different plant diseases is a practical and advantageous method from an environmental standpoint. Three significant fungi (PNF1, PNF2, and PNF3) were isolated from the soil of peanut plants' rhizospheres and examined for their capacity to encourage the development of sesame seedlings. In comparison to controls, PNF2 substantially increased the shoot length and fresh weight of seedlings in each of these isolates. In comparison to the other isolates, PNF2 contained a higher concentration of indole acetic acid in the fungal culture filtrate.

Plant primary and secondary metabolism is impacted by fungal interactions at all stages of development. A vital process and the primary source of energy for plants is photosynthesis. Its effectiveness is influenced by photosynthetic pigments like chlorophyll and carotenoids. Plants treated with fungus had more chlorophyll A in their leaves than untreated plants.Low pH or slightly acidic soils that are mostly undisturbed are ideal for fungi growth. The organic wastes are broken down by fungi, which enables a variety of microorganisms to digest and transform the leftovers into valuable products. About 90% of all plants develop symbiotic mycorrhizae fungal connections by developing hyphae networks. The mycorrhizae in the soil aid in the plant's uptake of phosphate and other minerals like zinc and copper. The root of the plant provides sugars and other nutrients to the fungus. A mycorrhizae network is a cooperative organisation.

Despite being able to thrive in a broad pH range, soil fungi are more prevalent in acidic soils owing to the fierce competition they face from bacteria at neutral pH levels. The bulk of fungi are aerobic, which means they like wet soil to develop in. These organisms play a very little part in the metabolic shift that occurs in environments with excessive moisture. The area around the foundation known as the rhizosphere is dominated by soil bacteria and is home to a number of different chemicals and organic chemistry activities. Fungi may make approximately 10% to 30% of the rhizosphere of soil. Fungi are able to produce a variety of extracellular enzymes. The fungus with vesicular arbuscular mycorrhiza belong to the class Glomeromycota (VAM). They are lower-level fungi that act as a support system for more advanced fungi (basidiomycetes). They create sporocarps, which are comparatively tiny microscopic structures (truffle- like). The majority of plants may form mycorrhizal connections with these fungi, despite the fact that there are only 200 species of them. The Greek words for "mushroom" and "root" are the source of the word "mycorrhiza." In a mycorrhizal connection, the subterranean mycellium comes into touch with plant roots without hurting the plant.

Mycorrhizal fungi are responsible for the improved development of host plant species due to increased nutrient intake, synthesis of growth-promoting compounds, resistance to salt and drought, and synergistic interactions with other helpful microbes. It is anticipated that AM fungus would thrive better in sustainable agricultural soil conditions than in standard agricultural soils. More than 80% of terrestrial plants, including ferns, woody gymnosperms, angiosperms, and grasses, have been linked to the AM fungus in both agricultural and natural settings.

Arbuscular mycorrhiza fungi (AMF) are advantageous fungi that coexist with a range of terrestrial plants. Arbuscular mycorrhizal fungi have the capacity to alter the soil's physical characteristics, hence promoting plant development in both calm and stressed situations. Arbuscular mycorrhiza fungi colonise plants, enhancing plant growth and modifying the morphological, nutritional, and physiological characteristics of plants to increase their resistance to abiotic stresses. Ocimum basilicum is shielded from salt stress by the arbuscular mycorrhiza fungal colonisation, which improves mineral absorption, chlorophyll production, and water usage efficiency. Compared to controls, tomato plants with arbuscular mycorrhiza fungus invaded show an increase in leaf area, nitrogen, potassium, calcium, and phosphorous contents to boost plant development rate.

It is possible to use fungi to produce vitamins, amino acids, and lipids, which will improve the nutritional value and attractiveness of food. For their fruit bodies, which are eaten directly as food, and yeast, mushrooms are grown. In fermenters, cells, mould mycelium, and mould mycelium are cultivated to produce edible single-cell protein. Plant responses are significantly influenced by soil phosphorus levels, and responses are often enhanced by low phosphorus levels. Host genotypes and fungal strains seem to have an impact on how plants react to inoculation. The worldwide field experiment has shown that efficient AM fungal endophytes boost soils lacking wheat, maize, barley, potatoes, and cowpea yields in soils with marginal P deficit. Increased zinc absorption has been seen in peach, maize, wheat, and potato that have been infected with the AM fungus in soils with low zinc levels. Citrus and avocado seedlings have shown AM connections with increased sulphur and calcium uptake, improved water

absorption, and plant tolerance to water stress. Additionally, it has been shown that plants infected with the AM fungus have increased concentrations of cytokinins and chlorophyll. As a consequence, several scientists have started testing out alternative strategies aimed at modifying or including microorganisms to enhance plant disease resistance. Producing antibiotics and parasitizing pathogens, antibiotic-producing bacteria (such as those in the genera visible radiation and Bacillus subtilis) and fungi (such as AMF and Trichoderma) compete with plant diseases for nutrients and cover.

Increasing nutrition exchange and absorption by using AM fungus:

Mycorrhizal association is a symbiotic interaction between fungus and the roots of higher plants that facilitates the intake of nutrients, especially those that are less mobile.

Ectotrophic mycorrhizae are those in which the mycelium develops within the cells of a mantle or sheath that the fungus forms over the surface of the root. Fungi like Boletus, Amenita, and others create this kind of bond.

Some AM species have been found in salty environments, and mycorrhizas may help plants survive in difficult environmental conditions, including saline environments. Some studies suggest that the proportion of plants with mycorrhizal connections in their root systems near shorelines is about 50%. Salt marsh plants have also been shown to have several types of AM.

There are AM species that can survive in settings that are very salty and have electrical conductivities more than 150 dS/m. AM fungus has a number of strategies to assist plants in overcoming salt stress. As shown by the addition of AM fungus to lettuce and onion plants, which led to increased phosphorus accumulation under salt stress, they may, for instance, boost soil nutrient absorption by plants. The ionic balance of plants may also be affected by AM, notably in the regions of Na+ and Cl-.

A further benefit of adding AM to tomato (Lycopersicon esculentum) grown in salt was an increase in the production of antioxidant enzymes that guarded against damage to cell membranes. Abscisic acid synthesis, among other hormones, may be increased by AM fungus. It is crucial to understand how mycorrhizal fungus affect hormones since these hormones may aid plants in withstanding a range of environmental challenges. When lettuce (Lactuca sativa) was inoculated with Glomus intraradices, for instance, the hormone levels in these plants' saline-stressed tissues rose, which had an effect on the control of stomatal closure. Because salt may make plants dry, AM fungus might be able to help them consume more water. Mycorrhizas enhanced the surface area of the roots of leek (Allium sativum), enabling them to absorb more water.

Most rice is produced in climates with abundant rainfall. Drought is one of the main issues since it may result in a severe reduction in productivity when the water supply is inadequate. Production of rice is restricted. Rice has drought-resistance mechanisms that work in conjunction with active soil organisms. One form of soil microorganism that may help rice endure drought is arbuscular mycorrhizal (AM) fungus. It facilitates the absorption of nutrients and water by plants. It has a part in regulation as well. In addition, intercropping is thought to increase the

activity of AM fungi and drought resistance. Intercropping may enhance rice root structure and AM fungal colonisation, both of which are crucial for drought resistance. This study's objective is to learn as much as possible about the mutualism that exists between the AM fungus and rice under drought stress. The impacts of AM fungus will be the main topic of the study.

Regarding rice drought resilience, intercropping, AM fungus, rice hormones, water potential, and rice growth Plant growth and survival under drought stress were both boosted by the establishment of mycorrhizal fungus. AMF has been shown to increase plants' ability to withstand drought.

Antifungal insecticides

In the past, the fungus were utilised to control insect problems. Microbes were initially used to control insects 100 years ago. Fungi, as opposed to infections brought on by bacteria, viruses, and protozoa, infect insects via the surface of the body. Those fungi that harm insects are called entomogenous ones. Conidia of fungi that feed on insects are adhered to the insect's integument, where they germinate and grow into germ tubes that may enter the insect's body at the ideal humidity and temperature. The body of the insect gets infected with the fungus, which causes mycelia and conidia to coat it. Conidia that have just been produced spread and cause new infections, perpetuating the cycle.

Negative insecticides

According to their characteristics, nematopathogenic fungi may be classified into three groups: nematodes, trapping fungi (Arthrobotrys, Dactylella), endoparasites (Hirsutella, Meria), and very specific egg parasites (Datylella). The most popular and frequently available myconematicides are Royal 300 R and Royal 350 R (Arthrobotrys robata) (Arthrobotrys suporba). The fungus changes the geometry of the roots, increasing the amount of root surface that can absorb nutrients, increasing the amount of nutrients that can be absorbed from the soil.

Associations between fungus hyphae and plant roots are known as mycorrhizae. Almost all terrestrial plants, including cultivated and wild crops, trees, and fungi in the soil, will develop mycorrhizal connections. These mycorrhizae are crucial for the development and well-being of the plants.Through the formation of mycorrhizal connections, fungi are crucial for the healthy growth of most plants, including crops. Most food chains start with plants, therefore if their development were restricted, all animal life, including humans, would suffer greatly from malnutrition. Mycorrhiza is the term used to describe the beneficial association (symbiotic) between the roots of higher plants and soil-borne fungus. The roots of the majority of deciduous and evergreen trees contain ectomycorrhizas, in which case fungi such as Boletus, Phallus, Scleroderma, Amantia, Tricholoma, etc. have grown around the roots on the outside of the tree. These fungus break down the organic stuff in the leaf litter and the soil. The ectomycorrhizal fungus encourages the development of old seedlings. The tissues of the roots are therefore readily supplied with nutrients such phosphorus, calcium, nitrogen, and potassium via dungbl hyphae. A symbiotic connection is created in this manner.

The fungus assist in supplying nutrients, particularly phosphorus, to the plant roots they are connected with via their extensive collecting network of hyphae in the soil. This can have incredible benefits on plant development, especially in less fertile soils. It has been shown that plants with mycorrhizae grow up to 20 times more quickly than those without them in soils with low levels of phosphorus. If mycorrhizae are present to aid plant seedlings in extracting nutrients and water from the soil, their chances of survival might increase by up to five times. Increasing plants' ability to absorb phosphorus from the soil may also boost their ability to withstand droughts, since this is one result of better phosphorus feeding.

Being saprophytes, they break down organic materials and increase the soil's fertility. Some fungus form symbiotic relationships with the roots of higher plants, such as Pinus, and aid in their nutritional uptake. Mycorrhizal fungi often develop connections with a variety of plants simultaneously. Therefore, it is possible that the fungal hyphae of various fungi act as a type of enormous subterranean network that links the majority of the plants in a habitat. This could make it feasible for various plants to exchange nutrients through the fungal hyphae, albeit it hasn't been verified. If this is the case, seedlings would have a far better chance of surviving since they would have access to the vast subterranean collecting network rather than just their limited root system.

Therefore, a healthy mycorrhizal fungus network in the soil is essential for optimum plant development in the majority of settings. With the exception of species that do not need mycorrhizae, plant development will be inhibited if the fungi are missing or just present in the soil as solitary spores. The majority of them are what are known as weed species. By producing a protein that helps bind smaller soil particles together to produce bigger ones, mycorrhizae also contribute to the development of sound soil structure. With larger air gaps and thus more air for soil organisms and plant roots, water may travel through the soil more readily as a result.

Mycorrhizae come in many different forms. In some, the fungi's hyphae actually reach the plant roots' cells. We refer to them as endomycorrhizae. These endomycorrhizae come in a variety of varieties, with the AM (Arbuscular Mycorrhizae) kind perhaps being the most well-known. Eighty percent of all plant species, including most agricultural plants, shrubs, flowers, and trees, establish AM relationships. Ectomycorrhiza is a different kind (ECM). This kind of fungus associates with plant roots but doesn't really penetrate the root cells. Among many other species of trees, this sort is mostly made up of pine, fir, spruce, and oak trees. Rhizomorphs, which are thick hyphal threads that ECM fungus may produce, are capable of transporting water and nutrients over quite large distances. Many of the fungi that are prevalent in woodlands are ectomycorrhizal fungi, having fruiting bodies that resemble mushrooms or toadstools.

Conclusion

As the fungus modifies the architecture of the roots, a greater root surface becomes accessible for nutrient absorption, leading to an increase in the uptake of nutrients from the soil. In addition to serving as an absorption surface, fungi also increase nutrient availability by solubilizing insoluble nutrients like phosphorus, making them available to plants. They also increase nutrient mobility by facilitating faster intracellular nutrient movement and mobilising nutrients from soil

masses that are not reached by roots but are instead travelled by mycorrhizal hyphae. By increasing the activity of antioxidant enzymes and osmolytes and controlling the production of phytohormones, which may conceivably link the multiple tolerance mechanisms for cumulative stress response, arbuscular mycorrhizal fungus protected plants. It has been shown that the key role of arbuscular mycorrhizal fungi in combating salinity is related to the roots' restricted ability to absorb salt and the maintenance of nutritional homeostasis.

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CHAPTER 18

ROLE OF FUNGI IN PHARMACEUTICAL INDUSTRY

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Abstract:

Fungi have a substantial impact on the treatment of disease in both humans and animals. Penicillin's foundation is fungus. Industrial fungus processing is used to produce more than 10 of the top twenty most profitable medicinal compounds. Research and development for new drugs are ongoing activities. Examples of how fungi are utilised in medicine include the antifungal drug micafungin, the chemical mycophenolate, which is used to prevent tissue rejection, and the cholesterol-lowering drug rosuvastatin.

Keywords:

Antibiotics, Ergot, Micafungin, Penicillin, Saccharomyces.

Introduction

In essence, fungi are plants without the green pigment chlorophyll. The classification of fungi as a distinct kingdom from plants and animals has since somewhat altered this original vision. They are regarded as the most significant living things on planet. Fungi are the subject of mycology. Fungi and certain bacteria work together to recycle, or change dead material into a form that is helpful to the earth. By creating mycorrhizal connections, fungi are crucial for the growth of most plants, including crops, but they can cause illnesses in humans, animals, and other plant and animal life. Humans eat edible mushrooms for their single nutritional benefit.

Single-celled yeast develops through budding. Examples include Saccharomyces, Cryptococcus, Histoplasma, and Candida albicans. Oval yeast Candida albicans has one bud. The term "yeast" describes a wide range of fungus with just one cell. *Saccharomyces cerevisiae* and S. ellipsoideus are examples of yeast that are utilised for both the manufacture of alcohol and baking bread (the later). Because Saccharomyces has a high concentration of B vitamins in its cytoplasm, yeast pills are beneficial nutritional supplements. One pharmaceutical business transforms yeast into "Ironized Yeast," which is advised for persons with low iron levels. Beer is produced when yeast ferment barley grains; wine is produced when grape juice is fermented.

Molds are made of lengthy filaments. (Hyphae is another name for the filaments.) Aspergillus, Penicillium, and Mucor, among other moulds, are examples. By producing spores, moulds reproduce. They may be seen sprouting on grass, jams, bread, leather goods, rotting oranges or other fruits, etc.

Some moulds and fungi are the original sources of several drugs, including Penicillin, Griseofulvin, Lovastatin, Streptomycin, and others. A common fungus called Penicillium is the source of penicillin, maybe the most well-known antibiotic medication. Actinomycetes is a

distinct type of bacteria that produces streptomycin as an antituberculous medication from the so-called soil fungi (Streptomyces). Other fungi also generate a variety of antibiotic compounds, which are now extensively employed to treat and prevent infections in both human and animal populations. The development of antibiotics altered global health care. Ergot is a disease that infects Rye crops and is brought on by a mould called Claviceps purpurea. Ergotamine and LSD are produced (Lysergic Acid Diethylamide).

The anticancer medication paclitaxel may be produced by several fungi, including Nodulisporium sylviforme and Taxomyces andreanae. The bacterium Penicillium griseofulvum may produce griseofulvin. Both paclitaxel and griseofulvin have anticancer properties because they bind to tubulin, which inhibits microtubule dynamics and prevents cell proliferation. This is also the anticancer medication vincristine's seventh mode of action.

A broad range of fungi may cause dermatomycosis, a fungal illness affecting the skin, hair, and nails. The disorders are sometimes referred to as tinea infections, from the Latin tinea, which means "worm," since in antiquity worms were believed to be the cause. The tinea diseases include tinea paedis (athlete's foot), tinea capitis (head ringworm), tinea corporis (body ringworm), tinea cruris (groyne ringworm or "jock itch"), and tinea unguium (ringworm of the nails). Trichophyton, Microsporum, and Dermatophytes are the most prevalent dermatophytes.

Epidermophyton. Aflatoxins are dangerous substances that Aspergillus flaevus generates. The agricultural goods it contaminates include peanuts, grains, cereals, maize, rice, sweet potatoes, and animal feeds.

These are many types (species) of fungi's fleshy, non-poisonous, and edible fruiting bodies. It is possible to observe their fruiting bodies with the unaided eye. They may appear above or below ground, where they can both be picked up by hands, respectively (hypogeous and epigeous). An item may be deemed edible if it meets certain requirements, which may include not being toxic to humans and having a pleasing flavour and scent.

Although edible mushroom species have been discovered in Chilean ruins dating back 1,300 years, the first conclusive evidence of mushroom eating comes from China, where it is said to have originated. Both the culinary and medicinal potential of mushrooms is highly regarded in China. Mushrooms were consumed by the aristocratic class in ancient Rome and Greece. Chinese soups and rice with mushrooms are well known around the globe. At least 60 nation’s farm mushrooms, with China, the United States, the Netherlands, France, and Poland being the top five producers. Its cultivation has lately begun at a modest level in a few private and excellent agricultural fields of Pakistan.

Like other fungi, mushrooms are not plants and do not engage in photosynthesis. Instead, they are fleshy, spore-bearing fruiting structures that are grown above ground on soil or on their food supply, organic humus. A mushroom's stipe, top, and bottom, as well as its subterranean portions, are covered with gills (lamellae, singular lamella) or pores. The spores are often raised on the gills or lamellae in such instances. The spores, which are unlike plant seeds, are in charge of reproduction. The body of a mushroom is made up of a single, two-mycellium-strong structure.

As stated in the paragraphs above, certain mushrooms may be eaten as food, while others are deadly if swallowed. More readily digestible than any other 24 veggies, mushrooms are a good source of protein. Mushrooms are a good source of B-complex vitamins, minerals, and other nutrients that are essential for good health. Some types of mushrooms have been discovered to stop the spread of cancer cells and AIDS, among other therapeutic properties. They are beneficial in the treatment of conditions including the common cold, headaches, and hepatitis B, it has been revealed. They may assist in lowering blood cholesterol levels, tiredness, and sleep issues. As well as helping to boost the immune system and preventing undesirable aspects of ageing, mushrooms may also be beneficial for ailments including arteriosclerosis, renal failure, high blood pressure, and kidney failure. The mushroom provides a suitable option for the condition of anaemia, which is brought on by a lack of folic acid. It also helps to control blood sugar levels.In contrast to other forms of protein like meat, mushrooms are often advised for patients with liver and renal illnesses as well as being a suitable source of protein for those with gout since they only produce a little quantity of uric acid at the conclusion of the digestion and metabolism.

Literature Review

Daley *et al.* studied the discovery of endophytes, some of which are fungus that have substantially influenced the pharmaceutical industry, was made possible by the traditional usage of plants in ethnomedicine. These fungi contain secondary metabolites, which are extracted, altered, and used in research. Such a contribution is comparable to their existence. The pharmaceutical, agricultural, and medical sectors all use these chemicals and have described them. Penicillin, an antibiotic, is the fungal metabolite that is well known to humans. Because fungus may exploit plant machinery to make secondary metabolites, they have both beneficial and harmful endophytic features. Beneficial: Fungi can give plants with essential nutrients. Disadvantageous: Fungi can have detrimental impacts[1].

Rocha-Miranda *et al.* studied on herbs and plants come in close touch with soil and are quickly colonised by fungus, there are worries about the public health implications of the growing use of plant-based products for health promotion or illness treatment. Numerous investigations have shown that mycotoxigenic fungus species are present in plant-based medications and supplements. With reports of the existence of several mycotoxigenic species, Aspergillus and Penicillium species have been identified as the most prevalent and dominating ones. Multiple mycotoxin incidence is a worry because to the diversity of species, and exposure is compounded by the rising popularity of these goods. Updated information on the prevalence of naturally occurring mycotoxigenic fungi and their mycotoxins in plant-based supplements and medications is included in this study[2].

In the context of contemporary life science, the word "fungi" has been recognised as a distinct area, making it important to clarify its meaning and vocabulary. As a result, the fungi that were formerly classified as phytomedicine in the Chinese materia medica (CMM) should now be referred to as "fungal medicine" and still fall under that classification, which will help to provide a more appropriate site for future fungi medicine research and development. There are two types of "fungi medicine": "fungi CMM" and "medicinal fungi." The first creates self-tissues of fungi that are used as medicines, such as Ganoderma and Poria, etc., while the latter is a particular

CMM with a special biopharmaceutical technique, such as Shenqu in ancient Chinese medicine and Huaier fungal material in current times. For the newly constructed bi-directional solid-state fungal fermentation engineering, based on pharmacological properties, significant progress has been made with products like "Linglei fungal substance" with detoxification on Tripterygium hypoglaucum and "Huaiqi fungal substance" with antiviral activity. The use of residues as a medicinal matrix has made the subsequent development and study of CMM as well as the associated environmental conservation very important to the pharmaceutical sector. In the review, recent developments are condensed[3].

Neuenkamp *et al.* discussed that Similar to people, different plant and animal species choose to dwell in certain environments, known as ecosystems. Similar to how humans avoid living in deserts because it is too hot and dry, if the ecology changes too much, certain species will vanish. Humans alter numerous ecosystems, often to the point that hardly any plants or animals can survive there. We often begin by planting trees or other plants in order to aid damaged ecosystems in recovering. Mycorrhizal fungi, small fungi that live within plant roots and in the soil, were discovered by biologists to hasten ecosystem recovery by causing plants to regrow more quickly and robustly. In this article, we discuss the role that mycorrhizal fungi play in ecosystem recovery and when they are most beneficial[4].

