COURSE INTRODUCTION

The objective of this course deals basic introduction to bioprocess in concerned to fermentation process. The aim is to provide brief introduction of bioprocess technology, and fermentation process. The course is organized into following blocks:

Block 1 It covers the bioprocess and fermentation

Block 2 It deals the Processing and industrial production.

Block 3 It describes food technology and isolation process.

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Block- I

Bioprocess and fermentation

Unit-1
Introduction to bioprocess technology

Unit-2
Types of fermentation process

Introduction

This is the first block on introduction to bioprocess technology and types of fermentation process. It consists of following two units as follows:

Unit 1: Bioprocess technology forms the backbone of the biotechnology industry. In a bioprocess living cells such as bacteria, enzymes, and chloroplast or their components are used to procure desired products. The process is leveraged to develop industrial products, processes and techniques to fulfill the needs of society. The different stages in bioprocess includes formulating raw materials, substrates and media, the conversion state, biocatalysts, downstream processing, volume production, purification and processing the final product.

Bioprocess technology finds application in end use industries for manufacture of biomaterials such as pharmaceutical supplements, antibiotics, food and agricultural products, vaccines, and enzymes. It also finds application in the production of alternative products for treating maladies, the creation and evaluation of safer food materials, and making of biodegradable and environmental-friendly chemicals. As techniques and instrumentation become further sophisticated, bioprocesses may find applications in other domains where chemical processes are now used.

Unit 2: Humanity has been fermenting food since the Neolithic age, long before people understood the science behind the process. Today, following the scientific discoveries of French microbiologist Louis Pasteur, who showed that living organisms initiate fermentation, we know why fermentation not only makes food like sourdough bread, cheese, and wine taste better, but also helps to keep us alive. Fermentation is any metabolic process in which microorganisms' activity creates a desirable change in food and beverages, whether it's increasing flavor, preserving foodstuffs, providing health benefits, or more. The word "ferment" comes from the Latin verb "fervere," which means "to boil." Ironically, fermentation is possible without heat.

During the fermentation process, these beneficial microbes break down sugars and starches into alcohols and acids, making food more nutritious and preserving it so people can store it for longer periods of time without it spoiling. Fermentation products provide enzymes necessary for digestion. This is important because humans are born with a finite number of enzymes, and they decrease with age. Fermented foods contain the enzymes required to break them down. Fermentation also aids in pre-digestion. During the fermentation process, the microbes feed on sugars and starches, breaking down food before anyone's even consumed it.

Unit- 1: Introduction to bioprocess technology

Structure

Objectives

1.1 Introduction

1.2 Global Bioprocess Technology Market: Trends and Opportunities

1.3 Global Bioprocess Technology Market: Regional Outlook

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1.4.2 The bioreactor, core of the process

1.4.3 Aeration of bioreactors using flow controllers

1.4.4 Bioreactor applications

1.5 Food & beverage industry

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1.8 Sustainable Energy

1.9 Isolation, preservation and maintenance of industrial organisms Isolation

1.10 Enrichment Methods for Isolation of Microorganisms:

1.10.1 Preservation

1.10.2 Maintenance

1.10.3 Kinetics of microbial growth and death

1.10.4 Growth kinetics

1.10.4.1 Growth associated

1.10.4.2 Non-growth associated

1.10.4.3 Mixed-growth associated

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1.10.4.4.1 Lag phase

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1.11 Media

1.12 Fermentation technology

1.13 Nutritional Control of Metabolite Production

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1.13.2 Nitrogen Source

1.13.3 Phosphate

1.1 Introduction

Bioprocess technology is a vital part of biotechnology that deals with processes combining the complete living matter or its components with nutrients to make specialty chemicals, reagents, and biotherapeutics. The processes form the backbone of translating discoveries of life sciences into useful industrial products. Various stages associated with the bioprocess technology include substrates and media, biocatalysts, volume production, downstream processing, purification, and final processing. Over the past few years, the application of bioprocess technology in the development of a variety of next-generation biopharmaceutical products is gaining traction in the bioprocess technology market.

One of the rapidly emerging areas of application is in the manufacturing of key oligosaccharides (OS)—galactooligosaccharides and fructooligosaccharides. These OS have promising uses in food ingredients owing to their several health benefits. These are confirmed to possess certain prebiotic functions and will help meet the growing demand for prebiotic for treating various chronic ailments. Recent technological advancements in bioprocessing methods have further expanded the scope of their applications. Conventionally, whole cells or enzymes that are partially purified are used to synthesize both the OS. Enzymes play a vital role in the synthesis process. Fructooligosaccharides can be synthesized by the degrading fructan using the enzyme Inulinase or by the

transglycosylation process of sucrose while galactooligosaccharides is produced using lactose. However, the complete conversion poses a challenge, since biocatalysis does not remove digestible carbohydrates found as a result of enrichment processes of oligosaccharides. Various bioengineering techniques for the removal of digestible carbohydrates are being developed. This includes downstream separation technologies, specific fermentation strategies, and further bioconversion using enzymes. Next-generation manufacturing strategies facilitate purification of sucrose-based fructooligosaccharides. Objectives under this block are as under.

This is the first block on bioprocess and fermentation**.** We have following objectives. These are as under:

Objectives

- To know about bioreactor and its types and applications.
- To know about kinetics of microbial growth and death.
- To know about Isolation, preservation and maintenance of industrial organisms.
- To know about fermentation technology.
- To know about sterilization methods.

1.2 Global Bioprocess Technology Market: Trends and Opportunities

The most prominent growth drivers in the global bioprocess technology market are the significant expansion in the biopharmaceutical industry, increasing thrust on research and development, higher demand for vaccine, and progress in the field of technology. Besides, bioprocesses steal a march over conventional chemical methods with the use of living materials for production. This is because bioprocesses typically need lower temperature, pressure, and pH, which is a measure of acidity. Second they can use renewable resources as raw materials and consume less energy.

Offsetting such benefits is the steep cost of instruments required for bioprocess. Another factor countering the growth in the global bioprocess technology market is the strict regulations. Depending upon the type, the global bioprocess technology market can be segmented into cell counting, cell culture, cell line development, cell expansion, single-use bioprocessing, virus filtration, flow cytometry, biologics safety testing, tangential flow

filtration, and pyrogen testing. Of these, the cell culture segment leads the market with a dominant share.

1.3 Global Bioprocess Technology Market: Regional Outlook

Geographically, the global bioprocess technology market can be divided into Asia Pacific, Europe, North America, and the Rest of the World. Among them, North America accounts for maximum share. The stellar growth in the North America market has been brought about by a strong demand for good quality biologics, and a very strong emphasis on research and development by prominent players in the region. Going forward, however, Asia Pacific is slated to expand at a good clip to outshine other regions vis-à-vis growth rate. The bioprocess technology market in the region will likely be propelled by the expansion in the biopharmaceutical industry, rising government initiatives, development in research and development, higher investments by key market players, and the trend of outsourcing production to Asia Pacific countries full of cheaper, high skilled manpower.

1.4 Bioreactors

Biotechnology is a technology that has been around for thousands of years but came into bright spotlight during the last 20 years. Why is that? Biotechnological processes are conducted in a bioreactor, where substances are being synthesized with the help of bacterial, yeast- and cell strains. The end product is used in many areas of our daily lives, like yoghurt and beer brewing processes. Besides in food and beverage applications, it is also used in the field of medical drug research and production. Flow controllers play an important role in these Bioreactors. The rise of biotechnology and its growing field of applications are enough reason to find out more about this fascinating technology.

A bioreactor refers to any manufactured device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel. It may also refer to a device or system designed to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical/bioprocess engineering.

The heart of the fermentation or bioprocess technology is the Fermentor or Bioreactor. A bioreactor is basically a device in which the organisms are cultivated to form the desired products. it is a containment system designed to give right environment for optimal growth and metabolic activity of the organism. A fermentor usually refers to the containment system for the cultivation of prokaryotic cells, while a bioreactor grows the eukaryotic cells (mammalian, insect cells, etc).

1.4.1 Types of Bioreactor

- Continuous Stirred Tank Bioreactor
- Airlift Bioreactor
- Fluidized Bed Bioreactor
- Packed Bed Bioreactor
- Photobioreactor
- Membrane Bioreactor

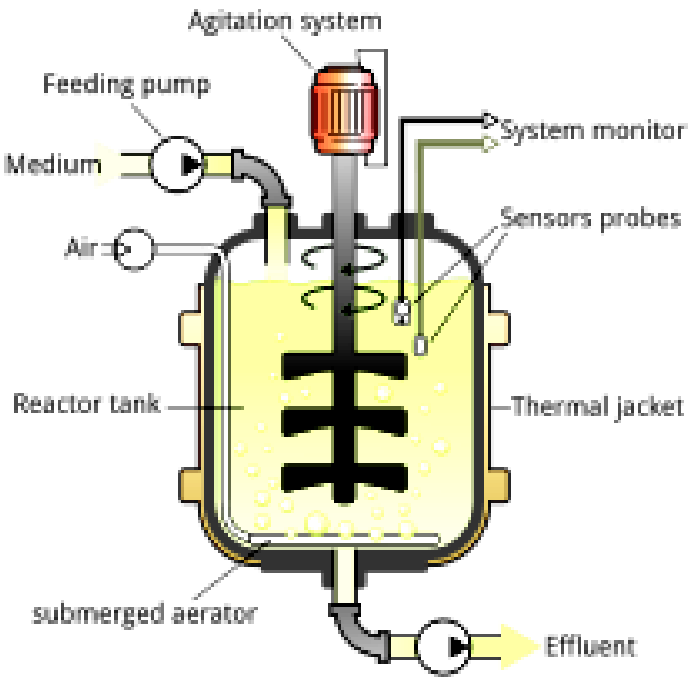


Fig. 1 General structure of a continuous stirred-tank type bioreactor

On the basis of mode of operation, a bioreactor may be classified as batch, fed batch or continuous (e.g. a continuous stirred-tank reactor model). An example of a continuous bioreactor is the chemostat. Organisms growing in bioreactors may be

submerged in liquid medium or may be attached to the surface of a solid medium. Submerged cultures may be suspended or immobilized. Suspension bioreactors can use a wider variety of organisms, since special attachment surfaces are not needed, and can operate at a much larger scale than immobilized cultures. However, in a continuously operated process the organisms will be removed from the reactor with the effluent. Immobilization is a general term describing a wide variety of methods for cell or particle attachment or entrapment. It can be applied to basically all types of biocatalysis including enzymes, cellular organelles, animal and plant cells. Immobilization is useful for continuously operated processes, since the organisms will not be removed with the reactor effluent, but is limited in scale because the microbes are only present on the surfaces of the vessel.

1.4.2 The bioreactor, core of the process

Simply said, a bioreactor is a vessel in which biological processes take place. The bioreactors are equipped with either a simple manual control or more complex, fully automated, PLC control. Typically, the bioreactor process is a batch process and the time between start and harvesting is called a campaign. The majority of bioreactors need to be supplied with gases and nutrients for growing bacteria, yeast or cells for the desired biological synthesis to take place. These additives are usually added continuously over a period of a few days to several weeks. Flow controllers play a significantly important role in the process control of Bioreactors. The differences in volume flow for either bacteria or cell cultures are significant. The campaign of a process containing cell cultures can take up to three or four weeks before harvesting, while a campaign with bacterial cultures is often just lasting for a few days. It is a challenge to carry out the process in a stable manner during this period of time and therefore, it is very important to accurately dose gases and nutrients. The differences in volume flow for either bacteria or cell cultures are significantly. The additive dosing is done in sterile conditions to prevent any contamination with unwanted bacteria that could compete with the microbial or cell culture. In short, **reliability** and **reproducibility** are key in **bioreactor processes**, especially for flow control.

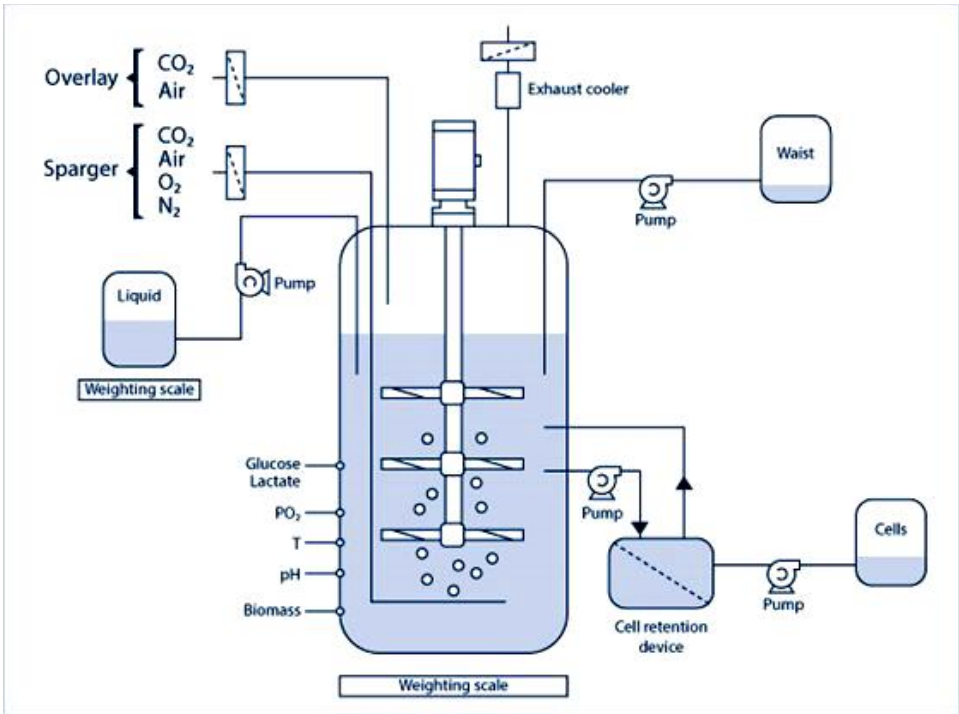


Fig. 2 Schematic overview of bioreactor process

1.4.3 Aeration of bioreactors using flow controllers

The gases that are commonly used for the aeration of bioreactors are: Air, O2 (Oxygen), N2 (Nitrogen) and CO2 (Carbon dioxide). N2 is used to calibrate the Oxygen sensor (pO2) and to reduce the O2-content in the bioreactor at the beginning of the process. The bigger the number of bacteria or cells, the bigger is the O2 requirement. CO2 is used to regulate the acidity (pH) in the liquid phase. A bioreactor is usually controlled by checking the partial oxygen pressure pO2 and the pH in the suspension. The cells' intake of oxygen and all other substances are taking effect in the liquid phase. The oxygen must therefore be present in the liquid. To ensure this, it is attempted to add the oxygen – possibly as a component of air – in the smallest bubbles possible. Stirring the liquid will help distribute and diffuse the added oxygen.

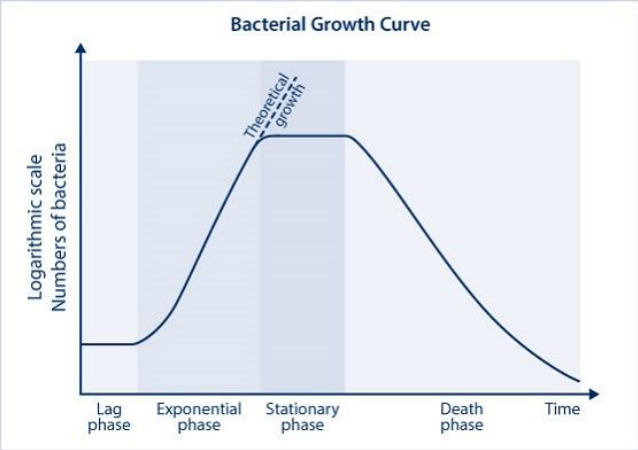


Fig. 3 The ideal growth curve of a bacterial culture in a bioreactor

The research and first production of microbiologically generated substances in medicines started during the second world war, when they discovered the advantages of using penicillin to treat the wounded soldiers. At that time, they discovered that using bacteria in microbiological processes have an advantage compared to the more conventional chemical synthesis. In chemical syntheses, many by-products are generated, some of them even in much larger proportions than the desired substance itself.

Fig. 4

In biological syntheses however, one sees much higher yields of the target substance. In addition, this synthesis often offers simpler separation methods. Besides, bacteria, as well as human or animal cells, synthesize specific substances that are difficult or even inaccessible with conventional chemical synthesis. In the last 20 years, powerful processes for isolating bacterial strains and other gene technological methods have allowed us to produce, isolate and multiply strains that do what they were developed for: synthesize

target substances specifically, selectively and efficiently. In most cases, these syntheses are carried out in the so-called bioreactors.

1.4.4 Bioreactor applications

Bioreactors come in many different sizes and shapes suitable for a wide variety of applications. From the smallest reactors with a capacity of a few milliliters to large bioreactors of up to 100 m^3. As a rule of thumb, one can assume that the oxygen flow is 0.1 to 0.15 times the working volume per minute for cell cultures and up to 2 times for bacterialcultures.

1.5 Food & beverage industry

Bioreactors are used in the production of foods and beverages for fermentation purposes, whether it is for addition of vitamins, colourants, flavourings, alcohol or antioxidants. Take yoghurt for example. This is a product produced from the fermentation of milk in bioreactors. Yoghurt cultures are lactic acid bacteria. Or beer… for beer brewing processes yeast cells are used for converting sugars into alcohol. And what about cheese; originally, cheese is produced from milk by adding natural existing rennet, which is an enzyme from a plant or an animal. Nowadays, rennet for making cheese is produced by yeast cells that are grown in a bioreactor. All examples of bioreactor applications. Micro-organisms have already been used for a long time in food production, but what are the reasons for the enormous increase in popularity of biotechnology since the second half of the 20th century?

1.6 Drug development and production

Biotechnology is becoming more and more important in drug development and production, as well as the multiplication of stem cells. Both are used for medical treatment. Time to market, cost reduction and consistent product quality are very important in designing and producing pharmaceutically active ingredients. Therefore, reliability of bioreactors and the possibility to scale-up the process from small to large sized bioreactors is very much desired.

1.7 Biobased chemicals and plastics

Other examples of biotechnological applications are biobased chemicals and plastics. Researchers are working on renewable plastics, which are made from organic materials with the help of enzymes and micro-organisms. There are already appealing examples of bio-based plastics such as toys, car parts and alternatives for PET bottles. A specific

example of bio-chemical production is using microalgae and sunlight for converting CO2. You can read our Application Note of a Belgium University about ‘Controlled CO2 supply for algae growth’

1.8 Sustainable Energy

The transition to sustainable energy is another driver that boosts the use of bioreactors. Biogas and biofuel in the form of biomethane, bioethanol and biodiesel are gaining popularity in our home, industrial and transport energy supply. The gas or fuel is created as a result of fermentation of organic material such as dung, sludge, organic waste, grass, corn, sugarcane. The fermentation tank, which is kept at a temperature of 38-40 ° C and is being stirred, is in fact a bioreactor.

1.9 Isolation, preservation and maintenance of industrial organisms

Isolation

In microbiology, the term **isolation** refers to the separation of a strain from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora, or from living beings with skin flora, oral flora or gut flora, in order to identify the microbe(s) of interest. Historically, the laboratory techniques of isolation first developed in the field of bacteriology and parasitology (during the 19th century), before those in virology during the 20th century. Methods of microbial isolation have drastically changed over the past 50 years, from a labor perspective with increasing mechanization, and in regard to the technology involved, and hence speed and accuracy.

In order to isolate a microbe from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora, or from living beings with skin flora, oral flora or gut flora, one has to separate it from the mix. Traditionally microbes have been cultured in order to identify the microbe(s) of interest based on its growth characteristics. Depending on the expected density and viability of microbes present in a liquid sample, physical methods to increase the gradient as for example serial dilution or centrifugation may be chosen. In order to isolate organisms in materials with high microbial content, such as sewage, soil or stool, serial dilutions will increase the chance of separating a mixture.

In a liquid medium with few or no expected organisms, from an area that is normally sterile (such as CSF, blood inside the circulatory system) centrifugation, decanting the supernatant

and using only the sediment will increase the chance to grow and isolate bacteria or the usually cell-associated viruses. If one expects or looks for a particularly fastidious organism, the microbiological culture and isolation techniques will have to be geared towards that microbe. For example, a bacterium that dies when exposed to air, can only be isolated if the sample is carried and processed under airless or anaerobic conditions. A bacterium that dies when exposed to room temperature (thermophilic) requires a pre-warmed transport container, and a microbe that dries and dies when carried on a cotton swab will need a viral transport medium before it can be cultured successfully. There are over a million species of microorganisms widely distributed in nature. Less than 1% of the world's microorganisms have been studied. In fact, only a few hundred species are important for industrial use. A selected list of organisms along with their products is given in the given table.

TABLE 19.3 A selected list of important microorganisms and their products

Microorganism	*Product*
Algae	
Chlorella sorokiniana	Single-cell protein
Spirulina maxima	Single-cell protein
Bacteria	
Acetobacter aceti	Acetic acid
Acetobacter woodii	Acetic acid
Bacillus subtilis	Bacitracin
B. brevis	Gramicidin
B. thuringiensis	Endotoxin
Clostridium aceticum	Acetic acid
Methylophilus methylotrophus	Glutamic acid
Pseudomonas denitrificans	Vitamin B_{12}
Actinomycetes	
Streptomyces aureofaciens	Tetracycline
S. griseus	Streptomycin
S. tradiae	Neomycin
Nocardia mediterranei	Rifamycin
Micromonospora purpurea	Gentamycin
Fungi	
Aspergillus niger	Citric acid
A. oryzae	Amylase, cellulase, single-cell protein
Candida lipolytica	Lipase
C. utilis	Single–cell protein
Penicillium chrysogenum	Penicillin
Saccharomyces cerevisiae	Ethanol, wine, single-cell protein
S. lipolytica	Citric acid, single-cell protein
Rhizopus nigricans	Steroids
Gibberella fujikuroi	Gibberellin
Trichoderma viride	Cellulase

Table. 1

1.10 Enrichment Methods for Isolation of Microorganisms:

The culture conditions can be appropriately modified to isolate certain types of microorganisms. The types of organisms that can be isolated by use of enrichment methods is given in given table For instance, thermophiles can be isolated by using high temperature while acidophilus can be isolated in acidic pH. Enrichment methods are certainly useful for quick isolation of specific types of organisms. Biotechnologists often prefer to isolate microorganisms from very extreme and unusual environments. This is done with a hope that such strains may be capable of producing new products of industrial importance. The unusual environments such as cold habitats, high altitudes, deserts, deep sea and petroleum fields are constantly being tried for this purpose. The enrichment methods described in given table. It will be very useful for isolating unusual strains.

TABLE 19.4 Types of microorganisms that can be isolated by enrichment methods

Type of organisms	*Enrichment method*
Thermophiles	High temperature (42–100°C)
Psychrotrophs	Low temperature (5–15°C)
Acidophiles	Low pH (2-4)
Halophiles	High NaCl concentration
Anaerobes	N_2 atmosphere
Actinoplanes	Pollen grains
Myxobacteria	Wood bark

1.10.1 Preservation

The preservation and maintenance of microbial cultures require special and careful attention, reliable preservation and appropriate quality control to ensure that recovered cultures perform in the same way as the original cultures. This requires a high degree of expertise in the maintenance and management of microbial cultures at ultralow temperatures, or as freeze-dried material, to secure their long-term integrity and relevance for future research, development, and conservation. This chapter outlines some of the important procedures and protocols involved in the conservation, preservation, and maintenance of microbial cultures. Microbial culture preservation aims at maintaining a microbial strain alive, uncontaminated, and without variation or mutation, as like original isolate. Many types of work require readily available microorganisms. The delays incurred

in acquiring them from other sources or trying to re-isolate them from their natural habitat can be unacceptable. Sometimes it is impossible to obtain the same isolate again. Sometimes repeated attempts of reisolation of the same organism have been failed.

1.10.2 Maintenance

The efficient practice of microbiology relies on the use of cultures of microorganisms. Authentic reference strains are required for comparison with laboratory isolates, for control cultures in standard methods of analysis, and for use in research and teaching. The great increase in number and size of industrial fermentations has accentuated the value of maintaining collections of microorganisms, especially of production strains, assay organisms and related species. Considerable work has been devoted to finding methods of maintaining cultures in a vigorous and stable condition. Industrially important microbes are also preserved for use in various industrial processes. The preservation of bacterial stock cultures to maintain viability and biochemical or virulence characteristics is an integral requirement for the continuity of microbiological research. Easy access to actively growing cultures is a requirement of most microbiological laboratories. Cultures are routinely required generally on a day-to-day basis for quality control, comparative testing, inoculum for bioassays and for various other reasons.

1.10.3 Kinetics of microbial growth and death

The kinetic model of cell growth is substantially capable to predict product formation. Mathematical models provide a strategy for solving problems encountered in fermentation process. A biochemical engineering approach to address this problem could be to develop a mathematical model which not only helps in the understanding of the system but also predicts various cultivation strategies to facilitate the optimization of a fermentation process, saving much of the time and cost for performing experiments. The presented overview indicates that many of the environmentally relevant aspects in growth kinetics are still waiting to be discovered, established, and exploited. A kinetic model that describes microbial growth, product formation and substrate consumption and the experimental data were fitted with modified logistic equation.

Cell growth implies increase in its mass and physical size controlled by physical, biological and chemical environments. Microbial growth is quantified by increase in the macromolecular and chemical constituents of the cell and growth pattern of each microbe

is unique. Cell growth and cell division are inseparable for microbes as bacteria divide by binary fission, yeast cells by budding and viruses divide intracellularly. Microbial growth during log phase is very important for the analysis of cells due to division by binary fission. A typical mammalian cell growth is influenced by nutrient availability and thus a threshold cell size is required for DNA synthesis and mitosis. Thus, each class of organisms have a different growth pattern based on their cell cycle and cell division. Understanding the growth kinetics of different classes of organisms forms the basis for fermentation process to achieve optimum product concentration.

Growth kinetics is an autocatalytic reaction which implies that the rate of growth is directly proportional to the concentration of cell. The cell concentration is measured by direct and indirect methods. Direct methods include measuring the cell mass concentration and cell number density by its dry weight, turbidity (optical density), plate counts etc. Whereas, indirect methods of measuring cell density are done by measuring the concentration of proteins, ATP or DNA content. Batch growth kinetics of a microbe follows a growth curve with lag phase as the initial phase during which cells adapt to a new environment. Multiple lag phases occur if the media is supplemented with more than one sugar and such type of growth is referred to as diauxic growth. Following the lag phase is the log phase in which the cell mass and cell number increases exponentially and then the depletion of nutrients starts which indicates the deceleration phase. The accumulation of toxic products results in deceleration phase after which stationary phase commences in which growth rate equals the death rate. The continuous growth kinetics accessed by a perpetual feeding process in which the growth is controlled by the concentration of the rate limiting nutrient.

Microbial growth kinetics explains the relationship between the specific growth rate of a microbe and its substrate concentration. Microbial growth kinetics largely depends on the laboratory culture conditions. In batch culture, microbial cell composition and its state change as a function of time and thus the rate of increase in biomass concentration was monitored. Alternatively, in continuous culture the concentration of substrate is at equilibrium and the culture grows at stable physiological state which provides more precise and reproducible data. However, the constant growth conditions represent an artificial growth environment which does not explain many microbial kinetic phenomena. Thus,

growth of microbial cells was performed under mixed substrates rather than single substrate to understand the growth kinetics of microorganisms in their natural environment.

1.10.4 Growth kinetics

Classified based on the relationship between product synthesis and energy generation in the cell:

- Growth associated
- Non-growth associated
- Mixed-growth associated

1.10.4.1 Growth associated

Growth linked products are formed by growing cells and hence primary metabolites. The given figure, clearly shows that product is formed simultaneously with growth of cells. That is product concentration increases with cell concentration. The formation of growth associated product may be described by the given equation.

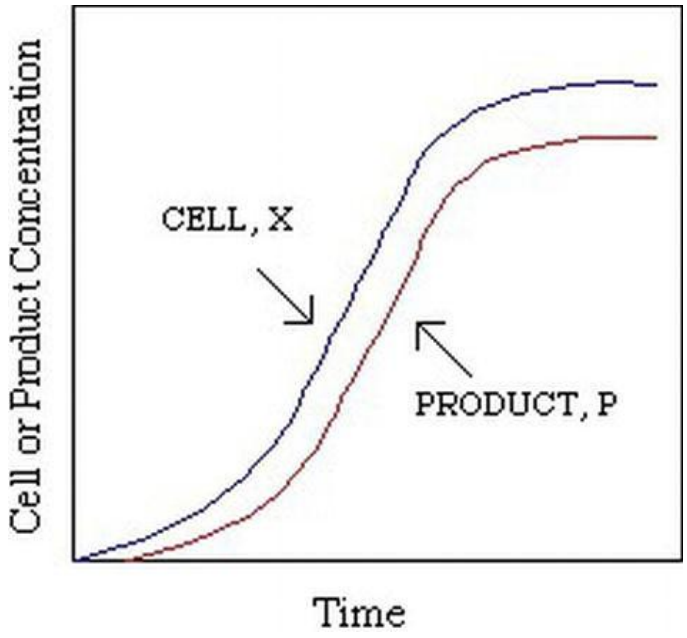


Fig. 6

Growth associated.

$dP/dt = rp = qpX$ E1

Where P = concentration of product

q_p = specific rate of product formation

X = biomass concentration.

1.10.4.2 Non-growth associated

They are formed by cells which are not metabolically active and hence are called secondary metabolites. The figure clearly shows that product formation is unrelated to growth rate but is a function of cell concentration. The formation of Non-growth associated product may be described by the given equation.

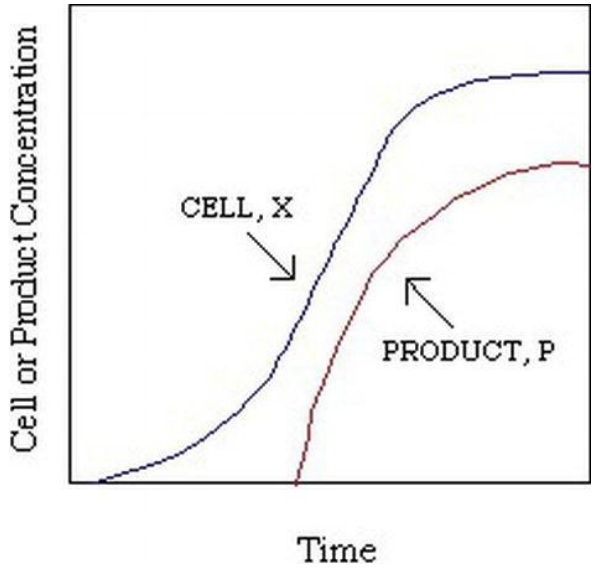


Fig. 7

Non-growth associated.

$q_p = \beta = \text{constant}$ E2

1.10.4.3 Mixed-growth associated

The product formation from the microorganism depends on both growth and Non-growth associated. It takes place during growth and stationary phases. In figure the , product formation is a combination of growth rate and cell concentration. The formation of Mixed-growth associated product may be described by given equation.

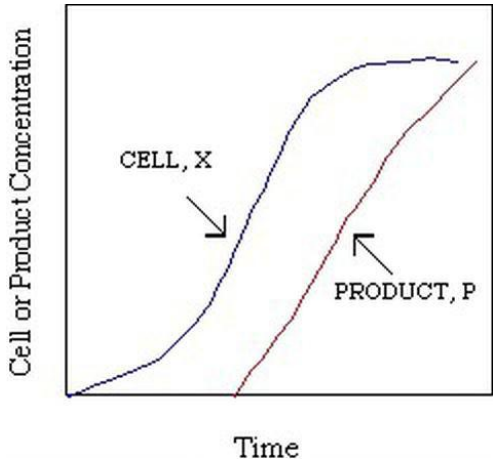


Fig. 8

Mixed growth associated.

$qp = \alpha\mu + \beta$ E3

1.10.4.4 Bacterial growth

Bacterial growth is proliferation of bacterium into two daughter cells, in a process called binary fission. Providing no event occurs, the resulting daughter cells are genetically identical to the original cell. Hence, bacterial growth occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry, direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.

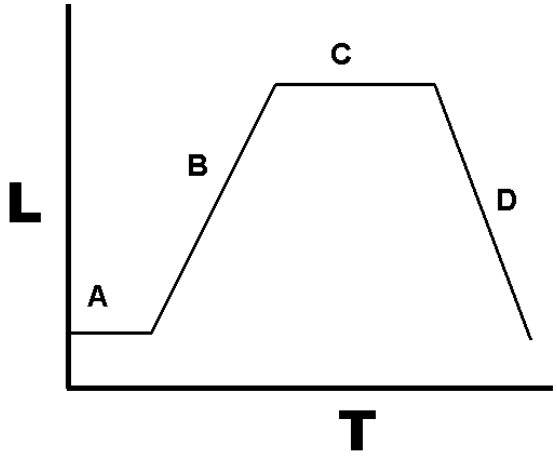


Fig. 9 Growth is shown as L = log(numbers) where numbers is the number of colony forming units per ml, versus T (time.)

In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: lag phase (A), log phase or exponential phase (B), stationary phase (C), and death phase (D).

1.10.4.4.1 Lag phase

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled

conditions, cyanobacteria can double their population four times a day and then they can triple their population. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

1.10.4.4.2 Stationary phase

The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase. Bridges et al. (2001) presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.

1.10.4.4.3 Death phase

At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions. This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations.

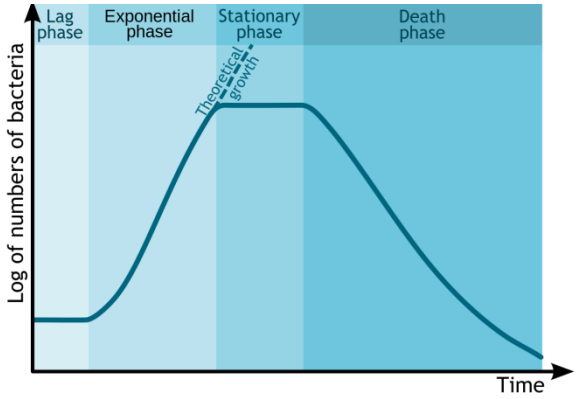


Fig. 10 Bacterial growth curve\Kinetic Curv

1.11 Media

Micro-organisms used for fermentation process grow on or in growth medium which satisfies the nutritional needs of microbes. Complete analysis is needed to be done to establish the most favourable medium for the growth of the microbe used for fermentation. Formulating medium at lab scale can be done by adding main ingredients like water, carbon source, nitrogen source, minerals and other supplements in pure form and in required quantities is very easy which supports the growth of the microbe whereas, the same may not support the satisfactory growth of the same organism at industrial level. Following criteria need to be satisfied for the material to be treated as medium at industrial level.

- It should give maximum yield of product.
- It should give minimum yield of undesired product. •
- It should be consistently available throughout the year. •
- It should be cheap.

Generally carbohydrates are used as “carbon sources” for fermentations at lab level. But, at industrial level cane molasses, corn steep liquor, sugar beet juice which are inexpensive sources are utilised. On contrary, some sensitive fermentation makes use of glucose, sucrose and other carbohydrates in their pure form which ensures the purity and quality of the final product. Sometimes starch will be added to the medium for the specific production of amylases. At lab level, peptone or tryptone or beef extract which is a partially digested hydrolysate, which is utilised in synthesis of proteins, components of nucleic acids and other essential cellular components. But at industrial level it is supplemented with soy meal or ammonia or nitrate salts to supplement the nitrogen source. Other elements include

growth factors, vitamins, anti-foaming agents, precursors, inducers chelating agents, trace elements such as Fe, Cu, Mn, Mo and Co, are added to the fermentation medium. Where growth factors, vitamins, precursors, inducers and trace elements directly supports the growth of microbe and anti-foaming agents are added to prevent the foam formation, in case of presence of higher concentrations of metal ions which is not preferable chelating agents are added.

1.12 Fermentation technology

Fermentation technology is widely used for the production of various economically important compounds which have applications in the energy production, pharmaceutical, chemical and food industry. Although, fermentation processes are used from generations, the need for sustainable production of products, meet the market requirements in a cost effective manner has put forward a challenging demand. For any fermentation based product, the most important thing is the availability of fermented product equal to that of market demand. Various microorganisms have been reported to produce an array of primary and secondary metabolites, but in a very low quantity. In order to meet the market demand, several high yielding techniques have been discovered in the past, and successfully implemented in various processes, like production of primary or secondary metabolites, biotransformation, oil extraction etc.

Medium optimization is still one of the most critically investigated phenomenon that is carried out before any large scale metabolite production, and possess many challenges too. Before 1970s, media optimization was carried out by using classical methods, which were expensive, time consuming, involving plenty of experiments with compromised accuracy. Nevertheless, with the advent of modern mathematical/statistical techniques, media optimization has become more vibrant, effective, efficient, economical and robust in giving the results. For designing a production medium, the most suitable fermentation conditions (e.g., pH, temperature, agitation speed, etc.) and the appropriate medium components (e.g., carbon, nitrogen, etc.) must be identified and optimized accordingly. Further, by optimizing the above said parameters, maximum product concentration could be achieved.