The need for colours in biotechnology-related products for food, textiles, medicine, and cosmetics is rising quickly. Industries are forced to use natural pigments due to the health risks of synthetic hues. Microbial pigments are the most significant and diversified kind of them all. The fungal pigments in this category are in great demand because they are mostly extracellular metabolites, are simple to extract, and have several biotechnological uses. The quantity of pigment produced by almost all genera of fungus varies and depends on the species. The most significant groups that generate valuable colours are yeast, basidiomycetes, zygomycetes, filamentous fungus, endophytic fungi, marine fungi, and halophiles. Melanin, phenazines, flavins, carotenoids, violacein, indigo, ankaflavins, canthaxanthin, prodigiosin, and moascins are the pigments that are most often found in the kingdom fungi. Industrial-scale extraction of these pigments is being done for a variety of biotechnological uses. The purpose of this review is to draw attention to the significance of fungi-produced pigments. The study of fungi that produce colours has the potential to advance the textile, food, pharmaceutical, and cosmetics sectors[5].

It has been suggested that some species of the ascomycete fungus Cordyceps are the teleomorphs of certain Metarhizium species. As insect biocontrol agents, the latter have been frequently used. The use of cordyceps species in traditional Chinese medicine is highly regarded, however the genes involved in the manufacture of bioactive substances, insect pathogenicity, and the regulation of sexuality and fruiting have not yet been identified. Results: The genomic sequence of the model species, Cordyceps militaris, is reported here. According to phylogenomic study, the Cordyceps/Metarhizium genus' diverse species have separately developed as insect pathogens, and their shared huge secretomes and gene family expansions are the result of convergent evolution. But compared to other fungus, such as Metarhizium spp., C. militaris has fewer protein families, which points to a more constrained environment. The Cordyceps genome does not include genes for any recognised human mycotoxins, which is consistent with its long

history of safe use as a medication. We demonstrate that C. militaris is sexually heterothallic, yet, very uncommonly, fruiting may take place without a partner who is the opposite of the mating type[6].

Chuxiong, sometimes referred to as "the City of Fungi," is home to a wealth of fungi and traditional knowledge about the variety of fungi. A variety of edible mushrooms thrive in the area's favourable habitat. The relevance of this resource for the Yi ethnic group was assessed. Twenty-two edible fungus species were noted, both as food and non-timber forest products (NTFPs), utilised to boost revenue. In summary, Chuxiong was found to have a significant edible fungal production chain as well as many and diversified wild genetic resources. But the risks to the wild edible fungus are growing as a result of overharvesting. The development of fungi cultivation to increase quality and supply and lessen harvest pressure is suggested as a way to enable the sustainable use of fungi resources. Other suggestions include enhancing public awareness of environmental protection and sustainable development, promoting eco-tourism, and developing fungi catering in rural agro- and slow-food tourism[7].

As antimicrobials, cytochrome bc 1 (also known as respiratory complex III) inhibitors of the mitochondrial respiratory chain have been produced. They are employed in medicine to combat human infections including the malaria parasite Plasmodium falciparum and Pneumocystis jiroveci as well as in agriculture to reduce plant harmful fungi (an opportunistic pathogenic fungus life-threatening in immuno-compromised patients). This makes these respiratory inhibitors useful against a variety of significant infections. Regrettably, the issue of acquired resistance has grown quickly. There are an increasing number of pathogen isolates that are resistant to inhibitor therapy, and this resistance is often correlated with mutations in cytochrome b, one of the critical catalytic subunits of the complex. *Saccharomyces cerevisiae* is a crucial model for determining how mutations affect a cell's ability to breathe, its susceptibility to medicines, and its fitness. This minireview provides a quick overview of the inhibitors, their mechanisms of action, and the resistance-related mutations discovered in yeast. The molecular foundation of resistance and the emergence of four mutations that are particularly significant in medicine and agriculture are briefly reviewed, more fully defined, and discussed[8].

Regarding the impressive ability of their secondary metabolites, marine fungi were thought to be the treasure of ocean medicines due to their beneficial pharmacological effects. To explore their potential as therapeutic agents, bioactive compounds produced from fungus have been extensively studied in the literature. Future treatments for life-threatening illnesses including cancer and various developing inflammations may be made possible by marine fungus. The biological effects of numerous secondary metabolites generated from marine fungus are still unknown, and here, detailed graphics are provided to explain how a variety of bioactive chemicals interact with human disorders. One of the key areas that must be created particularly for the efficient conversion of bioactive substances to medications is this knowledge. A wide variety of biochemical compounds with priceless carbon scaffolds are produced by marine fungus, and these compounds are the primary factor interacting with human illnesses at specific locations. A new trend that increases the likelihood of generating tailored medications is caused by some illnesses' propensity to attack certain bodily targets[9].

A poorly understood category of creatures with great biotechnological potential is the fungi. Fungus have evolved different survival strategies as a result of the wide variety of settings they occupy and the necessity to compete with a wide range of other fungi, bacteria, and animals. Thus, the special qualities of fungi portend enormous potential for their use in biotechnology and industry. Fungi may also be cultivated quite easily, making large-scale manufacturing feasible. The creation of a live fungus collection and the hunt for fungal biodiversity have enormous economic potential for finding organisms with unique industrial applications that will result in novel goods. This article examines fifty possible applications of fungus in biotechnology. In addition to giving examples from our own study and the work of other well-known academics, we also offer remarks and examples for each possible exploitation. We also provide a flowchart that may be used to persuade funding organisations of the value of mushrooms for biotechnological research and as future products. Penicillin, lovastatin, and other important drugs have been made possible by fungi, which are still mostly underutilised despite having immense industrial potential[10].

Discussion

We are all familiar with penicillin. We've all heard the tale of how it was found by accident when a research dunderhead by the name of Fleming came home from vacation to discover that his bacteria had been wiped out by a rogue fungus. Whether he immediately identified it as the first antibiotic varies depending on the retelling of the tale. Additionally, they disagree on whether and how Britain and the USA worked together to create the substance that would protect mankind from sickness. No one account of the events can, therefore, be completely accurate or completely false. But since I don't want to start here, I don't want to add to the range of tales. Instead, I'd want to begin by describing the scope of the lifestyle shift that antibiotics have wrought. Antibiotics are now taken for granted. A small sore throat is treated with an antibiotic, and a minor injury is treated with an antibiotic shot "in case of complication." All of it has become so unimportant. So typical. However, the alterations brought about by the quick accessibility of antibiotics were anything from minor. They gave independence from the worry of inescapable death due to different forms of "blood poisoning" for the first time in human history. Everyone will die for the most trivial of causes.

The Second World War was only getting started in 1940. Hitler's armies controlled much of Europe in the summer of 1940, stretching from the Pyrenees to the North Cape, a peninsula on an island off the coast of Norway. On May 10, Winston Churchill took over as Prime Minister of the United Kingdom, and some of the deadliest moments of World War II—Dunkirk, the fall of France, and the blitz followed. After France fell, the United States started the first peacetime conscription in its history. The British army had abandoned the majority of its armaments on the beaches at Dunkirk. The Battle of the Atlantic was started in June 1940 by German Navy u-boats, and it did not end until late 1943. But beginning in August 1940, when the German Air Force began conducting daytime attacks on ports and airfields, the Battle of Britain was fought in the air. The efficiency of Royal Air Force fighters was significantly boosted by the new technology known as radar, forcing the Germans to convert to night bombing at the end of September due to the severity of their casualties. There were 71 significant air attacks on London

between that time and May 1941, together with another 56 on other cities. Hitler delayed the invasion on September 17, 1940, admitting defeat in the Battle of Britain.

The first aircraft jet engines were being tested at top-secret sites in both Germany and England. In May 1941, a plane powered by Frank Whittle's engine made its maiden flight. The ram-jet powered pilotless V-1 and the ballistic rocket armament V-2 were both far along in development at the then-secret Peenemunde base in Germany. Two years after the first of more than 4,000 V-2s was test-fired, these ballistic missiles were detonating on their targets in England. The British had stopped their TV broadcasts, which had been in operation since a few years before the war began, but they were still using radar arrays to see German aircraft attacking from the Southern North Sea and English Channel. Alan Turing built Bletchley Park's first programmable digital computer, which was already routinely used to decrypt German military communications that were encrypted. Of course, the nuclear era was starting to emerge. The Austrian scientist Lise Meitner and her nephew, the (naturalised) British physicist Otto Frisch, described nuclear fission in 1939. Therefore, preparations were being made for the inevitable events that would result in the founding of the Manhattan Project in August 1942. I bring up each of these incidents to set the remainder of the narrative in its appropriate perspective. It's been more than 60 years, yet many modern conveniences, like jet flight, rockets, cruise missiles, telephones, computers, and atomic energy, were either in use or in the early stages of development. However, despite the fact that we are not discussing the Dark Ages.

The "penicillin narrative" is able to arouse curiosity and imagination more than any other scientific discovery of the modern era since its metabolic byproduct of a common green mould is capable of curing a range of illnesses which surrender slowly or not at all to other therapies. A single fungus substance has completely changed medicine. Although penicillin cannot treat every illness, it is effective in treating gonorrhoea, gangrene, and pneumonia. Diseases like septicaemia and osteomyelitis, which were deadly or severely disabling and were prevalent when penicillin was first used, have been consigned to medical history. There was no need for a war in the 1930s (or before) to always be at risk from deadly infections. Any wound where the skin has been damaged may get infected with airborne or soil bacteria, some of which may develop to a point where the immune system is unable to handle them.

When that occurred, the bacteria began to consume the patient and release poisons into the bloodstream, resulting in more extensive harm. A typically active adult might get small cuts or scratches when gardening, strolling, or climbing. How many bloody injuries to your shins did you sustain as a child? Osteomyelitis is a staphylococcus infection of the bone that was rather prevalent in kids. Any guy who routinely trims his beard risks developing an infection from the unavoidable nicks and cuts. This was the cause of Lord Caernarfon's death in 1923. He had lived in Egypt for 19 years before to discovering Tutankhamen's tomb in 1922. And it wasn't "The Mummy's Curse"—blood poisoning, or septicaemia—caused by an infected shaving cut that put an end to the noble Lord's life. Less severe infections, brought on by germs from shaved cuts invading previously healthy hair follicles, resulted in ugly boils and abscesses all over the face. Not fatal, but unpleasant, crippling, and most importantly, frequent. Women were at considerably greater danger. Even today, giving birth is a dirty process, and before there was

easy access to medications, an incredible number of new moms suffered from puerperal fever brought on by an internal infection, and thousands of them perished. And even when the kid survived, an equally startling percentage of newborns were infected at delivery with germs that were only weakly pathogenic but nonetheless resulted in blindness, deafness, and other lifelong impairments. Biologically, bacteria are incredibly successful. They are versatile and widely distributed. Human life was not simple. Penicillin had a significant role way to a revolutionary transformation in medical care, which revolutionised the way people live their lives to the point that illnesses that were formerly frequent causes of death and disability are now uncommon. The discovery and widespread use of penicillin, in my opinion, has dramatically altered how everyone on the earth lives their daily lives. The history of penicillin also includes a number of other noteworthy elements. One of them is supply. There was just a little more than one gramme accessible in the whole globe for Albert Alexander, one of the first patients to be treated, and it was not enough to save his life. Today, we generate enough of the completely pure substance to providegrammes to every person on the earth.

Conclusion

The basic cellular biology and metabolism of baker's yeast have been examined in addition to its function in baking. In 1929, Alexander Fleming isolated a substance from mould, which led to the discovery of penicillin. In 1941, the first of a series of antibiotics created exclusively from fungus, it successfully cured a bacterial disease and kicked off a revolution in medicine. Following that, several bacterial illnesses that were formerly fatal were now treatable. Numerous new groups of fungus have been discovered. The well-known antifungal drug Griseofulvin is made from fungi. With gliseofulvin, dermatophytes are treated. It accumulates in the skin and hair after topical therapy.

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CHAPTER 19

MEDIA STERILIZATION FOR INDUSTRIAL FERMENTATION

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Abstract:

All living things, including bacteria, viruses, spores, fungus, and other microbes, must be removed from the medium and the air in order to sterilise them for microbial fermentation. This is important to do in order to guarantee the sterility of the medium containing the necessary nutrients, the sterility of the incoming and exiting air, the sterility of the bioreactors, and the avoidance of contamination throughout the process. As a result, the filtration technique of air sterilization—which allows air to flow through filters rather than electrically heated equipment, which is less common but more expensive—is used the most often in businesses for fermentation reasons. Although the heat technique of media sterilisation is widely used, this is because of the kind and degree of contamination. The media's constituents, pH, and suspended particle sizes are all crucial factors in the efficiency of the sterilisation. Chemical and radiation sterilising techniques are thus hardly employed.

Keywords:

Autoclave, Fermentation, Media, Microwave, Sterilisation.

Introduction

In a lab, culture media are kept in petri dishes, test tubes, bottles with screw tops, flasks, magnetic stirrer bars, and silicon tubing made of glass or silicon.Whereas media are kept in kegs, drums, and tanks in enterprises. Nutritional media sterilisation all culture medium are properly labelled, distributed, sealed with a plastic closure, or plugged with cotton wool, and disinfected before use. Culture medium is steam sterilised in an autoclave for 30 minutes at 15 pounds per square inch of pressure at 121 degrees Celsius. It's also crucial to make sure the containers aren't completely full; otherwise, they might boil over in the autoclave or split or shatter. All containers should be marked before use with the following information: -the kind of culture medium they contain. Glassware sterilisation: Glassware, including petri plates, vials, culture tubes, flasks, pipettes, and metallic equipment, is sterilised in a hot air oven at 160 to 180 ^{0}C for two to four hours. Instrument sterilisation: Metallic tools such as forceps, scalpels, needles, spatulas, and so forth are flame sterilised. For example, immersing them in 75% ethanol, burning, and chilling (incineration)[1], [2].

Sterilization of the culture room and transfer area: The floor and table are cleaned with detergent first, then either 2% sodium hypochlorite or 95% ethanol, depending on the situation. By exposing lager surface area to UV radiation, it is disinfected[3], [4].

Prior to use, the laminar airflow cabinet is additionally disinfected by being exposed to UV radiation for 30 minutes and 95% ethanol for 15 minutes. Skin and eyes are both harmed by UV exposure.The significantly larger scale of the former is the primary distinction between media

sterilisation in laboratories and in industry. In the lab, a litre of medium would likely take ten minutes to reach the sterilising temperature, fifteen minutes at 1210C, and then another ten to fifteen minutes, for a total of forty to five minutes, whereas a 10,000 litre medium the equivalent period may well take several hours for each of the three periods.

Due to the much larger operation compared to laboratory operations, contamination by undesired MOS might pose major issues in the fermentation sector.Contamination in industrial microbiology might cause fermentation companies to suffer significant financial losses. Imagine that in the beer business, Pediococus streptococcus damnosus, a lactic acid bacterium, contaminates the fermenting wort and uses the sugars within to make undesired lactic acid, which turns the beer sour.

- The death of the generating organism may result from losses by lytic MOS such bacteriophages or Bdellovibrio.
- Contaminant may use fermentation's components to create undesirable byproducts and to change environmental conditions like pH or oxidation-reduction potential, which lowers final product yield. Since these by-products are not established in the manufacturing, removing them via the extraction process may be costly and time-consuming.

Sterilization is the process of eliminating or removing all live organisms from a habitat or an item, including viable spore viruses and viroids. A sterile thing is completely devoid of live microorganisms, spores, and other contagious agents.

Literature Review

Keller *et al.* investigated on a typical microwave oven was put to the test for sterilising phytoplankton growth material and equipment. For 1.5 vol. of saltwater in a Teflon container, contamination by bacteria, algae, and fungi is eliminated in under 10 min. Sterilization takes five minutes for empty culture tubes and containers. The process is rapid, simple, dependable, and neither precipitates nor contaminates with trace metals. There includes discussion of a sample methodology and several safety measures[5].

Hongzhang *et al.* studied about theIndustrial solid-state fermentation has encountered difficulties with large-scale solid medium sterilisation because the prolonged sterilising time (due to the solid medium's poor heat conductivity) causes the destruction of nutrients. A unique steam explosion sterilising technique is explored in this review. It is a high-temperature, brief-times technique that, when used in solid media with ideal operating settings, may totally inactivate microbes without producing inhibitors. Additionally, steam explosion sterilisation increases nutrients by breaking down macromolecules in solid medium and acts as a propulsion system for the industrial transportation of solid medium. The use of steam explosion sterilisation in large-scale industrial applications is thus promising[6].

da Costa Urtiga *et al.* carried out a study on the Low concentrations of sodium isocyanurate (ISO) as a medium steriliser were used to establish an optimization for a medium sterilising technique that might replace autoclaving. A concentration range of 0.04 to 0.005 g/L of sodium

isocyanurate in its dichloro form (sodium dichloroisocyanurate) was employed. The specimen of choice was Dianthus caryophyllus, which was seeded in Murashige and Skoog medium. In terms of the amount of time, materials used, and sterilising power, sodium isocyanurate sterilisation outperformed medium autoclaving. The D. caryophyllus sprouts developed at all successful ISO concentrations (0.04 g/L to 0.01 g/L) without exhibiting any phytotoxic effects and continued to grow until they filled their container to the utmost. The experiment was completed with contamination rates for values of 0.04 g/L, 0.02 g/L, and 0.01 g/L remaining below 5%. The findings reported and the methodology used in this study show that sodium isocyanurate may replace medium autoclaving for procedures involving D. caryophyllus seeds, which opens the door for future studies on the sterilisation of additional species using sodium isocyanurate[7].

Tiwari *et al.*In order to identify an efficient chemical agent for sterilising nutritive medium for in vitro micropropagation of sugarcane, three antimicrobial compounds—mercuric chloride ($HgCl_2$), sodium hypochlorite (NaClO), and bleaching powder—($CaOCl_2$)—were investigated at varied doses. At quantities below 0.1%, none of the compounds could completely prevent microbiological contamination. On medium enriched with 0.1% NaClO, shoot cultures could be micropropagated effectively for up to 10 subcultures (cycles), with no negative effects on shoot growth. It was also shown to be as effective to add NaClO to rooting media at a concentration of 0.1%. The findings indicated that chemical sterilisation of medium using sodium hypochlorite might be a more affordable alternative to autoclaving during sugarcane micropropagation on a commercial scale[8].

Agi *et al.* looked at the antifungal effects of fresh garlic (Allium sativum) and ginger (Zingiber officinale) on the development of three well-known pathogenic fungus. Candida albicans, Aspergillus spp., Penicillium spp., and other test organisms were used.Two distinct extract concentrations were employed. Concentration I had 100 g of crude extract in 100 ml of sterile distilled water, while concentration II included 100 g of extract in 50 ml of distilled water. Each 125 ml of SAB (saboraud dextrose agar) medium received ten ml of each extract both before and after sterilisation. Compared to extracts applied to the medium before sterilisation, extracts added after sterilisation significantly decreased the development of samples of harmful fungus. This suggests that the active components in the extracts that are responsible for the antifungal activity shown are adversely damaged and inactivated at 121oC for 15 minutes. It may be concluded that the therapeutic effects of ginger and garlic on fungus are to varying degrees[9].

Coté *et al.* in their study studied about the continual risk of microbial contamination in cell cultures is addressed in this course along with several strategies a lab might use to combat it. The first step is a protocol on aseptic technique. Any set of instructions for operations involving the maintenance of noncontaminating conditions will always use this general word. Aseptic technique, in actuality, includes all elements of environmental control, personal hygiene, equipment and media sterilisation, and related quality control processes required to confirm that an operation is being carried out with aseptic, noncontaminating method. The majority of cell culture work is done in a vertical laminar-flow biosafety cabinet or a horizontal laminar-flow clean bench, while it is possible to carry out cell culture on an open bench in a low-traffic area in theory[10].

It is typical to sterilise bioreactor medium by autoclaving, or heating with compressed steam, in order to kill the viability of the local microbial population. The high temperatures may also be predicted to cause simultaneous chemical changes in the medium. To estimate the attainment of sterility, a kinetic approach incorporating online computer heat input calculations, referred to as F0 values, was previously devised. Now, a comparable kinetic method has been developed to assess and regulate the impacts of temperature and heating duration on chemical processes taking place in the medium. Boeck *et al.* used method which is based on a general-purpose Arrhenius "pseudo" rate equation and is known as R0 values. According to the data provided, R0 may be a valuable parameter for minimising variability in culture metabolism and "scale-up" when these differences originate from various nutrient concentrations caused by non-standard heating during medium sterilisation in stirred bioreactors[11].

To test whether microwave (MW) irradiation can kill bacteria, various culture media in various volumes were subjected to the radiation for 2, 3, and 4 minutes, and the results were compared to medium sterilised using the traditional autoclaving approach. Microbial growth was examined on MW sterilised medium. All bacteria that were exposed to microwave radiation were destroyed in less than three minutes. It is feasible, quick, simple, and energy-efficient to sterilise many kinds of culture media using MW radiation, and the quality of the culture medium and microbial growth. after sterilisation are unaffected. a weak power current or an outage.

Discussion

This may be done via batch sterilisation, in which the medium is sterilised in batch quantities in the bioreactors at 121°C, either directly or indirectly, and continuous sterilisation, in which the sterilisation is done for a brief period of time (30 to 120 seconds) at 140°C. A constant and strong aeration of sterile air in the bioreactor is required for appropriate sterilisation in order to prevent contamination of the fermentation process. To do this, a variety of techniques may be utilised, including heat, radiation, chemical, and physical methods. They fall into two categories: physical and chemical approaches.

A. Physical Process

1. Asepsis is a practise that is often used in the pharmaceutical, food, and microbiological sectors. It entails maintaining general cleanliness, cleaning pipes, utensils, fermentation vats, and donning safety gear such gloves, masks, and lab coats. The load of germs is controlled and reduced, which also minimises the severity of the sterility measures used.

According to the autoclaves that run continuously in every microbiology lab, controlling microbial growth and sterilising things often involves the use of heat and other physical effects.

Heat, low temperature filtration, radiation, and other physical agents are the four that are used the most commonly.

2. Heat that may be applied either dry or wet.

Many items may be disinfected using dry heat when there is no water present. Glassware is sterilised using it, but various kinds of air filters are sterilised on a huge scale as well. It does not

damage metal equipment or glass the way wet heat does. Powders, oils, and other similar materials may be sterilised with it.

It is sluggish and unsuitable for goods made of various plastics and rubbers, which are heat-sensitive materials. The majority of labs sanitise pipettes and glass petri dishes. In order to sterilise at a temperature exceeding 1000C and eliminate bacterial endospores, saturated steam under pressure must be used.

1. Boiling: kills vegetative cells and the majority of viruses at 100^0C.

2. Autoclaving: Steam sterilisation pioneered by Chamberland in 1884 in which the air originally present in the chamber is driven out until the chamber is filled with saturated steam and the outlays are closed at 121^oC and 15 psi.

3. Tyndallization involves heating the substance for a half-hour over the course of three days. Vegetative cells are destroyed on the first day, germinated spores on the second day, and vegetative spores produced on the second day are destroyed on the third day. When the medium is too viscous for filtering, this procedure is employed to sterilise heat-labeled media.

4. Louis Pasteur invented pasteurisation in 1880. It involves exposing food or other materials to a temperature much below boiling for a long enough time to kill pathogenic or spoilage germs. There are two options:

Using the low temperature long time (LTLT) approach, batch heating at (63^0C for 30mins). When employed on a big scale, it is achieved by gradually raising the temperature, maintaining it at the pasteurisation temperature, and then evenly cooling everything down over a period of 90 minutes.

B. Continuous: Involves heating at around 72^0C for 15 seconds, followed by quick cooling. This technique is also known as the flash method or high temperature short time (HTST) pasteurisation.

C. The dairy sector uses ultra-high temperature (UHT) sterilisation, which involves heating milk and milk products at 140 to 150^0C for 1 to 3 seconds. Beer and wine are both treated with it (food industry). Without destroying the food, it destroys pathogens (vegetable cells) and food spoilage MOS. The main weakness of batch pasteurisation in comparison to continuous pasteurisation is that prolonged high temperature exposure might result in a "burnt" odour.

3. Steam under pressure is utilised in the industry and in laboratories to sterilise equipment (pipes, fermentors & media). This steam is under pressure since temperature increases with pressure. It eliminates viruses, endospores, and vegetal spore. Despite the fact that dry air heat is less efficient than most heat, at 121^0C, wet heat kills Clostridium botulinum spores in 5 minutes, whereas dry heat takes 2 hours to do the same. Heat is another application for it.

4. Low temperatures: Although we have focused on killing MOS, sometimes the most practical management method is to prevent growth and reproduction by using either;

At 200Cor below, microbial growth is halted and contaminating microorganisms are killed but not completely eliminated. Significantly slows down but does not stop microbial growth reproduction.

5. Filtration: The filter simply filters contaminated MOS, which is a good technique to lower the microbial population in solutions of heat-sensitive material. Particularly in the pharmaceutical industry where injections and vaccines are handled, large amounts of sterile air and other gases are needed. In most fermentations, they are used for aeration. There are two different kinds of filters: A thick layer of fibrous or granular materials that have been bonded together to form depth filters is filled with twist channels that have a tiny diameter.Under vacuum, MOS is drawn past this layer, and microbial cells are eliminated by physical screening or trapping. Additionally, through absorption onto the surface.

6. Radiations: Ultraviolet light, X-rays, and gamma rays are the radiations employed in sterilisation. X-rays and gamma rays are very high frequency electromagnetic waves with enough photon energy to ionise materials they travel through by knocking electrons off of atoms' outer orbits, creating positive and negative electrically altered atoms or portions of molecules. The electron changes cause the atoms that are losing electrons and those that are receiving them to become ionised.

Gamma rays are created by the spontaneous disintegration of radioactive metals like cobalt 60 and are produced by X-ray machines (C_0). They are used to sterilise rubber gloves, plastic syringes, and other materials that might be harmed by heat or chemicals.

Visible light's wavelength ranges from 400 to 700 nm in the case of ultraviolet light. UV light (ultraviolet light) has a wavelength range of 100 to 400 nm, with a germicidal peak at 254 nm between 200 and 300 nm. When DNA strands absorb a UV photon, it generates a dispersion in the DNA chain that forces nearby thyamine bases to dimerize or connect, which is how UV kills microorganisms. The organism's metabolism is messed up, and it might pass away.

UV radiation has the disadvantage of not penetrating, therefore it just affects the surface. As a result, it is utilised to sterilise the air in fermentation halls and other similarly large open areas as well as to introduce mutations to enhance culture.

B. Chemical Method: These fall into two categories.

i. Chemosterilants: A sterilant is a chemical that is used to accomplish sterilisation. It kills vegetative cells as well as spores of bacteria, fungus, viruses, and protozoa.

ii. Disinfectants: These are substances that at the at least eliminate pathogenic (MOS producing illnesses) or spoilage organisms from an inanimate item. But does not destroy certain vegetative cells or even spores.

Sanitization, on the other hand, is the process of reducing the microbial population to levels deemed safe by public health standards.

vii. Antisepsis is the use of antiseptics to stave against infection or sepsis.

1 Sterilization of Culture and Media

Vegetative cells and spores may get contaminated by the elements of culture medium, water, and containers. The medium must be clean before being used for fermentation (Marcel et al, 2010).

The most common method for sterilising media is heat, however other techniques are also sometimes utilised (physical methods, chemical treatment, and radiation).

1. Heat-based sterilisation

All of these methods—boiling at temperatures over 100°C for 20–50 minutes, autoclaving at 121°C for 15–20 minutes, dry heat at 160–170°C for 2–3 hoursare effective. The most popular sterilisation technique is heat, namely saturated steam under pressure (Autoclaving) or hot air sterilisation, which are both time-tested and trustworthy. Endospores from bacteria are the most thermos resistant cells, hence eliminating them typically results in sterility. The sort and load of microorganisms, the media's composition and pH, and the size of the suspended particles all have an impact on how well heat sterilisation works. At a lower temperature (about 60°C in 5–10 minutes), vegetative cells are often destroyed quickly On the other hand, the removal of spores requires a higher temperature and a longer period (about 80°C for 15-20 minutes). Geobacillus stearothermophilus spores are the most heat-resistant ones (Wells-Bennik, 2019). Actually, this bacteria is used to examine the sterility of fermentation apparatus. To make sure that steam is permeating, these biological markers—which come in the form of spores in glass vials with liquid medium or spores on strips of paper within glassine envelopes—are positioned in locations where steam is difficult to reach.

Mechanism

Utilizing a microorganism's thermal vulnerability, thermal/heat sterilisation stops its growth. Heat kills microbes because it denaturates their enzymes and proteins.

Because proteins coagulate and are damaged by wet heat, microorganisms are killed by it. After chilling for one to two hours, sterilisation follows, and it takes between 20 and 60 minutes to complete (William, 2008).

Industrially

The autoclave media in a bioreactor are heated to 140°C for continuous sterilisation and 121°C for batch sterilisation. It takes a few hours (between two and four hours) for the bioreactor's contents to achieve the necessary temperature (120°C). The actual sterilising procedure will take about another 20–60 minutes.

Batch sterilisation: A batch sterilizer's internal coils may be injected with steam (indirect approach). At 121 degrees Celsius, the culture medium are sterilised in batches. It is quite expensive and wastes a lot of energy.

Constant sterilisation

By directly injecting steam into the system or via a heat exchanger, continuous sterilisation may be done. Either way, the temperature is quickly raised to 140°C and maintained there for 30 to

120 seconds (2minutes). In the continuous sterilising process, three different kinds of heat exchangers are used: For 20 to 30 seconds, the first heat exchanger raises the temperature from 90 to 120 degrees Celsius.

The temperature is increased to 140 degrees Celsius by the second heat exchanger and maintained there for 30 to 120 seconds.In the next 20 to 30 seconds, the third heat exchanger lowers the temperature by cooling the system.Around 80 to 90 percent of the energy is preserved during continuous sterilisation due to the several phases that are used, including the fermenter, exchanger, heater, heat maintenance unit, residual heat recovery, and cooling.

When put in the middle of stacked crates, a single 100ml takes 19 minutes to reach 121 even though it takes 12 minutes to do so when placed in a crate with other bottles.When complex media including peptides, carbohydrates, minerals, and metals are heated, nutrients are destroyed. Chemicals that are sensitive to heat, such vitamins and antibiotics, denaturize as a consequence of direct heat breakdown or an interaction between the medium's constituents.

Since short-duration, high-temperature treatments are more lethal to microorganisms and less chemically damaging than longer-duration, lower-temperature procedures, it is essential to optimise the heating process as a whole. For instance, compared to 20 minutes at 115 degrees and 10 minutes at 126 degrees, 3 minutes at 134 degrees are preferable.Volumes (ml) and times (minutes) for sterilising media in glass bottles, such as 19 minutes for 100 ml

500ml in 18 minutes

2 litres take 27 minutes at 121°C and 5 litres take 37 minutes at 121°C to cook (5000ml). The "heat up" and "cool down" durations are often integrated into the 121°C holding time to prevent overheating huge volume media devices. Because autoclave performance varies, thermocouple tests with various medium volumes should be carried out to gauge the heat-up and cool-down timeframes.

The sterilising procedure is divided into four stages:

A recording probe is inserted into the air-discharge value located in the chamber base to measure the chamber's heat-up time.

Stage 1: A recording probe is inserted into the air-discharge value at the chamber's base to measure the chamber's heat-up time (20–120 degrees Celsius).

Stage 2: Using thermocouples in the middle of the innermost container, the heat penetration time of the medium containers is assessed at temperatures ranging from 100 to 121 degrees Celsius.

Stage 3: The holding period is between 121°C and 121°C at the stated temperature (Stanbury, 2017).

The following significant variables affect the outcome of heat sterilisation: The effectiveness of heat sterilisation is greatly influenced by the kind and amount of contaminants (the extent of the load and type of any contamination) The media's composition of heat-labile compounds, the type

of the products, the suspended matter's particle size, the media's pH level, and the circumstances under which the final product was produced are all factors.

The rules for great manufacturing practise must be adhered to. Regardless of the sterilisation technique used, it must be verified for each kind of product or material in terms of sterility assurance and cost in order to ensure that no unfavourable alterations have taken place inside the product. A product that is tainted or degrading might come from not strictly following a declared, established technique.This technique for sterilising animal cell cultures is the most popular and commonly utilised. Vitamins, blood components, and antibiotics are examples of culture media components that are heat labile and will be destroyed by heat sterilisation; as a result, these medium components are completely dissolved before being filtration sterilised. If they are not entirely destroyed, germwill be eliminated as well. The most common technique of air sterilisation is through filtration; however, air sterilisation by heat is no longer used because of its high cost. Dry objects like medical equipment and pharmaceuticals are the most frequent targets for sterilisation. Plate media used in clean rooms are commonly disinfected using gamma radiation during sterilisation (ionizing radiation). Bottled media, such as that used for sterility testing, may be irradiated when provided to the sterility test area double wrapped. Gamma radiation refers to the high-energy radiations emitted by certain radioactive isotopes.

The radiation dosage must be selected to be high enough to kill microorganisms. Microorganisms can regenerate from a single surviving cell, unlike more complex organisms, and some microbial species' individual cells are extremely resistant to the lethal effects of radiation, outperforming other biological species' resistance by orders of magnitude (for example, Deinococcus radiodura, formerly known as micrococcus 9 radiodurans). To ensure that no viable creatures are left to repopulate, radiation doses must be employed that are far higher than those that are lethal to animals. Cobalt-60 is used as the radiation source most often when gamma irradiating material. A dosage of 15 to 25 KGy is often enough for prepared culture media, but some manufacturers have used doses as high as 40 KGy for fried granules. In contrast to steam sterilisation, the technique for estimating the dosage is based on figuring out the radiation sensitivity of the medium bioburden (here radiation resistant biological indicators are not used). To provide the necessary sterility assurance safety margin, a statistically estimated higher dosage is employed after evaluating sensitivity to a low radiation dose and estimating the mean bioburden. Each batch's gamma dose is measured after an initial assessment using DOSIMETERS, which provide parametric release.

The pH of certain media may alter as a consequence of radiation doses that are too high or too low, weakening the substance (the outcome of partial degradation of the polymeric structures of the afar). Other media, like MacConkey what and CLED (Cysteine Lactose Electrolyte Deficient) media, call for the addition of additives to make them radiation sterilizable. Examples include adding sodium thioglycollate as a radioprotectant to MacConkey what and CLED (Cysteine Lactose Electrolyte Deficient) media (the latter of which is used in clinical microbiology to screen for urinary tract infections).

To avoid destroying the chemicals in the Media and making it worthless, care must be used while deciding on the dosage and duration for irradiation. When establishing the necessary

dosage, it is crucial to consider the medium's growth-promoting characteristics both before and after irradiation. Gamma radiation may be replaced with ultraviolet light, which is more often employed for cell culture medium. Yen and colleagues claim that UV radiation works in a non-thermal, non-adulterating manner. About 2650 nm in length in areas where nucleic acids absorb the most UV light, pyrimidine dimers that restrict microbial DNA replication are produced, providing the highest bactericidal effectiveness. However, UV light is not always necessary due to its weak penetrating capability. A second use is electron beam radiation, which is utilised when gamma sterilisation takes too long or results in the degradation of specific media components.

Chemical Sterilization. Sterilants, which may be gases or liquids, are used to sterilise, pasteurise, or disinfect materials that are vulnerable to other techniques like radiation (gamma, electron beam, X-ray), heat (wet and dry), or other chemicals. It functions with a variety of materials. The fact that it can oxidise the overwhelming majority of organic compounds is a benefit. However, it is inappropriate for many uses since it is a dangerous and unstable gas that must be produced on site.

Glutaraldehyde and formaldehyde are suitable liquid sterilising agents if the immersion duration is long enough for these solutions (also known as fixatives). Hydrogen Peroxide—another chemical sterilising agent that is available in liquid and vaporised forms is hydrogen peroxide (VHP). A potent oxidant, hydrogen peroxide may destroy a variety of microorganisms.Ethylene oxide is the most often used chemical for sterilising, pasteurising, or disinfecting objects that are delicate to processing with other techniques like radiation (gamma, electron beam, X-ray), heat (wet and dry), or other Chemicals. It is compatible with a variety of materials. The sterilisation methods that are used in industry the most often include heat and filtration. Sometimes, filtration and heat sterilisation are used in conjunction. For instance, although the concentrated nutrient solution is heat sterilised, the water used to make the medium is filtered. To adequately dilute the medium, water is now added. Both chemical (disinfectants) and radiation (U.V. rays, y-gamma) techniques are used, however seldom.

Physical Techniques

Some of the physical processes employed include filtration, centrifugation, and adsorption (to activated carbon or ion exchangers). Of these, filtering is the most often used. Because they are heat labile, some culture medium components (vitamins, blood components, and antibiotics) are destroyed during heat sterilisation. Such medium components are thoroughly dissolved (required, else germs wouldn't be eliminated), and then filter sterilisation is applied.

Conclusion

To guarantee the sterility of the fermentation, all live organisms in the microbiological medium and the air required for microbial fermentation must be completely destroyed. The most generally used techniques for guaranteeing sterility are to guarantee the sterility of the fermentation, all live organisms in the microbiological medium and the air required for microbial fermentation must be completely destroyed. The most generally used techniques for guaranteeing sterility are filtration and heat sterilisation, respectively, for both air and media sterilisation. This

is because these techniques are the most practical and cost-effective, which will be of great help in the industries.filtration and heat sterilisation, respectively, for both air and media sterilisation. This is because these techniques are the most practical and cost-effective, which will be of great help in the industries.

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CHAPTER 20

ANALYSIS ON MICROBIAL BIOMASS PRODUCTION

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Abstract:

The conversion of a part of the substrate to biomass occurs incidentally in the majority of the fermentation processes previously covered, or it may be purposefully repressed for certain goals. The fundamental objective has been the transformation of substrate into a valuable primary or secondary metabolic product, such antibiotics, ethanol, and organic acids. The organisms created in these situations, after the ideal quantity of the desired product has been reached, are often just waste materials that must be disposed of properly and expensively, or are just utilised as a cheap source of animal feed. However, in specialised biomass production, the end products are the cells created during the fermentation process. The fermentation is then tuned to produce the highest possible concentration of microbial cells.

Keywords:

Acids, Biomass, Molasses, Microbial Protein.

Introduction

The conversion of part of the substrate to biomass occurs incidentally or may even be purposefully repressed for certain objectives in the majority of the fermentation processes previously covered. The fundamental goal has been the transformation of substrate into a useful primary or secondary metabolic product, such antibiotics, ethanol, and organic acids. When this occurs, the organisms created are often just garbage that must be disposed of properly and at a cost, or they are just employed as a cheap source of animal feed[1], [2]. The cells generated during the fermentation process. As a result, the fermentation is set up to produce the highest possible concentration of microbial cells. The three main uses of microbial biomass are as follows: 1 - viable microbial cells are prepared as starter cultures and inocula for food and beverage fermentations waste treatment procedures, silage production, agricultural inoculants, mineral leaching, and as biopesticides; 2 - as a source of protein for human food because it is frequently flavourless and odourless and can therefore be formulated into a variety of food items;

Microbial protein makes up a very small fraction of the total protein ingested in human diets. This largely originates from ingestible macrofungi (such as mushrooms, truffles, etc.), with a tiny amount coming from yeast in bread.To produce the enormous amount of baker's yeast needed to make bread and related bakery goods, a significant fermentation industry has emerged. One of the first techniques used for the industrial generation of *Saccharomyces cerevisiae* baking strains was known as the "skimming method." Similar to the fermentation procedures used in brewing and distilling, this technique utilised media made from cereal grains. Here, the yeast floated to the top of the fermentation and was skimmed off, cleaned, and press-dried. However,

the yeast industry looked for alternative fermentation sources during World War I owing to a lack of cereal grains. Molasses, ammonia, and ammonium salts were utilised in a technique developed in Germany to replace cereal-based medium[3], [4]. This still serves as the foundation for contemporary manufacturing techniques, which currently produce 2 109 kg globally year with some automation and modifications.Production of baker's yeast starts with the multiplication of a starter culture, which comes from a pure freeze-dried sample or an agar-medium culture. Before eventually being employed to inoculate the huge production fermenters of 50-350m3 capacity, yeast cells are first transported to tiny liquid culture flasks, followed by larger intermediate containers. To achieve the required final inoculum volume, this process may need up to eight scale-up steps[5], [6].

Molasses is often used as the carbon and energy source in the production fermentation medium. Molasses may also be processed with acid to eliminate sulphides and heated to precipitate proteins. Certain amino acids are often lacking in molasses, necessitating the need for biotin and pantothenic acid supplementation. The pH is then changed to 4.0-4.4 and other nitrogen sources (such as urea or ammonium salts) may also be added.The major goal of the procedure is to produce a large amount of biomass that has an excellent balance of features, such as a high fermentation activity and good storage qualities. Because nearly half of the carbon that is available for fermentation might possibly be converted to biomass, aerobic fermentation favours a high biomass output. The highest theoretical growth yield (Ys) is 0.54 g/g, however under anaerobic circumstances, this value is lowered to around 0.12 g/g.

Thus, keeping the cells aerobic is the goal. This is largely accomplished by vigorous oxygenation of the fermentation broth using an aeration system that must reach an oxygen transfer rate of 150mmol/L/h and is often raised as the fermentation develops. Additionally, the fermentation is carried out as a fed-batch method. To avoid the Crabtree effect, which occurs when substrate inhibits aerobic respiration (see Chapter 3), nutrients are given at a specified pace. This feeding schedule restricts anaerobic metabolism and ethanol synthesis, which otherwise would lead to reduced biomass outputs. Cell concentrations of up to 60g/L may be attained by maintaining a specific growth rate (m) of 0.2-0.25/h at 28–30°C.

The aeration is kept going for a further 30 minutes to "ripen" the yeast towards the conclusion of the development phase, when all the nutrients have been used up. In addition to decreasing protein and RNA synthesis and stabilising the cells to prolong storage life, this promotes the formation of the storage carbohydrate trehalose.The yeast is taken out of the fermentation broth using centrifugal separators, which must be spun at a minimum speed of 5000 revolutions per minute. Following several water washes and cooling to a temperature of 2-4°C, harvested cells are eventually dried using vacuum filter dehydrators to a moisture content of around 70–75% (w/w). Typically, yeast is stored in a refrigerator and is packaged in 1 kilogramme bricks. As an alternative, the yeast may be further dried to a moisture content of 7–10% (w/w), resulting in dry yeast, which can then be kept for extended periods of time without refrigeration. In all, the cycle of activities takes around 2 weeks, going through 60 generations from the first pure yeast sample to the yeast blocks that are ready for sale.

Literature Review

The developments in the area of sterilising in supercritical (SC) media during the last ten years are compiled in this overview. New methods for performing the sterilising process are detailed in particular, and the potential use of a variety of SC media is shown. Additionally, some information on the process of microbial inactivation is provided, and novel applications for SC sterilisation are described[7].

Öner *et al.* discussed various microbial polysaccharide production processes that make use of inexpensive biomass resources like syrups and molasses, wastewater from olive mills, cheese, various vegetable and fruit pomace, pulp, and kernels, as well as carbon dioxide and lignocellulosic biomass like rice hull and bran, sawdust, and fibres. A particular emphasis is placed on the pretreatment techniques that are used in these processes[8].