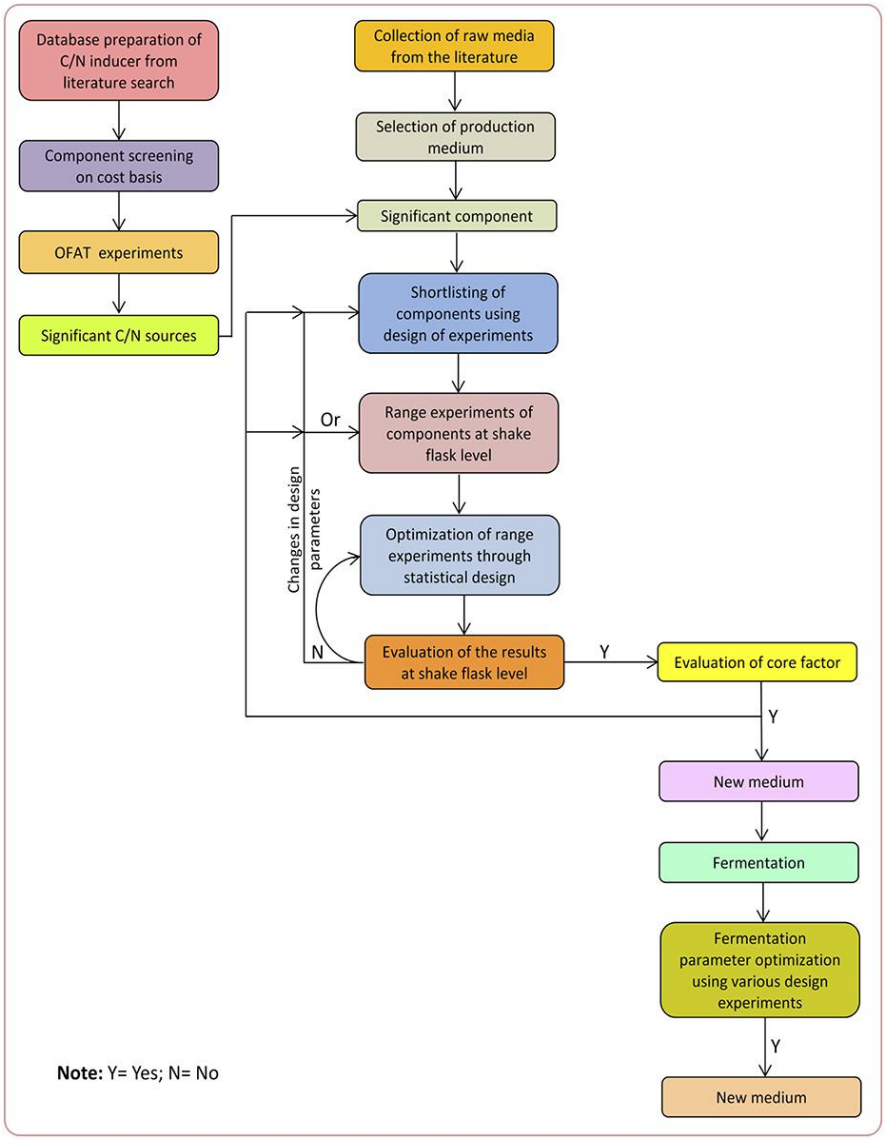


Fig. 11 Schematic diagram of a systematic approach of fermentation medium designing.

1.13 Nutritional Control of Metabolite Production

Fermented products that are used in our daily life are either primary or secondary metabolites produced during the trophophase and idiophase of the microbial growth, respectively. High productivity titer is the pre-requisite for the industrial production of any type of metabolite. The production of specific metabolites in high titer could be possible by maintaining proper control and regulation at different levels via transport and metabolism of extra-cellular nutrients, precursor formation and accumulation of intermediates. Fermentation processes, where the precursor(s) of the specific products are not added in the medium, carbon and nitrogen sources present in the medium during their metabolism may initiate the biosynthesis of precursors that regulate the metabolism and influence the end

product synthesis. Given this in view, nutrients type and their concentrations in the medium play an important role in commencing the production of primary and secondary metabolites as limited supply of an essential nutrient can restrict the growth of microbial cells or product formation. Generally, carbon and nitrogen sources present in the medium can influence the metabolite production.

1.13.1 Carbon Source

Carbon is the most important medium component, as it is an energy source for the microorganisms and plays an important role in the growth as well as in the production of primary and secondary metabolite. The rate at which the carbon source is metabolized can often influence the formation of biomass and/or the production of primary or secondary metabolites. Marwick et al. while studying antibiotics production from marine bacteria noticed that the gradually assimilating carbon sources, like, galactose generally enhances the production of secondary metabolites (antibiotics). A classic example for this is, penicillin production, where glucose is found to have repression effect. Later, it was found that lactose is a slowly assimilating carbon source and helped in the production of secondary metabolites (i.e., penicillin). Hence, in order to overcome the carbon catabolite repression phenomenon, the production process was established using lactose fermentation.

1.13.2 Nitrogen Source

Like carbon, the selection of nitrogen source and its concentration in the media also play a crucial role in metabolite production. The microorganism can utilize both inorganic and/or organic sources of nitrogen. Use of specific amino acids can increase the productivity in some cases and conversely, unsuitable amino acids may inhibit the synthesis of secondary metabolites during the optimization of actinomycin V production by *Streptomyces triostinicus* found that biosynthesis of actinomycin V involves tryptophan pathway and addition of amino acid tryptophan to the medium enhances the production. On the contrary, the same amino acid showed inhibitory effect in the production of candicidin from *Streptomyces griseus*.

1.13.3 Phosphate

Phosphate is another basic component which is required for the production of phospholipids present in the microbial cell membranes, and for the production of nucleic acids. The amount of phosphate which must be added in the fermentation medium depends upon the composition of the broth and the need of the organism, as well as according to the

nature of the desired product. For instance, some cultures will not produce secondary metabolites in the presence of phosphate, e.g., phosphatase, phytases etc. Sanchez and Demain reported that various secondary metabolites' production such as, actinorhodin, cephalosporin, clavulanic acid, streptomycin, tetracycline, vancomycin etc. is highly influenced by inorganic phosphate concentration present in the production medium.

1.14 Sterilization of Air

- In general, the industrial fermentations are carried out under vigorous and continuous aeration.
- For an effective fermentation, the air should be completely sterile, and free from all microorganisms and suspended particles.
- There is a wide variation in the quantity of suspended particles and microbes in the atmospheric outdoor air.
- On average, the microorganisms may range from 10-2,000/m^3 while the suspended particles may be 20-100,00/ m^3.
- Among the microorganisms present in the air, the fungal spores (50%) and Gram-negative bacteria (40%) dominate.
- Air or other gases can be sterilized by filtration, heat, UV radiation and gas scrubbing.
- Among these, heat and filtration are most commonly used.

1.14.1 Air sterilization by heat:

- In the early years, air was passed over electrically heated elements and sterilized. But this is quite expense, hence not in use these days.

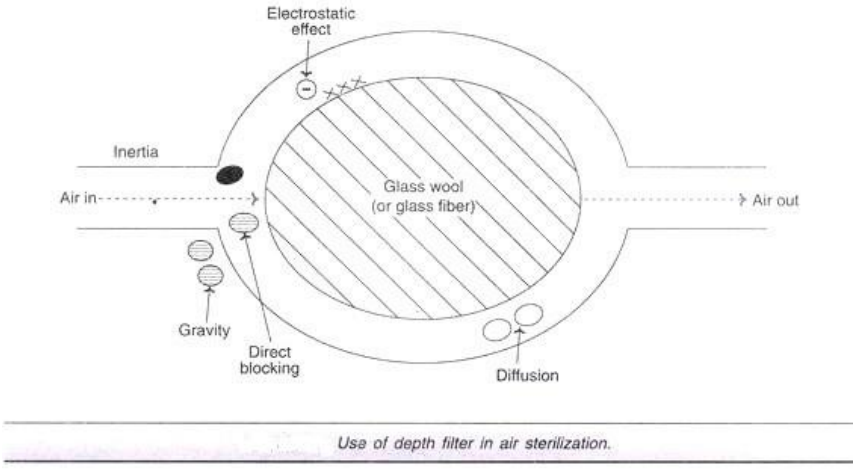
1.14.2 Air sterilization by filtration:

- Filtration of air is the most commonly used sterilization in fermentation industries.

1.14.3 Depth filters:

- When the air is passed through glass wool containing depth filters the particles are trapped and removed (Fig).
- This filtration technique primarily involves physical effects such as inertia, blocking, gravity, electrostatic attraction, and diffusion.
- Glass wool filters can be subjected to steam sterilization and reused.

- But there is a limitation in their reuse since glass wool shrinks and solidifies on steam sterilization.
- In recent years, glass fiber filter cartridges (that do not have the limitations of glass wool filter) are being used.



Use of depth filter in air sterilization.

Fig. 12

1.14.4 Membrane cartridge filters:

- These are removable pleated membrane filters made up of cellulose ester, nylon or polysulfone.
- Membrane cartridge filters are smaller in size, simpler for operation and replacement.
- The most important limitation of air sterilization is that there is no filter that can remove bacteriophages.
- Bacteriophages are capable of crippling the industrial fermentation.
- g., bacteriophages interfere in the production of glutamic acid by Corynebacterium glutamicum.

1.15 Other sterilization methods

1.15.1 Ionizing Radiation

Sterilization by ionizing radiation, primarily by cobalt 60 gamma rays or electron accelerators, is a low-temperature sterilization method that has been used for a number of medical products (e.g., tissue for transplantation, pharmaceuticals, medical devices). There are no FDA-cleared ionizing radiation sterilization processes for use in healthcare facilities. Because of high sterilization costs, this method is an unfavorable alternative to ETO and plasma sterilization in healthcare facilities but is suitable for large-scale sterilization. Some

deleterious effects on patient-care equipment associated with gamma radiation include induced oxidation in polyethylene and delamination and cracking in polyethylene knee bearings.

1.15.2 Dry-Heat Sterilizers

This method should be used only for materials that might be damaged by moist heat or that are impenetrable to moist heat (e.g., powders, petroleum products, sharp instruments). The advantages for dry heat include the following: it is nontoxic and does not harm the environment; a dry heat cabinet is easy to install and has relatively low operating costs; it penetrates materials; and it is noncorrosive for metal and sharp instruments. The disadvantages for dry heat are the slow rate of heat penetration and microbial killing makes this a time-consuming method. In addition, the high temperatures are not suitable for most materials. The most common time-temperature relationships for sterilization with hot air sterilizers are 170°C (340°F) for 60 minutes, 160°C (320°F) for 120 minutes, and 150°C (300°F) for 150 minutes. *B. atrophaeus* spores should be used to monitor the sterilization process for dry heat because they are more resistant to dry heat than are *G. stearothermophilus* spores. The primary lethal process is considered to be oxidation of cell constituents.

1.15.3 Microwave

Microwaves are used in medicine for disinfection of soft contact lenses, dental instruments, dentures, milk, and urinary catheters for intermittent self-catheterization. However, microwaves must only be used with products that are compatible (e.g., do not melt). Microwaves are radio-frequency waves, which are usually used at a frequency of 2450 MHz. The microwaves produce friction of water molecules in an alternating electrical field. The intermolecular friction derived from the vibrations generates heat and some authors believe that the effect of microwaves depends on the heat produced while others postulate a nonthermal lethal effect. The initial reports showed microwaves to be an effective microbicide. The microwaves produced by a “home-type” microwave oven (2.45 GHz) completely inactivate bacterial cultures, mycobacteria, viruses, and *G. stearothermophilus* spores within 60 seconds to 5 minutes depending on the challenge organism. Another study confirmed these resuIts but also found that higher power microwaves in the presence of water may be needed for sterilization. Complete destruction of *Mycobacterium bovis* was obtained with 4 minutes of microwave exposure (600W, 2450

MHz). The effectiveness of microwave ovens for different sterilization and disinfection purposes should be tested and demonstrated as test conditions affect the results (e.g., presence of water, microwave power).

1.16 Media Sterilization

The prevention of microbial contamination is fundamental to many industrial food fermentation processes. Media sterilization is the destruction or removal of all forms of microbial life from the aqueous feedstock. In industrial fermentations, components such as vessels, pipework, media, inlet air, and exhaust gases are frequently sterilized by a combination of wet-heat and filtration methods. Wet-heat methods are less expensive and more effective than dry-heat methods, and thus are employed commonly in fermentation industries to destroy unwanted microorganisms. The wet-heat sterilization conditions typically used to kill all microorganisms, including bacterial spores, are listed in given table. These conditions may be achieved in an autoclave in an atmosphere of saturated steam.

Temperature (°C)	Time (min)	Pressure (kPa)
121	15	103.4
126	10	137.8
134	3	206.7
140	0.67	261.8

Table1. Wet-heat sterilization conditions

Most heat treatments of industrial fermentation media are designed to selectively kill only those microorganisms of particular concern. Pasteurization is the method commonly employed for destroying frequently encountered pathogenic bacteria. Table 9 gives the pasteurization conditions used for common food fermentation media.

Strategies for the bulk sterilization of fermentation media include *in situ* steam injection of a full charge of nonsterile medium in the fermenter, or steam conduction through attemperation jackets in agitated fermenters. Alternatively, the media and vessels may be sterilized separately before fermentation. Antifoam agents, especially those that are oil

based, often are difficult to sterilize. Inert, silicone-based antifoams may be used, which, although expensive, are nontoxic toward microorganisms.

The loss of available carbon and the buildup of potentially toxic or inhibitory compounds may occur during heat treatments employed to sterilize or pasteurize growth media. For example, the excessive heating of molasses may generate undesired caramelization products, following the Maillard reaction between reducing sugars and the free amino groups in proteins. Heat also may destroy vitamins and other growth factors essential for microbial growth. Some of the problems that may be encountered during media sterilization, and their possible avoidance measures, are listed in given table.

Problem	Solutions
Sugar caramelization	Sterilize sugars separately and add aseptically
Metal precipitation	Sterilize phosphate source and metal salts separately
Unsuccessful sterilization of particulate and viscous media	Ensure sufficient agitation in fermenter to achieve heat transfer
Very high initial bioburden	Good housekeeping, cleaning in place, elimination of residues, sterilization of pipework dead legs

Table 2 Problems and solutions relating to media sterilization

1.17 Summary

Bioprocessing is loosely defined as being the production of a value-added material from a living source. The key component in the system is that the source organism is alive and responsive to its environment. As such, the paradigm is that it will adjust its physiology to maximize efficiency in response to comparatively minor changes in its physico-chemical environment. This translates to potential variability in the nature of the system output – in our case, the product. For the bioprocess engineer, the goal is to minimize such changes in physiology by understanding and controlling the production process. The archetypical bioprocess is based on growth of a microorganism under conditions which encourage the production of a product that can be recovered at an economically viable yield and in a format which permits its use. The product is, almost by definition, of limited or no value to the producing organism. Therefore, bioprocessing can be argued to run counter to the

evolutionary drive of the organism itself. Any reduction in wastage on the part of the production organism (either by limiting production of the product or by enhancing ability to recycle the product) will lead to increased fitness and population shift to the lower yielding variant. This is the second paradigm of bioprocessing – that the system will tend to move toward a state of lower productivity.

Terminal questions

Q.1. Define bioreactor. Explain various types of bioreactors.

Answer:----------

Q.2. Explain biobased chemicals and plastics.

Answer:----------

Q.3 Explain Food & beverage industry.

Answer:----------

Q.4 Describe kinetics of microbial growth and death.

Answer:----------

Q.5 Explain industrially important microorganisms.

Answer:----------

Q.6 Write a short notes on bacterial growth.

Answer:----------

Q. 7 What do you mean by fermentation?

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-2: Types of fermentation process

Structure

2.1 Introduction

2.2 Process of fermentation

Fermentation is an anaerobic biochemical process. In fermentation, the first process is the same as cellular respiration, which is the formation of pyruvic acid by glycolysis where net 2 ATP molecules are synthesised. In the next step, pyruvate is reduced to lactic acid, ethanol or other products. Here NAD+ is formed which is re-utilized back in the glycolysis process.

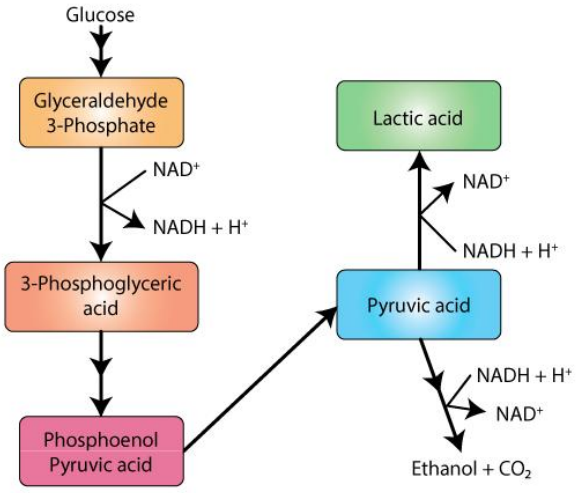


Fig. 3

2.2.1 Types of fermentation

- **Homo fermentation:** only one type of product formation
- **Hetero fermentation:** more than one product formed

On the basis of the end product formed, fermentation can be categorized as follows:

2.2.1.1 Lactic Acid Fermentation

Lactic acid is formed from pyruvate produced in glycolysis. NAD+ is generated from NADH. Enzyme lactate dehydrogenase catalyses this reaction. Lactobacillus bacteria prepare curd from milk via this type of fermentation. During intense exercise when oxygen supply is inadequate, muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Lactate.dehydrogenase]{Lactobacillus} 2CH_3CHOHCOOH(LacticAcid)$$

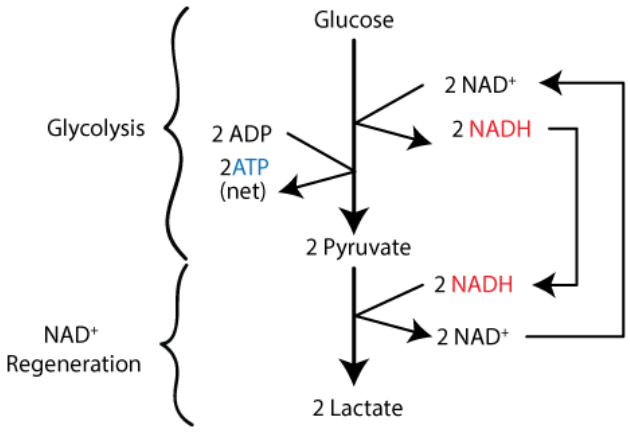


Fig. 4

2.3 Alcohol fermentation

This is used in the industrial production of wine, beer, biofuel, etc. The end product is alcohol and CO_2. Pyruvic acid breaks down into acetaldehyde and CO_2 is released. In the next step, ethanol is formed from acetaldehyde. NAD+ is also formed from NADH, utilized in glycolysis. Yeast and some bacteria carry out this type of fermentation. Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Decarboxylase]{Dehydrogenase} 2CH_3CHO(Acetaldehyde) + 2CO_2 \rightarrow 2C_2H_5OH(Ethanol)$$

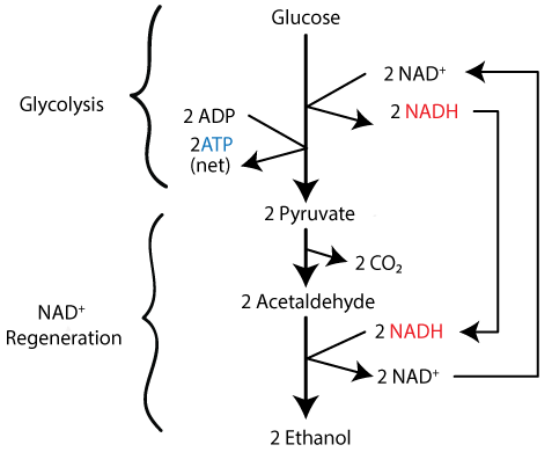


Fig. 5

2.4 Acetic acid Fermentation

Vinegar is produced by this process. This is a two-step process. The first step is the formation of ethyl alcohol from sugar anaerobically using yeast. In the second step, ethyl alcohol is further oxidized to form acetic acid using acetobacter bacteria. Microbial oxidation of alcohol to acid is an aerobic process.

$$C_2H_5OH\,(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH(acetic\ acid) + H_2O$$

2.5 Butyric acid Fermentation

This type of fermentation is characteristic of obligate anaerobic bacteria of genus clostridium. This occurs in retting of jute fibre, rancid butter, tobacco processing and tanning of leather. Butyric acid is produced in the human colon as a product of dietary fibre fermentation. It is an important source of energy for colorectal epithelium. Sugar is first oxidized to pyruvate by the process of glycolysis and then pyruvate is further oxidized to form acetyl-CoA by the oxidoreductase enzyme system with the production of H_2 and CO_2. Acetyl-CoA is further reduced to form butyric acid. This type of fermentation leads to a relatively higher yield of energy. 3 molecules of ATP are formed.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$

2.6 Advantages of Fermentation:

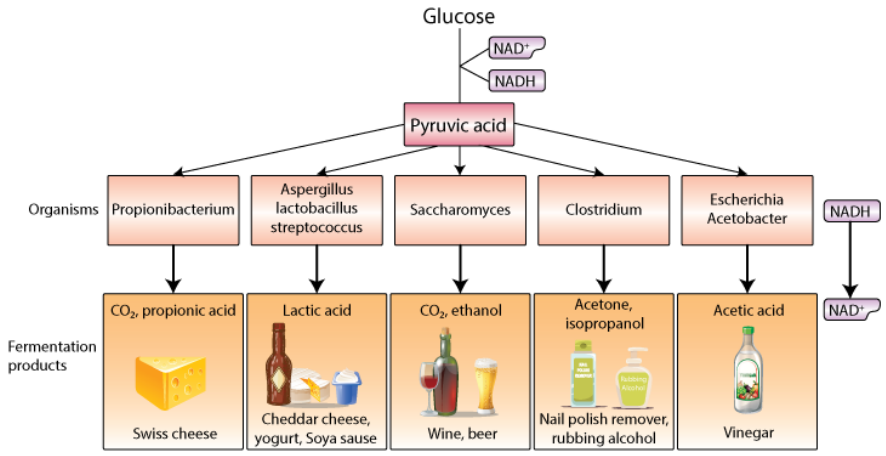


Fig. 6

Fermentation is suitable for all kinds of environments. It is one of the oldest metabolic processes which is common to prokaryotes and eukaryotes. Fermentation is widely used in various industries. Using suitable microorganisms and specified conditions different kinds of products are formed namely:-

- Wine
- Beer
- Biofuels
- Yoghurt
- Pickles
- Bread
- Sour foods containing lactic acid
- Certain antibiotics and vitamins

Fermentation can make food nutritious, digestible and flavoured. There are many benefits of consuming fermented food.

- It improves digestion and helps to maintain intestinal bacteria
- It has an anti-cancer effect.
- Improves immune system
- Reduces lactose intolerance

Other than the food industry, there are many other areas where the fermentation process is used. Methane is produced by fermentation in the sewage treatment plants and freshwater sediments.

2.7 Analysis of batch, fed-batch and continuous bioreactors

2.7.1 Analysis of batch

Batch fermentation is a process where all the substrate and nutrients are added at zero time or soon after inoculation takes place, and the vessel is allowed under a controlled environment to proceed until maximum end product concentration is achieved. More than 84% of all alcohol in North America is made by batch fermentation – mainly due to the flexibility in a batch plant, the higher product concentrations possible, the ability of industry to minimize infection/loss of yield, the ease of the practice, and low maintenance costs. Despite of above-noted advantages, batch fermentation requires frequent cleaning, sanitizing, and filling of fermentors, resulting in loss of productivity. To maintain the same productivity, the initial (i.e., capital) investment for a batch fermentation is higher than its continuous counterpart.

Batch fermentations are the most commonly used and simplest models to study the fermentation activity of colonic microbes. These batch fermentors are usually anaerobic sealed bottles with pure cultures, defined mixed cultures or faecal slurry, and are used to study the effects of added NSP on the microbes and their fermentation activity. They model a certain part of the gastrointestinal tract and the run-times in batch fermentations range from 2 to 24 hours (Mäkivuokko & Nurminen 2006; Rumney & Rowland 1992). The accumulation of fermentation products (e.g. SCFA) and the depletion of nutrients can alter the conditions and microbiota balance in the fermentor, thus affecting the *in vivo* relevance in longer simulations. This can be avoided by using more complex simulation models with several vessels and fluid transitions.

In batch fermentation, all nutrients necessary for cell growth and l-lysine production are present in the medium from start, and the batch fermentation is started by inoculation with biomass from a seed reactor. The batch process is finished when nutrients (typically the carbon source) have been consumed. A disadvantage with batch fermentations is that only a limited amount of the carbon source can be used in the growth medium, and this will limit the maximum product concentration of l-lysine in the process. High initial carbon source concentration will lead to high osmolarity that may prolong the lag phase of the production strain and thus reduce the productivity. Although batch fermentation is no longer the preferred method for industrial l-lysine production, it is still used in many smaller fermentation plants worldwide.

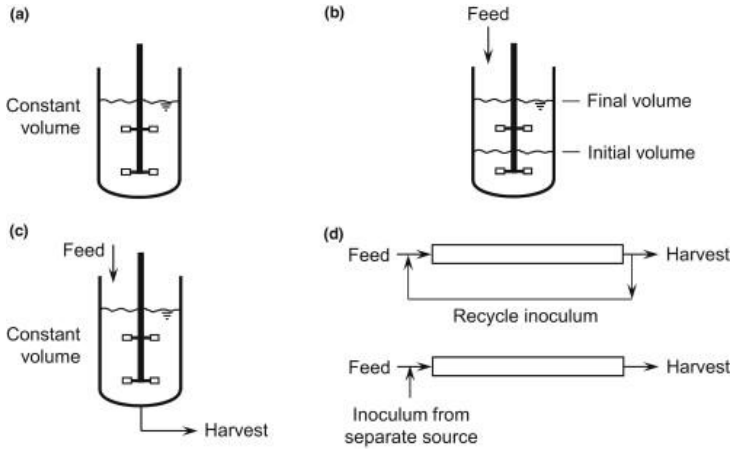


Fig. 7

In batch fermentation, substrate and microorganism are loaded into the fermenter batchwise, and this is the most popular and simple method for ethanol production. Batch fermentation has the advantages of low investment costs, simple control and operations, and easy-to-maintain complete sterilization. However, seed culture is needed for each new batch. Bioethanol from corn in the USA is almost entirely produced using batch fermentation. A higher initial sugar concentration is required in order to achieve more efficient ethanol production; however, a high sugar concentration will inhibit the growth and function of fermenting microorganisms due to excessive osmosis to result in a low fermentation yield with a prolonged fermentation period. The batchwise fermentation method will alleviate this drawback. Fermentation is started with a low initial sugar concentration to allow robust growth of the microorganisms at an early stage, and sugar is added periodically when it is consumed.

2.7.2 Analysis of fed-batch

Fed-batch fermentation, which is also called semicontinuous fermentation, refers to a method of feeding fresh medium or certain nutrients intermittently or continuously during the process of batch fermentation. It is applied broadly in the entire fermentation industry. He et al. found that cultivation under fed-batch fermentation mode could enhance butyric acid production ($P < .01$) by *Clostridium butyricum* ZJUCB significantly. They compared the difference between batch and fed-batch fermentation of butyric acid production at pH 6.5. It was found that maximum value of butyric acid concentration (16.74 g/L) was obtained in the fed-batch fermentation compared with 12.25 g/L in batch fermentation.

In addition, the fed-batch fermentation can be used for producing lactate production from corn cob hydrolysate by *Rhizopus oryzae*. The final l-lactate concentration, yield, and productivity were 132.4 g/L, 94.6%, and 1.38 g/(L·h), respectively, when using the fed-batch fermentation at feeding rate of 20 mL/h. In general, it has a wide range for the use of fed-batch fermentation technology for production and research including the cell protein, amino acids, growth hormone, antibiotics, vitamins, enzymes, organic solvents, organic acids, nucleotides, and polymers. It is widely used in liquid fermentation, solid fermentation, and mixed culture fermentation. With the intense research work and the application of microcomputer in the automatic control of fermentation process, the fed-batch fermentation technology will play an effective role.

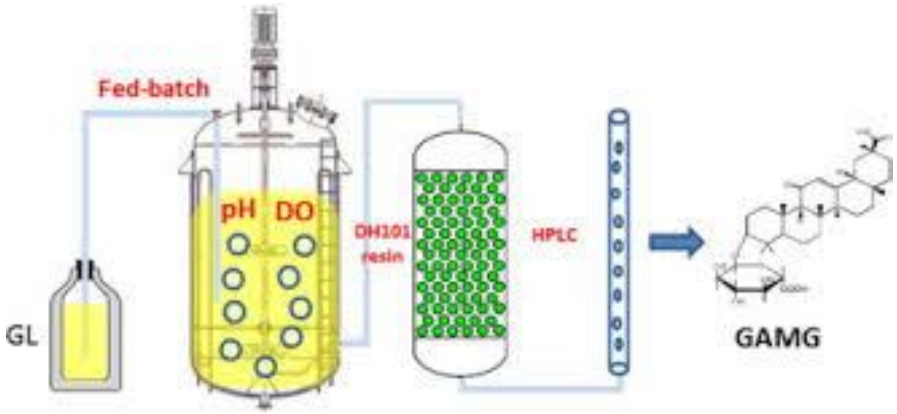


Fig. 8

Fed-batch fermentation is applied when a high concentration of substrate is toxic to the microbial culture. With the aim of keeping the substrate concentration below toxic levels, substrate is gradually added at a slow rate as the substrate is consumed by the culture. Fermentation is stopped and products are recovered only when the volume of the fermentation broth reaches 75% of the fermenter volume.

2.7.3 Analysis of continuous fermentation

There are three main types of continuous process biogas digesters: the fixed dome (Chinese type), the floating cover (Indian type) and the plug flow or bag type. The fixed dome consists of an air-tight container constructed of brick, stone or concrete, the top and bottom being hemispherical and the walls straight. Sealing is achieved by building up several layers of mortar on the digester's inner surface. Gas leakage through the dome is often a major problem in this kind of design. The digester is fed on a semi-continuous basis (usually about once per day) and the gas produced rises to be stored under the upper dome.

Gas pressures in the dome can reach 1–1.5 m of water pressure. Typical feedstock for these digesters is animal manure, “nightsoil” (human excreta) and agricultural waste. Gas production from the digester is commonly in the order of 0.1–0.2 volumes of gas to volume of feedstock per day, retention time in the digester being 60 days at 25°C.

Continuous fermentation is a microbial process with a constant flow of culture medium through the reactor. The main difference compared with an animal cell perfusion process is that no device prevents the biomass from staying in the culture vessel in a continuous fermentation.[1] The volume in a continuous fermentation is usually constant in industrial applications, but it can fluctuate in specific processes such as waste water treatment. The concept of continuous fermentation processes is closely linked to the chemostat, where one nutrient is growth limiting and used to determine the growth rate. However, there are several other, less common ways by which a continuous fermentation can be controlled: through constant pH (pH-auxostat), constant optical density (turbidostat), and constant substrate.

It starts as a batch process. At a certain point, for example, when the culture reaches the exponential growth phase, or when the culture becomes substrate limited, a feed with fresh growth medium is started, and an equal volume of culture broth is removed. Continuous fermentation is a superior tool in research, but the number of industrial applications is limited. Reasons for this include an increased risk for contamination, risk for genetic drift in the culture, and difficulties to control the process.

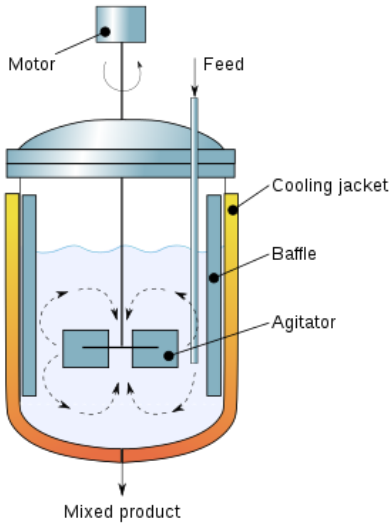


Fig. 9

2.8 Applications

The use of continuous fermentation is very extensive. The productivity of continuous culture of bacteria is higher than that of batch culture. Industrial production of single cell protein usually uses continuous culture. For example, British ICI Company uses the method of continuous cultivation to produce single-cell protein with a volume of 1500 m^3 giant fermentation tank, which makes the annual output reach 70 thousand tons. In addition, continuous fermentation can be used for the production of metabolic products and multistage continuous culture for cell growth and the synthesis of product. For example, when using glucose galactose as the carbon source to produce β-galactosidase by *Monascus* spp., cell growth is conducive using glucose and enzyme production was induced by galactose. But the glucose and galactose have a competitive inhibitory effect. Brown et al. produced α2 interferon by *E. coli* recombinants with two stages of continuous culture: the first level is for cell growth and interferon synthesis.

In the second level, they join the ampicillin to promote cell lysis and the release of intracellular interferon. Wen-Chien et al. successfully enhance the ethanol production with continuous fermentation in a two-tank system. In their search, continuous fermentation with the feeding sugar concentration of 160 g/L at r = 0.9 and dilution rate of 0.2 per h achieved the highest productivity with less than 2% of the unconverted sugar in the product steam. Under the same cell recycling ratios, a productivity range of 6.9–7.5 g/L h could be achieved with feeding concentrations of 80–200 g/L, while batch fermentation at these sugar concentrations led to productivities of 3.85–4.48 g/L h. In short, compared with other fermentation models, continuous fermentation has more advantages and effects.

2.9 Analysis of mixed microbial populations

Microbes have played an integral role in the evolution & operation of biosphere. Primitive algae capable of increasing the oxygen level by about 1% mixed microbial population can grow in air, soil, bodies of water and inside higher organisms. Like

- The symbioses with ruminant animals such as cattle, sheep, goat etc. The microbial activity in rumen is decomposition of cellulose and other complex carbohydrates of plant material into simpler substances which can be absorbed by blood stream.
- The natural flora of microorganisms which inhabit the human body.

- Dental plaque consists of several microorganisms.
- Production of wine and beer by fermenting fruits and grains.

A range of autotrophic and heterotrophic enrichment cultures were established to determine the cultural bacterial diversity present in samples obtained from the acidic runoff of a chalcocite overburden heap and from laboratory-scale (1- to 4-liter) batch and continuous bioreactors which were being used for the commercial assessment of the bioleachability of zinc sulfide ore concentrates. Strains identified as Thiobacillus ferrooxidans, Thiobacillus thiooxidans, "Leptospirillum ferrooxidans," and Acidiphilium cryptum were isolated from both the natural site and the batch bioreactor, but only "L. ferrooxidans," a moderately thermophilic strain of T. thiooxidans, and a moderately thermophilic iron-oxidizing bacterium could be recovered from the continuous bioreactor running under steady-state conditions. Sequence analysis of the 16S rRNA genes of 33 representative strains revealed that all of the strains were closely related to strains which have been sequenced previously and also confirmed the phylogenetic diversity of bacteria present in bioleaching environments.

Microbial population biology, in practice, is the application of population ecology and population genetics toward understanding the ecology and evolution of bacteria, archaebacteria, microscopic fungi (such as yeasts), additional microscopic eukaryotes (e.g., "protozoa" and algae), and viruses. Microbial population biology also encompasses the evolution and ecology of community interactions (community ecology) between microorganisms, including microbial coevolution and predator-prey interactions. In addition, microbial population biology considers microbial interactions with more macroscopic organisms (e.g., host-parasite interactions), though strictly this should be more from the perspective of the microscopic rather than the macroscopic organism. A good deal of microbial population biology may be described also as microbial evolutionary ecology. On the other hand, typically microbial population biologists (unlike microbial ecologists) are less concerned with questions of the role of microorganisms in ecosystem ecology, which is the study of nutrient cycling and energy movement between biotic as well as abiotic components of ecosystems.

Microbial population biology can include aspects of molecular evolution or phylogenetics. Strictly, however, these emphases should be employed toward understanding issues of

microbial evolution and ecology rather than as a means of understanding more universal truths applicable to both microscopic and macroscopic organisms. The microorganisms in such endeavors consequently should be recognized as organisms rather than simply as molecular or evolutionary reductionist model systems. Thus, the study of RNA *in vitro* evolution is not microbial population biology and nor is the *in silico* generation of phylogenies of otherwise non-microbial sequences, even if aspects of either may in some (especially unintentional) manner be analogous to evolution in actual microbial populations.