According to recent findings, a metabolic uncoupler may be helpful in lowering excessive sludge generation during the activated sludge process. Hiraishi *et al.* carried out an investigation was done to learn more about how various chemical uncouplers affected the growth of microbial biomass, metabolic activity, and community structure in a lab-scale activated sludge system fed with synthetic sewage. Results indicated that in all instances, the quantity of biomass generated reduced significantly as uncoupler concentrations rose. Respiratory quinone profiling at this point showed a considerable alteration in the organisation of the microbial population. According to these findings, administering an uncoupler for a brief period of time may help reduce excess sludge creation in the activated sludge process without significantly lowering BOD removal efficiency, although the long-term sustainability of this beneficial impact is in doubt[9].

Wang *et al.* carried out a research which intends to investigate the links between plant variety and soil microbial function, as well as the variables that mediate the correlations. Comprising natural and mine tailing soils, respectively, artificial plant communities with 1, 2, 4, and 8 kinds of plants were created. In both soil settings, the plant species richness had a favourable effect after a year on the functional diversity of the soil's microorganisms, while the natural soil's microbial biomass and basal respiration had a negative impact. The Dmix (over-function index) of aboveground biomass showed a positive correlation with the richness of plant species in the mine tailing soil, supporting the stress-gradient concept. The impacts of plant variety on the microbiological functions of the soil are influenced, according to the results, by the generation of root biomass. Due to root biomass production, which is regulated by other variables, soil microbial activity may respond to growing plant variety in different ways[10].

Discussion

In more recent years, the use of microbial protein has been looked at as a possible solution to the pressing demand for affordable protein in certain areas of the globe that agriculture there may not be able to provide. The rising population in emerging nations is the cause of the rising demand. Despite the clear need for and benefits that microbial protein offers over traditional protein sources, this goal has not been met. Producing luxury goods, meat replacements for the western diet, or animal feed has received more attention. Interest in microbial protein for animal feed is strongly influenced by production costs in comparison to the market leaders' average

prices, especially soy protein and fish meal. The current cheap cost of these conventional protein sources is the reason why less microbial protein is now generated for fodder. This might change, however, since soy and fish meal shortages have been predicted in the future.

In the 1960s and 1970s, microbial protein synthesis saw rapid advancements. Due to sharp rises in the cost of traditional animal feed, much research was done on a variety of microbes as potential substitute protein sources.The Massachusetts Institute of Technology initially used the term single cell protein (SCP) at this time. SCP, which refers to the whole cells of bacteria, yeasts, filamentous fungus, or algae, is not pure protein; it also includes carbohydrates, lipids, nucleic acids, mineral salts, and vitamins.

The particular microbe used and the fermentation procedure have a significant impact on the protein content and quality. Due of their high productivity and yields, aerobic microorganisms develop quickly are typically utilised. Compared to yeasts or filamentous fungus, bacteria often have a quicker rate of growth, a larger temperature range of growth, and a greater protein content. In comparison to filamentous organisms, yeasts develop very quickly and, like bacteria, have less fermentation issues due to their unicellular nature.However, many filamentous fungi can break down a variety of materials, and like yeasts, they can withstand low pH levels, lowering the possibility of microbial contamination. In comparison to yeasts or bacteria, they are also simpler to harvest at the conclusion of fermentation.The following factors must be taken into consideration when choosing an appropriate microbial strain for SCP production: performance (growth rate, productivity, and yields) on the specific, preferably inexpensive substrates to be used; temperature and pH tolerance; oxygen requirements, heat generation during fermentation, and foaming characteristics; growth morphology and genetic stability in the fermentation; ease of SCP recovery and requirements for furtility

Safety and acceptance are further important aspects

Currently, the majority of SCP products are utilised as animal feed rather than for human consumption. However, these goods must adhere to strict safety standards. The selection of the producing organism is undoubtedly influenced by the longer and more costly procedure of obtaining regulatory permission for the synthesis of proteins for human use. The nucleic acid composition of all SCP products must be taken into account while evaluating safety. The issue is made worse by the fact that many bacteria already have high levels, and fermentation conditions that favour quick growth and high protein content also encourage raised RNA levels. This might be a concern since purine molecules are produced when nucleic acids are digested by both humans and animals. As a consequence of their continued metabolism, uric acid levels in the blood are increased, which may cause kidney stones or cause gout-like symptoms when uric acid crystallises in the joints. It is important to check for any sluggish or indigestion of certain microbial cells in the stomach as well as any sensitivity or allergic responses to the microbial protein. The potential for aflatoxin formation in filamentous fungus must be ruled out. The ingestion of poisonous or cancer-causing chemicals, such as polycyclic aromatic compounds, which may be generated from certain growth substrates, is a further cause for worry.

Methods used in one-cell protein synthesis

Over the last 30 years, several pilot plants that make use of a variety of substrates and microorganisms have been created. Due to scaling-up challenges or for financial reasons, only a few number have really operated commercially. The physiological issues that are frequently encountered during scale-up include challenges with the following factors: 1) oxygen requirements and oxygen transfer rates; 2) nutrient and temperature gradients; 3) effects of CO2, as high levels may inhibit respiration in certain microorganisms; and 4) hydraulic pressure in deep fermenters.

In some cases, the economics of production can be improved by either raising the value of the product or lowering the production costs through: 1 the use of less expensive substrates; 2 increases in the organism's efficiency; 3 improved nutritional value/composition of the microbial protein; 4 marketing the protein as a premium product for human consumption rather than animal consumption; and 5 the production of other valuable byproducts, i.e. the development of a multiproduct procedure. Regardless of the carbon substrate or microbe employed, the SCP synthesis methods fundamentally have the same fundamental phases.

It could be necessary to physically or chemically prepare the major carbon source before usage. Before adding supplies of nitrogen, phosphorus, and other crucial nutrients, polymeric substrates are often hydrolyzed. According to the specific goals, the fermentation may be aseptic or conducted as a "clean" process. To fully use the higher productivity of continuous culture, continuous fermentations are often utilised, which are run at near to the organism's maximum growth rate (mmax). The cells may be treated to lower the amount of nucleic acids after being filtered or centrifuged from the used media. To render cellular proteases inactive, this often entails a heat shock. Retaining RNase activity causes RNA to be broken down into nucleotides that escape the cells. Prior to pasteurisation, dehydration, and packing, further purification, such a solvent wash, may be necessary depending on the growing medium utilised.

The different methods listed below have had some degree of commercial success and/or significant technical advancement. Over 80 million tonnes of whey are produced annually by the dairy industry globally. With a chemical oxygen demand of 60g oxygen per litre, this cheese manufacturing waste has a significant pollution burden. Therefore, it often has to be disposed of, which comes at a considerable capital expense for the dairy business. 10g/L of protein and 45g/L of lactose are both found in whey.Although efforts to culture other species, such as Penicillium cyclopium, have also been attempted, it is especially ideal for the manufacture of SCP using lactose-utilizing yeast. For the purpose of using the lactose in milk whey, many procedures have been devised.

Some of the most prosperous ones have been run by Bel Industries in France. The Bel method was created with the intention of creating a protein-rich product while simultaneously lowering the environmental burden caused by waste from the dairy sector. Protibel, a protein utilised in both human and animal consumption, is produced at a variety of facilities employing *Kluyveromyces lactis* or *K. marxianus* (formerly *K. fragilis*).

In Sweden, the Symba method was created to turn potato manufacturing byproducts into SCP for animal feed. As a standalone enterprise, it lacks economic appeal. However, since they have COD levels of over 20g of oxygen per litre and up to 3% solid content, alternative methods for the filtration of these waste fluids are challenging and costly. Starch makes up a large amount of the available substrate, which many bacteria cannot directly use. Two microorganisms that grow in symbiotic relationship were used in the process development to get around this issue. The hydrolytic enzymes required for starch breakdown are produced by the yeasts Candida utilis and Saccharomycopsis fibuligera, respectively.

The procedure is conducted in two parts. In the first step, a tiny reactor is used to cultivate S. fibuligera, which is fed with a nitrogen source and phosphate. The rate-limiting stage of the whole process occurs at this moment, when the starch is hydrolyzed. The resultant broth is then transferred into a second, 300 m3 capacity fermenter with both organisms already present. But C. utilis takes over the second stage and makes up to 90% of the final product. The Symba process runs constantly, and after 10 days, the trash has a 90% lower pollutant burden. The resulting biomass, which contains 45% protein, is concentrated by centrifugation before being spray- or drum-dried.

It was the first commercially successful, constantly running method for growing filamentous fungi, and it started working in 1975. It had to get through the unique challenges brought on by the pseudoplastic rheological behaviour of submerged cultures of fungal mycelium, which has a distinctive impact on oxygen transfer rates. The method was created in Finland to make use of acetic acid and monosaccharide-containing waste sulphite liquid from the processing of wood. Prior to the Paecilomyces variotii inoculation, additional carbon sources, often molasses, whey, and hydrolyzed plant wastes, may be introduced. Two 360m3 fermenters are used in this continuous process, which generates more than 10,000 tonnes of SCP annually. To create compounded animal feed, the resulting dried Pekilo protein, with up to 59% crude protein, is employed.

In the late 1960s and early 1970s, when the cost of traditional feed proteins was high and the price of oil was low, a large portion of the work was carried out by a number of oil corporations. These substances do, however, come with certain technological issues.Methane, in particular, is explosive when combined with oxygen because they are not miscible with water. Some of these substrates need to be purified as well, or the protein product that is made from them has to be treated to eliminate harmful substances that have been adsorbed. Furthermore, since these highly exothermic processes need a lot more oxygen than when carbohydrates are employed, these fermentations pose cooling and aeration concerns.

On these substrates, the majority of procedures were never developed beyond the pilot stage. But at least one such technique that was created more subsequently has shown to be rather effective. Methane-rich natural gas serves as the only source of carbon and energy for Methylococcus capsulatus development in the Bioprotein process, which Norferm invented in the 1990s. In order to stabilise the process, a variety of heterotrophic microorganisms are also present. The production facility was finished in 1998 and has a 10000-ton capacity per year. It is located in Tjedbergodden, Norway. This highly aerobic continuous fermentation is carried out in a loop-

fermenter using a mixture combining minerals, ammonia, and methane that is derived from North Sea gas sources. Centrifugation and ultrafiltration are used to continually capture biomass before being heated up and dried using a spray gun. Pronin is the name of the final product, which has a protein content of 70%. It has been given EU approval for use as fish and animal feed, yet it might one day be added to diets for humans.

The puppeteering process

The fact that methanol is entirely miscible with water and is accessible in a relatively pure state gives it significant benefits over methane and many other carbon sources. As a consequence, purification is not required for the final protein. Many oil and gas corporations created methods based on methane in the 1970s because it can be easily turned to methanol. Methanol as a substrate does, however, have significant drawbacks. The microorganisms that use it can tolerate it in only relatively low concentrations, 0.1-1.0% (v/v), and certain methylotrophic yeasts produce pseudo-mycelium when growing on methanol. Both the oxygen consumption and the fermentation's heat are considerable during the process. Even yet, compared to when methane or other hydrocarbons are used, the oxygen requirements are a little lower.

In the former Soviet Union, Japan, the USA, and Europe, attempts were made to create methanol-based processes. In addition to yeasts (*Candida boidinii, Pichia angusta,* and *Pichia pastoris*), they also included filamentous fungi (*Gliocladium deliquescens, Paecilomyces variotii,* and *Trichoderma lignorum*), bacteria (*Hyphomicrobium species, Methylococcus species*, and *Methylophilus methylotrophus*), and bacteria (Hyphomicrobium The technique created by ICI in the UK, which began manufacturing in 1980, was the most technically challenging. Pruteen, a feed protein for pigs, veal calves, and chickens, was created using the methylotrophic bacteria *M. methylotrophus* in this technique. Due to the rising cost of methanol, which accounted for 59% of manufacturing expenses, and the declining cost of soy meal, which was a competitor, production was stopped in 1987 for financial reasons. But given the improvements in fermentation design and technology that were achieved throughout its development, this procedure merits further study. Other than a few wastewater treatment systems, this was the biggest continuous aerobic bioprocess system ever built. It was a 3000m3 pressurecycle airlift fermenter with an inner loop and a working fluid capacity of 1.5 106 L that could produce up to 50 000 tonnes of pruteen annually. The fermenter, which cost US$80 million in 1979, has a pressure differential of 5 atm from top to bottom, is over 60 metres high, weighs more than 600 tonnes, and has such characteristics.

All streams entering the fermenter were disinfected, and compressed air that had been through a filter was utilised for both agitation and oxygenation. Using just commercial-grade inorganic fertilisers, the fermentation was carried out at a pH range of 6.5 to 6.9 and between 34 and 37°C. The methanol was fed via several distribution sites within the fermenter, and it was run as a chemostat with a methanol limit. A unique separation method was used to recover the bacterial cells. It included flocculation, which was aided by acid and heat shock, and starting concentration from 3% (w/w) to 12% (w/w). Centrifugal dewatering with water recycling and air drying were performed after that. There were 16% nucleic acids and more than 70% crude protein in the dried, unprocessed product.

Mushrooms

While certain mushrooms and other filamentous fungi's fruiting bodies are edible and an excellent source of protein, others are severely poisonous and have narcotic effects. There are several that have been historically used as food, but only a few number are raised economically. In actuality, less than 10 of the hundreds of edible species are commercially cultivated. A regulated non-axenic solid-substrate fermentation is required for mushroom production. Currently, it is the only lignocellulose fermentation product that is commercially viable.

In contrast to many other single cell proteins, these fruiting fungi are directly edible and many are regarded as delicacies due to their distinctive texture and flavour; (3) harvesting of fruiting bodies is the simplest method of separating edible biomass from the substrate in the production of edible biomass; and (4) they are examples of the most efficient conversion of plant wastes into edible food.

The agaricus bisporus

Over 90% of the overall value of mushroom output in Europe and the USA is attributable to Agaricus bisporus (the button mushroom). Agarics are naturally occurring organisms that break down plant detritus in meadows and forests. They are cellulosic material decomposers. Commercial cultivation of them takes place in temperate areas utilising decomposed straw as a substrate. When compared to other mushrooms, which might take months or even years to bear fruit, a crop is produced in only six weeks. Agaricus bitorquis, a species that is closely similar, is also cultivated in certain places. It is less susceptible to several viruses and the Pseudomonas tolaasii-caused bacterial blotch disease of mushrooms. The following phases make up the Agaricus manufacturing process.

A. Growing spawn (the inoculum) on sterile cereal grains is how the inoculum is prepared.
B. Composting straw, dung, and fertilisers at 60–70°C for two weeks constitutes the preparation of solid substrates (see Chapter 15, Composting).
C. Compost "peak heating," or substrate "sterilisation," for 5-7 days.

Conclusion

The transformation of nitrogen, phosphorus, sulphur, potassium, calcium, magnesium, manganese, and zinc into forms that plants can utilise depends on the microbial biomass. Plant nutrients would be "locked up" in dead plant and animal tissue if it weren't for soil bacteria. The generation of yeast for the baking industry and the creation of microbial cells for use as human food or animal feed are the two main operations that make up the commercial production of microbial biomass (single-cell protein).

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CHAPTER 21

COMPREHENSIVE STUDY ON INDUSTRIAL MATERIAL PURIFICATION

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Abstract:

The most crucial purification stage is to create reasonable standards. The elimination of all contaminants is often not required nor even desired, and a hasty attempt to do so may undermine attempts to remove impurities that are crucial for the intended application. For instance, potassium chloride is occasionally required that is sodium-free but not oxygen-free, and vice versa for other uses. The best way to remove salt is to provide oxygen, and vice versa; it is crucial to ascertain what is really required and prevent attempts that clash with one another. Purification may potentially backfire on itself if too complex techniques are used. Every used container and reagent adds new pollutants; in particular, the organic content of inorganic reagents is often disregarded and difficult to detect. To ensure that no step adds more than it subtracts, each one in the process must be carefully scrutinised. Here is a quick description of the typical purifying techniques. The detection of trace element contaminants in solid samples may be done using a variety of analytical techniques.

Keywords:

Contaminants, Chromatography,Microalgae, Purification.

Introduction

Purification seeks to restore the substance in an incredibly pure condition.The following steps are used to purify something: Sublimation: Some solids may go from the solid state to the vapour state directly, skipping the liquid phase. Sublimation is the term for the purification method that makes use of this feature. It is useful for distinguishing between compounds that are and are not sublimable. Crystallization: A chemical is said to crystallise when it goes from a liquid solution to a solid crystalline form. For low molecular weight compounds like antibiotics, this is employed. Crystallization that occurs as a result of evaporation, cooling, or crystallisation from a melt[1], [2].

Distillation is the method of utilising selective boiling and condensation to remove the constituents or compounds from a liquid combination. The separation produced by distillation might be virtually perfect (components are nearly pure), or it can be partial (the concentration of certain components in the mixture is increased). In either scenario, the procedure takes advantage of variations in the relative volatility of the mixture's constituent parts. Most often, fermented

products are separated via distillation. Chromatographic techniques are based on the idea that molecules in a mixture applied to a surface or a solid and a fluid stationary phase (stable phase) are separating while moving with the help of a mobile phase often used to separate low molecular mass compounds from mixtures of homologous antibiotics and macromolecules, for example (enzymes)[3]–[5].

Following are the several chromatographic techniques:

1. **Ion exchange chromatography:** Ion exchange chromatography relies on the electrostatic interactions between charged protein groups and solid support material (matrix). Protein affinity to the column is accomplished by ionic connections, and matrix contains an ion load that is opposite to that of the protein to be separated. Changes in pH, ion salt concentration, or ionic strength of the buffer solution are used to separate proteins from the column. Negatively charged proteins are adsorbed by anion-exchange matrices, which are positively charged ion exchange materials. Cation-exchange matrices, which adsorb positively charged proteins, are matrices coupled with negatively charged groups.
2. **Affinity chromatography**: Enzymes, hormones, antibodies, nucleic acids, and certain proteins may all be purified using this chromatography method. The column filler is bound by a ligand that may form a compound with a particular protein (such as dextran, polyacrylamide, cellulose, etc.). The particular protein that forms a compound with the ligand is bound to the matrix and kept in the column, whilst free proteins pass through the column. The bound protein then escapes the column by having the pH adjusted or by adding a salt solution, which alters the protein's ionic strength.
3. **Gas chromatology:**The stationary phase in this approach is a column that is inserted into the apparatus and contains a liquid stationary phase that has been adsorbed onto the surface of an inert solid. Gas-liquid chromatography is the same as gas chromatography. Gases such as HeL or N2 make up its carrier phase. An inert gas called mobile phase is transported through a column at intense pressure. The analysis sample vapourizes and transitions into the gaseous mobile phase. On the solid support, the sample's constituent parts are spread between the mobile phase and stationary phase. For the very good separation of very tiny molecules, gas chromatography is a straightforward, versatile, highly sensitive, and quickly implemented technology. The separation of very small quantities of analytes is accomplished using it.

Literature Review

Tang *et al.* highlighted that theMicroalgae are autotrophic organisms that make a variety of high-value bioactive substances from light energy, including proteins, lipids, and polysaccharides. Microalgae are used in many industrial fields nowadays because of their quick pace of development and ability to endure severe conditions. The selection of an appropriate microalgae strain, culture, and downstream processing of the biomass are the first steps in the process of generating microalgae-based biomolecules (i.e., pre-treatment, harvesting, extraction and purification). Biofuels and other important bioproducts are the operations' final byproducts. The increasing constraints in the scaling up of chemicals derived from microalgae biomass, however,

include poor production yield and expensive downstream procedures. To sum up, significant work is needed to overcome these obstacles in order to transform microalgae into an unique and environmentally friendly factory of various bioactive chemicals for industrial purposes to meet and fulfil worldwide demands[6].

In a study by Abubakar *et al.* they discussed onthe first and most important stage in producing high-quality research results is the preparation of medicinal plants for experimentation. Before moving further with the desired biological testing, it entails the extraction and assessment of the quality and amount of bioactive elements. This study's main goal was to assess the different approaches utilised in our routine research to prepare and screen medicinal plants. Finally, compounds are identified using a variety of identification methods, including nuclear magnetic resonance spectroscopy, infrared spectroscopy, ultraviolet spectroscopy, and mass spectroscopy. In order to direct young researchers and help them become more focused, the various approaches outlined above might be categorised and discussed in accordance with the anticipated biological testing[7].

In a study by Nachtigall *et al.*Streptomyces sp. C38, a strain isolated from a hyper-arid soil gathered from the Atacama Desert in the north of Chile, developed three novel 22-membered macrolactone antibiotics known as atacamycins A-C. The metabolites were extracted from the mycelium by extraction and chromatographic purification procedures after being found during our HPLC-diode array screening. Mass spectrometry and NMR tests were used to identify the structures. While atacamycin A shown a modest antiproliferative activity against adenocarcinoma and breast cancer cells, atacamycins A, B, and C showed considerable inhibitory effects against the enzyme phosphodiesterase (PDE-4B2)[8].

In Van den Meersche study, an ultra-high performance liquid chromatographic-tandem mass spectrometric (UHPLC-MS/MS) method was developed and validated for the rapid, straightforward, and selective simultaneous detection and quantification of colistin, sulfadiazine, trimethoprim, doxycycline, oxytetracycline, and ceftiofur as well as the detection of tylosin In swine dung samples taken from ten separate manure pits on farms where the chosen antibiotics were utilised, this approach was employed to detect the presence and concentration of the seven antibiotic residues. With the exception of ceftiofur, which is injected at low dosages and swiftly degrades in swine faeces and was not recovered in any of the samples, a connection between the antibiotics administered and identified was discovered. As far as we are aware, this is the only approach that can simultaneously extract and measure colistin while also measuring other antibiotic classes. Furthermore, colistin has never previously been taken out of swine dung. This method's simultaneous detection and measurement of five separate types of antibiotic residues in swine manure is another novel feature[9].