Microbial population biology can (and often does) involve the testing of more-general ecological and evolutionary hypotheses. Again, it is important to retain some emphasis on the microbe since at some point this "question-driven" microbial population biology becomes instead population biology using microorganisms. Because the point of departure of these potentially disparate emphases can be somewhat arbitrary, there exist vague and not universally accepted delimits around what the discipline of microbial population biology does and does not constitute.

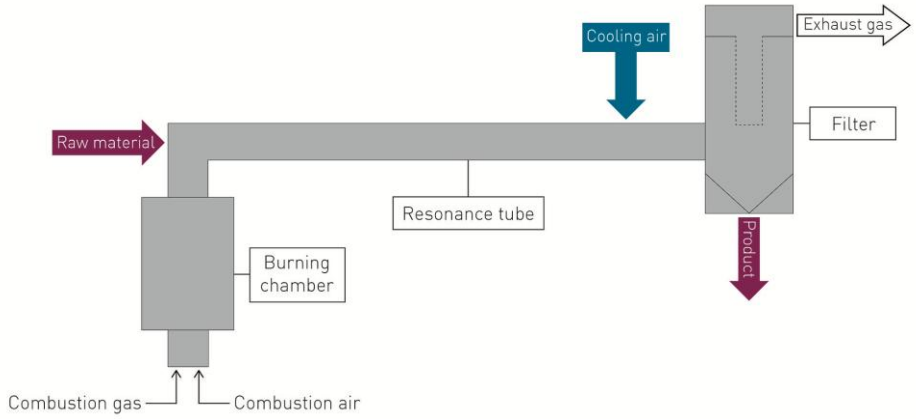
Bioprocess technology/engineering deals with the development and applications of competent strains of microorganisms for their optimum metabolite production in a specialized bioreactor, depending on the growth kinetics and nature of metabolite production at their suitable physico-chemical and nutritional levels. However, three important components are taken under consideration involving several microbiological and biochemical engineering skills, also known as bio-molecular engineering along with architecture and design of bioreactor systems, since the design and architecture of bioreactor is solely based on the nature of microorganisms, growth and metabolite production along with the elimination of toxic substances during fermentation. In the current scenario, the development of bioreactor technology can change any process parameters economically with greater productivity and quality of microbial products, therefore, the design and architecture of a bioreactor is important, and can make a new revolution in bioprocess engineering.

The mutational and recombinant DNA technology has developed several beneficial microorganisms, and their large scale economical production requires fermentation

technology mainly in the development of a suitable bioreactor systems and process parameters. Much work have been done by microbiologists and biochemists for the production of various microbial metabolites like enzymes, hormones, polysaccharides, organic acids vitamins etc at laboratory scale through flask culture experiments, while commercial scale production requires several correction factors as well as a specialized bioreactors for continuous production of microbial metabolites with minimum energy consumption. The importance of bioreactor systems and its evaluation for the architecture requires optimization of fermentation parameters by bench top fermentor, and then a bioreactor should be fabricated on the guidelines of microbiologist adopting the corrective measures of several growth parameters for optimum production of microbial metabolites.

Pulsation reactor technology is a thermal procedure with a special functional principle that results in reaction parameters and a reaction medium, and which ultimately leads to other property parameters in terms of surface, reactivity, homogeneity and particle size of the powder material. The technology has proven particularly effective in the manufacture of ceramic and submicroscale powders, as well as in the production of highly active catalysts. Also, simple oxides such as zirconium oxide with doping elements or mixed oxides like spinel can be produced in the pulsation reactor.

Fundamentally, a pulsation reactor can be described as a periodically transient tube-type reactor that can be used to thermally treat gas-borne matter. The pulsating flow of hot gas is generated within a hot gas generator in the reactor by burning natural gas or hydrogen with ambient air. The hot gas flows through the so-called “resonance tube” into which reactants in powder, liquid or gas form can be added. The reactant is treated by hot gas flowing through the resonance tube and this process ends through suitable cooling. The finished product is separated in a cleanable filter. The product can be removed throughout the ongoing process using a sluice system and collected in barrels or big bags. The risk of the product contaminating the environment can be completely excluded through the vacuum present in the reactor, including the filter.



Schematic structure of a pulsation reactor

Fig. 10

An almost tube-like flow with an almost constant temperature across the pipe diameter is generated in the resonance tube (the treatment area for the reactant) through the pulsating flow of hot gas. This tube-shaped flow results in a narrow residence time distribution. Furthermore, the pulsating hot gas flow results in an increased convective heat and mass transfer to and/or from the particles. Hot gas can be generated in two different ways. Either the hot gas generator works with a high level of excess air ($\lambda \geq 2$) or the hot gas atmosphere can be generated with little oxygen or none at all. The hot gas temperatures in the pulsation reactor range from 250° - 1,350 °C.

However, the actual treatment temperature may differ significantly from these values after the reactant has been added. The necessary treatment temperature can be determined through systematic experiments with temperature variation. In addition to the treatment temperature and the type of hot gas atmosphere, pulsation reactors also provide the option

of adjusting the frequency and amplitude of the pulsation (i.e. the spatially oscillating flow of hot gas) according to the material to be treated, without changing the geometry of the plant.

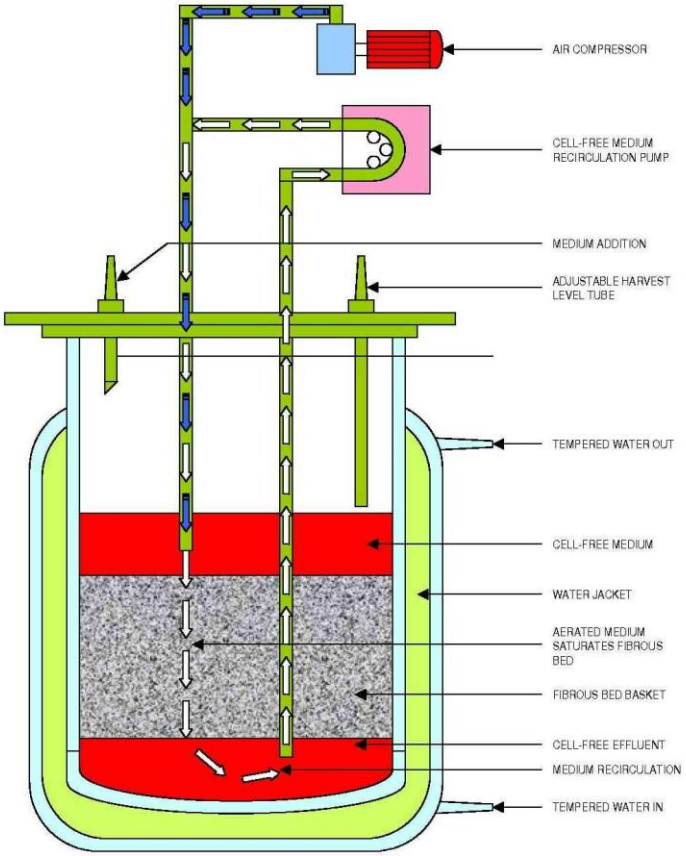


Fig. 11 Different Types of Fermentors / Bioreactors

2.10 Photobioreactor

Photobioreactor design is equally as important as supplemental nutrients in optimizing H_2 production from microalgae, especially through the direct biophotolysis method. A good photobioreactor should have the following characteristics: (1) effective illumination area, (2) optimal gas–liquid transfer rate, (3) easy to operate, (4) low potential for contamination, (5) low capital and production costs, and (6) minimal land area requirements. There are various type of photobioreactors designed mainly for microalgae biomass production, such as (1) open system, which is normally known as raceway pond, and (2) closed system, which include tubular, bubble column, flat plate, and fermentor.

Fig. 12

To date, the raceway pond appears to be the most feasible method for mass cultivation of microalgae biomass. The system consists of a closed-loop recirculation channel (oval shape) in which mixing is provided by paddle wheels. The depth of the pond is usually 0.2–0.5 m to ensure the microalgae receive sufficient exposure to sunlight. The system is relatively low-cost compared to a closed system and requires a minimal energy input to operate. However, it is impossible to create an anaerobic environment in the raceway pond for H_2 evolution from microalgae. Even if H_2 evolution is possible, H_2 collection would become problematic because of the large cultivation area involved. In this case, an extremely high energy input to operate air pump is expected, which could potentially result in a net negative energy balance in the life cycle of microalgae H_2 production.

A closed photobioreactor is an alternative for scaling-up H_2 production from microalgae. Generally, a closed photobioreactor offers several advantages compared to raceway ponds, including better contamination control, higher microalgae biomass yield per unit reactor volume, and the possibility of using single-strain culture for a prolonged duration. A closed photobioreactor has a high surface area to volume (A/V) ratio that allows higher solar energy conversion efficiency by microalgae to H_2 through direct biophotolysis. Among all the closed photobioreactors, the horizontal tubular/air-lift, helical tubular, and α-shaped photobioreactor exhibited a relatively higher A/V ratio and is currently being tested under lab-scale conditions for H_2 production from cyanobacteria and microalgae.

However, recent LCA studies have shown that closed photobioreactors used substantial amounts of energy and resulted in an unsustainable practice for producing biofuel from microalgae. For example, it was estimated that the energy input to operate air-lift photobioreactor was around 350% higher compared to raceway pond. Furthermore, an energy assessment carried out by Stephenson et al. indicated that using an air-lift photobioreactor to cultivate microalgae for biodiesel production could lead to net negative energy balance in the associated life cycle boundary. Most of the energy is used for powering pumps so that sufficient mixing and optimum gas–liquid transfer is achieved. Based on current technology maturity, an air-lift photobioreactor is not up to the commercialization stage yet, unless proper modifications are made to reduce the overall operating energy consumption.

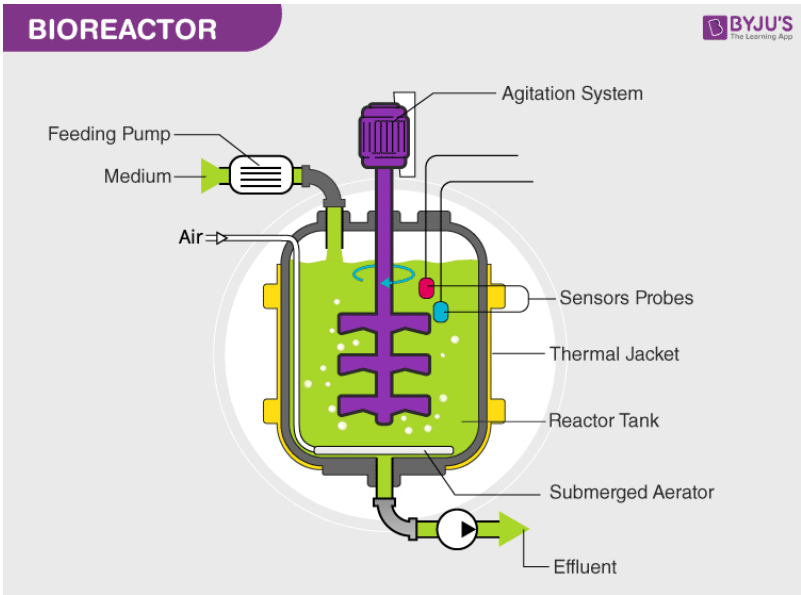


Fig. 13

To overcome the limitations of open and closed photobioreactors, integrating both types of photobioreactors into one system may be a possible solution for commercial H_2 production from microalgae. For the first stage, microalgae are cultivated in a nutrient-rich medium in raceway pond to promote the growth of microalgae until a certain quantity of cells is achieved. In the subsequent stage, the microalgae cells are harvested and immediately transferred to a sulfate-deficient medium in a closed photobioreactor to permit H_2 production under anaerobic conditions. To achieve anaerobic conditions, the cultivation must be initially flushed with N_2 and incubated in a dark environment for several hours

before exposing to light sources to initiate H_2 evolution. Mixing of microalgae culture utilizing air bubble is no longer possible in the second stage because of the need to have an anaerobic environment. Thus a mechanical agitator in the closed photobioreactor is needed to provide homogenous mixing and to allow microalgae to receive sufficient light.

In this regard, a vertical column-type photobioreactor is the most plausible option, as it allows easy installation of a mechanical agitator in the center of the column and facilitates H_2 collection at the headspace. Hence, choosing a suitable agitator and optimizing the agitation speed are important in minimizing the shear stress on microalgae cells that may subsequently affect the H_2 production rate. Other types of closed photobioreactors, such as flat-plate, helical tubular, and α-shaped, are also suitable for cultivating microalgae, but is expected to consume more energy because of the difficulty of installing a mechanical agitator in these photobioreactors, resulting in the need for a heavy-duty water pump to circulate the culture to avoid from settling.

2.10.1 Advantages of Photobioreactor

- Cultivation of algae is in controlled circumstances, hence potential for much higher productivity
- Large surface-to-volume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity. Typically the culture density of algae produced is 10 to 20 times greater than bag culture in which algaeculture is done in bags – and can be even greater.
- Better control of gas transfer.
- Reduction in evaporation of growth medium.
- More uniform temperature.
- Better protection from outside contamination.
- Space saving – Can be mounted vertically, horizontally or at an angle, indoors or outdoors.
- Reduced Fouling – Recently available tube self cleaning mechanisms can dramatically reduce fouling.

2.10.2 Bioprocess parameters

Bioprocess technology is the application and use of enzymes for different purposes and production under different circumstances. Cellulose polymer is biologically degraded by microorganisms by virtue of the production of cellulases. These are the enzymes that bring

about the hydrolysis of β-1,4-glycosidic linkages in cellulose polymers and produce glucose. In nature, the original microorganisms may produce low levels of enzyme cellulase.

Therefore once an organism producing cellulase is isolated and identified, extensive effort must be expended to increase the fermentation yield and minimize the production cost. Different strategies can be employed to improve the production and yield of a fermentation process, but generally it can be attained by modifying the various nutritional and physicochemical parameters. Enzyme cellulase yield is influenced by various parameters such as media, pH, temperature, incubation time, agitation/rpm, moisture level, inoculum size, media components such as C and N_2 sources, trace minerals, inducers, medium additives, etc. Hence it becomes essential to optimize the various parameters in a fermentation process to develop feasible technologies for maximum production of cellulase enzymes at lower cost. This chapter focuses on the different factors influencing the production of cellulase enzymes and defines the optimized bioprocess parameters for improving enzyme yield.

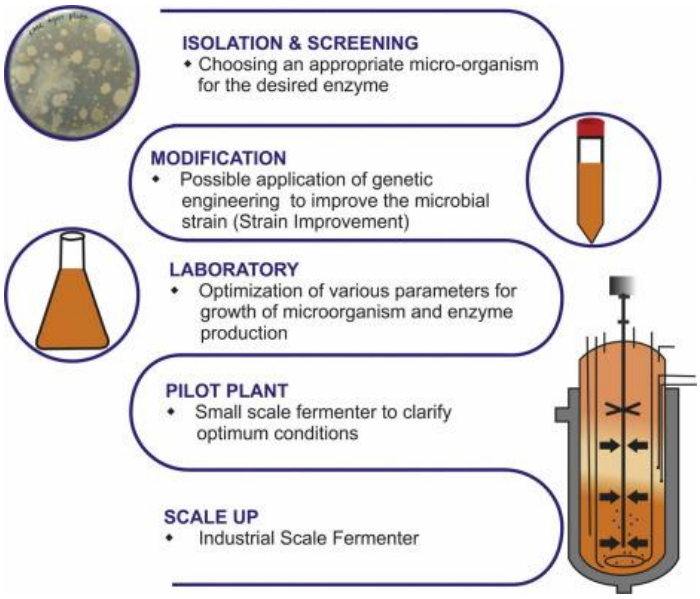


Fig. 14

There are a large number of physical, chemical and biological parameters that can be measured during fermentation/bioprocessing (Table 19.6) for data analysis and appropriate control. Some special sensors have been developed to carry out measurements in the bioreactors. The basic requirement of all the sensors is that they must be sterilizable. The

measurements of the parameters (listed in Table) can be done either directly in the bioreactor or in the laboratory.

2.10.3 Physical Parameters

Temperature

Pressure

Flow rates

iscosity

Turbidity

Power consumption

2.10.4 Chemical Parameters

pH

Substrate concentration

Product concentration

O_2 concentration (dissolved)

Waste gases concentration (e.g. CO_2)

Ionic strength

2.10.5 Biological Parameters

Activities of specific enzymes

Protein concentration

Energetics (ATP concentration)

DNA/RNA content

2.10.6 pH measurement:

There are pH electrodes that can withstand high temperature (sterilization) pressure and mechanical stresses, and yet measure the pH accurately. Combination electrodes (reference electrode, glass electrode) are being used. In fact, electrodes are also available for measuring several other inorganic ions.

2.10.7 O_2 and CO_2 measurement:

Oxygen electrodes and CO_2 electrodes can be used to measure O_2 and CO_2 concentrations respectively. The electrodes are amperometric in nature. They are however, susceptible for damage on sterilization. In a commonly used technique, O_2 and CO_2 respectively can be

measured by the magnetic property of O_2 and the infrared absorption of CO_2. This can be done by using sensors.

2.11 Summary

Fermentation is a process in which an agent (typically bacteria or yeast) causes an organic substance to break down into simpler substances. Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, lactose, and hydrogen. Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produces ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, ethanol fermentation is classified as anaerobic.

Fermentation is the heart of an ethanol process, in which the sugars are converted to ethanol by a variety of microorganisms. The raw materials or substrate for the fermentation is generally a solution containing natural sugars such as sugarcane or beet sugar juices, "molasses," the byproduct of the sugar industry or any other residual or low-value products such as fruit juice byproducts and residuals. Another category of raw materials for fermentation is the sugar solution produced from a prior hydrolysis process of, for example, grains or lignocelluloses. These hydrolysis steps are generally carried out by enzymes or acids. However, to have an efficient hydrolysis, a pretreatment is usually necessary, which could be by physical, chemical, thermal, or biological means. In summary, the raw materials used for ethanol production, as well as the steps prior to fermentation, can contain or create some chemical compounds that reduce the microorganism's ability of ethanol production. These "inhibitors" may reduce the yield or productivity of ethanol, reduce the viability of the microorganisms, or completely stop the fermentation. Furthermore, the carbon source of the fermentation process, that is, sugars, or the major product of the fermentation, that is, ethanol, can also act as an inhibitor.

Terminal questions

Q.1 Define fermentation technology. Explain types of fermenters.

Answer:----------

Q. 2 Explain alcohol and acetic acid fermentation.

Answer:----------

Q. 3 Write a short note on advantages of fermentation.

Answer:----------

Q. 4 Describe batch, fed-batch and continuous bioreactors.

Answer:----------

Q. 5 Write a short note on photobioreactor.

Answer:----------

Q. 6 Explain advantages of fermentation.

Answer:----------

Q. 7 What are bioprocess parameters.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.

2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Block- II

Processing and industrial production

Unit-3
Downstream processing

Unit-4
Industrial production of chemicals

Introduction

This is the second block on processing and industrial production**.** It consists of following two units as under:

Unit 3: Downstream processing involves all unit operations after fermentation that improves the purity of the final ethanol product. Ethanol is typically purified using combinations of distillation and molecular sieving. The fermented broth from the beer well is sent to a traditional distillation column to obtain 95% ethanol. Since this ethanol contains 5% moisture, it is dehydrated using azeotropic distillation. The presence of water increases the molecular polarity of ethanol, making it separate when mixed with gasoline. In azeotropic distillation, a third chemical called an entrainer (e.g., benzene or cyclohexane) is added. Since azeotropic distillation is complicated and expensive, molecular sieving methods have been implemented recently in large-scale ethanol purification. The molecular sieves let the smaller water molecules (0.28 nm) pass through while retaining dehydrated ethanol (0.44 nm). It is possible to regenerate molecular sieves by heating or applying vacuum.

Downstream processing equipment covers a vast range of systems varying in size and complexity. The scales covered include laboratory systems, pilot-scale equipment, and production scale. The complexity of equipment decreases with the scale, but in general, even larger-scale equipment needs to offer a certain degree of flexibility to be able to address various production scenarios. Downstream equipment by itself does not contribute to the actual purification event, but wrongly designed/chosen equipment can have a deterioration effect on the process yield and economy. For instance, in the case of a chromatography system, system contribution to zone broadening because of additional mixing and/or no-flow zones will result in high buffer consumption and lower yield or lower purity for a predefined set of operating conditions.

Unit 4 The chemical industry produces many intermediate compounds that are used as the basis for many chemical products. The chemical industry produces more than 50 000 chemicals and formulations. For example, ethylene, one of the most important bulk chemicals from an energy point

of view, is used to produce products varying from solvents to plastics. Also, many processes in the chemical industry produce different coproducts. Chemical industries consume fuels and electricity as energy and feedstock. This makes energy analysis of the chemical industry more complicated compared than that of other industries. A small number of bulk chemicals are responsible for the largest part of the energy consumption in the chemical industry. These are the so-called basic chemicals that are used as building blocks for many chemicals down the production chain. The most important basic chemicals are the family of petrochemicals (ethylene, propylene, butadiene, and benzene) from the organic chemical industry as well as ammonia and chlorine/caustic soda from the inorganic chemical industry.

Unit- 3: Downstream processing

Structure

3.1 Introduction

Objectives

3.2 Removal of microbial cells and solid matter

3.3 Foam separation

3.4 Precipitation

3.5 Properties of precipitates and precipitation Reaction

3.6 Filtration

3.7 Physical process

3.8 Centrifugation

3.8.1 Centrifugation in biological research

3.8.1.1 Microcentrifuges

3.8.1.2 Ultracentrifugations

3.8.1.3 Low-speed centrifuges

3.8.1.4 High-speed centrifuges

3.8.1.5 Preparative ultracentrifugation

3.8.1.6 Analytical ultracentrifugation

3.8.1.7 Applications of centrifuges

3.8.1.8 Cell disruption

3.1 Introduction

Downstream processing (DSP) describes the series of operations required to take biological materials such as cells, tissue culture fluid, or plant tissues, and derive from them a pure and homogeneous protein product. Because the product is initially trapped in a biological matrix with a vast and diverse array of nontarget molecules, DSP is a multistep process that incrementally increases the purity of the target by exploiting the physical and chemical properties that make it distinct from contaminants. DSP involves harvesting and clarification steps to remove bulk contaminants such as particulates, carbohydrates, and oils followed by capturing and polishing steps that refine the feed stream until only the target product remains. This article presents an overview of the technologies used in DSP, including recent

developments and industry trends that have been introduced to reduce the costs involved. This unit covers recent advances in filtration and chromatography technologies, as well as traditional operations such as precipitation, and discusses the requirements for process-scale protein purification.

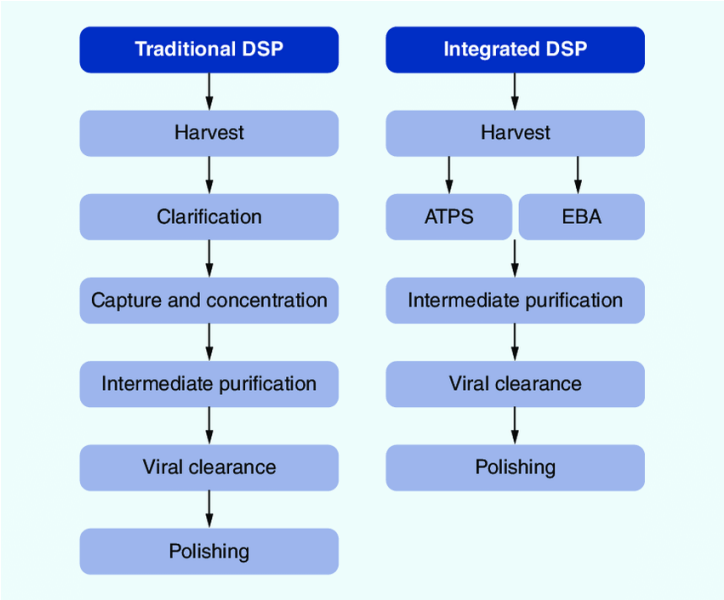


Fig. 1 Process flow sheet comparison between a traditional downstream processes

Downstream processing is an integral part of the entire production process and is influenced by many factors. Not only the bioprocess itself, but also the raw materials used, the specifications of the final product or the operations that may follow directly after DSP, such as formulation or direct *in situ* use, such as polymerization, need to be considered. Further, in some instances, at least part of the DSP sequence may be closely integrated with the fermentation step, whereby *in situ* separation of products can be performed. DSP make up a significant part of the total production costs, ranging between 50% and 90% of the total production process. The type of microorganism (filamentous fungi, yeast, bacteria), product (intra-/extracellular, chemical and physical properties), fermentation substrate, fermentation process (submerged, solid state) as well as target application (purity specifications) influence the decision for the specific choice of DSP.

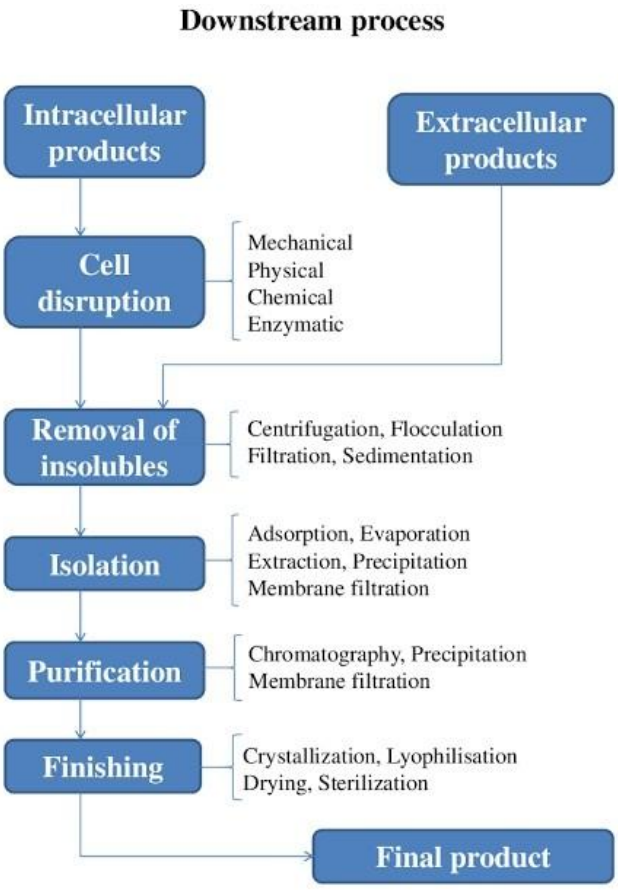


Fig. 2 Downstream processing and its steps

In most cases, different combinations of steps and different unit operations have to be used. As mentioned earlier, various by-products may arise from succinic acid fermentation, which have similar structures and properties, including malic acid and lactic acid, and their selective separation has a significant impact on DSP costs. The use of recombinant strains may allow for less production of metabolic by-products. Bacterial fermentation for the production of succinic acid is usually performed at neutral pH. Due to constant release of organic acids, the pH decreases during fermentation, and the effect of product inhibition usually occurs, unless pH control is implemented, normally through the use of calcium, magnesium, sodium or potassium hydroxides, carbonates, or ammonium salts or simply ammonia. This leads to the production of a succinate salt, which then needs to be converted into succinic acid through acidification with sulfuric acid, generating gypsum as a by-product.

Yeasts are more tolerant to acidic conditions and may be used in processes at significantly lower pH. At a pH below 3, many dicarboxylic acids are predominantly present in their

undissociated form (e.g. succinic acid pK_{a1} = 4.2, and pK_{a2} = 5.6 at 25°C. In this case, the requirements of pH-controlling elements are smaller, resulting in lower consumption of chemicals, and less waste produced since the undissociated free acid is directly recovered. Generally, carbon sources are typically provided by fermentation substrates containing easily fermentable carbohydrates such as mono- or disaccharides. An increasing diversity of low-cost but complex raw materials, such as agro-industrial wastes, are being considered by the chemical industry as it replaces its petroleum based production processes with industrial biobased fermentation processes. Pretreatment of material (e.g. digestion, removal of inhibitors) becomes necessary, allowing for a cleaner broth, better fermentation results, and consequently lower DSP costs. Yet, fermentation based on these alternative raw materials includes a more diverse initial mix of complex components, which need to be separated after the fermentation in order to meet product specifications.

This is the second block on processing and industrial production**.** We have following objectives. These are as under:

Objectives

- To know about centrifugation.
- To know about different types of centrifuges.
- To know about chromatography and its types.
- To know about crystallization and whole cell immobilization.
- To know about microcentrifuges and ultracentrifugation.

3.2 Removal of microbial cells and solid matter

Microbial cells are immobilized by mixing them with a polysaccharide such as sodium alginate or κ-carrageenan. The mixture is dropped into salt solutions to form gel particles. Trace dissolved oxygen in the solution will enable the microorganisms to grow prolifically near the surface of the gel particles. Therefore, the overall cell density in the reactor during fermentation becomes about 10 times higher than that in batch fermentation. As a result, the fermentation period can be shortened and the productivity of ethanol becomes highly efficient. Advantages of this method are smaller reactor size, greater feasibility of continuous processing, and shorter startup time. The reactor behaves as a fluidized bed that is agitated by the carbon dioxide produced during fermentation in the reactor. However, there are risks of contamination and poor activities for the microbial cells trapped in the gel.

Microbial cells are able to take up a broad range of metals referred to as “bioaccumulation.” Following biosorption, essential metals that are required for microbial metabolism can be transported across the cell membrane, and these processes can also uptake nonessential (and potentially toxic) metals. In some cases, it has been suggested that some ions may penetrate the cell membrane due to increased permeability, e.g., as a result of chemotoxicity. This is the case, e.g., for uranium, which has no known biological function, and the large size and stereochemistry of the UO_2^{2+} ion and its associated hydroxyl groups render it largely unable to enter the cytoplasmic compartment of living cells. Despite this, intracellular bioaccumulation of uranium has been reported in several species, including *Pseudomonas* spp

Microbial cells and other insoluble materials are normally separated from the harvested broth by filtration or centrifugation. Because of the small size of many microbial cells, it will be necessary to consider the use of filter aids to improve filtration rates, while heat and flocculation treatments are employed as techniques for increasing the sedimentation rates in centrifugation. Flocculation can also be utilized in other downstream processing operations to aid product recovery. Hao, Xu, Liu, and Liu report the use of the flocculants chitosan and polyacrylamide on cell debris and soluble protein in the fermentation broth, to enhance the recovery of 1,3-propanediol by reactive extraction and distillation. The methods of cell and cell debris separation described in the following sections have been practiced for many years.

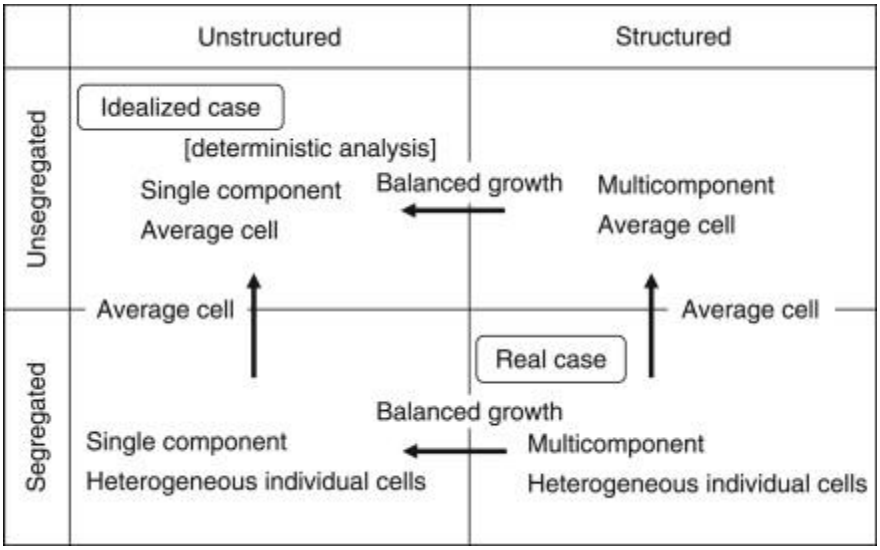


Fig. 3 Microbial cell- an overview

Bowden, Leaver, Melling, Norton, and Whittington review some potential developments in cell recovery. These include the use of electrophoresis and dielectrophoresis to exploit the charged properties of microbial cells, ultrasonic treatment to improve flocculation characteristics and magnetic separations. Although not necessarily for the removal of cells, other downstream operations, which involve the application of an electrical field are also showing potential. One such process is electrodialysis that involves the transfer of ions from a dilute solution to a concentrated one through a semipermeable membrane by applying an electrical field. Lopez and Hestekin report the use of electrodialysis in the separation of organic acids in aqueous solution where the product, sodium butyrate, was successfully transferred from the aqueous phase into an ionic liquid phase through electrodialysis. A recovery rate of 99% was obtained with reduced energy input compared to traditional processing.

In microbial, animal, and plant cells, there are various intracellular enzyme reactions. To produce any material by utilizing these intracellular reactions, all biochemical reactions involved in the production should be considered in principle. However, each individual cell is a complicated multicomponent system, and quantitative clarification of the enzyme reactions occurring in the microbial cell is much more complicated and difficult than that of the single enzyme reaction. The multi- and single-component representations are the concepts in structured model and in unstructured model, respectively. In unstructured model, balanced growth in which all intracellular components simultaneously proliferate is approximated. Furthermore, there are diversities of different cells in the population in regard to cell phase. The cells in different cell phase often show different features of metabolic functions and activities. From this point of view, the entire cell is hitherto dealt with as a black box and the individual cells in the microbial population are considered to be homogeneous without respect to their variability in shape, size, physiological function, and so on. The heterogeneous individual cells and homogeneous (average) cells are segregated and unsegregated viewpoints, respectively. In the actual case of cell, cellular representations should be described by a structured and segregated model. Generally, however, an unstructured and unsegregated model, the most idealized case, is applied to analyze cell population with a deterministic approach.

3.3 Foam separation

Foam separation is a process of removing a component of a liquid mixture using a bubbled gas and a surfactant to collect the material. A surfactant is a chemical that helps produce foam without chemically reacting with the solution. Many materials can be removed from liquids using foam separation methods, including precious metals, proteins, and impurities from water. There are a number of chemical methods for extracting gold from ore, but many of them include toxic chemicals and waste products. Gold can be extracted from a liquid mixture using air or nitrogen gas and a foam-producing surfactant. The foam exiting the equipment is enriched in gold, which can then be separated by centrifuges that spin out the gold and remove the foam.

Separation of proteins and oils can be accomplished with foam separation equipment. The surfactants attract the desired product onto the foam interface by preferring the organic or oil molecules to water molecules. Foam systems may use non-reactive nitrogen gas rather than compressed air to prevent any oxidation reactions of air with the desired materials. Wastewater treatment systems often use foam separation steps to remove solids from the water stream. Larger solids can be removed by gravity or centrifuge operations, but fine solids must also be removed to meet treated water standards. Air can be bubbled along with a surfactant to create a foam layer that rises to the top of the foam tank. The fine solids will be adsorbed onto the foam, which can overflow the tank or be skimmed from the top.

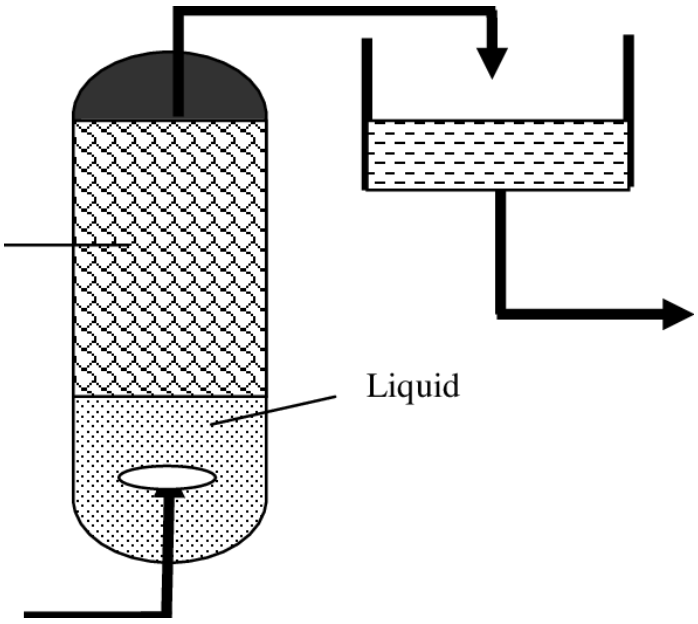


Fig. 4 Schematic representation of the foam separation process

Foam separation can be operated as batch or continuous operations.Batch processes will place the liquid mixture into a processing tank and produce foam until the majority of the product is removed. Product analysis can determine when the processing is complete, and the equipment can be re-filled and the process repeated. Continuous production systems add the mixture either into the bottom of the vessel or at a point on the side. Air or other gas and surfactant are added at the bottom and are mixed as the gas moves upward. The foam will be carried to the top of the tank or a collection point on the side above the liquid level. Further processing can remove the surfactant from the desired product stream.

Continuous foam separation is a chemical process closely related to foam fractionation in which foam is used to separate components of a solution when they differ in surface activity. In any solution, surface active components tend to adsorb to gas-liquid interfaces while surface inactive components stay within the bulk solution. When a solution is foamed, the most surface active components collect in the foam and the foam can be easily extracted. This process is commonly used in large-scale projects such as water waste treatment due to a continuous gas flow in the solution. There are two types of foam that can form from this process. They are wet foam (or *kugelschaum*) and dry foam (or *polyederschaum*). Wet foam tends to form at the lower portion of the foam column, while dry foam tends to form at the upper portion. The wet foam is more spherical and viscous, and the dry foam tends to be larger in diameter and less viscous. Wet foam forms closer to the originating liquid, while dry foam develops at the outer boundaries. As such, what most people usually understand as foam is actually only dry foam.

The setup for continuous foam separation consists of securing a column at the top of the container of solution that is to be foamed. Air or a specific gas is dispersed in the solution through a sparger. A collecting column at the top collects the foam being produced. The foam is then collected and collapsed in another container. In the continuous foam separation process a continuous gas line is fed into the solution, therefore causing continuous foaming to occur.

Continuous foam separation may not be as efficient in separating solutes as opposed to separating a fixed amount of solution.

3.4 Precipitation

Chemical reactions involve chemical changes that result in the formation of new compounds under some specific conditions. Chemical equations can help us understand the chemical reactions between various elements or compounds. Chemical equations show the reactants and the products that are involved in these reactions. The compounds that participate in a chemical reaction are called reactants. The reactants might be in the solid, gaseous, or liquid phase. Many chemical reactions occur in our daily lives. Common examples of such reactions are burning, corrosion, cooking of food and digestion. One important class of chemical reactions are *precipitation reactions*. In such reactions, two different soluble salts (which are in aqueous solutions) combine to form two products. One of these products is insoluble in the solution and is precipitated out.

The term 'precipitation reaction' can be defined as **"**a chemical reaction occurring in an aqueous solution where two ionic bonds combine, resulting in the formation of an insoluble salt**".** These insoluble salts formed in precipitation reactions are called precipitates. Precipitation reactions are usually double displacement reactions involving the production of a solid form residue called the precipitate. These reactions also occur when two or more solutions with different salts are combined, resulting in the formation of insoluble salts that precipitate out of the solution.

Most samples of interest are mixtures of many different components. Sample preparation, a key step in the analytical process, removes interferences that may affect the analysis. As such, developing separation techniques is an important endeavor not just in academia, but also in industry. One way to separate mixtures is to use their solubility properties. In this short paper, we will deal with aqueous solutions. The solubility of a compound of interest depends on

- Ionic strength of solution,
- pH, and

- Temperature. By manipulating with these three factors, a condition in which the compound is insoluble can be used to remove the compound of interest from the rest of the sample.

One of the best examples of precipitation reactions is the chemical reaction between potassium chloride and silver nitrate, in which solid silver chloride is precipitated out. This is the insoluble salt formed as a product of the precipitation reaction. The chemical equation for this precipitation reaction is provided below.

$$AgNO_3\,(aqueous) + KCl\,(aqueous) \text{——} AgCl\,(precipitate) + KNO_3\,(aqueous)$$

In the above reaction, a white precipitate called silver chloride or AgCl is formed which is in the solid-state. This solid silver chloride is insoluble in water. Precipitation reactions help in determining the presence of different ions present in a particular solution. The other example of a precipitation reaction is the reaction between calcium chloride and potassium hydroxide, resulting in the formation of an insoluble salt Called calcium hydroxide. The chemical equation for this reaction is below-

$$2KOH\,(aqueous) + CaCl_2\,(aqueous) \text{—-} Ca(OH)_2\,(aqueous) + 2KCl\,(aqueous)$$

Some more examples of chemical equations of on precipitation reaction are as below-

$$AgNO_3\,(aqueous) + NaCl\,(aqueous) \text{—-} AgCl\downarrow + NaNO_3\,(aqueous)$$

$$Mg(OH)_2\,(s) + 2HCl\,(aqueous) \text{———} MgCl_2\,(aqueous) + 2H_2O(l)$$

3.5 Properties of precipitates and precipitation Reaction

Some of the properties of precipitates and the reaction are as below-

- The precipitation reaction undergoes in aqueous solutions or medium in an ionic state.
- The reaction takes place between ions present in the aqueous solutions, forming the product
- The products formed at the end of precipitation reaction are the precipitates which are insoluble in aqueous solutions
- Precipitation reactions are known as ionic reactions since the ions actively take part in the reaction and form the product.

- These reactions depend on the temperature, concentration of the solution, buffer solution, etc.

Precipitation reaction helps in determining a particular element present in the given solution. These reactions also monitor the formation of a precipitate when some chemical is added to solutions. These are used for the extraction of magnesium from the seawater. The human body also encounters these reactions existing between antigens and antibodies. Precipitation reaction can be used in wastewater treatment. When a contaminant forms an insoluble solid, then we can use this reaction to precipitate out the contaminated ions. In wastewater, a frequent presence of heavy metals can be found such as compounds of sulphide and hydroxide. So, we can add a source of hydroxide that is soluble (NaOH Or Na_2S) that will result in a precipitation reaction.

3.6 Filtration

Filtration is a physical or chemical separation process that separates solid matter and fluid from a mixture using a *filter medium* that has a complex structure through which only the fluid can pass. Solid particles that cannot pass through the filter medium are described as *oversize* and the fluid that passes through is called the *filtrate*.[1] Oversize particles may form a filter cake on top of the filter and may also block the filter lattice, preventing the fluid phase from crossing the filter, known as *blinding*. The size of the largest particles that can successfully pass through a filter is called the effective *pore size* of that filter. The separation of solid and fluid is imperfect; solids will be contaminated with some fluid and filtrate will contain fine particles (depending on the pore size, filter thickness and biological activity). Filtration occurs both in nature and in engineered systems; there are biological, geological, and industrial forms.

Filtration is also used to describe biological and physical systems that not only separate solids from a fluid stream, but also remove chemical species and biological organisms by entrainment, phagocytosis, adsorption and absorption. Examples include slow sand filters and trickling filters. It is also used a general term for microphagy in which organisms use a variety of means to filter small food particles from their environment. Examples range from the microscopic *Vorticella* up to the Basking shark one of the largest fishes, and the baleen whales, all of which are described as Filter feeders.

3.7 Physical process

- Filtration is used to separate particles and fluid in a suspension, where the fluid can be a liquid, a gas or a supercritical fluid. Depending on the application, either one or both of the components may be isolated.
- Filtration, as a physical operation enables materials of different chemical composition to be separated. A solvent is chosen which dissolves one component, while not dissolving the other. By dissolving the mixture in the chosen solvent, one component will go into the solution and pass through the filter, while the other will be retained.
- Filtration is widely used in chemical engineering. It may be combined with other unit operations to process the feed stream, as in the biofilter, which is a combined filter and biological digestion device.
- Filtration differs from sieving, where separation occurs at a single perforated layer (a sieve). In sieving, particles that are too big to pass through the holes of the sieve are retained. In filtration, a multilayer lattice retains those particles that are unable to follow the tortuous channels of the filter. Oversize particles may form a cake layer on top of the filter and may also block the filter lattice, preventing the fluid phase from crossing the filter (blinding). Commercially, the term filter is applied to membranes where the separation lattice is so thin that the surface becomes the main zone of particle separation, even though these products might be described as sieves.
- Filtration differs from adsorption, where separation relies on surface charge. Some adsorption devices containing activated charcoal and ion-exchange resin are commercially called filters, although filtration is not their principal mechanical function.
- Filtration differs from removal of magnetic contaminants from fluids with magnets (typically lubrication oil, coolants and fuel oils), because there is no filter medium. Commercial devices called ‘magnetic filters’ are sold, but the name reflects their use, not their mode of operation.
- In biological filters, oversize particulates are trapped and ingested and the resulting metabolites may be released. For example, in animals (including humans), renal filtration removes waste from the blood, and in water treatment and sewage treatment, undesirable constituents are removed by adsorption into a biological film grown on or in the filter medium, as in slow sand filtration.

There are many different methods of filtration; all aim to attain the separation of substances. Separation is achieved by some form of interaction between the substance or objects to be

removed and the filter. The substance that is to pass through the filter must be a fluid, i.e. a liquid or gas. Methods of filtration vary depending on the location of the targeted material, i.e. whether it is dissolved in the fluid phase or suspended as a solid. There are several laboratory filtration techniques depending on the desired outcome namely, hot, cold and vacuum filtration. Some of the major purposes of getting the desired outcome are, for the removal of impurities from a mixture or, for the isolation of solids from a mixture.

3.8 Centrifugation

Centrifugation is a mechanical process that utilizes an applied centrifugal force field to separate the components of a mixture according to density and/or particle size. The principles that govern particle behaviour during centrifugation are intuitively comprehensible. This may, in part, explain why centrifugation is seldom a part of post-secondary science curricula despite the broad range of scientific, medical and industrial applications in which this technique has been employed for well over 100 years. Applications that range from the mundane, industrial-scale dewatering of coal fines to the provision of an invaluable tool for biomedical research.

Centrifugation is a mechanical process which involves the use of the centrifugal force to separate particles from a solution according to their size, shape, density, medium viscosity and rotor speed. The more dense components of the mixture migrate away from the axis of the centrifuge, while the less dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force of the test tube so that the precipitate (pellet) will travel quickly and fully to the bottom of the tube. The remaining liquid that lies above the precipitate is called a supernatant or supernate.

There is a correlation between the size and density of a particle and the rate that the particle separates from a heterogeneous mixture, when the only force applied is that of gravity. The larger the size and the larger the density of the particles, the faster they separate from the mixture. By applying a larger effective gravitational force to the mixture, like a centrifuge does, the separation of the particles is accelerated. This is ideal in industrial and lab settings because particles that would naturally separate over a long period of time can be separated in much less time.

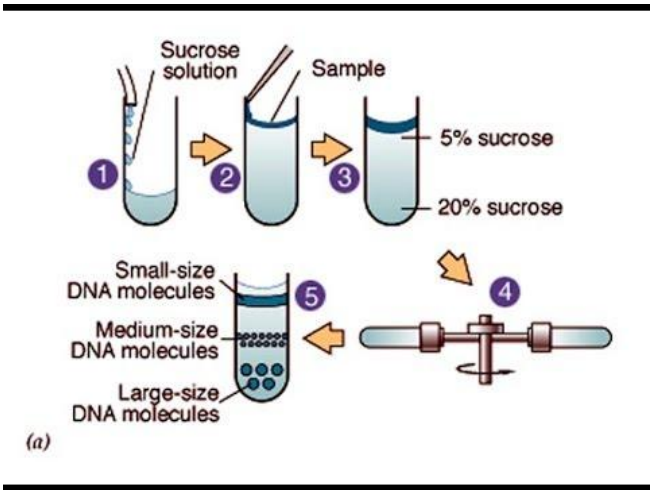


Fig. 5 Centrifugation

The rate of centrifugation is specified by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as *g*. The conversion factor between RPM and *g* depends on the radius of the centrifuge rotor. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity. The most common application is the separation of solid from highly concentrated suspensions, which is used in the treatment of sewage sludges for dewatering where less consistent sediment is produced.

Fig. 6 Centrifugation- Principle, Types, and Application

The centrifugation method has a wide variety of industrial and laboratorial applications; not only is this process used to separate two miscible substances, but also to analyze the hydrodynamic properties of macromolecules. It is one of the most important and commonly used research methods in biochemistry, cell and molecular biology. In the chemical and food industries, special centrifuges can process a continuous stream of particle-

laden liquid. Centrifugation is also the most common method used for uranium enrichment, relying on the slight mass difference between atoms of U-238 and U-235 in uranium hexafluoride gas.

3.8.1 Centrifugation in biological research

3.8.1.1 Microcentrifuges

Microcentrifuges are specially designed table-top models with light, small-volume rotors capable of very fast acceleration up to approximately 17,000 rpm. They are lightweight devices which are primarily used for short-time centrifugation of samples up to around 0.2–2.0 mL. However, due to their small scale, they are readily transportable and if necessary, can be operated in a cold room. They can be refrigerated or not. The microcentrifuge is normally used in research laboratories where small samples of biological molecules, cells, or nuclei are required to be subjected to high RCF for relatively short time intervals. Microcentrifuges designed for high speed operation can reach up to 35000 rpm, giving RCF up to 30000×g, and are called high-speed microcentrifuges.

3.8.1.2 Ultracentrifugations

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles at exceptionally high speeds. Current ultracentrifuges can spin to as much as 150,000 rpm (equivalent to 1,000,000 x g). They are used to harvest all membrane vesicles derived from the plasma membrane, endoplasmic reticulum (ER) and Golgi membrane, endosomes, ribosomes, ribosomal subunits, plasmids, DNA, RNA and proteins in fixed-angle rotors. Compared to microcentrifuges or high-speed centrifuges, ultracentrifuges can isolate much smaller particles and, additionally, while microcentrifuges and supercentrifuges separate particles in batches (limited volumes of samples must be handled manually in test tubes or bottles), ultracentrifuges can separate molecules in batch or continuous flow systems.

Ultracentrifugation is employed for separation of macromolecules/ligand binding kinetic studies, separation of various lipoprotein fractions from plasma and deprotonisation of physiological fluids for amino acid analysis. They are the most commonly used centrifuge for the density-gradient purification of all particles except cells, and while swinging buckets have been traditionally used for this purpose, fixed-angle rotors and vertical rotors are also used,

particularly for self-generated gradients and can improve the efficiency of separation greatly. There are two kinds of ultracentrifuges: the analytical and the preparative.

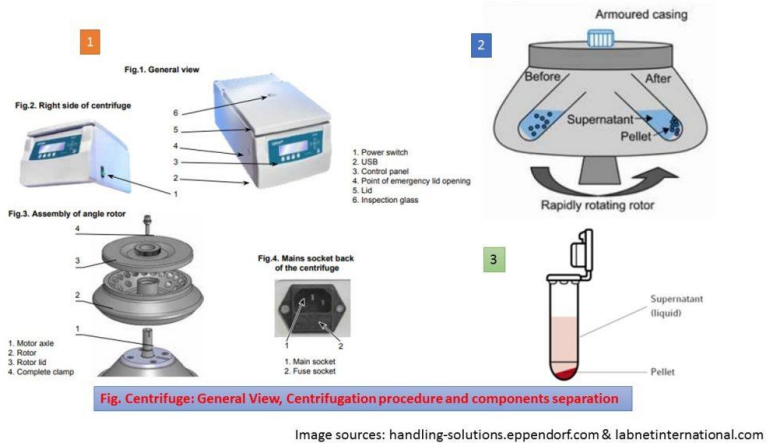


Fig. 7 Centrifuge: introduction, principle, types, handling procedure and uses

3.8.1.3 Low-speed centrifuges

Low-speed centrifuges are used to harvest chemical precipitates, intact cells (animal, plant and some microorganisms), nuclei, chloroplasts, large mitochondria and the larger plasma-membrane fragments. Density gradients for purifying cells are also run in these centrifuges. Swinging-bucket rotors tend to be used very widely because of the huge flexibility of sample size through the use of adaptors. These machines have maximum rotor speeds of less than 10 000 rpm and vary from small, bench-top to large, floor-standing centrifuges.

3.8.1.4 High-speed centrifuges

High-speed centrifuges are typically used to harvest microorganisms, viruses, mitochondria, lysosomes, peroxisomes and intact tubular Golgi membranes. The majority of the simple pelleting tasks are carried out in fixed angle rotors. Some density-gradient work for purifying cells and organelles can be carried out in swinging-bucket rotors, or in the case of Percoll gradients in fixed-angle rotors. High-speed or superspeed centrifuges can handle larger sample volumes, from a few tens of millilitres to several litres. Additionally, larger centrifuges can also reach higher angular velocities (around 30,000 rpm). The rotors may come with different adapters to hold various sizes of test tubes, bottles, or microtiter plates.

3.8.1.5 Preparative ultracentrifugation

Preparative ultracentrifuges are often used for separating particles according to their densities, isolating and/or harvesting denser particles for collection in the pellet, and clarifying suspensions containing particles. Sometimes researchers also use preparative ultracentrifuges if they need the flexibility to change the type of rotor in the instrument. Preparative ultracentrifuges can be equipped with a wide range of different rotor types, which can spin samples of different numbers, at different angles, and at different speeds.

3.8.1.6 Analytical ultracentrifugation

Analytical ultracentrifugation (AUC) can be used for determination of the properties of macromolecules such as shape, mass, composition, and conformation. It is a commonly used biomolecular analysis technique used to evaluate sample purity, to characterize the assembly and disassembly mechanisms of biomolecular complexes, to determine subunit stoichiometries, to identify and characterize macromolecular conformational changes, and to calculate equilibrium constants and thermodynamic parameters for self-associating and hetero-associating systems. Analytical ultracentrifuges incorporate a scanning visible/ultraviolet light-based optical detection system for real-time monitoring of the sample's progress during a spin.

Samples are centrifuged with a high-density solution such as sucrose, caesium chloride, or iodixanol. The high-density solution may be at a uniform concentration throughout the test tube or a varying concentration ("gradient"). Molecular properties can be modeled through sedimentation velocity analysis or sedimentation equilibrium analysis. During the run, the particle or molecules will migrate through the test tube at different speeds depending on their physical properties and the properties of the solution, and eventually form a pellet at the bottom of the tube, or bands at various heights.

3.8.1.7 Applications of centrifuges

A centrifuge can be used to isolate small quantities of solids retained in suspension from liquids, such as in the separation of chalk powder from water. In biological research, it can be used in the purification of mammalian cells, fractionation of subcellular organelles, fractionation of membrane vesicles, fractionation of macromolecules and macromolecular complexes, etc. Centrifugation is used in many different ways in the food industry. For example, in the dairy industry, it is typically used in the clarification and skimming of milk,

extraction of cream, production and recovery of casein, cheese production, removing bacterial contaminants, etc. This processing technique is also used in the production of beverages, juices, coffee, tea, beer, wine, soy milk, oil and fat processing/recovery, cocoa butter, sugar production, etc. It is also used in the clarification and stabilization of wine.

In forensic and research laboratories, it can be used in the separation of urine and blood components. It also aids in separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation. Centrifugation is also an important technique in waste treatment, being one of most common processes used for sludge dewatering. This process also plays a role in cyclonic separation, where particles are separated from an air-flow without the use of filters. In a cyclone collector, air moves in a helical path. Particles with high inertia are separated by the centrifugal force whilst smaller particles continue with the air-flow.

Centrifuges have also been used to a small degree to isolate lighter-than-water compounds, such as oil. In such situations, the aqueous discharge is obtained at the opposite outlet from which solids with a specific gravity greater than one are the target substances for separation.

3.8.1.8 Cell disruption

Cell disruption is energy-intensive and costly process, which is required to prepare microalgae for extracting its lipid content. Different cell disruption processes were used to facilitate the release of products inside the cells. The disruption process depends on the microalgae specifications, and there are several methods for this purpose including the following:

- Physical and mechanical techniques (such as ultrasonication, bead milling, autoclave, homogenization, and microwave).
- Chemical and biological techniques (such as enzyme, resin, cationic surfactant with nanoparticles, acid treatment, and osmotic shock.

Biological methods of cell disruption involve the application of enzymes, antibiotics, and phage treatment. Among these, the enzyme-based method is well-known in the literature. Use of cellulolytic enzymes to break down the cell wall of microalgae is one strategy to enhance the cell disruption of microalgae cells that are resistant to other methods of cell disruption. The enzyme can be used alone or in combination with other methods like solvent extraction or bead milling. Due to the high cost of enzymes, a process depending solely on enzymatic

hydrolysis is expensive, and hence this method is viable only when combined with other cell disruption methods.

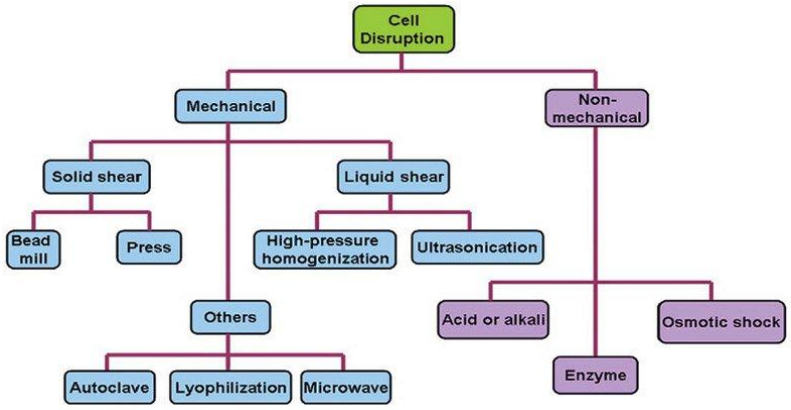


Fig. 8 Cell disruption methods

Cell disruption is required when the product protein is not expressed extracellularly and the host cell has a tough cell wall (i.e., *E. coli* or yeasts). Several unit operations are available to disrupt the cells and release their contents. Among the most common large-scale cell disruption operations are direct physical disruption methods, including high-pressure homogenization, grinding in ball mills, and cell wall breakage due to ice crystal formation through freeze/thaw of a cell paste. Of these three methods, high-pressure homogenizers offer efficiency, high throughput, and cleanability. These elements make them an attractive platform option, particularly for therapeutic protein production. Chemical or enzymatic lysis approaches also exist, but can be costly both from a raw material perspective and due to the potential need to introduce additional purification operations to remove the lysis additives. Solvent addition can simultaneously disrupt cells and extract the target molecule, as in the case of certain antibiotics.

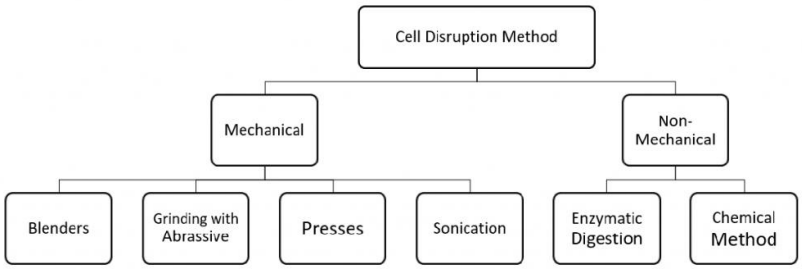


Fig. 9 Cell disruption technique

Enzymatic cell disruption can be an effective method for thoroughly releasing inclusion bodies in a gentler and potentially selective manner. Enzymatic cell disruption relies upon the addition of common cell membrane disrupting enzymes such as lysozyme or other muramidases which act by digesting the peptidoglycan layer of bacteria. As the peptidoglycan layer is digested, the cell membrane is weakened causing cell disruption or death when the cell is exposed to a more “hypoosmotic environment” or by the addition of specific cytoplasmic membrane disrupting chemicals. The addition of pretreatment steps with chemical additives, such as Tween, to help remove the more rugged outer LPS rich cell membrane has been shown to increase disruption efficiency, and may be necessary for some enzyme based cell disruption.

In addition to the enzymes intended for direct weakening of the cell membrane, DNAse enzymes may also be introduced to reduce the lysate viscosity resulting from the substantial amount of DNA released upon cell disruption. The digestion of DNA significantly benefits harvest- or membrane-based recovery operations by reducing feed stream viscosity [90,91]. Additionally, separation steps can be applied to selectively remove the digested DNA fragments from the inclusion body proteins to improve purity. The benefits of enzymatic cell lysis include:

(1) Specificity,
(2) High rate of product release, and
(3) Mild nature of cell disruption.

The specificity allows for selective release of protein which may be localized to one of the cell compartments thus can improve purity, the high rate of product release can improve overall step yield, while the mild nature can ensure product quality as compared to harsher physical disruption methods. The challenges with enzymatic cell lysis operations include:
(1) The large quantity of enzyme often required,
(2) The associated enzyme cost,
(3) The need for demonstrated removal of the enzyme downstream,
(4) Potential impact on product quality, and
(5) The formation of fine cell debris.
Additionally, for the most common enzyme applied, lysozyme, the high pI of ~ 11 creates a highly positively charged enzyme under most disruption conditions. As a result, lysozyme

may be prone to self-associate with inclusion bodies creating an additional challenging impurity complex to remove during purification.

3.9 Liquid-liquid extraction

Liquid–liquid extraction (LLE), also known as solvent extraction and partitioning, is a method to separate compounds or metal complexes, based on their relative solubilities in two different immiscible liquids, usually water (polar) and an organic solvent (non-polar). There is a net transfer of one or more species from one liquid into another liquid phase, generally from aqueous to organic. The transfer is driven by chemical potential, i.e. once the transfer is complete, the overall system of chemical components that make up the solutes and the solvents are in a more stable configuration (lower free energy). The solvent that is enriched in solute (s) is called extract. The feed solution that is depleted in solute (s) is called the raffinate. LLE is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to countercurrent distribution equipment called as mixer settlers. This type of process is commonly performed after a chemical reaction as part of the work-up, often including an acidic work-up.

The term *partitioning* is commonly used to refer to the underlying chemical and physical processes involved in *liquid–liquid extraction*, but on another reading may be fully synonymous with it. The term *solvent extraction* can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix.

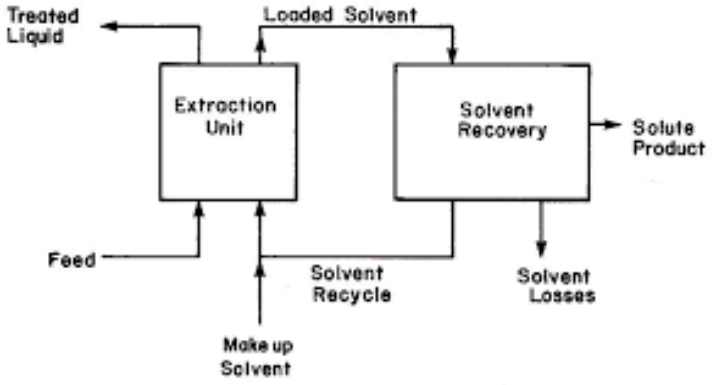


Fig. 10 Liquid–liquid extraction (LLE)

From a hydrometallurgical perspective, solvent extraction is exclusively used in separation and purification of uranium and plutonium, zirconium and hafnium, separation of cobalt and

nickel, separation and purification of rare earth elements etc., its greatest advantage being its ability to selectively separate out even very similar metals. One obtains high-purity single metal streams on 'stripping' out the metal value from the 'loaded' organic wherein one can precipitate or deposit the metal value. Stripping is the opposite of extraction: Transfer of mass from organic to aqueous phase. LLE is also widely used in the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries. It is among the most common initial separation techniques, though some difficulties result in extracting out closely related functional groups.

Liquid–liquid extraction is possible in non-aqueous systems: In a system consisting of a molten metal in contact with molten salts, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry greatly. For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase.

3.10 Chromatography

Chromatography, technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.

Kinetic molecular motion continuously exchanges solute molecules between the two phases. If, for a particular solute, the distribution favours the moving fluid, the molecules will spend most of their time migrating with the stream and will be transported away from other species whose molecules are retained longer by the stationary phase. For a given species, the ratio of the times spent in the moving and stationary regions is equal to the ratio of its concentrations in these regions, known as the partition coefficient. (The term *adsorption isotherm* is often used when a solid phase is involved.)

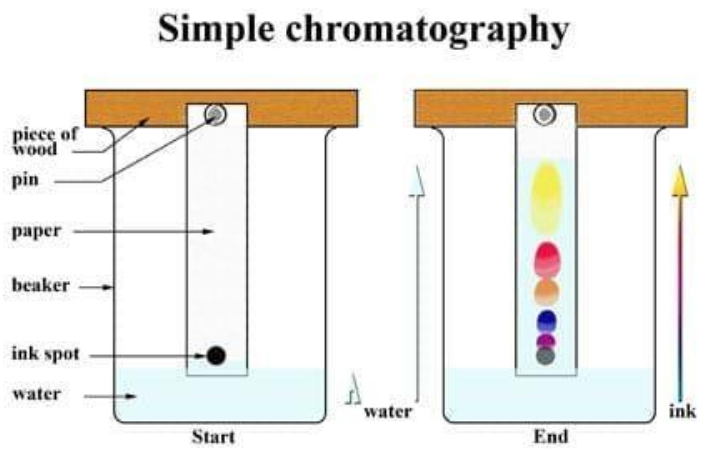


Fig. 11 Diagram of chromatography

A mixture of solutes is introduced into the system in a confined region or narrow zone (the origin), whereupon the different species are transported at different rates in the direction of fluid flow. The driving force for solute migration is the moving fluid, and the resistive force is the solute affinity for the stationary phase; the combination of these forces, as manipulated by the analyst, produces the separation.

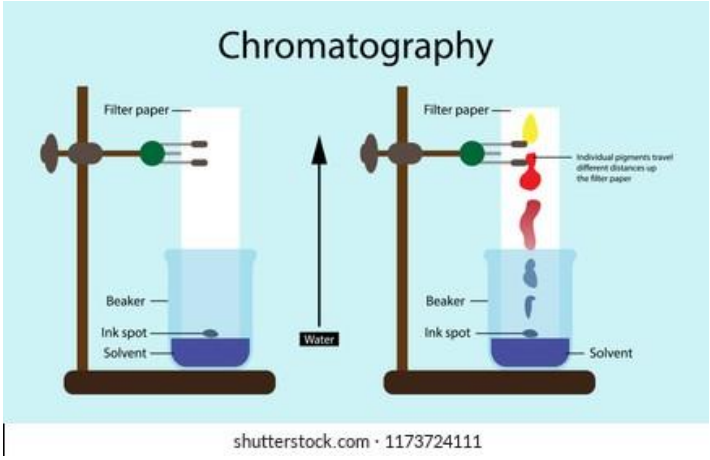


Fig. 12 Diagram of chromatography

Chromatography is one of several separation techniques defined as differential migration from a narrow initial zone. Electrophoresis is another member of this group. In this case, the driving force is an electric field, which exerts different forces on solutes of different ionic charge. The resistive force is the viscosity of the nonflowing solvent. The combination of these forces yields ion mobilities peculiar to each solute.

Chromatography has numerous applications in biological and chemical fields. It is widely used in biochemical research for the separation and identification of chemical compounds of biological origin. In the petroleum industry the technique is employed to analyze complex mixtures of hydrocarbons.

3.10.1 Different Types of chromatography

Chromatography is a technique in which substance are separated, purified and identified from a mixture for qualitative and quantitative analysis. On the basis of hydrophobic interactions, Polarity, enzymes and net charges are separated by using chromatography. Chromatography is a physical method of separation of compound. Chromatographic techniques can be classified into main categories:

- ❖ Partition chromatography
- ❖ Adsorption chromatography

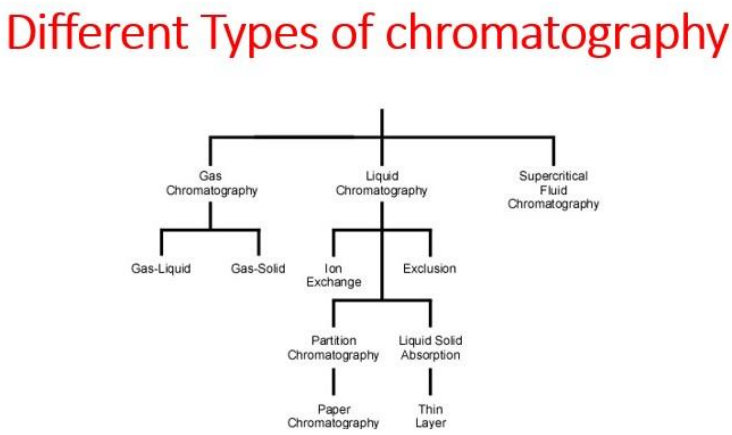


Fig. 13 Types of chromatography

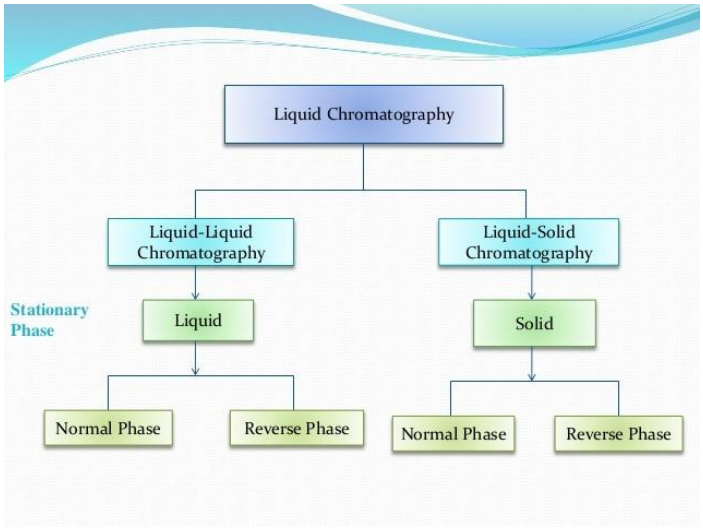


Fig. 14 Classification of chromatography

3.10.2 Adsorption chromatography

- It is a type of chromatography in which is the separation of components presents in a mixture is based on the relative differences in adsorption of components to the stationary phase present in the chromatographic column.
- The component of mixture travels with different rates due to differences in their non-covalent interactions with stationary phase.
- adsorption chromatography consists of solid stationary phase (known as adsorbent). The mobile phase is either liquid or gas.
- Adsorbents can be polar or non-polar molecule.

3.10.3 Partition chromatography

- In this type of chromatography, the components of the mixture are distributed into two phases due to differences in partition coefficient (K_d), which is the ratio of concentration of solutes in two phases.

$$K_d = \frac{\text{Concentration of solute in phase A}}{\text{Concentration of solute in phase B}}$$

- The distribution of solutes between the two phases is most primarily based on solubility differences.
- In the partition chromatography, the stationary phase is liquid and mobile phase is either a liquid or a gas.

3.10.4 Liquid-liquid partition chromatography

- In this type of partition chromatography, separation is based on solute partitioning between stationary liquid phase and mobile liquid phase.
- Substance which are more soluble in the mobile phase will pass rapidly through the system, while those which favor the stationary phase will be retarted.

It is of two types:

1. **Normal-phase partition chromatography:** an elution chromatography in which the stationary phase is more polar than the mobile phase. During elution least polar analyte is eluted first and the most polar last.
2. **Reversed-phase partition chromatography:** an elution procedure in which the mobile phase is significantly more polar than the stationary phase. In this case, most polar solutes elute first and least polar elute last.

3.10.5 Size exclusion chromatography

- They are also known as molecular sieve chromatography.
- It involves the separation of molecules on the basis of size and shape.
- In this, a column matrix is filled with porous gel beads, which is made up of an insoluble and hydrated polymer such as polyacrylamide or dextran or agarose acts as stationary phase.

3.10.6 Size exclusion chromatography is of two types:

I. Gel permeation chromatography.
II. Gel filtration chromatography

- Gel permeation chromatography generally uses organic mobile solvent while gel filtration chromatography uses aqueous mobile solvent to separate and characterize molecules.

3.10.7 Affinity chromatography

- Affinity chromatography is a technique involves purification of a biomolecule with respect to biological function or individual chemical structure.
- The substance to be purified is a specifically and reversibly adsorbed to a ligand, which is immobilized by a covalent bond to a chromatographic bed material.

3.10.8 Operation of affinity chromatography

The operation of affinity chromatography includes the following steps:

- Choice of an appropriate ligand.
- Immobilization of the ligand onto a support matrix.

- Binding of the molecules of interest with the ligand.
- Removal of non-specifically bound molecules.
- Elution of the molecules of interest in a purified form.

3.10.9 High performance liquid chromatography

- PLC is a type of column chromatography.
- It is a liquid chromatographic technique i.e. mobile phase is liquid.
- In this technique stationary phase may be solid or liquid.
- This technique of chromatography can function in several chromatographic modes: size exclusion.
- Adsorption, ion-exchange and partition.
- Instead of a solvent being allowed to drip through column under gravity, it is forced through under high pressure. It yields high performance and high speed compared with traditional column chromatography technique because mobile phase is pumped with high pressure.

3.10.10 Ion exchange chromatography

- It is employed for the separation of charged molecules.
- In this type of chromatography, ionic solutes display reversible electrostatic interactions with a charged stationary phase.
- The stationary solid phase generally consists of covalently attached anions or cations with an insoluble matrix called ion exchangers.

3.10.10.1 Ion exchangers

- It is made up of two parts- an insoluble matrix and chemically bonded charged groups within an on the surface of the matrix.
- **Cation exchanger:** also known acidic ion exchanger, it is used for the separation of cation.
- **Anion exchanger:** also known basic ion exchanger, it is used for the separation of anion.