In the study by Noppe *et al.*, multi-residue chromatographic techniques for the detection of steroid hormones in edible matrices are reviewed. The most relevant EU regulation concerning the residual control of these chemicals is then provided after a short introduction to steroid hormones and their usage in animal fattening. The measurement of steroid hormones (oestrogens, gestagens, and androgens) uses a variety of multi-residue analytical techniques, including sample extraction and purification, chromatographic separation, and several detection

methods. This review highlights common themes and method variability. There was a focus on food matrices, particularly beef, liver, kidney, fat, and milk. Additionally, the prospects for cutting-edge analytical techniques are highlighted. The review gives particular focus to the identification of natural steroids. The analytical potential of phytosterols, naturally occurring steroid analogues derived from plants, and a particular class of steroid hormones with hemi-endogenous status are emphasised in the last section[10].

Discussion

Excellent analysis can only be accomplished with the right extraction, purification, and preconcentration given the existence of surfactants in environmental matrices and trace concentrations. The chromatographic approach is the favoured analytical technique despite these pre-treatment steps because of its progress and advantages over competing methods. According to the literature, the selection of remediation methods for surfactants is heavily influenced by factors such as cost, energy use, regeneration capacity, and the amount and composition of created sludge. The three most popular extraction processes are: liquid-liquid extraction; solid-phase extraction (also known as solid/liquid extraction); and acid-base extraction (also known as a chemically active extraction).

Supercritical fluid extraction, ultrasound-assisted extraction, heat-reflux extraction, and mechanochemical-assisted extraction are some more forms.

A. Extraction with microwave assistance
B. Instantaneous controlled pressure drop extraction (DIC; Détente instantanée contrôlée in French)
C. Representation

Liquid-liquid extraction (LLE) is a technique for separating substances or metal complexes depending on how well they dissolve in two distinct immiscible liquids, often water (polar) and an organic solvent (non-polar). There is a net transfer of one or more species, often from an aqueous to an organic liquid phase. The transfer is fueled by chemical potential, meaning that when it is finished, the system's chemical constituents, which include the solutes and solvents, are in a more stable configuration (lower free energy). Extract is the name for the solvent that has been enhanced with solute. The feed solution that lacks one or more solutes is referred to as the caffeinate.

In chemical labs, LLE is a fundamental procedure that is carried out using a range of tools, such as mixer settlers and separator funnels for countercurrent distribution. This kind of procedure is often carried out as part of the work-up after a chemical reaction, sometimes including an acidic work-up. Partitioning is a phrase that is often used to describe the underlying chemical and physical processes involved in liquid-liquid extraction, although it might also be construed as being completely synonymous with it. The process of separating a component from a mixture by dissolving it preferentially in a suitable solvent is sometimes referred to as solvent extraction. In such situation, an insoluble component or a complex matrix is separated from a soluble compound.

SPE is an extractive method that allows chemicals that are suspended or dissolved in a liquid mixture to be separated from other compounds in the mixture based on their physical and chemical characteristics. In the liquid/solid examples of coffee and tea, a chemical (caffeine) is separated from a solid combination using a liquid extraction solvent (water).

Solid phase extraction is a technique used in analytical labs to concentrate and purify materials for examination.From a broad range of matrices, such as urine, blood, water, drinks, soil, and animal tissue, interested analytes may be isolated via solid phase extraction.

SPE divides a mixture into desirable and unwanted components by using the affinities of solutes suspended or dissolved in a liquid (referred to as the mobile phase) and a solid (referred to as the stationary phase) through which the sample is passed. As a consequence, the stationary phase either retains the intended analytes of interest or undesirable sample contaminants. Depending on whether it includes desired analytes or undesirable contaminants, the part that goes through the stationary phase is either collected or discarded. If the required analytes are present in the part that was kept on the stationary phase, they may then be extracted from the stationary phase for collection in a subsequent step in which the stationary phase is washed with the appropriate eluent.

In order to separate acids and bases from mixtures depending on their chemical composition, an acid-base extraction process is used.In order to isolate chemicals and natural products like alkaloids from crude extracts, acid-base extraction is often carried out during the work-up after chemical syntheses. Most neutral, acidic, and basic contaminants are absent from the finished product. This straightforward approach cannot be used to distinguish between chemically identical acids or bases.

The basic idea behind this method is that neutral molecules are not often water soluble whereas ionic ions are more likely to be.

An organic base mixed with an acid will result in the base being protonated to produce a salt while the acid will stay unaltered. The self-ionization of the organic acid, such as a carboxylic acid, may be prevented by the additional acid if the organic acid is sufficiently weak.

In contrast, when an organic acid and base are combined, adding a base causes the base to stay unaltered while the acid is deprotonated to produce the equivalent salt. Once again, the extra base prevents a strong base from self-ionizing.

If the difference in the pKa (or pKb) constants of the extremely weak acids and bases is sufficiently great, the acid-base extraction process may also be employed to separate the weak acids from the stronger acids.In accordance with the extraction principle, extraction procedures include solvent extraction, the distillation process, pressing, and sublimation. The most used technique is solvent extraction. The following phases are involved in the extraction of natural products:

The solute diffuses out of the solid matrix after being dissolved in the solvents, which happens in steps one through four. The extracted solutes are then collected. The extraction will be made easier by any element that increases the solubility and diffusivity in the aforementioned

processes. The qualities of the extraction solvent, raw material particle size, solvent to solid ratio, extraction temperature, and extraction time all have an impact on how well materials are extracted.

In order to extract solvent, the solvent must be carefully chosen. In choosing a solvent, factors including selectivity, solubility, cost, and safety should be taken into account. According to the rule of resemblance and intermiscibility (like dissolves like), solvents with polarity values close to the polarity of the solute are probably going to perform better, and vice versa. In solvent extraction for phytochemical research, alcohols (EtOH and MeOH) are the standard solvents.

The extraction often produces better results the finer the particle size. Small particle size will increase extraction efficiency since it will allow for more solute diffusion and solvent penetration. However, if the particle size is too small, the solid will absorb too much solution, making the following filtering problematic.Diffusion and solubility are both increased at high temperatures. However, too high temperatures may result in solvent loss, which can remove unwanted contaminants and cause thermolabile components to disintegrate.With an increase in extraction time over a certain time period, extraction efficiency rises. Once the solute has achieved equilibrium both within and outside of the solid substance, extending the extraction period will not have any impact.The higher the solvent to solid ratio, the higher the extraction yield; however, a high ratio will result in surplus extraction solvent and take a long time to concentrate.The traditional extraction techniques, such as maceration, percolation, and reflux extraction, often involve organic solvents, call for a considerable amount of solvent, and take a long time to complete.

Modern or environmentally friendly extraction techniques, such as super critical fluid extraction (SFC), pressurised liquid extraction (PLE), and microwave assisted extraction (MAE), have also been used to extract natural products. These techniques have the advantages of using less organic solvent, taking less time, and having higher selectivity. However, several extraction techniques, such as effleurage, expeller pressing, and sublimation, are hardly ever utilised in modern phytochemical research.

Chromatography. Several possible commercial uses for contemporary biotechnology have failed in this field, either because the extraction process outsmarted the designers' inventiveness or—more likely—because it consumed so much energy that it was no longer feasible.For commercial distribution, the downstream purification processes' final products have to be somewhat stable. For the majority of items, some kind of drying is the best way to ensure stability. In actuality, spray drying, fluidized-bed drying, or freeze drying are used to accomplish this.

The product and cost factors influence the technique of selecting. A large number of products are available in dry form, including organic acids, amino acids, antibiotics, polysaccharides, enzymes, SCP, and many more. Many items must be marketed in liquid formulations instead of being simply available in dry form. It is important to take precautions to prevent microbiological degradation and contamination, as well as proteinaceous products' denaturation.

One of the trickiest and most demanding aspects of many biotechnological processes will always be the function of downstream processing. Most high-value biotechnological products stand out

for their purity and stability.It can be claimed that biotechnological processes will, for the most part, need to be confined inside a certain area or bioreactor, and, to a significant degree, the proper selection and management of these systems will determine the eventual success of most of the processes. For the majority of high-value goods, the producer organism will often be grown in a monoculture, necessitating full asepsis to maximise product production. The scale of the operation will almost always be very large on the industrial side due to economic considerations, highlighting the truly interdisciplinary nature of biotechnological processes. This will almost always necessitate close collaboration between the bioscientist, the chemist, and the process or biochemical engineer.

The last phase of downstream processing is this. It entails the elimination of tiny contaminants and is only used when an extremely high degree of purity is needed. This step involves the removal of small contaminants such denatured proteins and non-functional isoforms. The device performs lyophilization, sterile filtering, and desiccation (drying in a frozen state under vacuum) also spray drying Depending on the product, this phase could also include steps to sterilise it and eliminate or deactivate any trace pollutants. It offers the required product in a shape that is appropriate for final formulation and mixing.

The important steps are: separation of microbial cells (biomass / pellet) from the fermentation broth, concentration, metabolite-specific purification and final purification. Isolation of cells from the fermented broth is, in general, carried out by either centrifugation or ultra-filtration. Some cells rapidly settle out of suspension once aeration and agitation of the fermented broth ceases. The settling of cells may also be assisted by the addition of certain flocculating agents. Where cell settling does not occur, cell removal can be effected by centrifugation. An alternative to centrifugation is ultra-filtration. The term ultra-filtration describes processes in which particles significantly greater in size than the solvent are retained when the solution is forced through a membrane of very fine pore size, usually less than 0.5 mm. Microbial cells can be concentrated using ultra filtration so that the fermented broth is separated from cells.

The clarified fermentation liquor will contain microbial metabolites and extra cellular enzymes. Several methods are available for recovery of metabolites such as precipitation, solvent extraction and ion exchange chromatography.

Different downstream operations are available for concentration as well as purification of the metabolite. But it is always advisable to use lesser number of steps to achieve desired purity of the metabolite or product. This is because, more the number of steps involved, more will be the cost of the production and lower would be the yield.In order to get the desired metabolite once fermentation has finished, recovery is required. The fermentation broth and cells will need to be separated as a minimum in order to do this. However, it may also include the purification of the metabolite, either with or without causing damage to the cells; if the metabolite is intracellular, cell disruption will be essential. Upstream processing describes these activities. One must first separate the cells from the fermented broth, then either disturb the cells if the product is intracellular or concentrate the broth if it is extracellular to isolate the desired microbial product. (3) Initial metabolite purification, (4) Metaboite-Specific Purification, where the target

metabolite is highly purified, and (5) Polishing of the target metabolite (bringing it to 98–100% purity), where it is further concentrated and prepared for usage.

Conclusion

It could be challenging and expensive to extract and purify fermentation products. The ideal situation is to acquire a high-quality product as rapidly as feasible, with an effective recovery rate, and with the least amount of plant investment, run at the lowest possible cost. Sadly, the recovery costs of microbial products might range from 15% to 70% of the entire production expenses. The particular product will, of course, determine the procedure that is used and, therefore, how much it costs. 80–90% of the entire processing expenses may be attributed to the extraction and purification of products such recombinant proteins and monoclonal antibodies. The ultimate goal in certain fermentations will be impacted by the high (and sometimes dominating) expense of downstream processing.

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CHAPTER 22

A REVIEW ON BIOPROCESS TECHNOLOGY

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Abstract:

Modern bio-process technology is an extension of ancient techniques for developing useful products by taking advantage of natural biological activities. Earliest example of bioprocess is alcoholic beverages that are a combination of yeast cells and nutrients (cereal grains) to form a fermentation system in which the organisms consumed the nutrients for their own growth and produced by-products (alcohol and carbon dioxide gas) that helped to make the beverage.

Keywords:

Bioprocessing, Fermentation,Microorganisms, Technology.

Introduction

Bioprocessing is the use of biological materials (organisms, cells, organelles, enzymes) to carry out a process for commercial, medical or scientific reasons. Bioprocess operations should ideally manufacture new products and destroy harmful wastes. Use of microorganisms to transfer biological material for production of fermented foods has been an essential part of many foods, chemical and pharmaceutical industries. Since then, bioprocesses have been developed for an enormous range of commercial products, from relatively cheap materials such as industrial alcohol and organic solvents to expensive specialty chemicals such as antibiotics, therapeutic proteins and vaccines[1], [2].

Advantages of bioprocessing:

A. They are specific in their action
B. They are extremely efficient
C. They are biodegradable
D. Safer
E. They work in mild conditions and thus energy saving.

Tools of modern biotechnology such as recombinant DNA, gene probes, cell fusion and tissue culture offer ways to produce new products or improve bioprocessing methods. Modern Biotechnology has allowed us to envision sophisticated medicines, cultured human tissues and organs, biochips for new-age computers, environmentally-compatible pesticides and powerful pollution-degrading microbes[3], [4].

Modern biotechnology was started in the 19th century when knowledge about biological system, their components, and interaction between them grew. In the first half of the 20th century the first large scale fermentation processes, namely citric acid and penicillin, were realized. The process of recombinant gene technology then led to a substantial increase in the number of bioprocesses and their production volume starting with insulin, the first product manufactured with recombinant technology, in the early 1980s.

The development of genetic engineering and monoclonal antibody technology, which started in the 1970s, has led to the introduction of a large number of new products with application in many different areas. The most highly visible applications have been in the area of human health care, with product such as human insulin, interferon's, tissue plasminogen activator, erythropoietin, colony-stimulating factors, and monoclonal antibody-based products.New products for agriculture, food industry, fine chemicals and the environmental protection are also under intense development.

Today, the bio-industries have reached a critical size and are additionally based on a broad understanding of genomics, proteomics, bioinformatics, genetic transformation and molecular breeding. However, this knowledge waits to be transfer to technology and market products. The knowledge of molecular breeding, stem cell technology and pharmacogenomics might lead to strongly personalized therapies and therapeutics.

Industrial Fermentation Products & Producer Organisms

The word fermentation comes from the Latin verb fevere, which means to boil. It originated from the fact that early at the start of wine fermentation gas bubbles are released continuously to the surface giving the impression of boiling. It has three different connotations when used in industrial microbiology

a) The first meaning relates to microbial physiology. In strict physiological terms, fermentation is defined in microbiology as the type of metabolism of a carbon source in which energy is generated by substrate level phosphorylation and in which organic molecules function as the final electron acceptor (or as acceptors of the reducing equivalents) generated during the break-down of carbon-containing compounds or catabolism.
b) The second usage of the word is in industrial microbiology, where the term 'fermentation' is any process in which micro-organisms are grown on a large scale, even if the final electron acceptor is not an organic. Thus, the production of penicillin, and the growth of yeast cells which are both highly aerobic, and the production of ethanol or alcoholic beverages which are fermentations in the physiological sense, are all referred to as fermentations.
c) The third usage concerns food. A fermented food is one, the processing of which microorganisms play a major part. Microorganisms determine the nature of the food through producing the flavor components as well deciding the general character of the food, but microorganisms form only a small portion of the finished product by weight.

Types of En Products of Fermentation

Types of end products of fermentation include:

1. Microbial cells (e.g. bacteria, yeast, fungal spores)
2. Microbial enzymes (e.g. milk clotting enzymes or rennets, recombinant fungal and bacterial rennets for cheese manufacture)
3. Microbial metabolites (e.g. alcohols– ethanol, butanol, 2, 3-butanediol, isopropanol; chemicals– lactate, propionate, proteins, vitamins, antibiotics; and fuels– methane)
4. Recombinant products (e.g. hormones)

Products of industrial microorganisms may also be divided into two broad groups, those which result from primary metabolism and others which derive from secondary metabolism.

Products of primary metabolism

Primary metabolism is the inter-related group of reactions within a microorganism which are associated with growth and the maintenance of life. Primary metabolism is essentially the same in all living things and is concerned with the release of energy, and the synthesis of important macromolecules such as proteins, nucleic acids and other cell constituents. When primary metabolism is stopped the organism dies. Products of primary metabolism are associated with growth and their maximum production occurs in the logarithmic phase of growth in a batch culture. Primary catabolic products include ethanol, lactic acid, and butanol while anabolic products include amino-acids, enzymes and nucleic acids. Single-cell proteins and yeasts would also be regarded as primary products.

Literature Review

In a study by Neethirajan*et al.* it was discussed thatNanotechnology has the potential to address a number of intricate technical and scientific problems in the food and bioprocessing sectors that must be resolved in order to produce high-quality, safe food in an effective and sustainable manner. Examples of recent developments in nanotechnology's use in the food business include the use of biosensors for bacteria detection and food quality monitoring, intelligent, active, and smart food packaging systems, and the nanoencapsulation of bioactive food components. We examine the history of nanotechnology's potential, provide a summary of its present and prospective uses in the food and bioprocessing sectors, and discuss the social ramifications of its successful deployment[5].

Ibrahim *et al.* talked about the Interest in replacing non-renewable and decreasing gasoline fuel with sustainable and renewable biobutanol is growing. Butanol is in demand as a chemical substitute for a variety of sectors in addition to its advantages over bioethanol as a transportation fuel. As a result, a lot of researchers have looked at ways to make biobutanol at a cheap cost by taking into account appropriate feedstock materials and bioprocessing technology. The synthesis of biobutanol often uses renewable resources including starch, lignocellulosic, and algal biomass, each of which offers benefits of its own. Multiple fermentation processes and integrated bioprocessing technologies have been used to address the drawbacks of standard batch fermentation, increasing the efficiency of biobutanol synthesis. The adaptability of the

fermentation operation to correlate with the microbial behaviour as well as bioprocessing techniques is necessary to optimise the whole process and make it sustainable on an industrial scale. This study thus analyses the bioprocessing technologies and appropriate approaches that have attempted to increase the production of biobutanol from renewable biomass[6].

Mahar *et al.* investigated the the separation, isolation, and purification of the biotherapeutics, whether they be recombinant proteins, vaccines, gene therapy, or cell therapy products, need the use of filtration as a conventional unit operation or in combination with other unit operations. This article will discuss the various filtering techniques used in operations involving both direct flow and tangential flow, with an emphasis on handling both liquids and gases. The operational facets of these filtering procedures will be covered in great depth. Filtration, direct flow, tangential flow, integrity testing, depth filtration, membrane filtration, spiral wound, cassettes, plate and frame, hollow fibre, monolith, alternating tangential flow, microfiltration, diafiltration, nanofiltration, and reverse osmosis are some of the words that are important to know[7].

Zaidi *et al.* studied the Tyrosinase which is a naturally occurring enzyme that is frequently only minimally purified and involved in a number of processes, the main ones of which are the o-hydroxylation of monophenols into their corresponding o-diphenols and the oxidation of o-diphenols to o-quinones with the use of molecular oxygen, which polymerizes to produce brown or black pigments. Tyrosinase has garnered a lot of interest with regard to industrial applications due to its potential use as a catalyst for the production of o-diphenols. This study combines the most current information on the molecular and biochemical characteristics of microbial tyrosinases, highlighting the significance of these characteristics for the commercial use of these enzymes. Then, the most exciting uses of these technologies in the sectors of pharmaceutical, food processing, and environmental science are discussed[8].

Yadav *et al.* investigated the Cheese, yoghurt, bread, wine, and beer may all be produced by food producers using a natural, secure, and efficient technique called bioprocessing. In bioprocessing, new products are made using live things and their parts. They employed bacteria and enzymes in bioprocessing technology, and these conditions-neutral pH, normal air pressure, and temperatures around room temperature-brought forth the greatest results. As a result, bioprocessing may significantly reduce energy consumption in the food sector, particularly when it replaces the need to heat items to high temperatures. Additionally, bioprocessing may improve flavour and texture. Additionally, the biodegradable bacteria utilised in bioprocessing help to further lower the carbon footprint of food production. The quality and flavour of food are developed through conventional food bioprocessing techniques like drying, fermentation, salting, and various cooking methods like roasting, frying, smoking, steaming, and oven baking, but these techniques are unable to protect against the microorganisms that cause food spoilage. Therefore, nanotechnology has become a priority in the food industry today, which lengthens the period before food spoils. Additionally, nanotechnology shown potential uses in the storage, quality control, food processing, and packaging stages of the food chain[9].

In two studies, groups of Friesian bull calves (average live weight at the start of the study: 142 or 150 kg) were given Winfred or Wairoa forage brassica (62 and 67% leaf, respectively) at 3, 6, 9, 12 or 15% body weight over a period of six weeks. Each week, calves received new allowances

based on their weekly weight. Between January and March of the same year, the two trials were carried out. Compared to calves given greater quantities of brassica (1.2 kg/day vs. 0.9 kg/day), calves fed Winfred brassica at 3% body weight saw poorer liveweight growth. Calves eating the meagre allotment of Winfred brassica (3% body weight) consumed 77% of the crop overall but just 43% of the stem. Calves who were given a modest amount of Wairoa brassica to graze on used 90% of the entire crop and 72% of the stem, and they gained liveweight at a rate that was comparable to that of calves that were given bigger amounts (on average, they gained 1.03 kg per day). Brassicas seemed to need a four-week acclimatisation time. Calves that were grazing on Winfred and Wairoa saw daily growth rates of 1.06 and 0.54 kilogrammes during the first four weeks and 1.50 and 1.94 kilogrammes per day during the last two weeks. If short grazing/finishing times are needed, the rate of adaption to brassica may be a crucial factor[10].