3.11 Gas chromatography

- In this type of chromatography, a carrier gas is the mobile phase and the stationary phase is either a solid adsorbent which is termed as gas-solid chromatography, or a liquid on an inert support termed as gas-liquid chromatography.
- This technique is commonly used to analyze volatile substances in the gas phase.

- Gas chromatography is only type that does not utilize the mobile phase for interacting with the analyte.

3.12 Membrane process

In water treatment, membranes are barriers that allow water to pass through but stop unwanted substances from passing through with it. Working much like the cell walls in our bodies, technical membranes filter out salts, impurities, viruses, and other particles from water. A membrane process is any method that relies on a membrane barrier to filter or remove particles from water. Fluid is passed through the membrane because of the pressure difference between one side of the membrane and the other. Contaminants remain on one side. Although many types of filtering media are used for water treatment — for instance, clay, silt, and sand — one of the properties that distinguishes membranes is their ability to separate smaller substances such as salts and ions from a liquid.

Membranes were first applied to water treatment processes in the 1960s, but in the next decade, they became increasingly used for desalination. Now, the list of membrane processes used in water treatment has lengthened to include:

- Forward osmosis
- Reverse osmosis
- Microfiltration
- Ultrafiltration
- Nanofiltration

Different processes require different types of membrane, broadly speaking, either functioning as a sieve or separating water from impurities on a molecular level. Membranes are made from polymer-based films, ceramics, and other materials. Research is underway on materials block polymers, aluminum oxide, graphene, and other nanomaterials like carbon nanotubes. Membranes have varying degrees of permeability: MF membranes have the largest pore size at 0.1 to 10 microns, followed by UF at 0.1-0.01 microns, NF at 0.01-0.035-microns, and RO membranes, which effectively are nonporous at 0.0001 of a micron.

3.13 Drying

Drying is a mass transfer process consisting of the removal of water or another solvent by evaporation from a solid, semi-solid or liquid. This process is often used as a final

production step before selling or packaging products. To be considered "dried", the final product must be solid, in the form of a continuous sheet (e.g., paper), long pieces (e.g., wood), particles (e.g., cereal grains or corn flakes) or powder (e.g., sand, salt, washing powder, milk powder). A source of heat and an agent to remove the vapor produced by the process are often involved. In bioproducts like food, grains, and pharmaceuticals like vaccines, the solvent to be removed is almost invariably water. Desiccation may be synonymous with drying or considered an extreme form of drying.

In the most common case, a gas stream, e.g., air, applies the heat by convection and carries away the vapor as humidity. Other possibilities are vacuum drying, where heat is supplied by conduction or radiation (or microwaves), while the vapor thus produced is removed by the vacuum system. Another indirect technique is drum drying (used, for instance, for manufacturing potato flakes), where a heated surface is used to provide the energy, and aspirators draw the vapor outside the room. In contrast, the mechanical extraction of the solvent, e.g., water, by filtration or centrifugation, is not considered drying but rather draining.

3.14 Crystallization

Crystallization or **crystallisation** is the process by which a solid forms, where the atoms or molecules are highly organized into a structure known as a crystal. Some of the ways by which crystals form are precipitating from a solution, freezing, or more rarely deposition directly from a gas. Attributes of the resulting crystal depend largely on factors such as temperature, air pressure, and in the case of liquid crystals, time of fluid evaporation.

Crystallization occurs in two major steps. The first is nucleation, the appearance of a crystalline phase from either a supercooled liquid or a supersaturated solvent. The second step is known as crystal growth, which is the increase in the size of particles and leads to a crystal state. An important feature of this step is that loose particles form layers at the crystal's surface and lodge themselves into open inconsistencies such as pores, cracks, etc. The majority of minerals and organic molecules crystallize easily, and the resulting crystals are generally of good quality, i.e. without visible defects. However, larger biochemical particles, like proteins, are often difficult to crystallize. The ease with which molecules will crystallize strongly depends on the intensity of either atomic forces (in the case of mineral

substances), intermolecular forces (organic and biochemical substances) or intramolecular forces (biochemical substances).

3.15 Whole cell mmobilization

The **immobilized whole cell** system is an alternative to enzyme immobilization. Unlike enzyme immobilization, where the enzyme is attached to a solid support (such as calcium alginate or activated PVA or activated PEI), in immobilized whole cell systems, the target cell is immobilized. Such methods may be implemented when the enzymes required are difficult or expensive to extract, an example being intracellular enzymes. Also, if a series of enzymes are required in the reaction; whole cell immobilization may be used for convenience. This is only done on a commercial basis when the need for the product is more justified. Multiple enzymes may be introduced into the reaction, thus eliminating the need for immobilization of multiple enzymes. Furthermore, intracellular enzymes need not be extracted prior to the reaction; they may be used directly. However, some enzymes may be used for the metabolic needs of the cell, leading to reduced yield of the cell.

Immobilized whole cells have been widely used in the production of industrially important chemicals as well as pharmaceutical important compounds. Generally, immobilization of cells could be carried out by either entrapment of the microorganisms in porous polymers or microcapsules or binding to an organic or inorganic support matrix. Adsorption in addition to its simplicity, has the possible advantages of reducing or eliminating the mass transfer problems associated with polymer entrapped cells. Nowadays, there has been considerable interest in the production of antibiotics by immobilized cells. Different antibiotics have been produced by immobilized cells such as nikkomycin, oxytetracycline, penicillin, rifamycins, cyclosporin, and other antibiotics. We have shown that the production of oxytetracycline increased by about 1.7-2.5 times after immobilization of *Streptomyces rimosus* cells in calcium alginate gels in comparison with free cells. In a previous paper, the usage of immobilized cells adhered on glass wool for both rifamycins and oxytetracycline production for 5 repeated batches had been studied. The purpose of the present work was to describe the optimal conditions for repeated batch production of oxytetracycline by immobilized cells, and the productivity of immobilized cells for 10 repeated batches in comparison with free cells.

3.16 Industrial applications

The major application of microbial biosensors is in the environmental field. The use of immobilized whole cells (usually bacteria) as the recognition element for biosensor has been described for chemical residues, such as phenols, pesticides, benzene, toluene, xilene, endocrine disruptors and the water biological oxygen demand (BOD). A large number publications have been appeared describing BOD microbial biosensors, and different devices are commercial available. However, a fewer number of applications have been developed for food toxicant analysis. Typically, electrochemical transduction methods have been used, particularly, the Clark oxygen electrode. These sensor systems rely on the interaction of a particular microorganism in the presence of a target analyte. By monitoring the respiratory activity of the microorganism, it has proved possible to detect and quantify the target analyte in a range of food matrices.

Recombinant organisms are an exploited alternative, that exhibits a number of important traits, such as the expression of cellular degradative enzymes, specific binding proteins against a target analyte, and reporter enzymes, for example bacterial luciferasa which is induced by target analytes with light production. Examples of microbial based biosensors application is the detection of antibiotic residues in milk, residues of pesticides in fish meat, or in aqueous solutions.

3.17 Summary

Downstream processing in pharmaceutical industries is very important because an ineffective downstream process can significantly increase the cost of the whole manufacturing process. Degerman et al. suggested that recovery and purification of solutes/products are the most expensive processes in pharmaceutical industries, and in some cases, its cost is higher than the whole bioprocess. Several techniques such as crystallization, chromatography, membrane technology, and distillation are available for downstream recovery and purification of pharmaceuticals. Due to ease of operation and scalability, several membranes such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis have been used for downstream processing of pharmaceuticals. Notably, ultrafiltration has been commonly studied for downstream processing in pharmaceutical industries. Li et al. (2004) used 5-,20-, and 50-kDa spiral wound ultrafiltration membranes for the removal of bio-emulsifiers from the fermentation broth containing benzyl penicillin antibiotic to improve the efficacy of solvent extraction process.

Ethylene and its derivatives are feedstocks for many plastics and resins as well as for fibers and detergents. Global ethylene production is estimated at more than 80 Million tons and growing. The United States is the world's largest ethylene producer, accounting for less than 30% of the world capacity. Since 1974, ethylene production has grown by 3% annually, while propylene has grown by more than 4% annually. Propylene has grown more rapidly – 5% per year – during the past decade or so. Ethylene and other coproducts are produced through cracking of hydrocarbon feedstocks. In the presence of steam, hydrocarbons are cracked into a mixture of shorter unsaturated compounds. In the cracking process, hydrocarbon feedstocks are preheated to 650 °C (using fuel gas and waste heat), mixed with steam, and cracked at a temperature of approximately 850 °C. A series of separation steps produce fractions consisting of ethylene, propylene, a C_4 fraction (e.g., butadiene), and pyrolysis gasoline (containing benzene, toluene, and xylenes).

The gas mixture is rapidly cooled to 400 °C (or quenched) to stop the reaction, during which process high-pressure steam is produced. Injection of water further decreases the temperature to approximately 40–50 °C, and a condensate that is rich in aromatics is formed. The liquid fraction is extracted, while the gaseous fraction is fed to a series of low-temperature, high-pressure distillation columns. Feedstocks used are ethane, LPG, naphtha, gas oils (GOs), and (sometimes) coal-derived feedstocks. Many of the installations used today can handle different (if not all) types of feedstock.

Terminal questions

Q.1. What do mean by precipitation?

Answer:----------

Q. 2 What do mean by centrifugation? Explain various types of centrifuges.

Answer:----------

Q. 3 Explain applications of centrifuges.

Answer:----------

Q. 4 Describe chromatography and its types.

Answer:----------

Q. 5 Explain ion exchange chromatography.

Answer:----------

Q. 6 Write a short note on whole cell immobilization.

Answer:----------

Q. 7 Explain filtration and separation.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-4: Industrial production of chemicals

Structure

4.1 Introduction

4.2 Industrial production of chemicals

4.3 What is Alcohol?

4.3.1 Ethanol

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4.3.3 Uses & Benefits

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4.4.2 Other biological roles

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4.6.1.1 Glycerol

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4.7.2 Medical, pharmaceutical and personal care applications

4.7.3 Acetone

4.8 Biosynthesis

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4.10.1 Industrial

4.10.2 Solvent

4.10.3 Acetylene carrier

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4.10.4.1 Chemical research

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4.10.4.3 Low-temperature bath

4.10.4.4 Histology

4.10.4.5 Lewis base properties

4.11 Medical

4.11.1 Drug solvent and excipient

4.11.2 Skin defatting

4.11.3 Butanol

4.12 Antibiotics

4.12.1 Resistance

4.12.2 Side effects

4.12.3 Penicillin

4.12.4 Penicillins (P, PCN or PEN)

4.12.5 Medical usage

4.1 Introduction

One of the first chemicals to be produced in large amounts through industrial processes was sulfuric acid. In 1736, the pharmacist Joshua Ward developed a process for its production that involved heating saltpeter, allowing the sulfur to oxidize and combine with water. It was the first practical production of sulphuric acid on a large scale. John Roebuck and Samuel Garbett were the first to establish a large-scale factory in Prestonpans, Scotland, in 1749, which used leaden condensing chambers for the manufacture of sulfuric acid.

In the early 18th century, cloth was bleached by treating it with stale urine or sour milk and exposing it to sunlight for long periods of time, which created a severe bottleneck in production. Sulfuric acid began to be used as a more efficient agent as well as lime by the middle of the century, but it was the discovery of bleaching powder by Charles Tennant that spurred the creation of the first great chemical industrial enterprise. His powder was made by reacting chlorine with dry slaked lime and proved to be a cheap and successful product.

4.2 Industrial production of chemicals

Soda ash was used since ancient times in the production of glass, textile, soap, and paper, and the source of the potash had traditionally been wood ashes in Western Europe. By the 18th century, this source was becoming uneconomical due to deforestation, and the French Academy of Sciences offered a prize of 2400 livres for a method to produce alkali from sea salt (sodium chloride). The Leblanc process was patented in 1791 by Nicolas Leblanc who then built a Leblanc plant at Saint-Denis. He was denied his prize money because of the French Revolution.

However, it was in Britain that the Leblanc process really took off. William Losh built the first soda works in Britain at the Losh, Wilson and Bell works on the River Tyne in 1816, but

it remained on a small scale due to large tariffs on salt production until 1824. When these tariffs were repealed, the British soda industry was able to rapidly expand. James Muspratt's chemical works in Liverpool and Charles Tennant's complex near Glasgow became the largest chemical production centres anywhere. By the 1870s, the British soda output of 200,000 tons annually exceeded that of all other nations in the world combined.

These huge factories began to produce a greater diversity of chemicals as the Industrial Revolution matured. Originally, large quantities of alkaline waste were vented into the environment from the production of soda, provoking one of the first pieces of environmental legislation to be passed in 1863. This provided for close inspection of the factories and imposed heavy fines on those exceeding the limits on pollution. Methods were soon devised to make useful byproducts from the alkali.

The Solvay process was developed by the Belgian industrial chemist Ernest Solvay in 1861. In 1864, Solvay and his brother Alfred constructed a plant in the Belgian town of Charleroi and in 1874, they expanded into a larger plant in Nancy, France. The new process proved more economical and less polluting than the Leblanc method, and its use spread. Mond was instrumental in making the Solvay process a commercial success; he made several refinements between 1873 and 1880 that removed byproducts that could slow or halt the mass production of sodium carbonate through use of the process.

Manufacture of chemical products from fossil fuels began at scale in the early nineteenth century. The coal tar and ammoniacal liquor residues of coal gas manufacture for gas lighting began to be processed in 1822 at the Bonnington Chemical Works in Edinburgh to make naphtha, pitch oil (later called creosote), pitch, lampblack (carbon black) and ammonium chloride). Ammonium sulphate fertiliser, asphalt road surfacing, coke oil and coke were later added to the product line.

Speciality chemicals are particular chemical products which provide a wide variety of effects on which many other industry sectors rely. Some of the categories of speciality chemicals are adhesives, agrichemicals, cleaning materials, colors, cosmetic additives, construction chemicals, elastomers, flavors, food additives, fragrances, industrial gases, lubricants, paints, polymers, surfactants, and textile auxiliaries. Other industrial sectors such as automotive, aerospace, food, cosmetics, agriculture, manufacturing, and textiles are highly dependent on such products.

Speciality chemicals are materials used on the basis of their performance or function. Consequently, in addition to "effect" chemicals they are sometimes referred to as "performance" chemicals or "formulation" chemicals. They can be unique molecules or mixtures of molecules known as formulations. The physical and chemical characteristics of the single molecules or the formulated mixtures of molecules and the composition of the mixtures influences the performance end product. In commercial applications the companies providing these products more often than not provide targeted customer service to innovative individual technical solutions for their customers. This is a differentiating component of the service provided by speciality chemical producers when they are compared to the other sub-sectors of the chemical industry such as fine chemicals, commodity chemicals, petrochemicals and pharmaceuticals.

In the USA the speciality chemical manufacturers are members of the Society of Chemical Manufacturers and Affiliates (SOCMA). In the United Kingdom such companies are members of the British Association for Chemical Specialties (BACS). SOCMA state that "Specialty chemicals differ from commodity chemicals in that each one may have only one or two uses, while commodities may have dozens of different applications for each chemical. While commodity chemicals make up most of the production volume (by weight) in the global marketplace, specialty chemicals make up most of the diversity (number of different chemicals) in commerce at any given time. Basic chemicals or commodity chemicals are a broad chemical category including polymers, bulk petrochemicals and intermediates, other derivatives and basic industrials, inorganic chemicals, and fertilizers. Polymers are the largest revenue segment and includes all categories of plastics and man-made fibers. The major markets for plastics are packaging, followed by home construction, containers, appliances, pipe, transportation, toys, and games.

- The largest-volume polymer product, polyethylene (PE), is used mainly in packaging films and other markets such as milk bottles, containers, and pipe.
- Polyvinyl chloride (PVC), another large-volume product, is principally used to make piping for construction markets as well as siding and, to a much smaller extent, transportation and packaging materials.
- Polypropylene (PP), similar in volume to PVC, is used in markets ranging from packaging, appliances, and containers to clothing and carpeting.

- Polystyrene (PS), another large-volume plastic, is used principally for appliances and packaging as well as toys and recreation.
- The leading man-made fibers include polyester, nylon, polypropylene, and acrylics, with applications including apparel, home furnishings, and other industrial and consumer use.

Principal raw materials for polymers are bulk petrochemicals like ethylene, propylene and benzene. Petrochemicals and intermediate chemicals are primarily made from liquefied petroleum gas (LPG), natural gas and crude oil fractions. Large volume products include ethylene, propylene, benzene, toluene, xylenes, methanol, vinyl chloride monomer (VCM), styrene, butadiene, and ethylene oxide. These basic or commodity chemicals are the starting materials used to manufacture many polymers and other more complex organic chemicals particularly those that are made for use in the specialty chemicals category.

Other derivatives and basic industrials include synthetic rubber, surfactants, dyes and pigments, turpentine, resins, carbon black, explosives, and rubber products and contribute about 20 percent of the basic chemicals' external sales. Inorganic chemicals (about 12 percent of the revenue output) make up the oldest of the chemical categories. Products include salt, chlorine, caustic soda, soda ash, acids (such as nitric acid, phosphoric acid, and sulfuric acid), titanium dioxide, and hydrogen peroxide. Fertilizers are the smallest category (about 6 percent) and include phosphates, ammonia, and potash chemicals.

4.3 What is Alcohol?

Humans have been drinking alcohol for thousands of years. Alcohol is both a chemical and a psychoactive drug. In chemistry, an alcohol exists when a hydroxyl group, a pair of oxygen and hydrogen atoms, replaces the hydrogen atom in a hydrocarbon. Alcohols bind with other atoms to create secondary alcohols. These secondary alcohols are the three types of alcohol that humans use every day: methanol, isopropanol, and ethanol. Alcohol-induced brain disorders have been reported for many decades. Ethanol (EtOH) presents amphiphilicity properties that permit the fluidibility across the biological barriers. The EtOH kinetics depends on the gender and age that elicit different brain susceptibility damage. The exact mechanisms that underlie such central nervous system (CNS) disruption due to EtOH is not well understood; however, neuroinflammation, oxidative stress, and neurotransmitter system

disorder processes may play a role. Therefore, the principal CNS effects described are anxiety, depression, motor disorder, and cognitive impairment after chronic ethanol exposure. This chapter summarizes many of the alcohol kinetics differences, the possible mechanisms of CNS damage, the main behavioral/CNS function, and the structures affected by chronic alcohol exposure.

4.3.1 Ethanol

Ethanol (also called ethyl alcohol, grain alcohol, drinking alcohol, or simply alcohol) is an organic chemical compound. It is a simple alcohol with the chemical formula C_2H_6O. Its formula can be also written as $CH_3{-}CH_2{-}OH$ or C_2H_5OH (an ethyl group linked to a hydroxyl group), and is often abbreviated as EtOH. Ethanol is a volatile, flammable, colorless liquid with a slight characteristic odor. It is a psychoactive drug, recreational drug, and the active ingredient in alcoholic drinks. Ethanol is naturally produced by the fermentation of sugars by yeasts or via petrochemical processes such as ethylene hydration. It has medical applications as an antiseptic and disinfectant. It is used as a chemical solvent and in the synthesis of organic compounds. Ethanol is a fuel source.

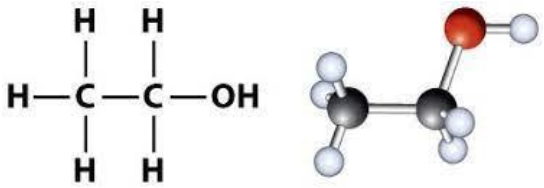


Fig. 1

There are two main processes for the manufacture of ethanol: the fermentation of carbohydrates (the method used for alcoholic beverages) and the hydration of ethylene. Fermentation involves the transformation of carbohydrates to ethanol by growing yeast cells. The chief raw materials fermented for the production of industrial alcohol are sugar crops such as beets and sugarcane and grain crops such as corn (maize). Hydration of ethylene is achieved by passing a mixture of ethylene and a large excess of steam at high temperature and pressure over an acidic catalyst.

Alcoholic Beverage	Alcohol Content (v/v)
Fruit juice	<0.1%
Ciders	4%–8%
American beer	4%–7%

Alcoholic Beverage	Alcohol Content (v/v)
Champagne	12%–13%
Table wine	8%–17%
Japanese Sake	14%–16%
Port wine	15%–22%
Whiskey, vodka, rum, and brandy	Usually 40% but much higher alcohol may be present in some brands
Tequila	45%–50%

Table 1

Ethanol produced either by fermentation or by synthesis is obtained as a dilute aqueous solution and must be concentrated by fractional distillation. Direct distillation can yield at best the constant-boiling-point mixture containing 95.6 percent by weight of ethanol. Dehydration of the constant-boiling-point mixture yields anhydrous, or absolute, alcohol. Ethanol intended for industrial use is usually denatured (rendered unfit to drink), typically with methanol, benzene, or kerosene. Pure ethanol is a colourless flammable liquid (boiling point 78.5 °C [173.3 °F]) with an agreeable ethereal odour and a burning taste. Ethanol is toxic, affecting the central nervous system. Moderate amounts relax the muscles and produce an apparent stimulating effect by depressing the inhibitory activities of the brain, but larger amounts impair coordination and judgment, finally producing coma and death. It is an addictive drug for some persons, leading to the disease alcoholism. Ethanol is converted in the body first to acetaldehyde and then to carbon dioxide and water, at the rate of about half a fluid ounce, or 15 ml, per hour; this quantity corresponds to a dietary intake of about 100 calories.

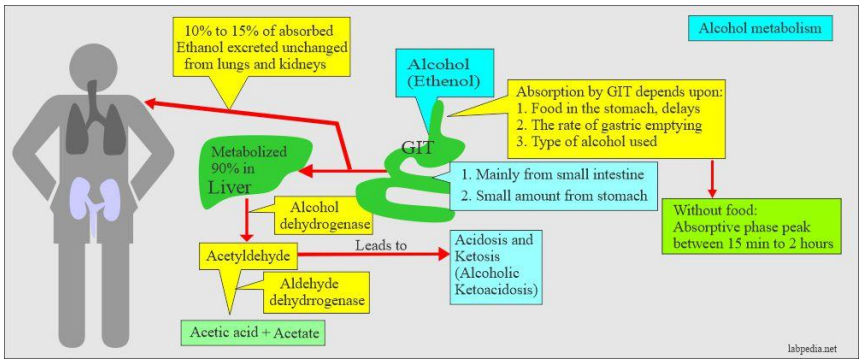


Fig. 2 Alcohol metabolism

4.3.2 Sources

Ethanol is made by fermentation of sugar or by the hydration of ethene. Ethanol is commonly found in households in the form of alcoholic beverages. The percentage of ethanol found in alcoholic beverages is one half of the value of the drink's proof value. Ethanol is also used for manufacturing paints and varnishes, as a carrier in various medications, as a disinfectant, in some types of thermometers, as a fuel substitute, and in some forms of antifreeze. The principal use of ethanol in veterinary medicine is in the treatment of ethylene glycol antifreeze poisoning. In humans and nonhuman primates, ethanol is sometimes used to treat methanol poisoning. Ethanol intoxication has been described in dogs ingesting alcoholic drinks intended for human consumption. Ethanol poisoning in dogs following ingestion of uncooked bread dough has also been reported. Additionally, ethanol intoxication has been reported in a dog from ingestion of rotten apples.

4.3.3 Uses & Benefits

Ethanol is one of the largest volume organic chemicals used in industrial and consumer products. The primary industrial uses of this aliphatic alcohol are as an intermediate in the production of other chemicals and as a solvent. Ethanol is used in the manufacture of drugs, plastics, lacquers, polishes, plasticizers, and cosmetics. Ethanol is used in medicine as a topical antiinfective, and as an antidote for ethylene glycol or methanol overdose. Commercial products containing ethanol include beverages, perfumes, aftershaves and colognes, medicinal liquids, mouthwashes, liniments, and some rubbing alcohols.

4.3.4 Personal Care Products

Ethanol is a common ingredient in many cosmetics and beauty products. It acts as an astringent to help clean skin, as a preservative in lotions and to help ensure that lotion ingredients do not separate, and it helps hairspray adhere to hair. Because ethanol is effective in killing microorganisms like bacteria, fungi and viruses, it is a common ingredient in many hand sanitizers. The U.S. Centers for Disease Control and Prevention (CDC) recommends the use of hand sanitizers in situations where soap and water are not available. Practicing hand hygiene is also an important part of helping to stop the spread of COVID-19. Using hand sanitizers or alcohol based hand rubs (ABHR) can help to inactivate SARS-CoV-2, the strain of coronavirus that causes COVID-19.

4.3.5 Household Products

Ethanol mixes easily with water and many organic compounds, and makes an effective solvent for use in paints, lacquers and varnish, as well as personal care and household cleaning products. As an additive to cleaning products, ethanol is also used as a preservative because it is effective in knocking out organisms that could pose a danger to consumers.

4.3.6 Food Additives

As a food additive, ethanol can help evenly distribute food coloring, as well as enhance the flavor of food extracts. For example, vanilla extract, a common food flavoring, is made by curing and processing vanilla beans in a solution of ethanol and water. In the United States, the Food and Drug Administration (FDA) only allows vanilla to be called "extract" when it has an alcohol or ethanol base.

4.3.7 Fuel

More than 97 percent of U.S. gasoline contains ethanol, typically in a mixture called E10, made up of 10 percent ethanol and 90 percent gasoline, to oxygenate the fuel and reduce air pollution. Ethanol has a higher octane number than gasoline, providing premium blending properties, according to the U.S. Department of Energy. Minimum octane number requirements prevent engine knocking and maintain drivability.

4.3.8 Safety measures

Ethanol is highly flammable and should not be used near open flames. Ethanol inhalation can cause coughing or headaches, according to the CDC. FDA has labeled ethanol as a Generally Recognized as Safe (GRAS) substance, which means that a panel of qualified experts determined that ethanol is safe to use in food products. Because ethanol is a very pure form of alcohol, its consumption and use in foods is regulated by the U.S. Food and Drug Administration (FDA) and the Bureau of Alcohol, Tobacco and Firearms.

4.3.9 Ethanol Safety

To discourage the drinking of pure ethanol from personal care or cleaning products, a "denaturant," such as a bitter flavoring, is usually added. Denaturants make alcohol unsuitable for human consumption, but does not change the other properties of the substance.

4.4.0 Acids

Acid, any substance that in water solution tastes sour, changes the colour of certain indicators (e.g., reddens blue litmus paper), reacts with some metals (e.g., iron) to liberate hydrogen, reacts with bases to form salts, and promotes certain chemical reactions (acid catalysis). Examples of acids include the inorganic substances known as the mineral acids—sulfuric, nitric, hydrochloric, and phosphoric acids—and the organic compounds belonging to the carboxylic acid, sulfonic acid, and phenol groups. Such substances contain one or more hydrogen atoms that, in solution, are released as positively charged hydrogen ions.

An **acid** is a molecule or ion capable of either donating a proton (i.e., hydrogen ion, H^+), known as a Brønsted–Lowry acid, or, capable of forming a covalent bond with an electron pair, known as a Lewis acid. The first category of acids are the proton donors, or Brønsted–Lowry acids. In the special case of aqueous solutions, proton donors form the hydronium ion H_3O^+ and are known as Arrhenius acids. Brønsted and Lowry generalized the Arrhenius theory to include non-aqueous solvents. A Brønsted or Arrhenius acid usually contains a hydrogen atom bonded to a chemical structure that is still energetically favorable after loss of H^+.

Aqueous Arrhenius acids have characteristic properties which provide a practical description of an acid. Acids form aqueous solutions with a sour taste, can turn blue litmus red, and react with bases and certain metals (like calcium) to form salts. The word *acid* is derived from the Latin *acidus/acēre*, meaning 'sour'. An aqueous solution of an acid has a pH less than 7 and is colloquially also referred to as "acid", while the strict definition refers only to the solute. A lower pH means a higher **acidity**, and thus a higher concentration of positive hydrogen ions in the solution. Chemicals or substances having the property of an acid are said to be **acidic**.

Common aqueous acids include hydrochloric acid (a solution of hydrogen chloride which is found in gastric acid in the stomach and activates digestive enzymes), acetic acid (vinegar is a dilute aqueous solution of this liquid), sulfuric acid (used in car batteries), and citric acid (found in citrus fruits). As these examples show, acids (in the colloquial sense) can be solutions or pure substances, and can be derived from acids (in the strict sense) that are solids, liquids, or gases. Strong acids and some concentrated weak acids are corrosive, but there are exceptions such as carboranes and boric acid.

4.4.1 Citric acid

Citric acid is an organic compound with the chemical formula $HOC(CO_2H)(CH_2CO_2H)_2$. Usually encountered as a white solid, it is a weak organic acid. It occurs naturally in citrus fruits. In biochemistry, it is an intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms. More than two million tons of citric acid are manufactured every year. It is used widely as an acidifier, as a flavoring, and a chelating agent.

A **citrate** is a derivative of citric acid; that is, the salts, esters, and the polyatomic anion found in solution. An example of the former, a salt is trisodium citrate; an ester is triethyl citrate. When part of a salt, the formula of the citrate anion is written as $C_6H_5O^{3-}_7$ or $C_3H_5O(COO)_3{}^{3-}$

Natural occurrence and industrial production

Fig. 3 Lemons, oranges, limes, and other citrus fruits possess high concentrations of citric acid

Citric acid exists in a variety of fruits and vegetables, most notably citrus fruits. Lemons and limes have particularly high concentrations of the acid; it can constitute as much as 8% of the dry weight of these fruits (about 47 g/L in the juices. The concentrations of citric acid in citrus fruits range from 0.005 mol/L for oranges and grapefruits to 0.30 mol/L in lemons and limes; these values vary within species depending upon the cultivar and the circumstances in which the fruit was grown.

Citric acid was first isolated in 1784 by the chemist Carl Wilhelm Scheele, who crystallized it from lemon juice. Industrial-scale citric acid production first began in 1890 based on the Italian citrus fruit industry, where the juice was treated with hydrated lime (calcium hydroxide) to precipitate calcium citrate, which was isolated and converted back to the acid using diluted sulfuric acid. In 1893, C. Wehmer discovered *Penicillium* mold could produce citric acid from sugar. However, microbial production of citric acid did not become industrially important until World War I disrupted Italian citrus exports.

In 1917, American food chemist James Currie discovered certain strains of the mold *Aspergillus niger* could be efficient citric acid producers, and the pharmaceutical

company Pfizer began industrial-level production using this technique two years later, followed by Citrique Belge in 1929. In this production technique, which is still the major industrial route to citric acid used today, cultures of *A. niger* are fed on a sucrose or glucose-containing medium to produce citric acid. The source of sugar is corn steep liquor, molasses, hydrolyzed corn starch, or other inexpensive, sugary solution. After the mold is filtered out of the resulting solution, citric acid is isolated by precipitating it with calcium hydroxide to yield calcium citrate salt, from which citric acid is regenerated by treatment with sulfuric acid, as in the direct extraction from citrus fruit juice.

In 1977, a patent was granted to Lever Brothers for the chemical synthesis of citric acid starting either from aconitic or isocitrate/alloisocitrate calcium salts under high pressure conditions; this produced citric acid in near quantitative conversion under what appeared to be a reverse, non-enzymatic Krebs cycle reaction. Global production was in excess of 2,000,000 tons in 2018.[16] More than 50% of this volume was produced in China. More than 50% was used as an acidity regulator in beverages, some 20% in other food applications, 20% for detergent applications, and 10% for applications other than food, such as cosmetics, pharmaceuticals, and in the chemical industry.

4.4.2 Other biological roles

Citrate can be transported out of the mitochondria and into the cytoplasm, then broken down into acetyl-CoA for fatty acid synthesis, and into oxaloacetate. Citrate is a positive modulator of this conversion, and allosterically regulates the enzyme acetyl-CoA carboxylase, which is the regulating enzyme in the conversion of acetyl-CoA into malonyl-CoA (the commitment step in fatty acid synthesis). In short, citrate is transported into the cytoplasm, converted into acetyl CoA, which is then converted into malonyl CoA by acetyl CoA carboxylase, which is allosterically modulated by citrate.

High concentrations of cytosolic citrate can inhibit phosphofructokinase, the catalyst of a rate-limiting step of glycolysis. This effect is advantageous: high concentrations of citrate indicate that there is a large supply of biosynthetic precursor molecules, so there is no need for phosphofructokinase to continue to send molecules of its substrate, fructose 6-phosphate, into glycolysis. Citrate acts by augmenting the inhibitory effect of high concentrations of ATP, another sign that there is no need to carry out glycolysis. Citrate is a vital component of bone, helping to regulate the size of apatite crystals.

4.5 Applications

4.5.1 Food and drink

Because it is one of the stronger edible acids, the dominant use of citric acid is as a flavoring and preservative in food and beverages, especially soft drinks and candies. Within the European Union it is denoted by E number **E330**. Citrate salts of various metals are used to deliver those minerals in a biologically available form in many dietary supplements. Citric acid has 247 kcal per 100 g. In the United States the purity requirements for citric acid as a food additive are defined by the Food Chemicals Codex, which is published by the United States Pharmacopoeia (USP).

Citric acid can be added to ice cream as an emulsifying agent to keep fats from separating, to caramel to prevent sucrose crystallization, or in recipes in place of fresh lemon juice. Citric acid is used with sodium bicarbonate in a wide range of effervescent formulae, both for ingestion (e.g., powders and tablets) and for personal care (*e.g.*, bath salts, bath bombs, and cleaning of grease). Citric acid sold in a dry powdered form is commonly sold in markets and groceries as "sour salt", due to its physical resemblance to table salt. It has use in culinary applications, as an alternative to vinegar or lemon juice, where a pure acid is needed. Citric acid can be used in food coloring to balance the pH level of a normally basic dye.

4.5.2 Cleaning and chelating agent

Citric acid is an excellent chelating agent, binding metals by making them soluble. It is used to remove and discourage the buildup of limescale from boilers and evaporators. It can be used to treat water, which makes it useful in improving the effectiveness of soaps and laundry detergents. By chelating the metals in hard water, it lets these cleaners produce foam and work better without need for water softening. Citric acid is the active ingredient in some bathroom and kitchen cleaning solutions. A solution with a six percent concentration of citric acid will remove hard water stains from glass without scrubbing. Citric acid can be used in shampoo to wash out wax and coloring from the hair. Illustrative of its chelating abilities, citric acid was the first successful eluant used for total ion-exchange separation of the lanthanides, during the Manhattan Project in the 1940s. In the 1950s, it was replaced by the far more efficient EDTA. In industry, it is used to dissolve rust from steel and passivate stainless steels.

4.5.3 Cosmetics, pharmaceuticals, dietary supplements, and foods

Citric acid is used as an acidulant in creams, gels, and liquids. Used in foods and dietary supplements, it may be classified as a processing aid if it was added for a technical or

functional effect (e.g. acidulent, chelator, viscosifier, etc.). If it is still present in insignificant amounts, and the technical or functional effect is no longer present. Citric acid is an alpha hydroxy acid and is an active ingredient in chemical skin peels. Citric acid is commonly used as a buffer to increase the solubility of brown heroin. Citric acid is used as one of the active ingredients in the production of facial tissues with antiviral properties.

4.5.4 Other uses

The buffering properties of citrates are used to control pH in household cleaners and pharmaceuticals. Citric acid is used as an odorless alternative to white vinegar for home dyeing with acid dyes. Sodium citrate is a component of Benedict's reagent, used for identification both qualitatively and quantitatively of reducing sugars. Citric acid can be used as an alternative to nitric acid in passivation of stainless steel. Citric acid can be used as a lower-odor stop bath as part of the process for developing photographic film. Photographic developers are alkaline, so a mild acid is used to neutralize and stop their action quickly, but commonly used acetic acid leaves a strong vinegar odor in the darkroom. Citric acid/potassium-sodium citrate can be used as a blood acid regulator. Soldering flux. Citric acid is an excellent soldering flux, either dry or as a concentrated solution in water. It should be removed after soldering, especially with fine wires, as it is mildly corrosive. It dissolves and rinses quickly in hot water.

4.5.5 Acetic acid (CH_3COOH)

Vinegar was known early in civilization as the natural result of exposure of beer and wine to air, because acetic acid-producing bacteria are present globally. The use of acetic acid in alchemy extends into the 3rd century BC, when the Greek philosopher Theophrastus described how vinegar acted on metals to produce pigments useful in art, including *white lead* (lead carbonate) and *verdigris*, a green mixture of copper salts including copper (II) acetate. Ancient Romans boiled soured wine to produce a highly sweet syrup called *sapa*. Sapa that was produced in lead pots was rich in lead acetate, a sweet substance also called *sugar of lead* or *sugar of Saturn*, which contributed to lead poisoning among the Roman aristocracy. In the 16th-century German alchemist Andreas Libavius described the production of acetone from the dry distillation of lead acetate, ketonic decarboxylation. The presence of water in vinegar has such a profound effect on acetic acid's properties that for centuries chemists believed that glacial acetic acid and the acid found in vinegar were two different substances.

Acetic acid is a colorless liquid organic compound with the chemical formula CH_3COOH, which is also the main component of vinegar apart from water. Familiar to everyone, acetic acid has a distinctive sour taste and pungent smell, which is common in many traditional fermentation foods, such as koumiss and fruit vinegar. As the second simplest carboxylic acid, acetic acid is not only an important chemical reagent in chemical industry, but also a useful material in the food industry, which is often used as acidity regulator or condiment. In addition, acetic acid contains an acetyl group that is fundamental to all forms of life. Although most people will believe that a large amount of acetic acid is used to produce vinegar, actually, compared with the consumption in the chemical industry, the total volume of acetic acid used for vinegar is comparatively small.

Therefore, the mainly utilization of acetic acid in the chemical industry will be introduced, which can be divided into three parts. 1. Vinyl acetate monomer: almost a third of the world's production of acetic acid is converted to vinyl acetate monomer, which is also the primary utilization of acetic acid. 2. Ester production: acetic acid can react with different alcohols to form esters, such as ethyl acetate, n-butyl acetate, isobutyl acetate, and propyl acetate, which are widely used as solvents for inks, paints, and coatings. 3. Acetic anhydride: approximately 25%–30% of the global production of acetic acid is used for the production of acetic anhydride, which can be used to synthesize cellulose acetate, heroin, and other compounds.

Acetic acid (CH_3COOH), also called ethanoic acid, the most important of the carboxylic acids. A dilute (approximately 5 percent by volume) solution of acetic acid produced by fermentation and oxidation of natural carbohydrates is called vinegar; a salt, ester, or acylal of acetic acid is called acetate. Industrially, acetic acid is used in the preparation of metal acetates, used in some printing processes; vinyl acetate, employed in the production of plastics; cellulose acetate, used in making photographic films and textiles; and volatile organic esters (such as ethyl and butyl acetates), widely used as solvents for resins, paints, and lacquers. Biologically, acetic acid is an important metabolic intermediate, and it occurs naturally in body fluids and in plant juices.

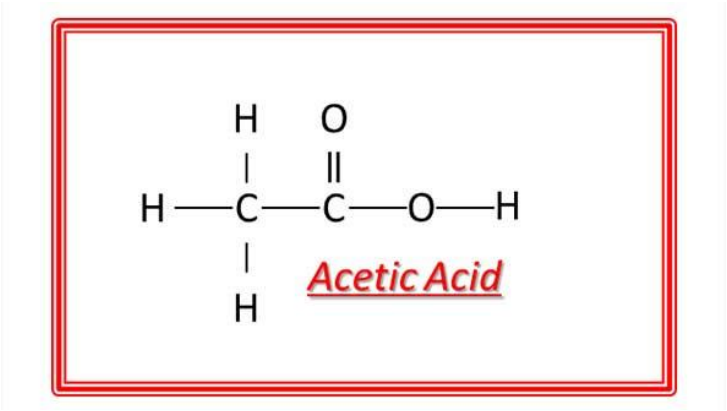


Fig. 4

Acetic acid has been prepared on an industrial scale by air oxidation of acetaldehyde, by oxidation of ethanol (ethyl alcohol), and by oxidation of butane and butene. Today acetic acid is manufactured by a process developed by the chemical company Monsanto in the 1960s; it involves a rhodium-iodine catalyzed carbonylation of methanol (methyl alcohol).

$$\underset{\text{methanol}}{CH_3OH} + \underset{\substack{\text{carbon}\\\text{monoxide}}}{CO} \xrightarrow{Rh/I_2} \underset{\text{acetic acid}}{CH_3COOH}$$

Pure acetic acid, often called glacial acetic acid, is a corrosive, colourless liquid (boiling point 117.9 °C [244.2 °F]; melting point 16.6 °C [61.9 °F]) that is completely miscible with water.

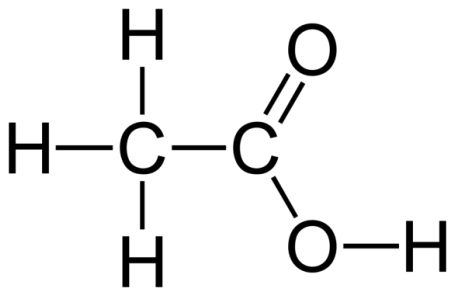


Fig. 5

4.5.6 Use as solvent

Glacial acetic acid is an excellent polar protic solvent, as noted above. It is frequently used as a solvent for recrystallization to purify organic compounds. Acetic acid is used as a solvent in the production of terephthalic acid (TPA), the raw material for polyethylene terephthalate (PET). In 2006, about 20% of acetic acid was used for TPA production. Acetic

acid is often used as a solvent for reactions involving carbocations, such as Friedel-Crafts alkylation. For example, one stage in the commercial manufacture of synthetic camphor involves a Wagner-Meerwein rearrangement of camphene to isobornyl acetate; here acetic acid acts both as a solvent and as a nucleophile to trap the rearranged carbocation. Glacial acetic acid is used in analytical chemistry for the estimation of weakly alkaline substances such as organic amides. Glacial acetic acid is a much weaker base than water, so the amide behaves as a strong base in this medium. It then can be titrated using a solution in glacial acetic acid of a very strong acid, such as perchloric acid.

4.5.7 Medical use

Acetic acid injection into a tumor has been used to treat cancer since the 1800s. Acetic acid is used as part of cervical cancer screening in many areas in the developing world. The acid is applied to the cervix and if an area of white appears after about a minute the test is positive. Acetic acid is an effective antiseptic when used as a 1% solution, with broad spectrum of activity against streptococci, staphylococci, pseudomonas, enterococci and others. It may be used to treat skin infections caused by pseudomonas strains resistant to typical antibiotics. While diluted acetic acid is used in iontophoresis, no high quality evidence supports this treatment for rotator cuff disease. As a treatment for otitis externa, it is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system.

4.5.8 Foods

Acetic acid has 349 kcal per 100 g. Vinegar is typically no less than 4% acetic acid by mass. Legal limits on acetic acid content vary by jurisdiction. Vinegar is used directly as a condiment, and in the pickling of vegetables and other foods. Table vinegar tends to be more diluted (4% to 8% acetic acid), while commercial food pickling employs solutions that are more concentrated. The proportion of acetic acid used worldwide as vinegar is not as large as commercial uses, but is by far the oldest and best-known application.

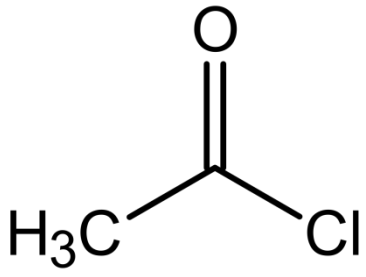


Fig. 6

Gluconic acid is an organic compound with molecular formula $C_6H_{12}O_7$ and condensed structural formula $HOCH_2(CHOH)_4COOH$. It is one of the 16 stereoisomers of 2,3,4,5,6-pentahydroxyhexanoic acid. In aqueous solution at neutral pH, gluconic acid forms the **gluconate ion**. The salts of gluconic acid are known as "gluconates". Gluconic acid, gluconate salts, and gluconate esters occur widely in nature because such species arise from the oxidation of glucose. Some drugs are injected in the form of gluconates. The chemical structure of gluconic acid consists of a six-carbon chain, with five hydroxyl groups positioned in the same way as in the open-chained form of glucose, terminating in a carboxylic acid group. In aqueous solution, gluconic acid exists in equilibrium with the cyclic ester glucono delta-lactone.

Gluconic acid is produced by oxidizing glucose. This can be accomplished in several ways:

- Via hydrogen peroxide
- Via bromine
- In a fermentation bath

Gluconic acid occurs naturally in fruit, honey, and wine. In 1929 Horace Terhune Herrick developed a process for producing the salt by fermentation. As a food additive, it is now known as an acidity regulator.

4.5.9 Gluconic acid

Gluconic acid, or pentahydroxycaproic acid ($C_6H_{12}O_7$), naturally occurs in plants, fruits, wine, honey, rice, meat, vinegar, and other natural sources. The alkali salt of gluconic acid such as calcium gluconate or sodium gluconate has multiple applications used in chemical, pharmaceutical, food, beverage, and construction industries. Due to its low toxicity, low corrosiveness, and high capability of forming water-soluble complexes with divalent and trivalent metal ions, sodium gluconate has been designated as GRAS by the US FDA. This acid is also recognized as a generally permitted food additive (E574) in the European Parliament and the Council Directive No. 95/2/EC. Like other organic acids, gluconic acid has diverse uses that range widely depending on their particular structure. Thus, gluconic acid and its derivatives (except glucono-lactone) are used mainly as additives by food, pharmaceutical,

hygiene, and building industries. Gluconic acid is produced either by chemical or biotechnology methods, although microbial processes are predominant at industrial scale. The production cost is estimated from US$1.20/kg to US$8.50/kg for calcium gluconate and glucono-δ-lactone. Sodium gluconate is the most widely marketed gluconic acid derivative, accounting for more than 80% of the world production.

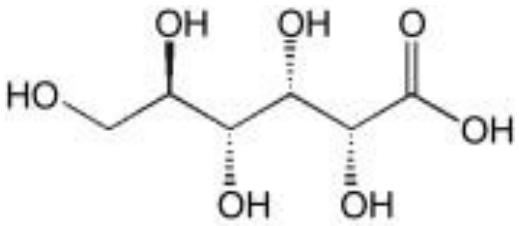


Fig. 7 Gluconic acid

Enzyme glucose oxidase (E.C. 1.1.3.4) from filamentous fungi involves two steps during conversion of glucose to gluconic acid. The first step is the dehydrogenation in which two hydrogen atoms are removed from b-d-glucopyranose to yield d-glucono-d-lactone. This reaction is favored in the presence of high levels of glucose and oxygen concentration in the medium and at pH of 5.5. The final step in the production of gluconic acid is the hydrolysis of the d-glucono-d-lactone by enzyme glucose dehydrogenase. Accumulation of the intermediate glucono-d-lactone has a negative effect on the rate of glucose oxidation. The spontaneous hydrolysis of d-lactone is quite rapid at neutral pH, which is brought by $CaCO_3$ or NaOH.

Initially, the acid was produced in surface fermentation by *Penicillium* fungi. This method was rapidly replaced by submerged fermentation cultures of *A. niger* or *Gluconobacter oxydans*, which are very efficient. Other reported gluconic acid producers are Filamentous fungi such as A. niger, Penicillium glaucum, Penicillium amagasakiense, Penicillium luteum purpurogenum, Penicillium chrysogenum*;* bacteria such as Pseudomonas savastanoi, G. oxydans (obligate aerobic bacterium), Acetobacter diazotrophicus, Acetobacter methanolicus, Pseudomonas ovalis, Pseudomonas fluorescens, *and* Zymomonas mobilis*;* and yeast such as Aureobasidium pullulans. The gluconate anion chelates Ca^{2+}, Fe^{2+}, Al^{3+}, and other metals, including lanthanides and actinides. It is also used in cleaning products, where it dissolves mineral deposits, especially in alkaline solution.

Calcium gluconate, in the form of a gel, is used to treat burns from hydrofluoric acid; calcium gluconate injections may be used for more severe cases to avoid necrosis of deep tissues, as well as to treat hypocalcemia in hospitalized patients. Gluconate is also an electrolyte present in certain solutions, such as "plasmalyte a", used for intravenous fluid resuscitation. Quinine gluconate is a salt of gluconic acid and quinine, which is used for intramuscular injection in the treatment of malaria. Zinc gluconate injections are used to neuter male dogs. Ferrous gluconate injections have been proposed in the past to treat anemia.

Gluconate is also used in building and construction as a concrete admixture (retarder) to slow down the cement hydration reactions, and to delay the cement setting time. It allows for a longer time to lay the concrete, or to spread the cement hydration heat over a longer period of time to avoid too high a temperature and the resulting cracking. Retarders are mixed in to concrete when the weather temperature is high or to cast large and thick concrete slabs in successive and sufficiently well-mixed layers.

4.6 Solvents

A **solvent** (from the Latin *solvō*, "loosen, untie, solve") is a substance that dissolves a solute, resulting in a solution. A solvent is usually a liquid but can also be a solid, a gas, or a supercritical fluid. Water is a solvent for polar molecules and the most common solvent used by living things; all the ions and proteins in a cell are dissolved in water within the cell.

The quantity of solute that can dissolve in a specific volume of solvent varies with temperature. Major uses of solvents are in paints, paint removers, inks, dry cleaning.[2] Specific uses for organic solvents are in dry cleaning (e.g. tetrachloroethylene); as paint thinners (toluene, turpentine); as nail polish removers and solvents of glue (acetone, methyl acetate, ethyl acetate); in spot removers (hexane, petrol ether); in detergents (citrus terpenes); and in perfumes (ethanol). Solvents find various applications in chemical, pharmaceutical, oil, and gas industries, including in chemical syntheses and purification processes.

When one substance is dissolved into another, a solution is formed. This is opposed to the situation when the compounds are insoluble like sand in water. In a solution, all of the ingredients are uniformly distributed at a molecular level and no residue remains. A solvent-solute mixture consists of a single phase with all solute molecules occurring

as *solvates* (solvent-solute complexes), as opposed to separate continuous phases as in suspensions, emulsions and other types of non-solution mixtures. The ability of one compound to be dissolved in another is known as solubility; if this occurs in all proportions, it is called miscible.

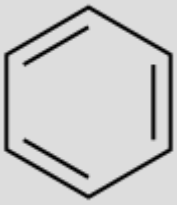
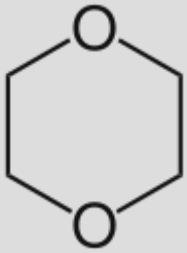
In addition to mixing, the substances in a solution interact with each other at the molecular level. When something is dissolved, molecules of the solvent arrange around molecules of the solute. Heat transfer is involved and entropy is increased making the solution more thermodynamically stable than the solute and solvent separately. This arrangement is mediated by the respective chemical properties of the solvent and solute, such as hydrogen bonding, dipole moment and polarizability. Solvation does not cause a chemical reaction or chemical configuration changes in the solute. However, solvation resembles a coordination complex formation reaction, often with considerable energetics (heat of solvation and entropy of solvation) and is thus far from a neutral process.

When one substance dissolves into another, a solution is formed. A solution is a homogeneous mixture consisting of a solute dissolved into a solvent. The solute is the substance that is being dissolved, while the solvent is the dissolving medium. Solutions can be formed with many different types and forms of solutes and solvents.

Solvents can be broadly classified into two categories: *polar* and *non-polar*. A special case is mercury, whose solutions are known as amalgams; also, other metal solutions exist which are liquid at room temperature. Generally, the dielectric constant of the solvent provides a rough measure of a solvent's polarity. The strong polarity of water is indicated by its high dielectric constant of 88 (at 0 °C). Solvents with a dielectric constant of less than 15 are generally considered to be nonpolar. The dielectric constant measures the solvent's tendency to partly cancel the field strength of the electric field of a charged particle immersed in it. This reduction is then compared to the field strength of the charged particle in a vacuum. Heuristically, the dielectric constant of a solvent can be thought of as its ability to reduce the solute's effective internal charge. Generally, the dielectric constant of a solvent is an acceptable predictor of the solvent's ability to dissolve common ionic compounds, such as salts.

4.6.1 Properties table of common solvent

The solvents are grouped into nonpolar, polar aprotic, and polar protic solvents, with each group ordered by increasing polarity. The properties of solvents which exceed those of water are bolded.

Solvent	**Chemical formula**	**B.P (°C)**	**Dielectric constant**	**Density (g/m)**	**Dipole moment (D)**
Hydrocarbon solvents					
Pentane	 $CH_3CH_2CH_2CH_2CH_3$	36.1	1.84	0.626	0.00
Hexane	 $CH_3CH_2CH_2CH_2CH_2CH_3$	69	1.88	0.655	0.00
Benzene	 C_6H_6	80.1	2.3	0.879	0.00
Toluene	C_6H_5-CH_3	**111**	2.38	0.867	0.36
Ether solvents					
1,4-Dioxane	 $C_4H_8O_2$	**101.1**	2.3	**1.033**	0.45
Diethyl ether	 CH_3CH_2-O-CH_2CH_3	34.6	4.3	0.713	1.15

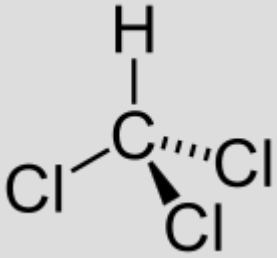
Tetrahydrofuran (THF)	 C_4H_8O	66	7.5	0.886	1.75
Chlorocarbon solvents					
Chloroform	 $CHCl_3$	61.2	4.81	**1.498**	1.04
Polar aprotic solvents					

Table 2

4.6.1.1 Glycerol

Glycerol is also called glycerine in British English or glycerin in American English) is a simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in lipids known as glycerides. Due to having antimicrobial and antiviral properties it is widely used in FDA approved wound and burn treatments. It can be used as an effective marker to measure liver disease. It is also widely used as a sweetener in the food industry and as a humectant in pharmaceutical formulations. Owing to the presence of three hydroxyl groups, glycerol is miscible with water and is hygroscopic in nature. Glycerol is generally obtained from plant and animal sources where it occurs in triglycerides, esters of glycerol with long-chain carboxylic acids. The hydrolysis, saponification, or transesterification of these triglycerides produces glycerol as well as the fatty acid derivative:

Triglycerides can be saponified with sodium hydroxide to give glycerol and fatty sodium salt or soap. Typical plant sources include soybeans or palm. Animal-derived tallow is another source. Approximately 950,000 tons per year are produced in the United States and Europe; 350,000 tons of glycerol were produced per year in the United States alone from 2000 to

2004. The EU directive 2003/30/EC set a requirement that 5.75% of petroleum fuels are to be replaced with biofuel sources across all member states by 2010. It was projected in 2006 that by the year 2020, production would be six times more than demand, creating an excess of glycerol.

Glycerol from triglycerides is produced on a large scale, but the crude product is of variable quality, with a low selling price of as low as 2-5 U.S. cents per kilogram in 2011. It can be purified, but the process is expensive. Some glycerol is burned for energy, but its heat value is low. Crude glycerol from the hydrolysis of triglycerides can be purified by treatment with activated carbon to remove organic impurities, alkali to remove unreacted glycerol esters, and ion exchange to remove salts. High purity glycerol (> 99.5%) is obtained by multi-step distillation; a vacuum chamber is necessary due to its high boiling point (290 °C).

4.7 Applications

4.7.1 Food industry

In food and beverages, glycerol serves as a humectant, solvent, and sweetener, and may help preserve foods. It is also used as filler in commercially prepared low-fat foods (e.g., cookies), and as a thickening agent in liqueurs. Glycerol and water are used to preserve certain types of plant leaves.[15] As a sugar substitute, it has approximately 27 kilocalories per teaspoon (sugar has 20) and is 60% as sweet as sucrose. It does not feed the bacteria that form a dental plaque and cause dental cavities. As a food additive, glycerol is labeled as E number E422. It is added to icing (frosting) to prevent it from setting too hard.

As used in foods, glycerol is categorized by the U.S. Academy of Nutrition and Dietetics as a carbohydrate. The U.S. Food and Drug Administration (FDA) carbohydrate designation includes all caloric macronutrients excluding protein and fat. Glycerol has a caloric density similar to table sugar, but a lower glycemic index and different metabolic pathway within the body, so some dietary advocates accept glycerol as a sweetener compatible with low-carbohydrate diets. It is also recommended as an additive when using polyol sweeteners such as erythritol and xylitol which have a cooling effect, due to its heating effect in the mouth, if the cooling effect is not wanted.

4.7.2 Medical, pharmaceutical and personal care applications

Glycerin is mildly antimicrobial and antiviral and is an FDA approved treatment for wounds. The Red Cross reports that an 85% solution of glycerin shows bactericidal and antiviral effects, and wounds treated with glycerin show reduced inflammation after roughly 2 hours. Due to this it is used widely in wound care products, including glycerin based hydrogel sheets for burns and other wound care. It is approved for all types of wound care except third degree burns, and is used to package donor skin used in skin grafts. There is no topical treatment approved for third degree burns, and so this limitation is not exclusive to glycerin.

Glycerol is used in medical, pharmaceutical and personal care preparations, often as a means of improving smoothness, providing lubrication, and as a humectant. Ichthyosis and xerosis have been relieved by the topical use of glycerin. It is found in allergen immunotherapies, cough
syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps, and water-based personal lubricants. In solid dosage forms like tablets, glycerol is used as a tablet holding agent. For human consumption, glycerol is classified by the U.S. FDA among the sugar alcohols as a caloric macronutrient. Glycerol is also used in blood banking to preserve red blood cells prior to freezing. Glycerol is a component of glycerin soap. Essential oils are added for fragrance. This kind of soap is used by people with sensitive, easily irritated skin because it prevents skin dryness with its moisturizing properties. It draws moisture up through skin layers and slows or prevents excessive drying and evaporation.

Taken rectally, glycerol functions as a laxative by irritating the anal mucosa and inducing a hyperosmotic effect, expanding the colon by drawing water into it to induce peristalsis resulting in evacuation. It may be administered undiluted either as a suppository or as a small-volume (2–10 ml) enema. Alternatively, it may be administered in a dilute solution, e.g., 5%, as a high volume enema. Taken orally (often mixed with fruit juice to reduce its sweet taste), glycerol can cause a rapid, temporary decrease in the internal pressure of the eye. This can be useful for the initial emergency treatment of severely elevated eye pressure.

Research on the effects of probiotic Lactobacillus reuteri on Clostridium difficile grown in a laboratory found that when the probiotic was supplemented with glycerol, it converted it into the broad-spectrum antimicrobial compound reuterin. The reuterin acted as an antimicrobial

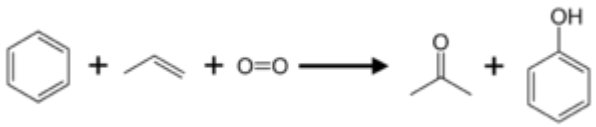
agent, and worked as well as vancomycin to inhibit C. difficile growth. In addition, the researchers found that glycerol or L. reuteri alone were not effective against C. difficile, and that the reuterin did not harm the good bacteria in the complex gut community. Glycerol has also been incorporated as a component of bio-ink formulations in the field of bioprinting. The glycerol content acts to add viscosity to the bio-ink without adding large protein, carbohydrate, or glycoprotein molecules.

4.7.3 Acetone

Acetone, or **propanone**, is an organic compound with the formula $(CH_3)_2CO$. It is the simplest and smallest ketone. It is a colourless, highly volatile and flammable liquid with a characteristic pungent odour. Acetone is miscible with water and serves as an important organic solvent in its own right, in industry, home, and laboratory. About 6.7 million tonnes were produced worldwide in 2010, mainly for use as a solvent and production of methyl methacrylate and bisphenol A. It is a common building block in organic chemistry. Familiar household uses of acetone are as the active ingredient in nail polish remover and as paint thinner. While it has volatile organic compound (VOC) exempt status in the United States, it is considered by the EU as a contributor to environmental pollution.

Acetone is produced and disposed of in the human body through normal metabolic processes. It is normally present in blood and urine. People with diabetic ketoacidosis produce it in larger amounts. Reproductive toxicity tests show that it has low potential to cause reproductive problems. Ketogenic diets that increase ketone bodies (acetone, β-hydroxybutyric acid and acetoacetic acid) in the blood are used to counter epileptic attacks in infants and children who suffer from refractory epilepsy.

Acetone is produced directly or indirectly from propylene. Approximately 83% of acetone is produced via the cumene process; as a result, acetone production is tied to phenol production. In the cumene process, benzene is alkylated with propylene to produce cumene, which is oxidized by air to produce phenol and acetone:



Other processes involve the direct oxidation of propylene, or the hydration of propylene to give 2-propanol, which is oxidized (dehydrogenated) to acetone.

4.8 Biosynthesis

Small amounts of acetone are produced in the body by the decarboxylation of ketone bodies. Certain dietary patterns, including prolonged fasting and high-fat low-carbohydrate dieting, can produce ketosis, in which acetone is formed in body tissue. Certain health conditions, such as alcoholism and diabetes, can produce ketoacidosis, uncontrollable ketosis that leads to a sharp, and potentially fatal, increase in the acidity of the blood. Since it is a byproduct of fermentation, acetone is a byproduct of the distillery industry.

Acetone can be produced from the oxidation of ingested isopropanol, or from the spontaneous/enzymatic breakdown of acetoacetate (a ketone body) in ketotic individuals.

4.9 Metabolism

Although some biochemistry textbooks and current research publications[39] indicate that acetone cannot be metabolized, there is evidence to the contrary. It can then be metabolized either by CYP2E1 via methylglyoxal to D-lactate and pyruvate, and ultimately glucose/energy, or by a different pathway via propylene glycol to pyruvate, lactate, acetate (usable for energy) and propionaldehyde.

4.10 Uses

4.10.1 Industrial

About a third of the world's acetone is used as a solvent, and a quarter is consumed as acetone cyanohydrin, a precursor to methyl methacrylate.

4.10.2 Solvent

Acetone is a good solvent for many plastics and some synthetic fibers. It is used for thinning polyester resin, cleaning tools used with it, and dissolving two-part epoxies and superglue before they harden. It is used as one of the volatile components of some paints and varnishes. As a heavy-duty degreaser, it is useful in the preparation of metal prior to painting or soldering, and to remove rosin flux after soldering (to prevent adhesion of dirt and electrical leakage and perhaps corrosion or for cosmetic reasons), although it attacks many electronic components (for example polystyrene capacitors) so it is unsuitable for cleaning many circuit boards.

4.10.3 Acetylene carrier

Although itself flammable, acetone is used extensively as a solvent for the safe transportation and storage of acetylene, which cannot be safely pressurized as a pure compound. Vessels containing a porous material are first filled with acetone followed by acetylene, which dissolves into the acetone. One litre of acetone can dissolve around 250 litres of acetylene at a pressure of 10 bar.

4.10.4 Laboratory

4.10.4.1 Chemical research

In the laboratory, acetone is used as a polar, aprotic solvent in a variety of organic reactions, such as S_N2 reactions. The use of acetone solvent is critical for the Jones oxidation. It does not form an azeotrope with water (see azeotrope tables). It is a common solvent for rinsing laboratory glassware because of its low cost and volatility. Despite its common use as a supposed drying agent, it is not effective except by bulk displacement and dilution. Acetone can be cooled with dry ice to −78 °C without freezing; acetone/dry ice baths are commonly used to conduct reactions at low temperatures. Acetone is fluorescent under ultraviolet light, and its vapor can be used as a fluorescent tracer in fluid flow experiments. Acetone is used to precipitate proteins. Alternatives for protein precipitation are trichloroacetic acid or ethanol.

4.10.4.2 Cleaning

Low-grade acetone is also commonly used in academic laboratory settings as a glassware rinsing agent for removing residue and solids before a final wash. Acetone leaves a small amount of residue on a surface when dried that is harmful to surface samples.

4.10.4.3 Low-temperature bath

A mixture of acetone and dry ice is a popular cooling bath that maintains a temperature of −78 °C as long as there is some dry ice left.

4.10.4.4 Histology

Acetone is used in the field of pathology to find lymph nodes in fatty tissues for tumor staging (such as looking for lymph nodes in the fat surrounding the intestines). This helps dissolve the fat, and hardens the nodes, making finding them easier. Acetone also used for destaining microscope slides of certain stains.

4.10.4.5 Lewis base properties

Acetone is a weak Lewis base that forms adducts with soft acids like I_2 and hard acids like phenol. Acetone also forms complexes with divalent metals.

4.11 Medical

4.11.1 Drug solvent and excipient

Acetone is used as a solvent by the pharmaceutical industry and as a denaturant in denatured alcohol. Acetone is also present as an excipient in some pharmaceutical drugs.

4.11.2 Skin defatting

Dermatologists use acetone with alcohol for acne treatments to chemically peel dry skin. Common agents used today for chemical peeling are salicylic acid, glycolic acid, 30% salicylic acid in ethanol, and trichloroacetic acid (TCA). Prior to chemexfoliation, the skin is cleaned and excess fat removed in a process called defatting. Acetone, Septisol, or a combination of these agents is commonly used in this process.

4.11.3 Butanol

Butanol is of interest as a fuel for internal combustion engines. Butanol has a higher energy density and lower vapour pressure than ethanol, which makes it more attractive as fuel or blending agent. Butanol is produced during fermentation by solvent producing bacteria (e.g. *Clostridia acetobutylicum*) in a process that is generally referred to as ABE (i.e. acetone, butanol, ethanol fermentation). Production of butanol and acetone from biomass via fermentation started during World War I, but declined in the course of the twentieth century primarily due the lower production cost of non-renewable butanol produced by the petrochemical industry.

However, with the increasing demand for renewable biofuels there is great renewed interest in fermentative production of butanol. Currently, a number of industrial facilities are producing butanol, although uniquely from starch and sugar feedstocks such as corn and molasses. Production of ABE from lignocellulosic feedstocks (i.e. cellulosic butanol) is currently at the R&D stages. One of the main advantages of cellulosic butanol fermentation is that most solvent-producing bacteria can convert both pentose sugars (a main component of lignocellulose) as well as hexose sugars to butanol.

Major challenges in further development of ABE processes at industrial scale are overcoming the low volumetric productivity of the fermentation, which requires development of new microorganisms for ABE fermentation that have a higher tolerance for the end products. In addition, a particular challenge in butanol fermentation is the efficient separation of the three end products acetone, butanol and ethanol. It is expected that with advances in cellulosic ethanol and, in particular, pre-treatment of lignocellulosic biomass, butanol production from lignocellulosic biomass will get further implemented.

4.12 Antibiotics

- Alexander Fleming discovered penicillin, the first natural antibiotic, in 1928.
- Antibiotics cannot fight viral infections.
- Fleming predicted the rise of antibiotic resistance.
- Antibiotics either kill or slow the growth of bacteria.
- Side effects can include diarrhea, an upset stomach, and nausea.

- Antibiotics are powerful medicines that fight certain infections and can save lives when used properly. They either stop bacteria from reproducing or destroy them.

- Before bacteria can multiply and cause symptoms, the immune system can typically kill them. White blood cells (WBCs) attack harmful bacteria and, even if symptoms do occur, the immune system can usually cope and fight off the infection.

- Sometimes, however, the number of harmful bacteria is excessive, and the immune system cannot fight them all. Antibiotics are useful in this scenario.

- The first antibiotic was penicillin. Penicillin-based antibiotics, such as ampicillin, amoxicillin, and penicillin G, are still available to treat a variety of infections and have been around for a long time.

- Several types of modern antibiotics are available, and they are usually only available with a prescription in most countries. Topical antibiotics are available in over-the-counter (OTC) creams and ointments.

4.12.1 Resistance

Some medical professionals have concerns that people are overusing antibiotics. They also believe that this overuse contributes toward the growing number of bacterial infections that

are becoming resistant to antibacterial medications. There are different types of antibiotic, which work in one of two ways:

- A bactericidal antibiotic, such as penicillin, kills the bacteria. These drugs usually interfere with either the formation of the bacterial cell wall or its cell contents.
- A bacteriostatic stops bacteria from multiplying.

4.12.2 Side effects

Antibiotics commonly cause the following side effects:

- Diarrhea
- Nausea
- Vomiting
- Rash
- Upset stomach
- With certain antibiotics or prolonged use, fungal infections of the mouth, digestive tract, and vagina

Less common side effects of antibiotics include:

- Formation of kidney stones, when taking sulphonamides
- Abnormal blood clotting, when taking some cephalosporins)
- Sensitivity to sunlight, when taking tetracyclines
- Blood disorders, when taking trimethoprim
- Deafness, when taking erythromycin and the aminoglycosides

Some people, especially older adults, may experience bowel inflammation, which can lead to severe, bloody diarrhea. In less common instances, penicillins, cephalosporins, and erythromycin can also cause inflamed bowels.

4.12.3 Penicillin

The term "penicillin" is defined as the natural product of *Penicillium* mould with antimicrobial activity. It was coined by Alexander Fleming on 7 March 1929 when he discovered the antibacterial property of *Penicillium rubens*. The name was "to avoid the repetition of the rather cumbersome phrase 'Mould broth filtrate,' the name 'penicillin' will be used," as Fleming explained in his 1929 paper in the *British Journal of Experimental Pathology*. The name thus refers to the scientific name of the mould, as described by Fleming

in his Nobel lecture in 1945: Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming as a crude extract of *P. rubens*. Fleming's student Cecil George Paine was the first to successfully use penicillin to treat eye infection (ophthalmia neonatorum) in 1930. The purified compound (penicillin F) was isolated in 1940 by a research team led by Howard Florey and Ernst Boris Chain at the University of Oxford. Fleming first used the purified penicillin to treat streptococcal meningitis in 1942. For the discovery, Fleming shared the 1945 Nobel Prize in Physiology or Medicine with Florey and Chain.

4.12.4 Penicillins (**P**, **PCN** or **PEN**) are a group of antibiotics originally obtained from *Penicillium* moulds, principally *P. chrysogenum* and *P. rubens*. Most penicillins in clinical use are chemically synthesised from naturally-produced penicillins. A number of natural penicillins have been discovered, but only two purified compounds are in clinical use: penicillin G (intravenous use) and penicillin V (given by mouth). Penicillins were among the first medications to be effective against many bacterial infections caused by staphylococci and streptococci. They are members of the β-lactam antibiotics. They are still widely used today for different bacterial infections, though many types of bacteria have developed resistance following extensive use.

About 10% of people report that they are allergic to penicillin; however, up to 90% of this group may not actually be allergic. Serious allergies only occur in about 0.03%. Those who are allergic to penicillin are most often given cephalosporin C (another β-lactam antibiotic) because there is only 10% crossover in allergy between the penicillins and cephalosporins.

Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming as a crude extract of *P. rubens*. Fleming's student Cecil George Paine was the first to successfully use penicillin to treat eye infection (ophthalmia neonatorum) in 1930. The purified compound (penicillin F) was isolated in 1940 by a research team led by Howard Florey and Ernst Boris Chain at the University of Oxford. Fleming first used the purified penicillin to treat streptococcal meningitis in 1942. For the discovery, Fleming shared the 1945 Nobel Prize in Physiology or Medicine with Florey and Chain.

4.12.5 Medical usage

The term "penicillin", when used by itself, may refer to either of two chemical compounds, penicillin G or penicillin V.

Common name	Chemical name	Method of administration
Penicillin V	phenoxymethylpenicillin	oral
Penicillin G	benzylpenicillin	intravenous intramuscular

4.13 Penicillin G

Penicillin G is destroyed by stomach acid, so it cannot be taken by mouth, but doses as high as 2.4 g can be given (much higher than penicillin V). It is given by intravenous or intramuscular injection. It can be formulated as an insoluble salt, and there are two such formulations in current use: procaine penicillin and benzathine benzylpenicillin, which are used only in the treatment of syphilis. When a high concentration in the blood must be maintained, penicillin G must be administered at relatively frequent intervals, because it is eliminated quite rapidly from the bloodstream by the kidney. Penicillin G is licensed for use to treat septicaemia, empyema, pneumonia, pericarditis, endocarditis and meningitis caused by susceptible strains of staphylococci and streptococci.

It is also licensed for the treatment of anthrax, actinomycosis, cervicofacial disease, thoracic and abdominal disease, clostridial infections, botulism, gas gangrene (with accompanying debridement and/or surgery as indicated), tetanus (as an adjunctive therapy to human tetanus immune globulin), diphtheria (as an adjunctive therapy to antitoxin and for the prevention of the carrier state), erysipelothrix endocarditis, fusospirochetosis (severe infections of the oropharynx, lower respiratory tract and genital area), *Listeria* infections, meningitis, endocarditis, *Pasteurella* infections including bacteraemia and meningitis, Haverhill fever; rat-bite fever and disseminated gonococcal infections, meningococcal meningitis and/or septicaemia caused by penicillin-susceptible organisms and syphilis.

4.13.1 Penicillin V

Penicillin V can be taken by mouth because it is relatively resistant to stomach acid. Doses higher than 500 mg are not fully effective because of poor absorption. It is used for the same bacterial infections as those of penicillin G and is the most widely used form of penicillin. However, it is not used for diseases, such as endocarditis, where high blood levels of penicillin are required.

4.13.2 Bacterial susceptibility

Because penicillin resistance is now so common, other antibiotics are now the preferred choice for treatments. For example, penicillin used to be the first-line treatment for infections with *Neisseria gonorrhoeae* and *Neisseria meningitidis*, but it is longer recommended for treatment of these infections.