Equipment for single-use bioprocessing has advanced significantly during the last ten years. These technologies now rule small- and medium-sized bioprocessing, and they are beginning to advance to larger-scale productionLanger *et al.*covered the advantages of single-use vs. fixed stainless steel for almost ten years of cumulative industry expertise. Most people agree that single-use systems make it possible to quickly set up bioprocessing at various sizes in the same production area. Concerns and considerable unknowns concerning single-use equipment existed ten years ago. Modern versions of these materials and devices have cutting-edge design, multilayer laminated plastic bags, and other integrated technology. This article analyses market dynamics and trends for single-use bioprocessing systems during the last ten years and makes predictions for the future. A large portion of this is factual and supported by information from the yearly survey of bioprocessing experts carried out by BioPlan Associates, Inc., now in its eleventh year. This yearly report contains considerable discussion and outside analysis of bioprocessing and associated trends in addition to quantitative survey data. The information in this article also comes from other original research sources. Capacity constraints are becoming less frequent as a result of single-use and modular technologies; in fact, evaluating present and forecast industrial capacity may become subjective and perhaps useless[11].

Discussion

At the most fundamental level of life, the genetic, we can now control it. People have engaged in selective breeding and genetic engineering for thousands of years. But today, with the molecular manipulation of DNA, it can be done in a deliberate, premeditated way. With the use of a technology that was unthinkable 25 years ago, we may now explore the secrets of life.With this intellectual revolution come new visions and hopes for the future, including a variety of consumer goods and industrial processes, semi-synthetic organs grown in large vats, abundant and nutrient-rich foods, computers based on biological molecules rather than silicon chips, superorganisms to degrade pollutants, and new medicines.Without effort, these goals would always stay unattainable. In order to make these ideals a reality, engineers will be crucial. Even though biological systems are very intricate and well-designed, they nonetheless adhere to the laws of physics and chemistry and may be studied using engineering principles. Living cells are predictable, and the commercial-scale procedures to utilise them may be logically designed. The task of the bioprocess engineer is to carry it out.

Old terminology are often inadequate to describe new disciplines that arise from new concepts. Examples are preferable to single words or brief sentences when describing biotechnology and what engineering in this discipline entails.

Biotechnology often refers to the application of or research into direct genetic alteration techniques for a socially acceptable outcome. These objectives might include making a specific chemical, but they could also include making better plants or seeds, using gene therapy, or using organisms created especially to break down trash. The employment of advanced methods outside the cell for genetic alteration is the crucial component for many professionals. Some people define biotechnology much more broadly and compare it to applied biology; they could classify engineering as a subset of biotechnology.

Engineers who deal with biotechnology have been referred to by a variety of terms

Agricultural, electrical, mechanical, industrial, environmental, and chemical engineers, among others, practise bioengineering, a vast field that encompasses work on medical and agricultural systems. Similar to chemical engineering, biological engineering focuses on applications to plants and animals. It has often been understood that biochemical engineering refers to the application of chemical engineering concepts to systems that use a biological catalyst to effect desired chemical transformations. Bioseparations and bioreaction engineering are two common divisions. Although the distinction between biochemical and biomedical engineering has traditionally been regarded to be absolute, it is becoming hazier, especially in the fields of cell surface receptors and animal cell culture. The National Institutes of Health has described biomolecular engineering as "research at the intersection of biology and chemical engineering and is concentrated at the molecular level," which is another pertinent word.

Biochemical engineering and bioprocess engineering are two distinct fields

Bioprocess engineering would also involve the effort of mechanical, electrical, and industrial engineers to apply the concepts of these fields to processes based on employing live cells or components of such cells. This is in addition to chemical engineering. Principles from these disciplines may be applied to the issues of intricate equipment design, sensor development, control algorithms, and production methods. In that it largely applies chemical engineering concepts, biochemical engineering is more constrained than chemical engineering, but it is also more expansive since it may be used to improve natural systems rather than being confined to well define artificial processes.

With an emphasis on systems using biotechnology, we will largely concentrate on how chemical engineering concepts may be applied to systems incorporating biological catalysts. The design and monitoring of bioprocesses now have new options thanks to the fast growing capacity to determine the whole sequence of the genes in an organism. Now, the cell itself may be designed as a part of the larger process.

Biologists and engineers have quite diverse educational backgrounds. In contrast to chemistry and physics, the development of mathematical theories and quantitative approaches (apart from statistics) has been less important in the biological sciences. Most advancements are a result of

better experimental instruments. Results are qualitative, and models for describing phenomena are developed and evaluated. Because of this, biologists often have weak mathematical backgrounds but are quite adept at using laboratory equipment and, more crucially, in interpreting data from complicated systems collected in the laboratory.

Engineers often have extensive training in the physical and mathematical disciplines. Mathematical formulations are often the result of a theory, and the accuracy of the theory is checked by contrasting expected results with experimental results. Even for complicated systems, quantitative models and methods have advantages. When it comes to creating testable hypotheses, designing experiments, and interpreting data from complicated systems, biologists often do better. The experimental methods and approaches utilised by life scientists are often unknown to engineers.

Engineer and life scientist talents complement one another. The combination of these abilities is necessary to translate molecular biology's promises into novel manufacturing procedures and new products. The engineer requires a firm grasp of biology and the techniques used in its experiments in order to perform at this level. The biological foundation we present in this book is adequate for you to grasp the chapters on applying engineering ideas to biosystems. To become a bioprocess engineer, you must complete additional courses in microbiology, biochemistry, and cell biology in addition to more difficult biochemical engineering tasks. These chapters may be utilised for review if you've previously taken these courses.

A bioprocess is a particular process that produces desired products by using whole live cells or parts of them (such as bacteria, enzymes, or chloroplasts).

Many biological and environmental systems depend on the movement of mass and energy. Understanding how energy and mass can be transported through materials is necessary for a variety of applications, including food processing (including the brewing of beer), thermal design of buildings, biomedical devices, the production of monoclonal antibodies, and pollution control and global warming (momentum, heat transfer, etc.).

Cell therapy bioprocessing, a branch of bioprocess engineering, unites the sciences of cell treatment and bioprocessing (i.e., the production of biopharmaceuticals). The establishment of reliable and repeatable manufacturing procedures for the production of therapeutic cells is the aim of cell therapy bioprocessing. Commercially viable bioprocesses will: Create goods that adhere to all biopharmaceutical medication quality standards. Throughout the different phases of development, provide therapeutic cells in both clinical and commercial numbers. Scalable manufacturing procedures and technology are required, andControl the cost of goods (CoGs) for the finished drug product. Building the basis for a commercially successful enterprise depends on this factor.

Upstream and downstream processes may be distinguished in the manufacture of therapeutic cells. The whole procedure from early cell isolation and cultivation through cell banking and culture multiplication of the cells to the last harvest is referred to as the upstream process (termination of the culture and collection of the live cell batch). In addition to technological difficulties with regard to the scalability of culture equipment, a variety of raw material supply

problems, such as the accessibility of GMP grade foetal bovine serum, have developed recently. The first stage of a bioprocess, during which microorganisms or cells are produced in bioreactors, such as bacterial or mammalian cell lines (see cell culture), is referred to as the upstream portion. Upstream processing includes all processes connected to inoculum creation, medium development, inoculum enhancement via genetic engineering, and growth kinetics optimization so that product development may be greatly enhanced. Upstream and downstream are the two phases of fermentation. Purification of the product for the required quality comes next after product development. For batch and fed-batch cultures, they are harvested and transferred to the downstream stage of the bioprocess when they achieve the necessary density.

Downstream bioprocessing

The portion of a bioprocess known as the downstream is where the cell mass from the upstream is treated to fulfil purity and quality standards. Cell disruption, purification, and polishing are the three basic divisions of downstream processing. By distilling the harvested culture without pre-treatment, the volatile compounds may be isolated. Continuous stills distil at decreasing pressure. Direct distillation of the product from the fermentor may be accomplished at low pressure. The stages of downstream processing are:

Biomass separation: Centrifugation or ultracentrifugation is often used to separate the biomass (microbial cells). If the product is biomass, it is collected for processing and the used medium is thrown away. The biomass will be thrown away if the product has excess cells. The alternative to centrifugation is ultra-filtration.Cell disruption: If the desired product is intracellular, it may be possible to disturb the cell biomass in order to release the product. Cell waste is eliminated after the solid-liquid mixture has been centrifuged or filtered.Broth concentration: If the final product is extracellular, the wasted medium is concentrated.Initial metabolite purification: Several procedures for recovering the product from the clarified fermentation broth were utilised, depending on the physico-chemical composition of the product molecule (precipitation, etc.)

De-watering: When a little quantity of product is present in a very high volume of used medium, the volume is lowered by eliminating water to concentrate the product. Reverse osmosis or vacuum drying are used to accomplish.Metabolites must be polished in order for the product to be 98–100% pure. Excipients are a group of inert substances that are combined with the purified product. The finished product is packaged and distributed to customers in the market.

Conclusion

The food, chemical, and pharmaceutical sectors all depend heavily on bioprocessing. To create new goods and eliminate dangerous waste, bioprocess operations utilise cells from microorganisms, animals, plants, and even parts of those cells like enzymes. It dates back to antiquity that biological elements were transformed by microbes for the formation of fermented meals. Since then, bioprocesses have been created for a huge variety of commercial goods, from inexpensive materials like industrial alcohol and organic solvents to pricy speciality compounds like antibiotics, therapeutic proteins, and vaccines. Commercial byproducts of bioprocessing also include live organisms like yeast and enzymes that are beneficial for industry.

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CHAPTER 23

EXPLORATION OF ANIMAL CELL CULTURE

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Abstract:

Today, cell culture is one of the most important methods utilised in the biological sciences. This manual aims to provide a fundamental introduction to animal cell culture. It is suitable for laboratory workers who are using it for the first time as well as for anyone who engage with cell culture experts and want to better comprehend the fundamental ideas and jargon in this fascinating and quickly developing subject.

Keywords: Animal cell culture, Cells, Cell lines, Suspension Culture, Vaccines.

Introduction

The process of removing cells, tissues, or organs from an animal or plant and putting them into a synthetic environment where they may develop is known as tissue culture. An appropriate glass or plastic culture vessel filled with a liquid or semisolid media that provides the nutrients necessary for survival and development often serves as this habitat. Organ culture is the process of growing complete organs or intact organ parts for the purpose of examining their ongoing growth or function. Cell culture refers to the process wherein the cells are separated from the organ pieces either before or during cultivation, breaking their natural interactions with one another[1], [2].

Ross Harrison successfully cultivated animal cells for the first time in 1907, but it wasn't until a number of advancements in the late 1940s and early 1950s that cell culture became a common tool for scientists. First, antibiotics were created, which made it simpler to avoid many of the contamination issues that hampered early efforts at cell culture. The creation of the methods required to produce continually growing cell lines, such as the use of trypsin to remove cells from culture containers, came in second (such as HeLa cells). Third, researchers were able to create uniform, chemically defined culture conditions that made it much simpler to grow cells using these cell lines. Many more scientists are now able to employ cell, tissue, and organ culture in their research because to the combination of these three fields. Commercialization of this technique had other effects on cell culture throughout the 1960s and 1970s that are still felt today. Companies like Corning started creating and marketing disposable glass and plastic cell culture hoods, liquid and powdered tissue culture medium, and enhanced filtration tools. Today, there are a growing number of labs and companies adopting cell culture as a consequence of these and other ongoing technical advancements[3], [4].

Primary Culture

A cell's ability to adhere, divide, and develop is determined by the environment it is put in after being surgically removed from the organism. A Primary Culture is what is meant by this. In order to achieve this, there are two primary approaches. Small fragments of tissue are first affixed to a glass or specially treated plastic culture vessel for explant cultures and then submerged in culture media[3]–[5].

Individual cells will eventually leave the tissue explant and go to the surface or substrate of the culture vessel, where they will start to divide and develop, after a few days. The second, more popular technique, quickens this process by incorporating digestive (proteolytic) enzymes into the tissue pieces to break down the connective tissue between the cells, including collagenase or trypsin. Single cells are then created as a suspension and put into culture vessels containing culture media where they are allowed to multiply. Enzymatic dissociation is the technique's name[6].

Subculturing

The main culture vessel's cells must be subcultured to provide space for more growth after they have expanded and consumed all of the culture substrate. The typical method for doing this is to use enzymes to gently remove them from the substrate.

They are used to dissolve the protein bonds holding the cells to the substrate and are identical to the enzymes utilised to create the primary culture. Gently scraping the cells off the bottom of the culture jar may be used to harvest certain cell lines. The cell suspension may be split into smaller pieces and added to fresh culture containers once it has been released. When there is an excess of cells, they may be treated with appropriate cryoprotective substances, such as dimethylsulfoxide (DMSO) or glycerol, properly frozen, and kept at cryogenic temperatures (below -130°C) until they are required. The Corning Technical Bulletin: General Guide for Cryogenically Storing Animal Cell Cultures covers the theory and procedures for cryopreserving cells.

Purchases and Lending

It is also possible to purchase pre-existing cell cultures from institutions like the ATCC (www.atcc.org) or the Coriell Institute for Medical Research as an alternative to starting cultures from scratch (ccr.coriell.org). The high-quality cell lines that are provided by these two nonprofit organisations are rigorously examined to confirm the cells' validity.

Researchers will commonly receive (borrow) cell lines from other labs. Although common, this approach has one significant flaw. The likelihood that the cells acquired in this way won't be healthy, practical cultures is quite high. Typically, this is the consequence of prior muddles, contamination with other cell lines, or contamination with pathogens such mycoplasmas, bacteria, fungus, or yeast. A Corning Technical Bulletin: Understanding and Managing Cell Culture Contamination addresses these issues in depth.

Cells display a vast diversity of behaviours, traits, and forms once they are in culture. The following describes some of the most typical ones.

Systems for Cell Culture

Cell growth is accomplished using two fundamental culture methods. These are essentially dependent on the cells' capacity to develop either attached to a glass or plastic substrate that has been treated (Monolayer Culture Systems) or dangling freely in the culture medium (Suspension Culture Systems).

Typically, suspension cultures are either:

1. In stationary culture containers like T-flasks and bottles where, while not being constantly agitated, the cells are unable to securely adhere to the substrate;
2. In magnetically spun spinner flasks or shaken Erlenmeyer flasks where the cells are kept actively suspended in the media.

Many cell lines are thought to be anchorage-dependent, meaning that they can only develop when connected to a suitable substrate. This is particularly true of those generated from normal tissues.

Some cell lines that have stopped being regarded as normal (sometimes referred to as Transformed Cells) may commonly grow either attached to a substrate or floating free in suspension; they are Anchorage-Independent. Additionally, certain healthy cells, like those in the blood, constantly develop in suspension and don't often adhere to substrates.

Variety of Cells

Usually, the morphology (form and appearance) or the functional properties of cultured cells are used to characterise them. Three fundamental morphologies exist:

1. Cells that resemble epithelial tissue are connected to a substrate and have a flattened, polygonal appearance.
2. Cells that resemble lymphoblasts: spherical cells that are suspended in suspension and do not adhere to a substrate ordinarily.
3. Cells that are linked to a substrate, seem elongated and bipolar, and typically form swirls in dense cultures are referred to as fibroblast-like cells.

It is crucial to keep in mind that shape is significantly influenced by the culture conditions and that many cell cultures may display a variety of morphologies.

It is also feasible to create hybrid cells by fusing cells from two separate parents utilising cell fusion procedures. These could show traits from one parent or both parents.

In 1975, cells that could produce monoclonal antibodies with a specific purpose were made using this method. These hybrid cells, also known as hybridomas, are created by merging two unrelated yet similar cells. The first is a lymphocyte generated from the spleen that can produce the necessary antibodies. The second is a myeloma cell, a kind of cancer cell that divides quickly and has the ability to generate antibodies but is not designed to do so. Large amounts of the required antibody may be produced by the ensuing hybridomas. Due to their purity, these

antibodies—known as monoclonal antibodies—have several significant therapeutic, diagnostic, and industrial uses and have an annual market worth of well over $1 billion.

Literature Review

Nema *et al.* investigated the animal cell culture is now a more important and versatile application tool for ongoing research streams. A wide range of fields, including stem cell biology, IVF technology, cancer cell biology, the creation of monoclonal antibodies, recombinant proteins, gene therapy, the manufacture of vaccines, and the selection and enhancement of innovative drugs, are all derived from animal cell culture. Animal cell culture and its prerequisites are discussed in this review's conclusion[7].

Kazemzadeh *et al.* demonstrated that larger production capacity, better flexibility, ability to minimise cross-contamination, lower cleaning costs, and shorter downtime, single-use stirred bioreactors are becoming more and more acknowledged as a competitive option in animal cell culture. In this study, the effects of impeller speed, volumetric gas flow rate, and impeller type on the volumetric gas-liquid mass transfer coefficient in a single-use unbaffled angled-shaft bioreactor used for animal cell culture were analysed and compared to results obtained for baffled vertical-shaft bioreactors. The simplified dynamic pressure approach was used to experimentally measure the volumetric gas-liquid mass transfer coefficient (KLa). Unbaffled angled-shaft bioreactors produced the lowest P/V and greatest KLa values. In terms of mass transfer, it was discovered that the Rushton impeller was the most effective impeller for this bioreactor. Additionally, a mass transfer coefficient empirical correlation was created[8].

Varley *et al.* demonstrated that in order to address the need for recombinant medicinal products, freely suspended animal cell culture has been expanding on a larger scale, and this expansion is anticipated to continue. Stirred tank reactors are the most often utilised reactor types. Although less often, air lift fermenters are also utilised. Reactor designs are often modelled on those used for microbial systems, and no particular standards have been published for large scale (10,000 L) animal cell growth. These designs, however, may not be the best ones given the significant energy input differences between microbial and mammalian cell systems. The significance of striking a balance between mixing, mass transfer, and shear effects is emphasised in this study. Meeting this equilibrium has ramifications for vessel design and operation. In particular, techniques to assure appropriate mixing to achieve pH and dissolved gas concentration uniformity are reviewed[9].

Kim *et al.* discussed about the Production of therapeutic proteins has often used animal cells. The culturing settings have a significant impact on how animal cells develop and how therapeutic proteins are expressed. To determine the ideal culturing conditions, a wide range of experimental permutations must be investigated. Due to their high-throughput parallel operation and lower reagent costs, miniature bioreactors are ideally suited for these kinds of applications. They may also be automated and connected to downstream analytical devices for real-time measurements of culture products. Based on the design categories of microtiter plates, flasks, stirred tank reactors, innovative designs with active mixing, and microfluidic cell culture devices, this study covers the present state of miniaturised bioreactors for animal cell growth. For

each system's batch or fed-batch modes, we compare the cell density and product titer. The monitoring/controlling equipment for engineering parameters that may be used in such systems, including as pH, dissolved oxygen, and dissolved carbon dioxide, is outlined. Finally, the following mini-scale instruments are presented for evaluating the process performance in animal cell cultures: total cell density, cell viability, product titer and quality, substrates, and profiles of metabolites[10].

Discussion

One of the most important techniques in cell and molecular biology nowadays is cell culture. The following is a quick description of some of the significant areas where cell culture is now playing a significant role:

Cell cultures provide an excellent model system for investigating

1. Fundamental cell biology and biochemistry,
2. Interactions between pathogens and cells,
3. Medication effects on cells,
4. The ageing process and its causes, and
5. Nutritional research.

Toxicity Evaluation

To examine the impact of novel medications, cosmetics, and chemicals on cell growth and survival in a broad range of cell types, cultured cells are often utilised alone or in combination with animal testing. Cell cultures produced from the liver and kidney are particularly significant.

Research on cancer

The fundamental distinctions between cancer cells and normal cells may be thoroughly researched since both types of cells can be cultivated in culture. Additionally, it is feasible to change healthy cultured cells into cancer-causing cells by utilising chemicals, viruses, and radiation. As a result, it is possible to research the change's causing processes. Cancer cell cultures are also used as a testing ground to find effective treatments and procedures for eradicating certain cancer kinds.

The morphology of these dyed roller bottles, which contain MRC-5 human fibroblasts, may be examined to determine if the cells are "happy." Too quickly rotating the container on the left led to highly "unhappy" cells, poor attachment and growth, and poor cell attachment.

Virology

The replication of viruses in cell cultures (instead of animals) for use in the creation of vaccines is one of the oldest and most important applications of cell culture. The clinical isolation and detection of viruses as well as fundamental studies into how viruses spread and infect living things both make extensive use of cell cultures.

Cell-Based Production

Three areas are sparking the greatest attention among the many useful items that may be produced using cultivated cells. The first is the industrial-scale exploitation of viruses for the creation of vaccines. Vaccines against polio, rabies, chicken pox, hepatitis B, and measles are among them.

The second is the mass creation of cells that have been genetically modified to generate useful or valuable proteins. These include insulin, hormones, and monoclonal antibodies. The third is the replacement of tissues and organs with cells. The first product on the market is artificial skin for healing burns and ulcers. However, testing on artificial organs such the pancreas, liver, and kidney is now taking place. Work being done with both adult and embryonic stem cells may result in a source of replacement cells and tissues. These cells are capable of differentiating into several distinct cell types. It is believed that understanding how to regulate the growth of these cells may lead to the development of novel therapeutic strategies for a broad range of medical diseases.

Genetic Guidance

The ability to collect and grow foetal cells from pregnant women via the diagnostic procedure known as amniocentesis has provided medical professionals a critical tool for the early detection of foetal abnormalities. Then, these cells may be analysed for chromosomal and genetic abnormalities using molecular methods like chromosome painting and karyotyping.

Genetic Modification

Molecular scientists who want to research the biological consequences of the expression of these genes now have a powerful weapon in the form of the capacity to transfect or reprogram cultured cells with new genetic material (DNA and genes) (new proteins). These methods may also be utilised to generate significant amounts of these novel proteins in cultivated cells for future research. In order to express significant amounts of proteins that they produce after being infected with genetically modified baculoviruses, insect cells are often utilised as tiny cell factories.

The use of cells for gene therapy has also been made possible by the capacity to genetically modify cells. A patient without a functioning gene may have his or her cells removed, and the damaged or absent gene can subsequently be restored. The cells may be cultivated in culture for a period before being infused back into the patient. A different strategy is to incorporate the lacking gene into a viral vector and then "infect" the patient with the virus in the hopes that the virus would cause the patient's cells to express the missing gene.