4.13.3 Streptomycin

Streptomycin is an antibiotic medication used to treat a number of bacterial infections, including tuberculosis, Mycobacteriumavium complex, endocarditis, brucellosis, B urkholderia infection, plague, tularemia, and rat bite fever. For active tuberculosis it is often given together with isoniazid, rifampicin, and pyrazinamide. It is administered by injection into a vein or muscle. Common side effects include vertigo, vomiting, numbness of the face, fever, and rash. Use during pregnancy may result in permanent deafness in the developing baby. Use appears to be safe while breastfeeding. It is not recommended in people with myasthenia gravis or other neuromuscular disorders. Streptomycin is an aminoglycoside. It works by blocking the ability of 30S ribosomal subunits to make proteins, which results in bacterial death.

Albert Schatz first isolated streptomycin in 1943 from Streptomyces griseus. It is on the World Health Organization's List of Essential Medicines. The World Health Organization classifies it as critically important for human medicine.

4.13.4 Medication

- Infective endocarditis: An infection of the endocardium caused by enterococcus; used when the organism is not sensitive to gentamicin.
- Tuberculosis: Used in combination with other antibiotics. For active tuberculosis it is often given together with isoniazid, rifampicin, and pyrazinamide. It is not the first-line treatment, except in medically under-served populations where the cost of more expensive treatments is prohibitive. It may be useful in cases where resistance to other drugs is identified.
- Plague (Yersinia pestis): Has historically been used as the first-line treatment. However streptomycin is approved for this purpose only by the US Food and Drug Administration.

- In veterinary medicine, streptomycin is the first-line antibiotic for use against gram negative bacteria in large animals (horses, cattle, sheep, etc.). It is commonly combined with procaine penicillin for intramuscular injection.
- Tularemia infections have been treated mostly with streptomycin.

Streptomycin is traditionally given intramuscularly, and in many nations is only licensed to be administered intramuscularly, though in some regions the drug may also be administered intravenously.

4.13.4 Pesticide

Streptomycin also is used as a pesticide, to combat the growth of bacteria beyond human applications. Streptomycin controls bacterial diseases of certain fruit, vegetables, seed, and ornamental crops. A major use is in the control of fireblight on apple and pear trees. As in medical applications, extensive use can be associated with the development of resistant strains. Streptomycin could potentially be used to control cyanobacterial blooms in ornamental ponds and aquaria. While some antibacterial antibiotics are inhibitory to certain eukaryotes, this seems not to be the case for streptomycin, especially in the case of anti-fungal activity.

4.13.5 Cell culture

Streptomycin, in combination with penicillin, is used in a standard antibiotic cocktail to prevent bacterial infection in cell culture.

4.13.6 Protein purification

When purifying protein from a biological extract, streptomycin sulfate is sometimes added as a means of removing nucleic acids. Since it binds to ribosomes and precipitates out of solution, it serves as a method for removing rRNA, mRNA, and even DNA if the extract is from a prokaryote.

4.13.7 Side effects

The most concerning side effects, as with other aminoglycosides, are kidney toxicity and ear toxicity. Transient or permanent deafness may result. The vestibular portion of cranial nerve VIII (the vestibulocochlear nerve) can be affected, resulting in tinnitus, vertigo, ataxia, kidney toxicity, and can potentially interfere with diagnosis of kidney malfunction.

Common side effects include vertigo, vomiting, numbness of the face, fever, and rash. Fever and rashes may result from persistent use. Use is not recommended during pregnancy. Congenital deafness has been reported in children whose mothers received streptomycin during pregnancy. Use appears to be okay while breastfeeding. It is not recommended in people with myasthenia gravis.

4.14 Mechanism of action

Streptomycin has two mechanism of action depending on what conformation (isomer) is at in the system in which it will work. Isomer A functions as a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome irreversibly, interfering with the binding of aminoacyl-tRNA to the 30S subunit. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 30S subunit association with the mRNA strand. This results in an unstable ribosomal-mRNA complex, leading to a frameshift mutation and defective protein synthesis; leading to cell death.[15] Humans have ribosomes which are structurally different from those in bacteria, so the drug does not have this effect in human cells. At low concentrations, however, streptomycin only inhibits growth of the bacteria by inducing prokaryotic ribosomes to misread mRNA.

Streptomycin isomer B is a peptidoglycan synthesis inhibitor much like lysozyme. It binds to the glycosidic linkages and breaks them through a SN2 mechanism. This leads to bacterial cell walls' integrity being compromised, ultimately resulting in death of microbial cells. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria, and is therefore a useful broad-spectrum antibiotic. Tetracycline is an antibiotic that fights infection caused by bacteria. Tetracycline is used to treat many different bacterial infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems. It is often used in treating severe acne, or sexually transmitted diseases such as syphilis, gonorrhea, or chlamydia. Tetracycline is also used to treat infections you can get from direct contact with infected animals or contaminated food.

In some cases, tetracycline is used when penicillin or another antibiotic cannot be used to treat serious infections such as Anthrax, Listeria, Clostridium, Actinomyces, and others. Tetracycline is used to treat a wide variety of infections, including acne. It is an antibiotic that

works by stopping the growth of bacteria.This antibiotic treats only bacterial infections. It will not work for viral infections (such as common cold, flu). Using any antibiotic when it is not needed can cause it to not work for future infections. Tetracycline can also be used in combination with anti-ulcer medications to treat certain types of stomach ulcers.

4.15 How to use Tetracycline HCL

Tetracycline works best when taken on an empty stomach 1 hour before or 2 hours after meals. If stomach upset occurs, ask your doctor if you can take this medication with food. Take each dose with a full glass of water (8 ounces or 240 milliliters) unless your doctor directs you otherwise. Do not lie down for at least 10 minutes after taking this medication. For this reason, do not take it right before bedtime. Take this medication 2-3 hours before or after taking any products containing magnesium, aluminum, or calcium. Some examples include antacids, quinapril, certain forms of didanosine (chewable/dispersible buffered tablets or pediatric oral solution), vitamins/minerals, and sucralfate. Follow the same instructions with dairy products (e.g., milk, yogurt), calcium-enriched juice, bismuth subsalicylate, iron, and zinc. These products bind with tetracycline, preventing its full absorption. Dosage is based on your medical condition and response to therapy. For use in children older than 8 years of age, the dosage is also based on weight. For the best effect, take this antibiotic at evenly spaced times. To help you remember, take this medication at the same time(s) every day.

4.15.1 Mechanism of action

Tetracycline inhibits protein synthesis by blocking the attachment of charged aminoacyl-tRNA at the P site peptide chain. Tetracycline blocks the P-site so that a hydrogen bond is not formed between the amino acids. Tetracycline binds to the 30S and 50S subunit of microbial ribosomes. Thus, it prevents the formation of a peptide chain. The action is usually not inhibitory and irreversible even with the withdrawal of the drug. Mammalian cells are less vulnerable to the effect of tetracyclines, despite the fact that tetracycline binds to the small ribosomal subunit of both prokaryotes and eukaryotes (30S and 40S, respectively). This is because bacteria actively pump tetracycline in, even against a concentration gradient, whereas mammalian cells are simply not affected by the mechanisms of tetracycline within the cytoplasm. This accounts for the relatively small off-site effect of tetracycline on human cells.

4.15.2 Mechanisms of resistance

Bacteria usually acquire resistance to tetracycline from horizontal transfer of a gene that either encodes an efflux pump or a ribosomal protection protein. Efflux pumps actively eject tetracycline from the cell, preventing the buildup of an inhibitory concentration of tetracycline in the cytoplasm. Ribosomal protection proteins interact with the ribosome and dislodge tetracycline from the ribosome, allowing for translation to continue.

4.15.3 Summary

The chemical industry currently depends almost exclusively on fossil sources such as petroleum oil. According to the finite availability of this consuetudinary resource and due to the increasing demand for energy from developing countries and the related rise in prices of oil, renewable resources must be considered as valuable alternatives. Therefore, this article is focused on alternative sources such as wood and other agricultural residues as feedstock for chemical synthesis processes. Especially, the utilization and pretreatment of the lignocellulose feedstock as well as the use of perennial grasses is described. A brief overview about the utilization of synthesis gas derived from gasified biomass is given, which can be used for fermentation processes.

The industry produces many intermediate compounds that are used as the basis for many chemical products. The chemical industry produces more than 50 000 chemicals and formulations. For example, ethylene, one of the most important bulk chemicals from an energy point of view, is used to produce products varying from solvents to plastics. Also, many processes in the chemical industry produce different coproducts. Chemical industries consume fuels and electricity as energy and feedstock. This makes energy analysis of the chemical industry more complicated compared than that of other industries. A small number of bulk chemicals are responsible for the largest part of the energy consumption in the chemical industry. These are the so-called basic chemicals that are used as building blocks for many chemicals down the production chain. The most important basic chemicals are the family of petrochemicals (ethylene, propylene, butadiene, and benzene) from the organic chemical industry as well as ammonia and chlorine/caustic soda from the inorganic chemical industry.

Terminal questions

Q. 1 Describe industrial production of chemicals**.**

Answer:----------

Q. 2 What is alcohol? Explain ethanol production sources uses & benefits.

Answer:----------

Q.3 Define fuel. Explain safety measures.

Answer:----------

Q.4 Describe citric acid and is uses.

Answer:----------

Q.5 Describe acetic acid and is uses.

Answer:----------

Q.6 Describe gluconic acid and is uses.

Answer:----------

Q.7 Define solvent. What are uses of solvent?

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

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Industrial Biotechnology

Block- III

Food technology & isolation process

Unit-5
Introduction to food technology

Unit-6
Isolation of important microorganisms

Introduction

This is the third block on food technology & isolation process**.** It consists of following two units as given below:

Unit: 5 Introduction to Food Science and Technology focuses on the importance of food science and food technology to humans. This book discusses the total sequence of operations in food technology, which includes the selection of raw materials, processing, preservation, and distribution. Comprised of nine chapters, this monograph starts with an overview of the processing and storage of food. This book examines how the food processor often controls the producer's operations by demanding a raw product of a certain type in order to satisfy a particular processing and consumer demand. Other chapters consider the primary concern of food scientists and technologists in the processing and preservation of raw agricultural products as nutritious and stable foods of acceptable quality. The final chapter deals with the variety of jobs available for those trained in the biological, physical, and behavioral sciences and their applications to food processing and food preservation. Food technologists, chemists, and scientists will find this book extremely useful.

It is a distinct field involving the application of basic sciences such as chemistry and physics, culinary arts, agronomics and microbiology. It is a broad discipline concerned with all the technical aspects of food, beginning with harvesting or slaughtering and ending with cooking and consumption. Food Scientists have to use the knowledge of biology, physical sciences and engineering to study the composition of foods, changes that occur at various stages from harvest through different processes and storage, causes of their spoilage and the principles underlying food processing. Food scientists deal with physico-chemical aspects of food, thus helping us to understand the nature and properties of food.

Unit: 6 Microorganisms occur in natural environment like soil. They are mixed with several other forms of life. Many microbes are pathogenic. They cause a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control the infectious agent. The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms. There are special techniques employed to obtain pure cultures of microorganisms. In few cases it is possible to secure pure

culture by direct isolation or direct transfer. This can be done only in those situations in which pure culture occurs naturally. Kinds of specimens taken for culturing will depend on the nature and habitat of microbes.

Different pathogens can be isolated from body tissues and fluids such as blood, urine, sputum, pus, faces, spinal fluid, bile, pleural fluids, stomach fluids etc. In the blood stream of a patient suffering with typhoid fever, the bacteria Salmonella typhosa may be present. A pure culture of this bacterium may be obtained by drawing blood sample using a sterilized hypodermic syringe and treating the blood with anticoagulant such as heparin and potassium oxalate. The presence of the anticoagulant prevents the pathogenic microbe from entrapping in fibrin clot. The sample of the blood may be inoculated into a suitable medium.

Unit-5: Introduction to food technology

Structure

Objectives

5.1 Introduction

5.2 Definition of Food Technology

5.3 Scope of Food Technology

5.4 Components of Food Technology

5.5 Elementary idea of canning

5.6 Packging

5.8 Applications

5.8.1 Foods

5.8.2 Medicine and surgery

5.8.3 Spacecraft

5.8.4 Heat Sterilization

5.8.4.1 Moist heat methods

5.8.4.1 Dry heat methods

5.9 Chemical methods

5.10 Pasteurization

5.1 Introduction

Food technology is a branch of food science that deals with the production, preservation, quality control and research and development of the food products. Early scientific research into food technology concentrated on food preservation. Nicolas Appert's development in 1810 of the canning process was a decisive event. The process wasn't called canning then and Appert did not really know the principle on which his process worked, but canning has had a major impact on food preservation techniques. Food Science can be defined as the application of the basic sciences and engineering to study the fundamental physical, chemical, and biochemical nature of foods and the principles of food processing. Food technology is the use of the information generated by food science in the selection, preservation, processing, packaging, and distribution, as it affects the consumption of safe, nutritious and wholesome food. As such, food science is a broad discipline which contains within it many specializations such as in food microbiology, food engineering, and food chemistry. Because food interacts directly with people, some food scientists are also interested in the psychology of food choice. These individuals work with the sensory properties of foods.

5.2 Definition of Food Technology

Food Technology is the application of food science to the selection, preservation, processing, packaging, distribution, and use of safe, nutritious, and wholesome food. **5.3 Scope of Food Technology**

- Food Technology developed as a discipline to systematically organize and link the various kinds of knowledge which are necessary to inform human activity in food handling, processing, distribution and marketing.
- Food Technology applies:

✓ The principles and concepts of engineering to problems of food handling and processing.

✓ Studies the interrelationships between the properties of materials and the changing methods of handling and manufacturing them.

Louis Pasteur's research on the spoilage of wine and his description of how to avoid spoilage in 1864 was an early attempt to apply scientific knowledge to food handling. Besides research into wine spoilage, Pasteur researched the production of alcohol, vinegar, wines and beer, and the souring of milk. He developed pasteurization—the process of heating milk and milk products to destroy food spoilage and disease-producing organisms. In his research into food technology, Pasteur became the pioneer into bacteriology and of modern preventive medicine.

Food Engineers deal with the conversion of raw agricultural products such as wheat into more finished food products such as flour or baked goods. Food processing contains many of the same elements as chemical and mechanical engineering. Virtually all foods are derived from living cells. Thus, foods are for the most part composed of “edible biochemicals,” and so biochemists often work with foods to understand how processing or storage might chemically affect foods and their biochemistry. Likewise, nutritionists are involved in food manufacture to ensure that foods maintain their expected nutritional content. Other food scientists work for the government in order to ensure that the foods we buy are safe, wholesome, and honestly represented.

5.4 Components of Food Technology

❖ Food analysis and chemistry

❖ Food Quality Factors and their Measurement

- Nutritive aspects of food constituents and effect of processing and handling.
- Food microbiology, mycology, and toxicology.
- Food processing and engineering Emerging trends in Food Technology Increased concern about the nutritional content of technologically derived, refined foods is expressed by both consumers and nutritionists.
- Dietary guidelines and nutrition education focus on partially replacing refined foods with whole grains, legumes, and other foods which retain their biochemical unity.
- Concern about food safety issues is very strong. Food scientists are responding to these nutritional and safety concerns in a variety of ways.
- Increased attention to food interactions and bioavailability of nutrients.
- Improved analytical and detection methods, and research and education in food safety.
- New product development, particularly in the area of reduced-fat and reduced-calorie products is predicted. New processing technologies such as high energy electric pulse processing, freeze concentration, and hydrostatic pressure processing (which are often not yet available in the U.S.) show promise.
- Biotechnology is a growing area. Impact of developments in other Technologies on Food Technology For the sake of completeness it should also be mentioned that development of food technology draws heavily on developments in other technologies, such as those in steel, tinplate, glass, aluminum, plastics, engineering, instrumentation, electronics, chemicals, and agriculture.

Objectives

This is the third block on Food technology & isolation process**.** We have following objectives. These are as under:

- To define food technology, scope and its components.
- To know applications of food technology.
- To know about pasteurization.
- To know about elementary idea of canning and packging.
- To know various types breads, cheese and idli.

5.5 Elementary idea of canning

Canning is a method of food preservation in which food is processed and sealed in an airtight container (jars like Mason jars, and steel and tin cans). Canning provides a shelf life that typically ranges from one to five years, although under specific circumstances, it can be much longer. A freeze-dried canned product, such as canned dried lentils, could last as long as 30 years in an edible state. In 1974, samples of canned food from the wreck of the *Bertrand*, a steamboat that sank in the Missouri River in 1865, were tested by the National Food Processors Association. Although appearance, smell, and vitamin content had deteriorated, there was no trace of microbial growth and the 109-year-old food was determined to be still safe to eat.

One of the most common methods for preserving foods today is to enclose them in a sterile container. The term "canning" refers to this method although the specific container can be glass, plastic, or some other material as well as a metal can, from which the procedure originally obtained its name. The basic principle behind canning is that a food is sterilized, usually by heating, and then placed within an air-tight container. In the absence of air, no new pathogens can gain access to the sterilized food.

In most canning operations, the food to be packaged is first prepared in some way—cleaned, peeled, sliced, chopped, or treated in some other way—and then placed directly into the container. The container is then placed in hot water or some other environment where its temperature is raised above the boiling point of water for some period of time. This heating process achieves two goals at once. First, it kills the vast majority of pathogens that may be present in the container. Second, it forces out most of the air above the food in the container.

After heating has been completed, the top of the container is sealed. In home canning procedures, one way of sealing the (usually glass) container is to place a layer of melted paraffin directly on top of the food. As the paraffin cools, it forms a tight solid seal on top of the food. Instead of or in addition to the paraffin seal, the container is also sealed with a metal screw top containing a rubber gasket. The first glass jar designed for this type of home canning operation, the Mason jar, was patented in 1858. The commercial packaging of foods frequently makes use of tin, **aluminum**, or other kinds of metallic cans. The technology for this kind of canning was first developed in the mid-1800s, when individual workers hand-sealed cans after foods had been cooked within them. At this stage, a single worker could seldom produce more than 100 "canisters" (from which the word "can" later came) of food a

day. With the development of far more efficient canning machines in the late nineteenth century, the mass production of canned foods became a reality.

As with home canning, the process of preserving foods in metal cans is very simple in concept. The foods are prepared and the empty cans sterilized. The prepared foods are then added to the sterile metal can, the filled can is heated to a sterilizing temperature, and the cans are then sealed by a machine. Modern machines are capable of moving a minimum of 1,000 cans per minute through the sealing operation.

5.6 Packging

Food packaging is an important part of food processing operations and food preservation. "Packaging" ensures safe product delivery to the ultimate consumer in a sound condition and at a minimum cost (Paine and Paine, 1983). In the last quarter of the twentieth century, many important developments in both materials and packaging systems led to the reduction of packaging costs and the development of novel and minimally processed foods.

Packaging serves a number of different functions including preservation, containment, and convenience. Preservation is one of its major roles: packaging protects the contents against environmental, physical, and mechanical hazards (oxygen, water/moisture, light, contamination from microorganisms, rodents, and insects, physical damage, chemical attack, etc.) during storage and distribution. Containment is another important function: packaging contains the food and keeps it secure until it is used. Packaging is also a means of providing useful information to the consumer; communication is its third important function. It provides a way of identifying the contents; attractive or eye-catching packaging helps to sell the product; and it provides a means of fulfilling any regulatory requirements concerning labeling of foods.

In addition, food packages provide convenience: they unitize or group products together in useful amounts, have features like easy opening, dispensing, resealing after use, and so on. Finally, a successful, effective food package should fulfill many other requirements. It should have good machinability (that is, it should be easily filled, closed, and processed at high speeds); it should be aesthetically pleasing, recyclable or reusable, nontoxic, tamper-resistant (or tamper-evident); it should have a functional size and shape, be disposed of easily, have low cost, and be compatible with the food it contains.

There are three levels of packaging: primary, secondary, and tertiary. A primary package is in direct contact with the product. Usually, primary packages provide the major protective barrier. A secondary package usually contains several primary packages, and provides the strength for stacking in the warehouse. Like the secondary, a tertiary package contains a number of secondary packages. Its function is to hold together the secondary packages during distribution.

Due to the large variety of food products, a great deal of packaging materials, container types, packaging systems, and techniques exist. The selection and development of a package depend on the nature of the food, the desired shelf life of the product, the storage conditions, and the cost. It is a difficult task and requires in-depth knowledge of the food product and its deterioration mechanisms, transportation hazards, market and distribution requirements, and, finally, the properties and characteristics of all available packaging materials, machines, and systems.

5.7 Sterilization

Sterilization refers to any process that removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents like prions present in a specific surface, object or fluid, for example food or biological culture media. Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic.

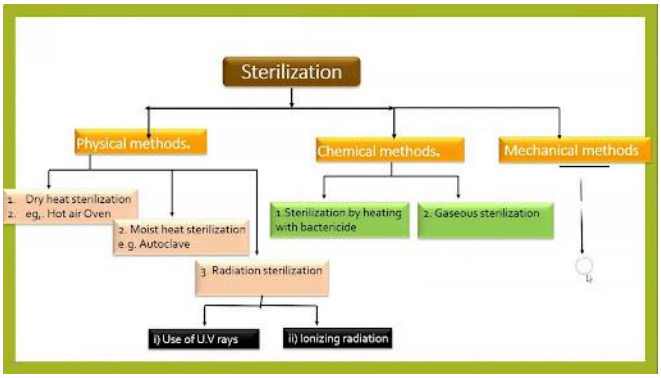

Fig. 1

Sterilization may be defined as the statistically complete destruction of all microorganisms including the most resistant bacteria and spores. It is a condition that is difficult to achieve and hard to prove. Whilst there are many chemicals, inorganic and organic, that kill microorganisms they may not be totally effective and can leave undesirable or toxic residues. Ultraviolet and Ionising radiations are also effective biocides, disrupting or modifying the DNA to prevent replication, but Ultraviolet will not produce the effective results and easy validation that moist heat (steam) sterilization can provide. If sterility is an absolute requirement then today's scientists turn, as their predecessors did, to steam.

Microorganisms tend to become more active as the temperature of their surroundings rises, - most, but not all, die at above 80^{o}C. In the case of Prions the temperature and time requirements for deactivation are much higher. Steam molecules condense on cooler microorganisms, and transfer 2500 joules per gram of steam, very efficiently heating the microorganisms to the temperature at which they are destroyed. Other methods of heating suffer from the much lower heat transfer of hot dry gases and boundary layer effects, which can insulate and protect the microorganisms. For maximum effect the Steam must be saturated, and this condition, and the temperature and pressure of the steam are easily monitored, facilitating proof that sterilization has occurred. By employing Steam Sterilization techniques a high level of sterility can be achieved and the most popular piece of equipment used in laboratories and hospitals is the steam sterilizer or autoclave.

5.8 Applications

5.8.1 Foods

One of the first steps toward modernized sterilization was made by Nicolas Appert who discovered that thorough application of heat over a suitable period slowed the decay of foods and various liquids, preserving them for safe consumption for a longer time than was typical. Canning of foods is an extension of the same principle and has helped to reduce food borne illness ("food poisoning"). Other methods of sterilizing foods include food irradiation and high pressure (pascalization). One process by which food is sterilized is heat treatment. Heat treatment ceases bacterial and enzyme activity which then leads to decreasing the chances of low quality foods while maintaining the life of non-perishable foods. One specific type of heat treatment used is UHT (Ultra-High Temperature) sterilization. This type

of heat treatment focuses on sterilization over 100 degrees Celsius. Two types of UHT sterilization are moist and dry heat sterilization. During moist heat sterilization, the temperatures that are used vary from 110 to 130 degrees Celsius. Moist heat sterilization takes between 20 and 40 minutes, the time being shorter the higher the temperature. The use of dry heat sterilization uses longer times of susceptibility that may last up to 2 hours and that use much higher temperatures compared to moist heat sterilization. These temperatures may range from 160 to 180 degrees Celsius.

5.8.2 Medicine and surgery

In general, surgical instruments and medications that enter an already aseptic part of the body (such as the bloodstream, or penetrating the skin) must be sterile. Examples of such instruments include scalpels, hypodermic needles, and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals. Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only sterility but also well-designed containers to prevent entry of adventitious agents after initial product sterilization.

Most medical and surgical devices used in healthcare facilities are made of materials that are able to go under steam sterilization. However, since 1950, there has been an increase in medical devices and instruments made of materials (e.g., plastics) that require low-temperature sterilization. Ethylene oxide gas has been used since the 1950s for heat- and moisture-sensitive medical devices. Within the past 15 years, a number of new, low-temperature sterilization systems (e.g., vaporized hydrogen peroxide, peracetic acid immersion, ozone) have been developed and are being used to sterilize medical devices. Steam sterilization is the most widely used and the most dependable. Steam sterilization is nontoxic, inexpensive, rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics.

5.8.3 Spacecraft

There are strict international rules to protect the contamination of Solar System bodies from biological material from Earth. Standards vary depending on both the type of mission and its destination; the more likely a planet is considered to be habitable, the stricter the requirements are. Many components of instruments used on spacecraft cannot withstand very high temperatures, so techniques not requiring excessive temperatures are used as tolerated,

including heating to at least 120 °C (248 °F), chemical sterilization, oxidization, ultraviolet, and irradiation. Sterilization is a process of eradicating live microorganisms from substances. It is done to preserve things for a long time and kill germs. If something is not sterilized, it may cause infection to those who use it. Therefore, it should not be taken for granted. There are several methods of sterilization, including:

5.8.4 Heat Sterilization

This is the most common type of sterilization because the heat used kills all microbes. The extent of sterilization is affected by the duration of heating and heat temperature. As the temperature goes up, the duration of heating goes up. The heat method of sterilization can be further divided into two:

5.8.4.1 Moist heat methods

Here, heat is applied through boiling and includes methods like pasteurization, using steam, and boiling. Boiling is done for metal devices such as surgical scissors, custom trays, and needles. The substances are boiled to kill any microbes. Pasteurization, on the other hand, is a method for heating milk to 60 or 72 degrees thrice or four times. When using steam, the substances being sterilized are subjected to steam in autoclave steam heating equipment. The process uses temperatures of up to 115 degrees for an hour. It is the most common method for sterilizing drugs because it can kill the bacterial spores, which are inert bacterial forms.

5.8.4.1 Dry heat methods

Substances are subjected to flaming, incineration, hot air ovens, or radiation sterilization. In flaming, metallic devices such as needles or scalpels are placed over a flame for several minutes. The flame will kill all microbes directly. Incineration is used especially for inoculating the loops utilized in microbe cultures. The loop’s metallic end is burnt red hot on a flame, killing all microbes. The radiation method involves the exposure of packed materials to radiation. There are two types of radiation: non-ionic and ionizing radiation sterilization. The former is safe to the person doing the procedure while the latter requires the operator to wear protective gear. The hot air method is ideal for dry materials such as glassware and powder. They are placed inside the racks of a hot air oven until sterilized.

5.9 Chemical methods

In this method, the items are subjected to sterilization through toxic gasses. When sterilizing heat sensitive liquids, you should use bacterial filters. Three types of filters are used in this type of sterilization:

- **Seitz filters** – they are made from materials such as asbestos and are pad-like and thicker than a membrane filter. Seitz filters do not rupture during the process of filtration. However, the solution can end up being absorbed by the filter. An alternative to Seitz filters are sintered glass filters, which are made of glass and hence cannot absorb liquids. However, they are fragile and breakable.
- **Membrane filters** – these thin filters are made of cellulose and can be used for online sterilization during injections. The membrane is placed between the needle and syringe. However, this type of filter can rupture easily causing improper sterilization.
- **Candle filters** – they are made from clay such as diatomous mud, which has small pores made by algae. The filters have many lengthy pores that trap the microbes as they travel through the candle.

The type of filter that you choose depends on the substance that you want to sterilize. When using gas to sterilize, cost factors as well as chances of explosion should be considered. The gasses used are usually very toxic and should be used with caution. If you want to sterilize surgical instruments, the most effective methods are autoclave, boiling, and incineration.

5.10 Pasteurization

Pasteurisation was invented by a French Scientist called Louis Pasteur during the nineteenth century. Pasteur discovered that heating milk to a high temperature and then quickly cooling it before bottling or packaging it could keep it fresh for longer. Today, the process of pasteurisation is widely used within the food and drink industry, and it is the most common form of heat treatment used on milk within Northern Ireland. Pasteurisation makes sure milk is safe to drink (by killing any bacteria) and also helps to prolong its shelf life. The process of pasteurisation involves heating milk to 71.7°C for at least 15 seconds (and no more than 25 seconds). Because of the nature of the heat treatment it sometimes referred to as the ‘High Temperature Short Time’ (HTST) process. Once the milk has been heated, it is then cooled very quickly to less than 3°C. The equipment which is used to heat and cool the milk is called a heat exchanger.

Pasteurization is the heat-treatment process that destroys pathogenic microorganisms in certain foods and beverages. It is named for the French scientist Louis Pasteur, who in the 1860s demonstrated that abnormal fermentation of wine and beer could be prevented by heating the beverages to about 57 °C (135 °F) for a few minutes. Pasteurization of milk, widely practiced in several countries, notably the United States, requires temperatures of about 63 °C (145 °F) maintained for 30 minutes or, alternatively, heating to a higher temperature, 72 °C (162 °F), and holding for 15 seconds (and yet higher temperatures for shorter periods of time). The times and temperatures are those determined to be necessary to destroy *Mycobacterium tuberculosis* and other, more heat-resistant, non-spore-forming, disease-causing microorganisms found in milk. The treatment also destroys most of the microorganisms that cause spoilage and so prolongs the storage time of food.

Ultra-high-temperature (UHT) pasteurization involves heating milk or cream to 138–150 °C (280–302 °F) for one or two seconds. Packaged in sterile, hermetically sealed containers, UHT milk may be stored without refrigeration for months. Ultrapasteurized milk and cream are heated to at least 138 °C for at least two seconds, but, because of less stringent packaging, they must be refrigerated. Shelf life is extended to 60–90 days. After opening, spoilage times for both UHT and ultrapasteurized products are similar to those of conventionally pasteurized products. Pasteurization of some solid foods involves a mild heat treatment, the exact definition of which depends on the food. Radiation pasteurization refers to the application of small amounts of beta or gamma rays to foods to increase their storage time.

5.11. History of Pasteurization

Pasteurization is named in honor of French chemist Louis Pasteur. In 1864, Pasteur developed a technique to heat wine to 50–60 °C (122–140 °F) before aging it to kill microbes and reduce acidity. However, the technique had been in use since at least 1117 AD in China to preserve wine. In 1768, Italian scientist Lazzaro Spallanzani demonstrated heating meat broth to boiling and immediately sealing the container kept the broth from spoiling. In 1795, French chef Nicolas Appert sealed foods in glass jars and immersed them in boiling water to preserve them (canning). In 1810, Peter Durand applied a similar method to preserve foods in tin cans. While Pasteur applied his process to wine and beer, it wasn't until 1886 that Franz von Soxhlet suggested pasteurization of milk. So, why is the process called "pasteurization," when it had been in use before Pasteur? The most likely explanation is that Pasteur's experiments demonstrated particles in the air, as opposed to pure air, caused food spoilage. Pasteur's

research pointed toward microorganisms as the culprit for spoilage and disease, ultimately leading to the Germ

5.12 Commonly Pasteurized Products

Pasteurization may be applied to both packaged and unpackaged solids and liquids. Examples of commonly pasteurized products include:

- Beer
- Canned goods
- Dairy products
- Eggs
- Fruit juices
- Milk
- Nuts
- Syrup
- Vinegar
- Water
- Wine

5.13 Pasteurization of food products

The objective of pasteurizing beers is to reduce the microbial and enzymatic activity found in the final product, thus extending its shelf life to more than 6 months (Deák, 2014). The method consists in the application of mild temperatures (approximately 60°C) during low times (a few minutes) for maintaining most of its nutritional value and organoleptic properties. Thereafter, the beer returns to ambient temperature gradually through a further cooling.Commonly, the pasteurization of bottled beers is carried out in pasteurization tunnels for assuring the microbiological control of the beverage. This kind of pasteurization uses large and inefficient equipment, which are composed of different zones with water nozzles at different temperatures. The processes depend on the conduction of heat from the surface of the vessel (can or bottle) to the liquid inside the vessel.

Recently, other technologies that provide a suitable elimination of microorganisms, such as flash pasteurization and particle retention, have been used. The flash pasteurization consists of applying slightly higher temperatures (typically 65–79°C) for a much shorter period (typically

17–19 s) to a continuous flow of liquid. This method has as advantages the speed with which the process is conducted and the low time of exposure to the temperatures, which can maintain the quality of the compounds of the beverage.Nevertheless, pasteurization has some drawbacks. According to Hyeronymus (2012), beers that have not undergone any thermal processing are much cooler, having a flavor intensity and much higher flavor than the pasteurized beers.

According to Stewart and Priest (2011), pasteurization reduces the colloidal stability of beer and breaks the link between high-molecular-weight proteins and polyphenols. Consequently, free molecules aggregate to other carbohydrate molecules and inorganic materials. Considering the aforementioned advantages and drawbacks, another technology that has been successfully applied to food processing (liquid state) is cold pasteurization through microfiltration membranes. For beers, this technology can replace the other previously mentioned because it integrates two unit operations in single equipment: filtration and pasteurization. Filtration and pasteurization of beers can avoid reducing nutritional and functional losses associated with heat treatments.

5.14 Technology of typical food/food product

Food items are being processed for various reasons. Since times immemorial, grains have been dried after harvest to increase their shelf life. Initially, foods were processed primarily to improve digestibility, palatability and to ensure a continuous supply. In India pickles, murabbas and papads are examples of preserved products made from certain vegetables/ fruits/ grains. With passage of time, improved transportation, communication and increasing industrialisation, the needs of consumers have become more diverse and there is now increasing demand for foods, ‘fresh’ and ‘organic’ foods, ‘safer and healthier’ foods and foods with adequate shelf life. Consumers expect better-quality foods with retention of nutrients, many a time having specific functional properties and taste/texture/consistency, while being shelf-stable and easy to package, store and transport. This has served as a stimulus to scientists to develop methods and techniques to process foods in a manner that the food products will meet the requirements and demands of the consumers. All of us eat readymade foods. These range from biscuits, bread, pickles/papads to foods such as ready-to-eat curries, meal items, snacks, etc. Such foods are manufactured using a variety of processes and

technologies. For some, simple traditional methods are still used while newer processes and technologies are employed to produce processed foods in bulk.

5.14.1 Bread

Bread baked food product made of flour or meal that is moistened, kneaded, and sometimes fermented. A major food since prehistoric times, it has been made in various forms using a variety of ingredients and methods throughout the world. The first bread was made in Neolithic times, nearly 12,000 years ago, probably of coarsely crushed grain mixed with water, with the resulting dough probably laid on heated stones and baked by covering with hot ashes. The Egyptians apparently discovered that allowing wheat doughs to ferment, thus forming gases, produced a light, expanded loaf, and they also developed baking ovens. Flat breads, the earliest form of bread, are still eaten, especially in much of the Middle East, Asia, and Africa. The principal grains used in such breads are corn (maize), barley, millet, and buckwheat—all lacking sufficient gluten (elastic protein) to make raised breads—and wheat and rye. Millet cakes and chapaties (crisp, whole-meal cakes) are popular types in India. Corn is used to make the small, flat cakes known as tortillas, important throughout much of Latin America; and in Brazil small cakes are made from cassava.

Although Far Eastern peoples have traditionally preferred rice, consumed as a grain, consumption of Western breads was increasing there in the latter half of the 20th century; and in Japan the bread-baking industry, using U.S. processes, expanded rapidly after World War II. Raised black bread, common in Germany, Russia, and Scandinavia, is made chiefly from rye. Lighter rye loaves, with wheat flour added, are popular in the United States. Raised wheat breads include white bread, made from finely sifted wheat flour; whole wheat bread, made from unsifted flour containing much of the outer and inner portions of the wheat kernel normally removed for white flour; gluten bread, lower in sugars because much of the starch is removed from the flour; and Vienna and French bread, long, narrow, crusty loaves. Other forms of raised breads include rolls and buns, chemically leavened quick breads, and yeast-leavened sweet goods that are rich in sugar and shortening.

Improvements in the commercial production of bread include better temperature control, handling methods, fuels, and refrigeration. Modern commercial bread making is highly mechanized. Mixing is performed by the straight-dough or sponge-dough methods or the newer continuous-mixing process. In the straight-dough method, frequently used in small

bakeries, all ingredients are mixed at one time. In the sponge-dough method, only some of the ingredients are mixed, forming a sponge that is allowed to ferment and is then mixed with the remaining ingredients to form the dough. The mixed dough is divided into appropriately sized pieces, deposited in bakery pans, and allowed to rise. The pans then pass through a travelling tray oven, baking the bread. The continuous-mixing process eliminates many individual operations.