Drug Development and Screening Cell-based assays are now more crucial than ever for the pharmaceutical industry's high throughput screening of chemicals with potential for use as medications, in addition to being used for cytotoxicity testing. Initially, 96-well plates were used for these cell culture assays, but 384- and 1536-well plates are now increasingly used.

The achievable cell density and practicable bioreactor capacity will be limited by factors such as low growth rate, metabolic inefficiency, catabolite inhibition, and shear-induced cell damage. High capital costs are associated with equipment and facilities that have effective

microbiological contamination protections. Costs for appropriately pure amino acids and protein growth agents are similarly high in the forecast. It is debated and needs further research if plant protein hydrolysates can substitute amino-acid media. Economic factors likely make it impossible for their products to be affordable as food, according to assessments of the capital- and operating-cost structures of hypothetical cell-mass manufacturing facilities.

The origin of cultured cells (liver, heart, etc.) and their degree of adaptability to the culture environment determine their properties. If cells are still performing specific tasks that they did *in vivo* (such as liver cells secreting albumin), biochemical markers may be utilised to confirm this. It is also possible to look at morphological or ultrastructural indicators, such as cells from a beating heart. Being in an artificial environment often causes these traits to be either lost or altered. Eventually, certain cell lines will stop proliferating and start to become old. Finite describes these lines. Other lines are immortal or will become eternal; they are known as continuous cell lines since they may divide endlessly. A "regular" finite cell line experiences a fundamental, irreversible alteration or "transformation" when it becomes immortal. This may happen on its own or be purposely caused by employing medications, radiation, or viruses. Transformed Cells may commonly be grown in suspension and contain extra or faulty chromosomes in addition to generally being easier and growing quicker. Aneuploid cells are ones that contain extra chromosomes, while diploid cells have the typical amount of chromosomes. When cells are put into animals and become tumours, they are said to have undergone neoplastic transformation.

Chemical and biological contamination are the two basic kinds of cell culture contamination. Since it is caused by invisible substances like endotoxins, plasticizers, metal ions, or remnants of chemical disinfectants, chemical contamination is the most challenging to detect. The Technical Bulletin: Endotoxins and Cell Culture covers the endotoxin effects on cell culture in great depth (Ref. 10). The impacts of fast-growing yeast, bacteria, and fungus on the culture are often observable (changes in medium turbidity or pH), making them simpler to spot (especially if antibiotics are omitted from the culture medium).

Mycoplasmas and viruses, two other biological contaminants, are difficult to see and often need specialised detection techniques.

To prevent contamination, two prerequisites are essential. First, the cell culturist has to be well trained in and practise appropriate aseptic procedure. Second, media, plastics, glassware, and equipment that has been appropriately developed, maintained, and sanitised. Using antibiotics intended for tissue culture with caution and discretion (and in moderation) may also assist prevent culture loss owing to biological contamination. Understanding and Managing Cell Culture Contamination by Corning Technical Bulletin goes into great length on these ideas (Ref. 7).

A "happy" environment is one that does more than merely enable cells to thrive in culture, according to cell culturists. It often refers to a setting that, at the very least, permits cell division, which increases the number of cells (mitosis). Better still, under the correct circumstances, some cultured cells will perform crucial in vivo physiological or biochemical processes like muscle

contraction or the release of hormones and enzymes as a way of expressing their "pleasure" with their surroundings. The right culture medium, the right temperature, and a suitable substrate for attachment are necessary to provide this environment for the cells. The Corning Technical Bulletin: General Guide for Identifying and Correcting Common Cell Culture Growth and Attachment Problems addresses many of the concerns and challenges related to maintaining "happy" cells.

The temperature is often set at the same level as the host from whence the cells were taken, which is the body temperature. A temperature range of 18° to 25°C is acceptable for cold-blooded animals; most mammalian cells need 36° to 37°C. Usually, to maintain this temperature range, incubators that have been precisely calibrated and periodically inspected are used.

Low-level yeast infection in a liver cell line (PLHC-1, ATCC # CRL2406) as seen under a microscope. Multiple locations have budding yeast cells (arrows). No medium turbidity would be seen at this low level of contamination, but in the absence of antibiotics, the culture medium would most likely get turbid in a day.

Rat liver-derived epithelial-like cell line (Cl-9). The presence of mitotic cells suggests that this culture is expanding.

CHO-K1 cells are a frequently used continuous (transformed) cell line that was created in 1957 from adult Chinese hamster ovary tissue.

A suitable substrate is also necessary for the attachment and development of anchorage-dependent cells. The most popular substrates are glass and specially processed plastics (which make the ordinarily hydrophobic plastic surface hydrophilic or wettable). However, substrate coatings made of attachment factors like collagen, gelatin, fibronectin, and laminin may be employed to enhance the development and functionality of normal cells originating from the brain, blood vessels, kidney, liver, skin, and other tissues. Ordinary anchorage-dependent cells often perform better when grown on porous or permeable surfaces. This enables them to polarise, as they do in the body (having a top and bottom via which items may enter and exit the cell). Transwell® inserts are membrane-based permeable Corning vessels with Corning vessels that enable these cells to acquire polarity and the capacity to display specialised capabilities, such as transport. Many specialised cells can only be cultured on a porous substrate in serum-free media with the right ratio of growth and attachment factors in order to actually be "happy" (operate properly).

On beads consisting of glass, plastic, polyacrylamide, and cross-linked dextran molecules, cells may also be cultured in suspension. This method has been utilised to allow the growth of anchorage-dependent cells in suspension culture systems, and it is crucial for the production of cell-based biologicals.

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CHAPTER 24

APPLICATIONS OF PLANT CELL CULTURE

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Abstract:

The term "plant cell, tissue, and organ culture" refers to a group of procedures for the growth and multiplication of cells and tissues in an aseptic and controlled environment utilizing nutritional solutions. With the aid of this technique, it is investigated how to create in vitro circumstances that encourage cell proliferation and genetic reprogramming. Plant tissue culture, which was primarily developed in the early 1960s, has evolved into a standard practice for contemporary biotechnology. Today, one can identify five key areas where in vitro cell cultures are currently applied: large-scale propagation of elite materials, generation of genetically modified fertile individuals, as a model system for fundamental plant cell physiology aspects, preservation of endangered species, and metabolic engineering of fine chemicals. The most recent advancements in these fields are reviewed in this chapter.

Keywords:

Plant cell, Plant Cell Culture,Cells, Cell lines.

Introduction

The technique of plant tissue culture is frequently employed for industrial-scale plant propagation. Plant tissue culture methods have recently gained significant industrial relevance in the fields of plant propagation, disease eradication, plant enhancement, and the synthesis of secondary metabolites, in addition to its use as a research tool. Explants are tiny bits of tissue that may be utilised to continuously grow tens of thousands of plants. Under regulated circumstances, a single explant may be reproduced into many thousand plants in a short amount of time and space, regardless of the time of year or the weather. Due to the high coefficient of multiplication and low requirements on the starting plant population and available space, endangered, threatened, and uncommon species have been successfully cultivated and preserved using micropropagation [1], [2].

In addition, the creation of somaclonal and gametoclonal variations makes plant tissue culture the most effective approach for crop development. The micropropagation technique has a significant potential for producing plants of higher quality, isolating beneficial variations in genotypes that are well-adapted, high yielding, and have greater capacity for coping with stress and disease. Due to the potential for somaclonal variability to arise, some types of callus cultures produce clones with inheritable features that are distinct from those of the parent plants. This results in the creation of economically significant enhanced varieties. Compared to more conventional methods of plant propagation such as seed, cutting, grafting, air-layering, etc., commercial plant production using micropropagation techniques offers a number of benefits.

Rapid plant reproduction techniques may result in the generation of virus-free plants. An essential medicinal plant called Coryodalisyanhusuo was multiplied through somatic embryogenesis from callus produced from tubers to create tubers free of disease. Banana plants with meristem tips that were free of the BBTV and BMV (brome mosaic virus) were created. Pathogen-free germplasm has been cultured in vitro to produce greater yields. Under controlled circumstances, a potato output increase of up to 150% of virus-free potatoes was achieved. This chapter's primary goal was to outline tissue culture methods, numerous advancements, current trends, and their applications in many sectors[3], [4].

Tissue culture is the in vitro aseptic cultivation of cells, tissues, organs, or whole plants under regulated nutritional and environmental conditions, which is often used to make plant clones. The clones that arise are faithful to genotype. The regulated circumstances provide an environment for the culture's development and proliferation. These requirements include appropriate nutrition supply, pH medium, acceptable temperature, and a suitable gaseous and liquid environment.Plant tissue culture technology is increasingly being employed on a big basis for plant multiplication. Plant tissue culture methods, in addition to their use as a research tool, have recently gained industrial relevance in the areas of plant propagation, disease removal, plant enhancement, and secondary metabolite synthesis. Explants are little bits of tissue that may be utilised to continuously create hundreds of thousands of plants. Under regulated circumstances, a single explant may be reproduced into thousands of plants in a very short amount of time and space, regardless of season or weather. Endangered, endangered, and uncommon species have been successfully produced and preserved using micropropagation because to the high coefficient of multiplication and low starting plant and space requirements[5], [6].

Furthermore, plant tissue culture is often regarded as the most effective method for crop improvement via the generation of somaclonal and gametoclonal variations. Micropropagation technique offers enormous promise for producing high-quality plants, isolating beneficial variations in well-adapted high-yielding genotypes, and improving disease resistance and stress tolerance. Because of the potential of somaclonal variability, some types of callus cultures give birth to clones with inheritable features distinct from those of parent plants, which leads to the production of economically relevant enhanced varieties. Commercial plant production using micropropagation techniques offers various benefits over conventional propagation methods like as seed, cutting, grafting, and air-layering, among others. Rapid propagation methods may result in virus-free plants. Somatic embryogenesis was used to grow Coryodalisyanhusuo, a significant medicinal plant, using tuber-derived callus to create disease-free tubers. Meristem tip culture of banana plants was created that were free of banana bunchy top virus (BBTV) and brome mosaic virus (BMV). Pathogen-free germplasm cultured in vitro produced higher yields. In controlled settings, virus-free potatoes produced up to 150% more yield. The primary goal of this chapter is to outline tissue culture methods, numerous advancements, current and future trends, and their use in many sectors.

Plant Tissue Culture History

The science of plant tissue culture stems from the discovery of the cell, which was followed by the formulation of cell theory. Schleiden and Schwann suggested the cell as the fundamental

structural unit of all living beings in 1838. They imagined that each cell is capable of autonomy, and that if given the right conditions, each cell might regenerate into a full plant. Based on this assumption, Gottlieb Haberlandt, a German biologist, tried for the first time in 1902 to cultivate isolated single palisade cells from leaves in knop's salt solution supplemented with sucrose. The cells survived for up to a month, grew in size, and stored starch but did not divide. Despite his failure, he is considered as the father of plant tissue culture for laying the groundwork for tissue culture technology

Literature Review

Eibl *et al.* studied that there is a long history of using plant cell and tissue cultures to produce medicines, cosmetics, and food. Over the last ten years, plant cell culture technology has seen a major change due to the rising trend of producing food and cosmetics in a natural and sustainable way. During this period, more than 50 products based on extracts from plant cell cultures have entered the cosmetics market, the bulk of which are developed using plant cell suspension cultures. Additionally, the first components for dietary supplements based on plant cell culture, such Teoside 10 and Echigena Plus, are already manufactured on a large scale. We go through the benefits, traits, and difficulties of plant cell culture-based manufacturing for the cosmetics and food sectors in this brief overview. It concentrates on the state of the art at the time in this area. Two instances of the most recent advancements in food production using plant cell cultures are also given, including foods that may be made in a lab or at home and superfoods that improve health[7].

To maintain their sessile existence, plants have developed a large chemical arsenal. Since the Neolithic era, man has taken use of this natural resource, and now there are several uses for chemicals obtained from plants. But since the majority of high-value natural products (NPs) are derived from plants rather than synthetic materials, their manufacturing cannot be done on a large-scale agricultural basis. Furthermore, the target chemical concentration is very changeable and often present at extremely low concentrations in these plant species, which all contribute to their populations being limited and slow-growing. For the industrial synthesis of plant NPs, plant cell and organ culture represents a sustainable, controlled, and environmentally beneficial technology. Increases in plant NP yields are also being fueled by developments in cell line selection, biotransformation, product secretion, cell permeabilization, extraction, and scale-up, among other areas. The commercial synthesis of high-value compounds from these sources still faces considerable challenges. Cambial meristematic cells (CMCs), which have just recently been isolated, cultured, and characterised, provide a promising new way to get around several of these possible obstacles[8].In the last 10 years, synthetic biology has advanced quickly, and many plant scientists are becoming more interested in it. However, only a few number of microorganisms can produce highly valuable secondary metabolites that are unique to plants. This may be an issue due to improper protein post-translational modification, variations in protein micro-compartmentation, substrate and chaperone availability, product toxicity, and cytochrome p450 reductase enzyme availability.

Caretto *et al.* carried out the study on Tocopherols, often known as vitamin E, are lipophilic antioxidants that are only produced by photosynthetic organisms and are crucial dietary

components for mammals. The main vitamin E form found in the tissues of green plants is -tocopherol, which also has the greatest vitamin E activity among the four forms. Being a racemic combination of eight distinct stereoisomers, synthetic -tocopherol is never as effective as the natural form (R, R,R). Due to this, getting this chemical from natural sources like plant cell cultures is becoming more appealing. Plant cell and tissue cultures have the capacity to generate and assemble useful metabolites that may be used as medicines, nutraceuticals, and food additives. Utilizing heterotrophic sunflower cell cultures, a feasible in vitro manufacturing method for natural tocopherol was developed. The tocopherol yields of these cultures were increased by elicitor application, precursor feeding, and optimization of culture conditions. Additionally, by examining the connection between -tocopherol biosynthesis and photomixotrophic growth conditions, these cell cultures were helpful in illuminating the potential to increase tocopherol production by favouring sunflower cell photosynthetic abilities. A regulatory influence on tocopherol metabolism may be inferred from the regulation of -tocopherol levels in plant cell cultures[9].

Roberts *et al.* highlighted that there has been a major advancement towards the utilisation of plant cell cultures on a large-scale. Elicitation has been shown to be efficient in boosting the product yields of a broad range of secondary metabolites, especially when used in conjunction with enhancement techniques like immobilisation and in situ extraction. The implementation of enhancement techniques is evolving from being purely empirical to being semi-rational as a result of rapid advancements in our knowledge of the control of the biosynthetic pathways of secondary metabolites. Work on paclitaxel (Taxol), whose yields have increased more than 100-fold in the last two years, serves as an excellent example of much of this improvement[10].

Discussion

Plant cell culture is the basis of many different technologies that are now proving to be of great benefit to many disciplines. The ability to culture plant cells or tissues is essential to the success of all of these techniques. This paper introduces the basics of plant cell culture, and discusses the techniques, which utilize the ability of plant cells to be cultured. All of these techniques have an agricultural application and are being used throughout the world to improve agricultural productivity. Many of the techniques discussed here are crucial for genetic transformation research. These techniques range from the ability to produce plant cells in a form in which they can be transformed, to the regeneration of those transformed cells. The effective and efficient use of such techniques all require a basic understanding of plant cell culture. For the biotechnological manufacture of recombinant proteins and small-molecule active components, such as biopharmaceuticals, plant cell cultures may be employed as a platform. To achieve high batch-to-batch repeatability throughout manufacturing campaigns, this necessitates the cryopreservation of well-characterized cell lines as master cell banks from which uniform working cell banks may be produced. Plant cells have limited vitality and poor regeneration following freezing, making cryopreservation of these cells difficult. The methods of gradual freezing, vitrification, and encapsulation-dehydration have all been devised. The methods are often modified repeatedly to account for the characteristics of various plant cell lines, devoting time and money while having a mediocre level of effectiveness.Microbial contamination and

cost reduction are two major challenges in commercial plant micropropagation. To decrease microbial contamination, new kinds of containers and culture systems are being designed and tested. One of the most costly components of cell culture is the manual labour required in cutting and sorting the plant material. To address this limitation, robotic or automated systems are being actively developed and tested, and some solutions seem to be possible.Perhaps the most fascinating aspect of plant cell culture is the possibility for genetically modified plants. Both the governmental and business sectors are working hard to uncover beneficial genes for agricultural enhancement and value-added crops. Cotton fibre with enhanced features for textile manipulation, vaccinations given in plants with edible fruit or vegetables, healthier oil composition from our oilseed crops, and oil more appropriate for industrial uses are all possibilities.

Plant tissues and organs are produced in vitro on artificial medium under aseptic and regulated conditions in plant cell culture. The approach is based mostly on the idea of totipotentiality of plant cells [9], which refers to a single cell's capacity to express the whole genome during cell division. Along with the totipotent potential of plant cells, the ability of cells to change their metabolism, growth, and development is equally vital and critical for the regeneration of the whole plant. Plant tissue culture media includes all of the nutrients needed for normal plant growth and development. It is mostly constituted of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon sources, and certain gelling agents in the case of solid medium. Murashige and Skoog media (MS medium) is widely used in vitro for vegetative growth of many plant species. The pH of the medium is also essential since it influences both plant development and the function of plant growth regulators. It has been changed to a value between 5.4 and 5.8. For culturing, both solid and liquid media may be employed. The composition of the media, notably the plant hormones and nitrogen supply, has a significant impact on the response of the first explant.

Plant growth regulators (PGRs) are critical in directing the development route of plant cells and tissues in culture media. The most prevalent plant growth regulators are auxins, cytokinins, and gibberellins. The kind and concentration of hormones employed are mostly determined by the plant species, tissue or organ cultivated, and experiment goal. Auxins and cytokinins are the most often utilised plant growth regulators in plant tissue culture, and their concentration determines the kind of culture formed or regenerated. A high quantity of auxins typically encourages root production, while a high concentration of cytokinins stimulates shoot regeneration. A balance between auxin and cytokinin causes the formation of a mass of undifferentiated cells known as callus.

Maximum root induction and proliferation were seen in Stevia rebaudiana when the medium was supplemented with 0.5 mg/l NAA. Cytokinins, in general, increase cell division and cause shoot development and axillary shoot growth. A high cytokinin-to-auxin ratio promotes shoot growth, while a high auxin-to-cytokinin ratio promotes root development. Shoot initiation and proliferation were observed to be greatest when the callus of black pepper was transferred to media supplemented with BA at a concentration of 0.5 mg/l. Gibberellins are utilised to stimulate

growth and cell elongation. Maximum branch length was reported in Phalaenopsis orchids cultivated in media containing 0.5 mg/l GA3 (unpublished).

Plant tissue culture, as an emerging technology, has a significant influence on agriculture and industry by producing plants required to fulfil the world's ever-increasing demand. It has made important contributions to the improvement of agricultural sciences in recent years, and it is now a vital instrument in contemporary agriculture. Biotechnology is being integrated into agricultural practise at an unprecedented pace. Tissue culture enables the creation and multiplication of genetically homogenous, disease-free plant material. In vitro cell and tissue culture is an effective method for inducing somaclonal variation. Tissue culture-induced genetic diversity might be exploited to generate novel stable genotypes. Biotechnological methods for in vitro regeneration, mass micropropagation techniques, and gene transfer experiments in tree species have proved promising. In vitro cultures of mature and/or immature zygotic embryos are used to recover plants from inter-generic crosses that do not generate viable seeds. A variety of enhanced crop types with high yield potential and pest resistance may be created by genetic engineering. Genetic transformation technology is based on the technical elements of plant tissue culture and molecular biology:

In vitro cell and organ culture provides an alternative source for the conservation of endangered genotypes. Germplasm conservation is becoming more important owing to the rapid extinction of plant species and the greater necessity to protect nations' floristic legacy. Tissue culture procedures may be used to preserve vegetative tissues when the aims for conservation are clones rather than seeds, to maintain a crop's genetic background, and to minimise the loss of preserved heritage owing to natural catastrophes, whether biotic or abiotic stress. Plant species that do not generate seeds (sterile plants) or have'recalcitrant' seeds that cannot be maintained for an extended length of time may be effectively conserved using in vitro procedures for the preservation of gene banks.

Cryopreservation is critical for the long-term in vitro preservation of crucial biological material and genetic resources. It entails storing in vitro cells or tissues in liquid nitrogen, which causes cryo-injury when the tissues are subjected to physical and chemical stressors. Cell and tissue survival, as well as the capacity to re-grow or regenerate into whole plants or create new colonies, are often used to determine cryopreservation success. It is preferable to analyse the genetic integrity of recovered germplasm to establish if it is 'true-to-type' after cryopreservation. The faithfulness of recovered plants may be tested at the phenotypic, histological, cytological, biochemical, and molecular levels, albeit each technique has benefits and limits. Cryobionomics is a novel way to studying genetic stability in cryopreserved plant materials. The embryonic tissues may be cryopreserved for future use or germplasm conservation.

Embryo culture is a form of plant tissue culture that is used to produce embryos from seeds and ovules in a nutritional media. In embryo cultivation, the plant grows directly from the embryo or indirectly via the creation of callus and subsequent production of branches and roots. The approach was created to break seed dormancy, assess the viability of seeds, and produce uncommon species and haploid plants. It is a successful approach for shortening the breeding cycle of plants by developing excised embryos, which leads in a decrease of the lengthy dormant

period of seeds. Intra-varietal hybrids of the commercially significant energy plant "Jatropha" have been successfully generated with the express goal of mass multiplication. Somatic embryogenesis and plant regeneration have been performed in Jucara Palm embryo cultures for fast cloning and improvement of chosen individuals. Furthermore, performing embryo cultivation method may help to save endangered species. Recently, an effective approach for in vitro multiplication of Khayagrandifoliola by excising embryos from mature seeds was devised. The plant has a great economic value for both timber wood and therapeutic reasons. This approach is useful in forestry because it allows for the propagation of elite individuals in situations where natural population selection and improvement is difficult.

Genetic transformation is the most recent element of plant cell and tissue culture that allows for the transfer of genes with desired traits into host plants and the recovery of transgenic plants [63]. The approach offers a high potential for genetic enhancement of diverse agricultural plants when integrated into plant biotechnology and breeding programmes. It has a prospective function in the introduction of agronomically significant features such as greater yield, improved quality, and enhanced resistance to pests and diseases. Plants may be genetically transformed using either the vector-mediated (indirect gene transfer) or vectorless (direct gene transfer) methods. Agrobacterium-mediated genetic transformation is the most extensively utilised vector-dependent gene transfer technique for expressing foreign genes in plant cells. The use of root explants for genetic transformation resulted in the successful introduction of agronomic characteristics in plants. Virus-based vectors provide an alternate method of steady and fast transient protein expression in plant cells, giving an effective means of large-scale recombinant protein synthesis.

Recently, effective transgenic Jatropha plants were grown by direct DNA delivery to mature seed-derived shoot apices through particle bombardment. This approach has a significant influence on the decrease of hazardous compounds in seeds, hence removing the barrier to seed consumption in numerous industrial sectors. The genetic transformation technology is increasingly used to regenerate disease or virus resistant plants. Researchers were successful in creating transgenic potato plants that are resistant to the potato virus Y (PVY), which is a severe threat to potato crops globally [70]. Furthermore, employing a multi-auto-transformation (MAT) vector system, marker-free transgenic Petunia hybrida plants were created. The plants were resistant to Botrytis cinerea, the causative agent of grey mould.

Somatic hybridization is an essential strategy for plant breeding and agricultural development because it produces interspecific and intergeneric hybrids. The process comprises the fusing of protoplasts from two distinct genomes, followed by the selection of appropriate somatic hybrid cells and the regeneration of hybrid plants. Protoplast fusion is an effective method of transferring desirable traits from one species to another and is having an increasing influence on crop development. Somatic hybrids were created by fusing protoplasts from rice and ditch reed utilising an electrofusion therapy for salt tolerance.

By overcoming the hurdles of sexual incompatibility, in vitro fusing of protoplasts allows for the development of unique hybrid plants. The approach has been used in the horticulture sector to generate novel hybrids with higher fruit output and disease resistance. When citrus protoplasts

were fused with other related citrinae species, viable hybrid plants were created. The generation of intergeneric hybrid plants among Brassicaceae member's best illustrates the possibility of somatic hybridization in major agricultural plants. To address the issue of chromosomal loss and limited regeneration ability, a viable procedure for the formation of somatic hybrid plants has been devised by employing two varieties of wheat protoplast as recipients and Haynaldiavillosa protoplast as fusion donors. It is also used as a major gene source for wheat enhancement.

Instead of traditional breeding, tissue culture methods allow for the production of homozygous plants in a very short amount of time using protoplast, anther, and microspore cultures. Haploids are sterile plants with a single pair of chromosomes that are turned into homozygous diploids by spontaneous or controlled chromosomal doubling. The doubling of chromosomes restores plant fertility, resulting in the formation of double haploids with the potential to become pure breeding new cultivars. The development of haploid plants from immature pollen cells without fertilisation is referred to as androgenesis. Sudherson *et al.* demonstrated haploid plant development of sturt's desert pea employing pollen grains as main explants. By speeding up the creation of inbred lines and circumventing the limits of seed dormancy and embryo non-viability, haploidy technology has now become a fundamental feature of plant breeding operations. The approach has a great use in genetic transformation by producing haploid plants with induced tolerance to diverse biotic and abiotic stressors. The introduction of desirable trait genes in a haploid state followed by chromosomal doubling resulted in the generation of double haploids inbred wheat and drought tolerance plants.

Plant cell biotechnology has grown as a new age in the area of biotechnology during the last several decades, focused on the development of a vast variety of secondary plant products. The development of genetic engineering and molecular biology methods in the second half of the twentieth century enabled the emergence of better and novel agricultural goods, which have occupied a rising demand in the productive systems of numerous nations worldwide. Nonetheless, this would not have been conceivable without the invention of tissue culture methods, which offered the instruments for introducing genetic information into plant cells. Transgenic plants are now one of the most promising techniques of manufacturing proteins and other therapeutic compounds such as antibodies and vaccinations. Transgenic plants are a cost-effective alternative to fermentation-based manufacturing methods. Plant-made vaccinations or antibodies are particularly intriguing since plants are devoid of human illnesses, lowering screening expenses for viruses and bacterial toxins. In 2008, 13.3 million farmers used transgenic plants in their agricultural systems, up from 11 million in 2007.

Conclusion

Tissue culture is the regulated nutritional and environmental cultivation of cells, tissues, organs, or complete plants in vitro, often to create plant clones. The resulting clones are faithful to the genotype that was chosen. The culture has a favourable environment for development and reproduction thanks to the regulated circumstances. These prerequisites include a suitable nutrition source, a pH-balanced environment, an appropriate temperature, and a suitable gaseous and liquid environment.

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CHAPTER 25

FUTURE ASPECTS OF INDUSTRIAL MICROBIOLOGY

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Abstract:

Microbiology, along with metallurgical processes, is a technical and scientific subject that has been employed since ancient times to enhance living circumstances and increase survival prospects by changing hazards into challenges. After humans became sedentary and agriculture began to modify food availability, the acquisition of new materials that either did not exist before (metallurgy) or existed at extremely low levels (microbiology) provided the stimulus for a new human activity: industry. Industry is often described as the art and skill of transforming raw resources into new and useful goods.

Keywords: Enzyme, Microbiology, Microbes, Penicillin.

Introduction

Food was perhaps one of the earliest products that mankind attempted to assure. While agriculture provided mankind with the capacity to have adequate food year round, weather and climate circumstances restricted its availability. As a result, the problem was to keep adequate foodstocks in excellent shape throughout the year until the next harvest. By coincidence, microbiology became engaged in this process, and as a consequence (after hundreds of unsuccessful efforts), bread, cheese, wine, and beer have been enjoyed for thousands of years. In truth, yeast was employed to make beer around Sumeria before 7000 BC, while the Assyrians were already making wine in 3500 BC. These drinks are produced by microbes, and the finished goods may be preserved for longer periods of time than the original raw components. Early industrial processes were distinguished by two major characteristics:

They arose as a result of a fortuitous observation (i.e. the fermentation of sugar contained in fruits or grains to generate alcohol), which human civilizations eventually learned to manage and benefit from[1]–[5].

Although procedures arising from these observations were optimised and grew more lucrative, knowledge of them was gained by experience, and the reasons why, for example, fruit must became wine or milk produced cheese, remained unknown. This was the state of the art until the second part of the nineteenth century, when two events ushered in industrial microbiology: The scientific phenomena underlying early industrial processes were more understood and comprehended. Pasteur identified the important function of yeast in the food and beverage industries about 1860. As a consequence, he is regarded as the founder of modern microbiology. The concept that microbes were living materials that could be employed as basic chemical reactors gained widespread acceptance. Thus, industrial microbiologists and engineering

chemists might address the same topics (especially ener-gy and mass balances). The capacity to manage and benefit from these processes (including the adoption of more efficient microbial species or strains) grew. This capacity stems from the realisation that the conditions under which microbial "machines" produce the highest output are regulated, external conditions (pH, temperature, pressure, feedflow) that do not impact the machine itself: the microorganisms utilised develop naturally in the environment. Furthermore, all procedures are focused on preserving the status quo, attempting to eliminate deviations that may influence yield. Currently, various laws for whisky and wine production are connected to the use of endogamic yeast. As a result, mutation, deterioration, or contamination of the functional microbial species must be prevented. That was the case until a few decades ago, and it remains the foundation of traditional industrial microbiology. Food, pharmaceutical, fine-chemistry, cosmetic, energy, and new-material sectors all use microbial processes. It is estimated that these procedures presently transfer more than US$70 billion globally. The substitution of petroleum-based fuels with yeast fermentation-obtained ethanol (bioethanol) will usher in a new era in the energy industry while also meeting environmental criteria. For a variety of reasons, microbiological processes with industrial applications are often much more beneficial than other industrial processes, which explains the continuous interest in this sector. First, the capacity of a certain microbe to create a specific chemical may be used. This molecule might be a secondary metabolite or, more directly, a byproduct of microbial metabolism. While chemical industrial techniques may produce the same product, the process is often more complicated, including more route stages and/or containing unwanted byproducts. Second, the compounds acquired from microbes are produced in a moderate environment[6], [7].

When compared to chemical synthesis methods, this results in lower energy and equipment costs. Nonetheless, industrial microbiology has certain restrictions since the working microbe must be completely operational and safeguarded from mutation, other microbial contaminants, competition, and environmental changes. Molecular biology and, later, genetic engineering have led to a better understanding of both the final mechanisms involved in industrial microbial activities and the primary factors influencing them during the previous several decades. For the first time, it has become able to modify these microbiological machines carefully and effectively; their native abilities have been enhanced or new ones acquired from other creatures (either bacteria or plants) have been added to them. As a result, these living machines have shown to be more productive and resistant to change, and may therefore be employed for novel biochemical processes. Over the past two decades, new or more efficient industrial processes employing microbes have been introduced, generating purer, less costly products or chemicals that were previously unavailable by traditional chemical procedures. In general, it is now possible to: convert secondary reactions into main metabolic pathways; optimise productions and yields; change the original metabolic pathways to allow the use of less expensive raw materials or to obtain previously unknown molecules; and use enzyme enantiomeric properties to obtain new chiral molecules. The number of items obtained via these procedures grows every year, and numerous industrial fields are engaged.

The most common products derived by industrial microbiology are: • Pharmaceutical proteins (human interferon, epidermal growth factor, and haemoglobin, antigens for hepatitis-B virus,

stabilisers for erythropoietin and human chorionic gonadotropin) derived from microorganisms such as Saccharomyces cerevisiae, Pichia pastoris, Hansenulapolymorpha, or Agrobacterium tumefaciens. In 2000, the industrial enzyme market for non-therapeutic applications such as food, detergents, textiles, leather, pulp and paper reached US $2 billion. Microbial lipases are of particular interest due to their stability in organic solvents, absence of a need for cofactors, wide substrate specificity, and excellent enantioselectivity. LipolaseTM from Novozyme was the first recombinant lipase, released in 1994, and was generated by cloning the Thermomyceslanuginosus (previously Humicula lanuginosa) lipase gene into the Aspergillus orzyae genome

Antibiotics, such as biosynthetic penicillin V and naturally occurring penicillin G. More than 20% of the 12,000 antibiotics known in 1995 can be generated by filamentous fungus. The market for biosynthetic and semisynthetic penicillins and cephalosporins is worth $15,4 billion

Immunosuppressive medicines derived from Tolypocladium nivenum, such as cyclosporin A, or mycophenolatemofetil derived from numerous *Penicillium species*.

1. Hypocholesterolemic agents, such as lovastatin derived from Aspergillus terreus and pravastatin derived from Penicillium citrium, have a $15 billion market.
2. Antitumoral compounds, such as taxol, which was identified in plants but was subsequently transferred to and generated by Taxomycesandreanae; in 2000, it accounted for 10% of total revenues for Bristol-Myers-Squibb, totaling US $1 billion
3. Mycotoxins, which include adrenalin inhibitors, oestrogens, and anabolic agents for cattle and sheep, as well as gibberellins utilised in the brewing and malting industries.
4. Pigments, such as astaxanthin from Phaffia rhodozyma and -carotenoid from Blakesleatrispora, are utilised in the food and textile sectors.
5. Polyunsaturated fatty acids, such as - acid derived from Mucor circinelloides and arachidonic acid derived from Mortierella isabellina. This journey is still in its early stages, and we encounter several hurdles. For example, technology that can synthesise all of these chemicals in a less costly and more efficient manner, using carbon dioxide as the carbon source, water as the electron acceptor, and sunlight as the driving energy, is required. Many scientists have spent years trying to answer this equation, ignorant that a similar mechanism, photosynthesis, already exists and has been employed by photosynthetic organisms (many bacteria and all plants) for millions of years. As an example, the previously indicated shift from petroleum-based fuels to bioethanol would usher in a new era in the energy industry and drastically alter international business connections. However, this is merely the first step towards the ultimate goal: a hydrogen-based battery. Microbiology will undoubtedly play a role in this job as well.

Literature Review

Adrio *et al.* studied about the discovery of the double-stranded structure of DNA and the development of recombinant DNA technologies spurred a revolution in industrial microbiology. Traditional industrial microbiology and molecular biology have been combined to provide enhanced recombinant techniques for the industrial production of primary and secondary metabolites, protein biopharmaceuticals, and industrial enzymes. Novel genetic approaches and

their adaptations, such as metabolic engineering, combinatorial biosynthesis, and molecular breeding techniques, are making significant contributions to the creation of better industrial processes. Furthermore, functional genomics, proteomics, and metabolomics are being used to find new important small compounds for therapeutics as well as enzymes for catalysis. Industrial microbal genome sequencing is now underway, which speaks well for future process optimization and the identification of novel industrial products[8].

Shah *et al.* carried out a study in which they talked about the Heavy metal contamination is a developing problem all over the globe. Heavy metals are mostly contributed to the soil by human activities such as industrial discharge, mining, smelting, hazardous solid waste disposal, and so on. Heavy metals that remain in the soil may be taken up by plant tissues, enter the biosphere, and accumulate in the trophic levels of the food web. For the cleaning of heavy metal polluted soil, many physical, chemical, and biological remediation approaches (both in-situ and ex-situ) are available. Phytoremediation is the most sustainable (both economically and environmentally). Though phytoremediation is less effective than physical approaches, it is still in its early stages, and hence research efforts must be redirected for commercial usage. This paper outlines the functions of soil chemistry, plant physiology, and microbiology (plant-microbiology interaction) in helping heavy metal polluted soil phytoremediation[9].

Schmidt *et al.* investigated the separate treatment has become much more cost-effective in recent years, since numerous techniques for biological ammonia removal from concentrated waste streams have become accessible. In this section, we will look at three processes that employ novel ideas in microbiology: partial nitrification, nitrifier denitrification, and anaerobic ammonia oxidation (the anammox process). These techniques aim to remove ammonia from gases as well as ammonium-bicarbonate from concentrated wastewaters (i.e. sludge liquor and landfill leachate). The review discusses microbiology, its implications for application, the existing state of application, and prospective advances[10].

Novel and possibly life-saving bioactive secondary metabolites are being produced by a rising variety of marine fungus. We've spoken about some of the unique antibacterial, antiviral, and antiprotozoal chemicals identified from marine-derived fungus, as well as their potential involvement in disease eradication. We also explored the potential commercialization of these molecules for medication development through metabolic engineering and post-genomics techniques.

Discussion

There are several definitions of biotechnology. One of the most comprehensive is the one delivered during the United Nations Conference on Biological Diversity (also known as the Earth Summit) in Rio de Janeiro, Brazil in 1992. This conference defined biotechnology as "any technical application that employs biological systems, live creatures, or derivatives thereof, to create or change goods or processes for specialised purpose." There are several instances of living organisms being utilised to create or change processes for specific purposes. Some of these include the use of microorganisms to generate the antibiotic penicillin or the dairy product

yoghurt; the use of microorganisms to manufacture amino acids or enzymes is also an example of biotechnology.

In the past two decades or more, advances in molecular biology have greatly enhanced our knowledge of the role of nucleic acids in genetic processes. This has led to the use of molecular biological modification in technologies such as genetic engineering. All parts of biological manipulations now include molecular biology dimensions, thus it is appropriate to split biotechnology between classical biotechnology, which does not directly involve nucleic acid or molecular manipulations, and nucleic acid biotechnology, which does.

Industrial microbiology is the study of the large-scale and profit-driven production of microbes or their products for direct consumption or as inputs in the creation of other items. Thus, yeasts may be generated for direct human consumption as food or animal feed, or for use in bread-making; their output, ethanol, can also be consumed in the form of alcoholic drinks, or employed in the creation of fragrances, medicines, and other products. Industrial microbiology is obviously a field of biotechnology that incorporates both conventional and nucleic acid components.

Medical microbiology, environmental microbiology, food microbiology, and industrial microbiology are some of the sub-disciplines of microbiology. The lines between these sub-divisions are often blurred and are just there for convenience.

With this qualification in mind, the traits of industrial microbiology may be emphasised by comparing them to those of another sub-division of microbiology, medical microbiology.

The first is the immediate motivation: profit and wealth creation are the immediate motivations in industrial microbiology. The primary concern of the microbiologist or laboratory technician in medical microbiology is to provide professional advice to the doctor concerning, for example, the spectrum of antibiotic susceptibility of bacteria isolated from a sick state in order to return the patient to good health. The development of riches is, of course, in the back of the medical microbiologist's mind, but the immediate focus is the return of the patient to good health.The second distinction is that the microorganisms employed in ordinary medical microbiology have little or no direct economic value other from the contribution they make to guarantee the patient's return to good health, who may then pay for the services. The microorganisms engaged in industrial microbiology or their products are very important and serve as the reason for the establishment's existence.

The scale at which the microorganisms are treated is the third distinction between the two sub-disciplines. The scale in industrial microbiology is huge, and organisms may be grown in fermentors as big as 50,000 litres or greater. In ordinary medical microbiology, the pathogen is treated on a loopful or a few millilitres scale. If a pathogen that has no economic value is treated on the massive scale utilised in industrial microbiology, it is most likely to develop a vaccine against the pathogen. Under such conditions, the pathogen would gain economic worth and profit potential; the activity would be correctly referred to as industrial microbiology.In contrast to many other areas of the study of microbiology, the microbiologist in an industrial setting does not work alone. He is generally merely one of many different officials with whom he must continually deal. These others may include chemical or production engineers, biochemists,

economists, attorneys, marketing specialists, and other high-level functionaries in a contemporary industrial microbiology organisation.

They all work together to fulfil the firm's goal, which is not altruism (at least not right away), but the development of profit or riches.Despite the need of teamwork, the microbiologist plays an important position in his company. Some of his responsibilities include:

A. Selecting the organism to be utilised in the processes.

B. Selecting the medium of growth for the organism.

C. Determining the environmental parameters for the organism's optimal productivity, such as pH, temperature, aeration, and so on.

D. During the actual production, the microbiologist must monitor the process for the absence of contaminants and participate in quality control to ensure uniformity of quality in the products.

E. Proper custody of the organisms, usually in a culture collection, to ensure that their desirable properties are retained; f. improvement of the microorganisms' performance through genetic manipulation or medium reconstitution.

Because business drives the development of industrial microbiology, less efficient technologies are abandoned when better ones are found. Indeed, a microbiological procedure may be completely abandoned in favour of a less expensive chemical method. This was the situation with ethanol, which was manufactured through fermentation until about 1930. Fermentation ethanol was practically abandoned as cheaper chemical alternatives utilising petroleum as the substrate became available about 1930. Petroleum prices have risen dramatically since the mid-1970s. It is now economical to manufacture ethanol via fermentation. Several nations, including Brazil, India, and the United States, have formally acknowledged the manufacture of ethanol through fermentation for use as gasohol in gasoline.

Many industrial microbiology processes do not become public property for a long time because the corporations that discover them either keep them secret or patent them. The unknown procedures are frequently referred to as 'know-how'. The rationale for the secrecy is obvious: the owner of the secret wants to stay one step ahead of his or her rivals. As a result, industrial microbiology textbooks often fall behind in explaining industrial microbiology methodologies.

The first meaning is related to microbial physiology. In microbiology, fermentation is defined as the type of metabolism of a carbon source in which energy is generated by substrate level phosphorylation and organic molecules function as the final electron acceptor (or as acceptors of the reducing equivalents) generated during the breakdown of carbon-containing compounds or catabolism. The process is known as respiration when the end acceptor is an inorganic molecule. Respiration is classified as aerobic when the end acceptor is oxygen and anaerobic when it is some other inorganic chemical other than oxygen, such as sulphate or nitrate.

In industrial microbiology, the term "fermentation" refers to any process in which microorganisms are grown on a large scale, even if the end electron acceptor is not an organic substance (i.e., even if the growth is carried out under aerobic circumstances). Thus, the

synthesis of penicillin, the development of yeast cells, both of which are extremely aerobic, and the generation of ethanol or alcoholic drinks, all of which are physiological fermentations, are all referred to as fermentations.

The third application is related to food. A fermented food is one in which microorganisms play a significant role in the processing. Microbes affect the nature of the food by manufacturing taste components as well as determining the overall character of the meal, however microorganisms constitute just a tiny fraction of the completed product by weight. Fermented foods include cheese, bread, and yoghurt.The structure of a fermentation industrial establishment will differ from one business to the next and will depend on what is produced. The culture is normally from the firm's culture collection, although it might have originated from a public culture collection and been tied to a patent. On the other side, it might have been segregated from the start by the company from soil, air, sea, or another natural body. The nutrients in the medium are derived from a variety of raw materials, sometimes after suitable preparation or modification, such as saccharification in the case of complex carbohydrates like as starch or cellulose. An inoculum is often made from a lyophilized vial, the quality of which must be verified on an agar plate. The organism is then cultivated in shaking flasks of increasing capacity until it reaches roughly 10% of the volume of the pilot fermentor. It is then injected into the pilot fermentor(s) before being transferred to the production fermentor(s).

The extraction method is determined by the final product. The approaches are plainly different depending on whether the organism itself or its metabolic product is the intended commodity. If the product is the desired substance, the method will be defined by its chemical composition. Quality control must be performed on a regular basis to guarantee that the proper material is produced. Sterility is vital in industrial microbiology operations and is maintained in a variety of ways, including the use of steam, filtration, or chemicals. Air, water, steam, and other utilities must be delivered and properly processed before usage.

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