5.15 Different Types of Bread

Fig. 2

- **Bara Brith** – Fruited bread from Wales of which there are many varieties, some made with yeast and others baking powder. Traditionally eaten sliced and buttered.
- **Barrel** – Usually made with a milk bread dough, baked in a ridged mould. Also known as a pistol.
- **Batch** – Loaf baked in a batch with others, rather than separately, wholemeal.
- **Bloomer** – Thick, long, white loaf, lightly cut across the top so that the cuts open out or ‘bloom’ to give a crisp crust. Sometimes sprinkled with poppy seeds.
- **Buttery Rowies** – Traditional Aberdeen butter yeast rolls. Shaped in a round or oval with a crisp crust and light flaky texture.
- **Cob** – Round smooth crusted loaf often topped with cracked wheat.

- **Coburg** – Round, crusty white loaf with a deeply cut cross on the top.
- **Cornish Splits** – Sweet, light yeasted buns enriched with butter and milk. Also called Devonshire splits. Often dusted with icing sugar and traditionally eaten filled with jam and clotted cream.
- **Cottage** – White loaf made from two round pieces of dough. One (smaller than the other) is secured on top of the larger piece. Often dusted with flour before baking.
- **Farmhouse** – White loaf baked in a special tin and cut lengthwise along the top, often dusted with flour.
- **Plait** – A special shape, usually plaited with three strands of white dough, sometimes enriched with eggs or milk.

5.16 More types of British breads

Fig. 3

- **Rolls** – Many different varieties, shapes and sizes ranging from crusty white rolls to soft wholemeal baps.
- **Sandwich** – Large flat-topped loaf baked in a lidded square tin.
- **Sliced wrapped** – With many different varieties including white, brown and wholemeal, the sliced wrapped loaf is a convenient bread which makes perfect toast and sandwiches.
- **Soda Bread** – Flat, round, heavy loaf usually marked into quarters and risen with baking powder, not yeast. Soda Bread comes originally from Ireland.
- **Stottie** – A flat round large bap from the North East of England. The Geordie stottie has a fluffy texture and was often traditionally eaten filled with bacon and pease pudding.

- **Tin** – Loaf baked in a rectangular open tin.

5.18 World breads

Fig. 4

5.19 Well known world breads

Baguette – Originally from France, the baguette is now sold around the world.

Bagel – Originally from Eastern Europe, the bagel is characterised by its ring shape and almost chewy texture

Brioche – Originally from France. A highly enriched French bread, noted for its high butter and egg content, commonly served as a component of French desserts.

Chapatti – A south Asian bread, usually eaten with cooked dhal (lentil soup), vegetable curry, chicken and mutton curry dishes; pieces are used to wrap around and pick up each bite of the cooked dish

Ciabatta – Originally from Italy. Loaf is somewhat elongated, broad and flattish and should be somewhat collapsed in the middle

Foccacia – Also from Italy. Often punctured with a knife to relieve surface bubbling, or dotted

Naan – From Northern India and Pakistan

Tiger bread – Originated in the Netherlands

Tortilla – A flatbread which originated in Mexico

5.20 More unusual world breads

Fig. 5

Balep Korkun – A flat, Tibetan bread made with Baking powder and fried in a frying pan.

Bazlama – A Turkish flatbread which is usually eaten fresh.

Cesnica – A Serbian soda bread.

Damper – An Australian soda bread.

Mantou – A steamed bun from China made with white flour and often slightly sweetened.

Melanpan – a Japanese bread made from enriched dough covered in a layer of cookie dough.

Pane Ticinese – This Swiss bread is distinguishable by its shape – it is composed of several small loaves or rolls made to be broken off by hand – and by the addition of oil to the dough, which makes the bread particularly soft.

Vánocka – Traditionally eaten in the Czech Republic and Slovakia, the dough is enriched with egg and milk to form a bread which is similar to a brioche.

5.21 Cheese

Cheese is nutritious food made mostly from the milk of cows but also other mammals, including sheep, goats, buffalo, reindeer, camels and yaks. Around 4000 years ago people have started to breed animals and process their milk. **Cheese** is a dairy product, derived from milk and produced in wide ranges of flavors, textures and forms by coagulation of the milk protein casein. It comprises proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. During production, the milk is usually acidified and the enzymes of rennet (or bacterial enzymes with similar activity) are added to cause the milk proteins (casein) to coagulate. The solids (curd) are separated from the liquid (whey) and

pressed into final form. Some cheeses have aromatic molds on the rind, the outer layer, or throughout. Most cheeses melt at cooking temperature.

Over a thousand types of cheese exist and are currently produced in various countries. Their styles, textures and flavors depend on the origin of the milk (including the animal's diet), whether they have been pasteurized, the butterfat content, the bacteria and mold, the processing, and how long they have been aged for. Herbs, spices, or wood smoke may be used as flavoring agents. The yellow to red color of many cheeses is produced by adding annatto. Other ingredients may be added to some cheeses, such as black pepper, garlic, chives or cranberries. A **cheesemonger**, or specialist seller of cheeses, may have expertise with selecting the cheeses, purchasing, receiving, storing and ripening them.

For a few cheeses, the milk is curdled by adding acids such as vinegar or lemon juice. Most cheeses are acidified to a lesser degree by bacteria, which turn milk sugars into lactic acid, then the addition of rennet completes the curdling. Vegetarian alternatives to rennet are available; most are produced by fermentation of the fungus *Mucor miehei*, but others have been extracted from various species of the *Cynara* thistle family. Cheesemakers near a dairy region may benefit from fresher, lower-priced milk, and lower shipping costs.

Cheese is valued for its portability, long shelf life, and high content of fat, protein, calcium, and phosphorus. Cheese is more compact and has a longer shelf life than milk, although how long a cheese will keep depends on the type of cheese. Hard cheeses, such as Parmesan, last longer than soft cheeses, such as Brie or goat's milk cheese. The long storage life of some cheeses, especially when encased in a protective rind, allows selling when markets are favorable. Vacuum packaging of block-shaped cheeses and gas-flushing of plastic bags with mixtures of carbon dioxide and nitrogen are used for storage and mass distribution of cheeses in the 21st century.

Cheese is one of the happiest accidents of food history, likely discovered when an enterprising herdsman decided to store some fresh milk in a pouch made from a sheep's stomach and later opened it to find the liquid transformed into curds of fatty goodness (along with some whey to drink). In ruminant animals like cows and sheep, the fourth stomach is home to rennet, a naturally occurring enzyme that curdles milk, separating it into solid curds and liquid whey. To make cheese, all you really need are three ingredients: milk, salt and live microbial cultures including rennet. The specific strains of microbes added to the milk play an important role in giving each variety of cheese its distinct flavor. When you mix those three ingredients

together, the rennet immediately goes to work curdling the milk. Simply drain off the whey, pack together the curds and you've got cheese!

Well, technically speaking, the process described above is how you would make a fresh cheese like cottage cheese, queso fresco or ricotta. But thanks to centuries of cheesemaking experimentation and innovation, there are dozens of varieties of different cheeses on the market, each with its own unique taste, texture and stink factor. If you've ever felt overwhelmed by the exhaustive selection of cheese at your local supermarket or cheesemonger, we're here to help. First, it's important to know that cheese can be classified in any number of different ways. These include:

- Texture (soft, semi-soft, hard)
- Flavor (mild, sharp, extra sharp)
- Age
- Preparation method (unripened, mold-ripened, bacteria-ripened)
- Type of milk used (cow, goat, sheep, buffalo)
- Color
- Country
- Region

To keep things simple, we're going to follow the lead of Marcella Wright, certified cheese professional and the popular cheese blogger behind Marcella the Cheesemonger. Wright divides cheese into eight major families (with some cheeses appearing in multiple categories) covering just about every cheese you'll find at a grocery store.

1. Fresh Cheese

Fresh cheeses are also known as "unripened" cheeses because they aren't aged at all. They are soft, spreadable cheeses with creamy textures and very mild flavors. Like other cheeses, fresh cheeses can be made from different types of milk and varying amounts of salt, which gives them distinct flavors. The texture of fresh cheese also depends on how much whey and moisture is drained from the final product, resulting in everything from soupy (cottage cheese) to crumbly (queso fresco). You can make fresh cheeses on your kitchen counter," says Wright, who makes her own cottage cheese by using lemon juice instead of rennet to curdle the milk. Some popular fresh cheeses are:

- Cottage cheese
- Queso fresco

- Cream cheese
- Mascarpone
- Ricotta
- Chevre

2. Pasta Filata

This category refers to the classic Italian stretched-curd cheese preparation made famous in Italy. Pasta Filata is Italian for "spun paste." Fresh cheese curds are steeped in a hot water bath and then stretched, spun or kneaded into different shapes. Mozzarella is arguably the most famous Pasta Filata. Other Pasta Filata cheeses like provolone are tied up and air-cured for weeks or months. Pasta Filata cheeses can also be smoked for added flavor. Examples of Pasta Filata cheeses are:

- Mozzarella
- Burrata
- Provolone
- Queso Oaxaca
- Scamorza affumicata
- Caciocavallo

5.22 Idli

Idli was invented in karnataka around 900CE. After that,A precursor of the modern idli is mentioned in several ancient Indian works. Vaddaradhane, a 920 CE Kannada language work by Shivakotiacharya mentions "iddalige", prepared only from a black gram (urad dal) batter. Chavundaraya II, the author of the earliest available Kannada encyclopaedia, Lokopakara (c. 1025 CE), describes the preparation of this food by soaking black gram in buttermilk, ground to a fine paste, and mixed with the clear water of curd and spices. The Western Chalukya king and scholar Someshwara III, reigning in the area now called Karnataka, included an idli recipe in his encyclopedia, Manasollasa (1130 CE). This Sanskrit-language work describes the food as iḍḍarikā. The food prepared using this recipe is now called uddina idli in Karnataka.

The recipe mentioned in these ancient Indian works leaves out three key aspects of the modern idli recipe: the use of rice (not just urad dal), the long fermentation of the mix, and the steaming for fluffiness. The references to the modern recipe appear in the Indian works only after 1250 CE. Food historian K. T. Achaya speculates that the modern idli recipe might have originated in present-day Indonesia, which has a long tradition of fermented food. According

to him, the cooks employed by the Hindu kings of the Indianised kingdoms might have invented the steamed idli there, and brought the recipe back to India during 800-1200 CE. Achaya mentioned an Indonesian dish called "kedli", which according to him, was like an idli. However, Janaki Lenin was unable to find any recipe for an Indonesian dish by this name. The Gujarati work Varanaka Samuchaya (1520 CE) mentions idli as idari, and also mentions its local adaption idada (a non-fermented version of dhokla). The earliest extant Tamil work to mention idli (as itali) is Maccapuranam, dated to the 17th century.

Fig. 6

Idli are a type of savoury rice cake, originating from the Indian subcontinent, popular as breakfast foods in Southern India and in Sri Lanka. The cakes are made by steaming a batter consisting of fermented black lentils (de-husked) and rice. The fermentation process breaks down the starches so that they are more readily metabolised by the body. Idli has several variations, including rava idli, which is made from semolina. Regional variants include *sanna* of Konkan. To make Idli, four parts uncooked rice (Idli rice or parboiled rice) to one part whole white lentil (black gram, *Vigna mungo*) are soaked separately for at least four hours to six hours or overnight. Optionally spices such as fenugreek seeds can be added at the time of soaking for additional flavour. Once done soaking, the lentils are ground to a fine paste and the rice is separately coarsely ground, then they are combined. Next, the mixture is left to ferment overnight during which its volume will more than double. After fermentation some of the batter may be kept as a starter culture for the next batch. The finished idli batter is put into greased moulds of an idli tray or "tree" for steaming. The

perforated molds allow the idlis to be cooked evenly. The tree holds the trays above the level of boiling water in a pot, and the pot is covered until the idlis are done (about 10–25 minutes, depending on size). A more traditional method is to use leaves instead of moulds.

There are several regional variations of Idlis made in South India and Sri Lanka. With the emigration of south Indians and Sri Lankans throughout the region and world, many variations on idli have been created in addition to the almost countless local variations. Hard-to-get ingredients and differing cooking customs have required changes in both ingredients and methods. Parboiled rice can reduce the soaking time considerably. Store-bought ground rice or cream of rice may also be used. Similarly, semolina or cream of wheat may be used for preparing rava idli (wheat idli). Dahi (yogurt) may be added to provide the sour flavor for unfermented batters. Prepackaged mixes allow for almost instant idlis.

5.23 Summary

Food Technology is a science which deals with the techniques and principles involved in processing and preserving the food substances. The application of food science helps in manufacturing safe, wholesome and nutritious food products. The study of food technology is to develop new methods and systems for keeping food products safe and resistant from natural harms such as bacteria and other micro-organisms. Food processing helps in preservation enhances the flavor and reduces the toxins in the food product which results in better distributional efficiency and easy marketing of the food product.

Advances in food science and technology have brought innovations and improvements in the analytical methods or techniques used to ensure food safety, quality, and nutrition-related research and actual applications along the food production chain. Fundamental discussions such as those on the comprehensive reviews on well-established conventional chemical, physical, and microbiological methods were included in various units; furthermore, recent advances in sensorial, bioanalytical, and instrumental analysis techniques and their applications have also been addressed. Applications and their pros and cons of these novel technologies in the analysis of the food components and additives including carbohydrates, proteins, lipids, trace elements, vitamins, toxins, contaminants, and residues have been discussed. Technologies related to the physical and chemical properties of food such as texture, rheology, and color are also important aspects for food-related research and development. Introduction to novel technologies and approaches, such as the noninvasive

and nondestructive methods such as NMR, ultrasound, biosensor and e-nose and e-tongue, is believed to provide insights into future trends in food analysis.

5.21Terminal questions

Q.1 Define food technology. Explain scope of food technology.

Answer:----------

Q.2 Explain components of food technology.

Answer:----------

Q.3 Explain elementary idea of canning.

Answer:----------

Q.4 What do you mean by pasteurization? Explain commonly pasteurized products**.**

Answer:----------

Q.5 Describe different types of breads.

Answer:----------

Q.6 Explain cheese and idli.

Answer:----------

Q.7 Explain elementary idea of packging.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-6: Isolation of important microorganisms

Structure

6.1 Introduction

6.2 Properties of enzymes

6.20 Summary

Terminal Questions

6.1 Introduction

6.2 Screening of industrially important microorganism

6.3 Primary screening:

6.4 Crowded plate technique

6.5 Enrichment isolation

6.6 Secondary screening

6.7 Giant Colony Technique

6.8 Biofuel

6.9 Types of biofuels

6.10 Economic and environmental considerations

6.11 Biofuels production

6.11 Ethanol

6.12 Biodiesel

6.1 Introduction

There are approximately 10,000 named species of microbes. It is estimated that there are between 10,000 and 100,000 more unidentified species for every identified one. Not only are there many types of bacteria, there are a lot of individual bacteria. A single spoonful of soil can have 100 million individual bacteria. A scraping of your gums can yield 1 million bacteria per cm^2 (a cm^2 is about the size of your little fingernail). The bacteria in and on our bodies makes up about 10% of our dry body weight. Most of the currently known species of bacteria have been identified using traditional microbiological techniques such as the gram stain reaction, morphology, and metabolic reactions. Bacteria rarely live alone but in communities with other bacteria. This is true both in the environment and in and on our bodies. This class focuses on the role of bacteria in disease. Isolating a single bacterium species is the first step in identifying the bacteria possibly responsible for a disease process. The first requirement for physically isolating a bacterium is that it can be cultured in the laboratory. This requires

knowledge of optimal temperature for growth, optimal oxygen requirements, and optimal nutritional needs.

There are special techniques employed to obtain pure cultures of microorganisms. In few cases it is possible to secure pure culture by direct isolation or direct transfer. This can be done only in those situations in which pure culture occurs naturally. Kinds of specimens taken for culturing will depend on the nature and habitat of microbes. Different pathogens can be isolated from body tissues and fluids such as blood, urine, sputum, pus, faces, spinal fluid, bile, pleural fluids, stomach fluids etc. In the blood stream of a patient suffering with typhoid fever, the bacteria Salmonella typhosa may be present. A pure culture of this bacterium may be obtained by drawing blood sample using a sterilized hypodermic syringe and treating the blood with anticoagulant such as heparin and potassium oxalate. The presence of the anticoagulant prevents the pathogenic microbe from entrapping in fibrin clot. The sample of the blood may be inoculated into a suitable medium.

In order to isolate a microbe from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora, or from living beings with skin flora, oral flora or gut flora, one has to separate it from the mix. Traditionally microbes have been cultured in order to identify the microbe(s) of interest based on its growth characteristics. Depending on the expected density and viability of microbes present in a liquid sample, physical methods to increase the gradient as for example serial dilution or centrifugation may be chosen. In order to isolate organisms in materials with high microbial content, such as sewage, soil or stool, serial dilutions will increase the chance of separating a mixture. In a liquid medium with few or no expected organisms, from an area that is normally sterile (such as CSF, blood inside the circulatory system) centrifugation, decanting the supernatant and using only the sediment will increase the chance to grow and isolate bacteria or the usually cell-associated viruses.

If one expects or looks for a particularly fastidious organism, the microbiological culture and isolation techniques will have to be geared towards that microbe. For example, a bacterium that dies when exposed to air, can only be isolated if the sample is carried and processed under airless or anaerobic conditions. A bacterium that dies when exposed to room temperature (thermophilic) requires a pre-warmed transport container, and a microbe that

dries and dies when carried on a cotton swab will need a viral transport medium before it can be cultured successfully.

6.2 Screening of industrially important microorganism

Success of fermentation depends upon the isolation of microorganism. The microorganisms are isolated from their natural habitats like soil, lakes, river mud or even in unusual habitats or environments such as extreme cold, high altitude, deserts, and deep sea and petroleum fields and are tested directly for the product formation and isolated or it can be genetically modified. Different types of microorganisms are isolated by different methods. Different microbes with desired activity are isolated using various culture techniques. The next step after isolation of microorganisms is the selection or screening. For the successful fermentation process, selection of microorganisms is the prime important step. Screening includes primary screening and secondary screening.

6.3 Primary screening:

The elementary steps that are performed to select the desired organisms and eliminate the undesirable organisms are termed as primary screening. Methods such as crowded plate technique, auxanography and enrichment culture technique are some of the techniques used in primary screening. For screening of antibiotic producing organisms crowded plate technique is given below.

6.4 Crowded plate technique

- Soil is serially diluted.
- The serially diluted sample is spread on the nutrient agar plates.
- The plates are incubated and the agar plate having 300 to 400 colonies are observed for antibiotic producing activity.
- The ability of a colony to exhibit antibiotic activity is indicated by the presence of a zone of inhibition surrounding the colony.
- The technique is improved by using test organism.
- The antibiotic produced by the organisms in the soil may inhibit the growth of test organism.

- The formation of inhibitory zones around certain colonies indicates their antibiotic sensitivity.
- The diameter of the zones of inhibition is measured in millimeters. Crowded plate technique is depicted in the given diagram.

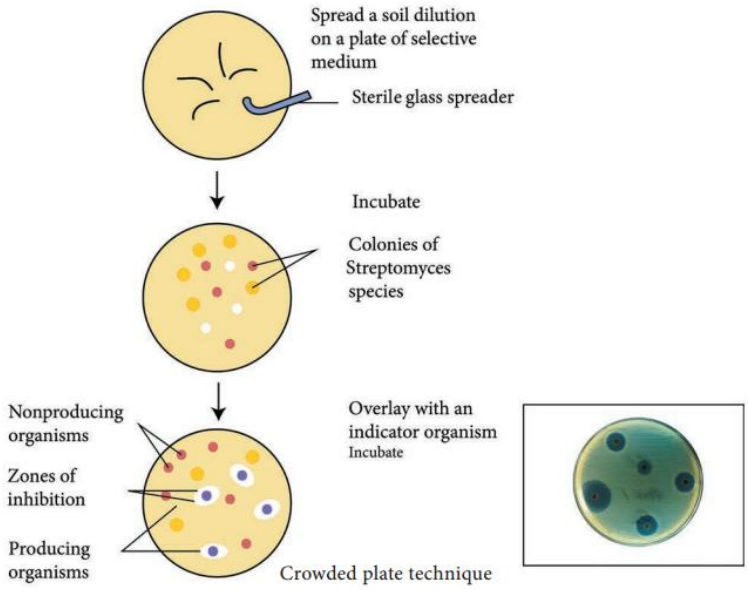

Fig. 1

6.5 Enrichment isolation

The process of enrichment provides a suitable condition to support the growth of microorganisms. It allows the growth of the specific microbe while inhibiting the other non-target microbe. The growth of target microorganisms is enriched by providing sole carbon source. For screening microorganisms degrading the compound, different inhibitors are employed which have the ability to block a specific metabolic pathway of the non-target microbe.

6.6 Secondary screening

It is very useful in sorting out microorganisms that have real commercial value from many isolates obtained during primary screening.

- ❖ As primary screening allows the detection and isolation of microorganisms which posses, potentially interesting industrial applications. It is further followed by

secondary screening, to check the capabilities and gain information about these organisms.

- Through primary screening only few or many microorganism that produce a industrially important product, are isolated. The information about the product formed is very less. So, through secondary screening, further sorting out is performed. In this method, only microorganisms with real commercial value are selected and those that lack the potential are discarded.
- Secondary screening should yield the types of information which are needed in order to evaluate the true potential of a microorganisms industrially usage.
- Secondary screening may be qualitative and quantitative in its approach.
- It is done by using paper, thin layer or other chromatographic techniques.
- The product's physical, clinical, and biological properties are determined.
- It detects gross genetic instability in microbial cultures.
- It gives information about the number of products produced in a single fermentation.
- It determines the optimum conditions for growth or accumulation of a product associated with particular culture.
- It gives information about the different components of the fermentation medium.

6.7 Giant Colony Technique

The Streptomyces culture is inoculated onto the central areas of petriplates containing a nutritious agar medium or they are streaked in a narrow band across the centre of plates. The plates are then incubated until growth and possibly, sporulation have occurred. Strains of micro organisms to be tested for possible sensitivity to the antibiotics (the test organisms) are then streaked from the edges of the plates up to but not touching the Streptomycete growth. The plates are further incubated to allow the growth of the test organism. The growth of the test organism inhibited by antibiotic in the vicinity of the Streptomycete is then measured in millimeters. These Streptomycetes that have produced antibiotics with observable microbial inhibition spectrum are retained for further testing as shown below.

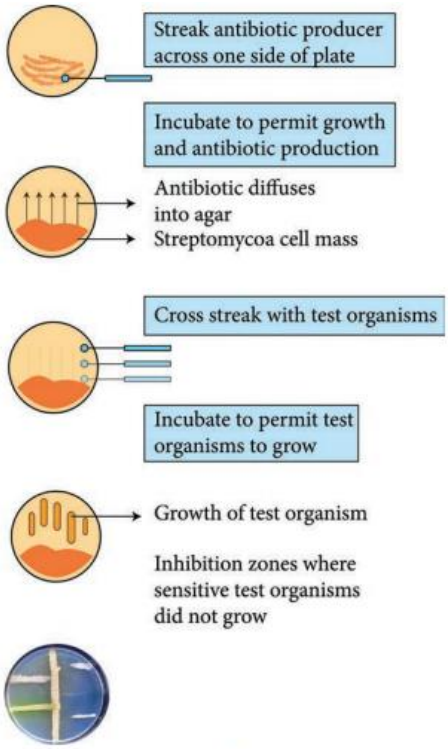

Fig. 2

The microbes used in the industrial microbiology should have following characters.

- The strain should be a high-yielding strain.
- The strain should have stable biochemical and genetical characteristics.
- It should not produce undesirable substances.
- It should be easily cultivated on large scale.

The strain should be in pure culture, free from other microorganisms including Bacteriophages. These characters are screened for the production of desirable products from microorganisms.

6.8 Biofuel

Fig. 3

Biofuel, any fuel that is derived from biomass—that is, plant or algae material or animal waste. Since such feedstock material can be replenished readily, biofuel is considered to be a source of renewable energy, unlike fossil fuels such as petroleum, coal, and natural gas. Biofuel is commonly advocated as a cost-effective and environmentally benign alternative to petroleum and other fossil fuels, particularly within the context of rising petroleum prices and increased concern over the contributions made by fossil fuels to global warming. Many critics express concerns about the scope of the expansion of certain biofuels because of the economic and environmental costs associated with the refining process and the potential removal of vast areas of arable land from food production.

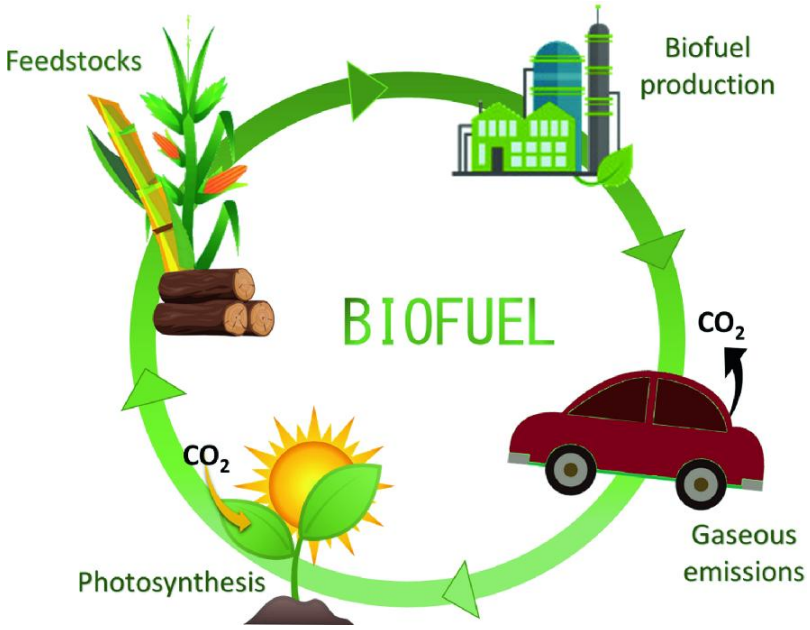

Fig. 4

6.9 Types of biofuels

Some long-exploited biofuels, such as wood, can be used directly as a raw material that is burned to produce heat. The heat, in turn, can be used to run generators in a power plant to produce electricity. A number of existing power facilities burn grass, wood, or other kinds of biomass. Liquid biofuels are of particular interest because of the vast infrastructure already in place to use them, especially for transportation. The liquid biofuel in greatest production is

ethanol (ethyl alcohol), which is made by fermenting starch or sugar. Brazil and the United States are among the leading producers of ethanol. In the United States ethanol biofuel is made primarily from corn (maize) grain, and it is typically blended with gasoline to produce "gasohol," a fuel that is 10 percent ethanol.

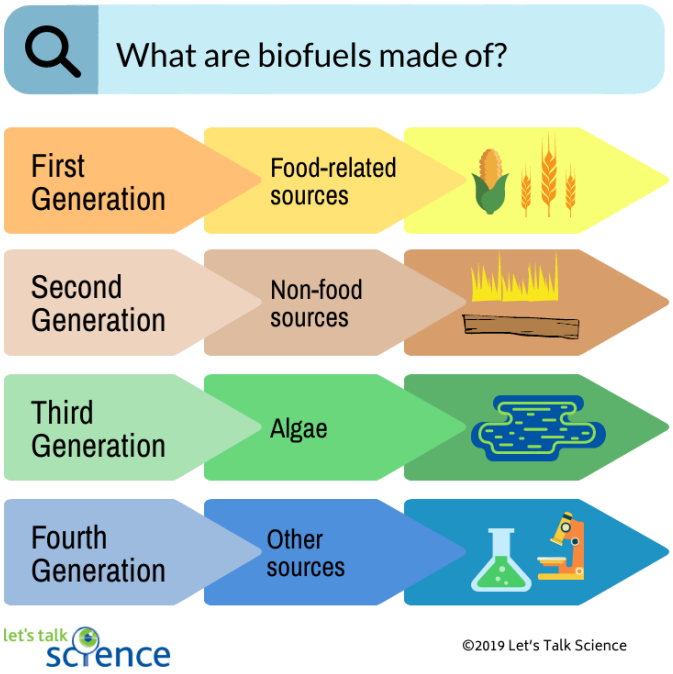

Fig. 5

In Brazil, ethanol biofuel is made primarily from sugarcane, and it is commonly used as a 100-percent-ethanol fuel or in gasoline blends containing 85 percent ethanol. Unlike the "first-generation" ethanol biofuel produced from food crops, "second-generation" cellulosic ethanol is derived from low-value biomass that possesses a high cellulose content, including wood chips, crop residues, and municipal waste. Cellulosic ethanol is commonly made from sugarcane bagasse, a waste product from sugar processing, or from various grasses that can be cultivated on low-quality land. Given that the conversion rate is lower than with first-generation biofuels, cellulosic ethanol is dominantly used as a gasoline additive.

The second most common liquid biofuel is biodiesel, which is made primarily from oily plants (such as the soybean or oil palm) and to a lesser extent from other oily sources (such as waste cooking fat from restaurant deep-frying). Biodiesel, which has found greatest acceptance in Europe, is used in diesel engines and usually blended with petroleum diesel fuel in various

percentages. The use of algae and cyanobacteria as a source of “third-generation” biodiesel holds promise but has been difficult to develop economically. Some algal species contain up to 40 percent lipids by weight, which can be converted into biodiesel or synthetic petroleum. Some estimates state that algae and cyanobacteria could yield between 10 and 100 times more fuel per unit area than second-generation biofuels. Other biofuels include methane gas and biogas—which can be derived from the decomposition of biomass in the absence of oxygen—and methanol, butanol, and dimethyl ether—which are in development.

6.10 Economic and environmental considerations

In evaluating the economic benefits of biofuels, the energy required to produce them has to be taken into account. For example, the process of growing corn to produce ethanol consumes fossil fuels in farming equipment, in fertilizer manufacturing, in corn transportation, and in ethanol distillation. In this respect, ethanol made from corn represents a relatively small energy gain; the energy gain from sugarcane is greater and that from cellulosic ethanol or algae biodiesel could be even greater.

Biofuels also supply environmental benefits but, depending on how they are manufactured, can also have serious environmental drawbacks. As a renewable energy source, plant-based biofuels in principle make little net contribution to global warming and climate change; the carbon dioxide (a major greenhouse gas) that enters the air during combustion will have been removed from the air earlier as growing plants engage in photosynthesis. Such a material is said to be “carbon neutral.” In practice, however, the industrial production of agricultural biofuels can result in additional emissions of greenhouse gases that may offset the benefits of using a renewable fuel. These emissions include carbon dioxide from the burning of fossil fuels during the production process and nitrous oxide from soil that has been treated with nitrogen fertilizer. In this regard, cellulosic biomass is considered to be more beneficial.

Land use is also a major factor in evaluating the benefits of biofuels. The use of regular feedstock, such as corn and soybeans, as a primary component of first-generation biofuels sparked the “food versus fuel” debate. In diverting arable land and feedstock from the human food chain, biofuel production can affect the economics of food price and availability. In addition, energy crops grown for biofuel can compete for the world’s natural habitats. For example, emphasis on ethanol derived from corn is shifting grasslands and brushlands to corn

monocultures, and emphasis on biodiesel is bringing down ancient tropical forests to make way for oil palm plantations. Loss of natural habitat can change the hydrology, increase erosion, and generally reduce biodiversity of wildlife areas. The clearing of land can also result in the sudden release of a large amount of carbon dioxide as the plant matter that it contains is burned or allowed to decay.

Some of the disadvantages of biofuels apply mainly to low-diversity biofuel sources—corn, soybeans, sugarcane, oil palms—which are traditional agricultural crops. One alternative involves the use of highly diverse mixtures of species, with the North American tallgrass prairie as a specific example. Converting degraded agricultural land that is out of production to such high-diversity biofuel sources could increase wildlife area, reduce erosion, cleanse waterborne pollutants, store carbon dioxide from the air as carbon compounds in the soil, and ultimately restore fertility to degraded lands. Such biofuels could be burned directly to generate electricity or converted to liquid fuels as technologies develop.

The proper way to grow biofuels to serve all needs simultaneously will continue to be a matter of much experimentation and debate, but the fast growth in biofuel production will likely continue. In the United States the Energy Independence and Security Act of 2007 mandated the use of 136 billion litres (36 billion gallons) of biofuels annually by 2022, more than a sixfold increase over 2006 production levels. The legislation also requires, with certain stipulations, that 79 billion litres of the total amount be biofuels other than corn-derived ethanol, and it continued certain government subsidies and tax incentives for biofuel production.

6.11 Biofuels production

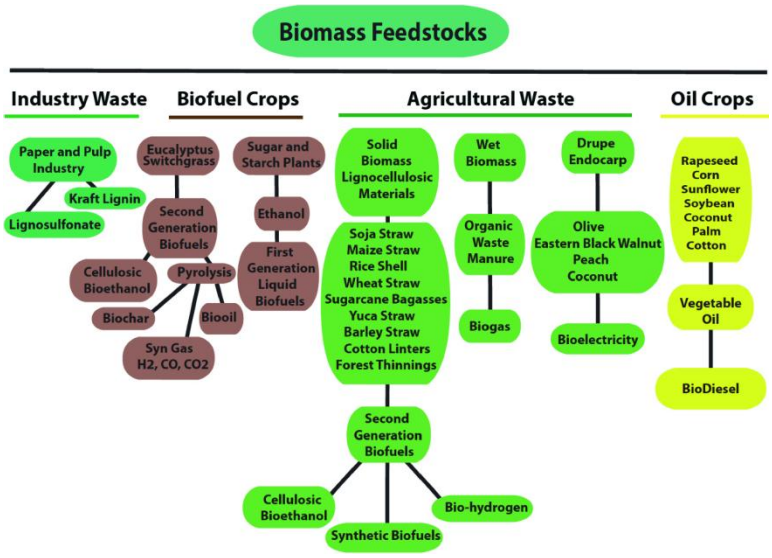

Fig. 6

6.11 Ethanol

Ethanol (CH_3CH_2OH) is a renewable fuel that can be made from various plant materials, collectively known as "biomass." Ethanol is an alcohol used as a blending agent with gasoline to increase octane and cut down carbon monoxide and other smog-causing emissions. The most common blend of ethanol is E10 (10% ethanol, 90% gasoline). Some vehicles, called flexible fuel vehicles, are designed to run on E85 (a gasoline-ethanol blend containing 51%–83% ethanol, depending on geography and season), an alternative fuel with much higher ethanol content than regular gasoline. Roughly 97% of gasoline in the United States contains some ethanol.

Most ethanol is made from plant starches and sugars, but scientists are continuing to develop technologies that would allow for the use of cellulose and hemicellulose, the non-edible fibrous material that constitutes the bulk of plant matter. In fact, several commercial-scale cellulosic ethanol biorefineries are currently operational in the United States. The common method for converting biomass into ethanol is called fermentation. During fermentation, microorganisms (e.g., bacteria and yeast) metabolize plant sugars and produce ethanol.

6.12 Biodiesel

Biodiesel is a liquid fuel produced from renewable sources, such as new and used vegetable oils and animal fats and is a cleaner-burning replacement for petroleum-based diesel fuel. Biodiesel is nontoxic and biodegradable and is produced by combining alcohol with vegetable

oil, animal fat, or recycled cooking grease. Like petroleum-derived diesel, biodiesel is used to fuel compression-ignition (diesel) engines. Biodiesel can be blended with petroleum diesel in any percentage, including B100 (pure biodiesel) and, the most common blend, B20 (a blend containing 20% biodiesel and 80% petroleum diesel).

6.13 Renewable able hydrocarbon “Drop-in fuels

Petroleum fuels, such as gasoline, diesel, and jet fuel, contain a complex mixture of hydrocarbons (molecules of hydrogen and carbon), which are burned to produce energy. Hydrocarbons can also be produced from biomass sources through a variety of biological and thermochemical processes. Biomass-based renewable hydrocarbon fuels are nearly identical to the petroleum-based fuels they are designed to replace—so they're compatible with today's engines, pumps, and other infrastructure. Currently one commercial scale facility (World Energy in Paramount, California) is producing renewable diesel from waste fats, oils, and greases. Several companies are interested in either retrofitting existing brown-field sites or building green-field facilities for renewable diesel and jet in the US.

6.14 Biofuels conversion process

6.14.1 Deconstruction

Producing advanced biofuels (e.g., cellulosic ethanol and renewable hydrocarbon fuels) typically involves a multistep process. First, the tough rigid structure of the plant cell wall—which includes the biological molecules cellulose, hemicellulose, and lignin bound tightly together—must be broken down. This can be accomplished in one of two ways: high temperature deconstruction or low temperature deconstruction.

6.14.2 High-Temperature Deconstruction

High-temperature deconstruction makes use of extreme heat and pressure to break down solid biomass into liquid or gaseous intermediates. There are three primary routes used in this pathway:

- Pyrolysis
- Gasification
- Hydrothermal liquefaction.

During pyrolysis, biomass is heated rapidly at high temperatures (500°C–700°C) in an oxygen-free environment. The heat breaks down biomass into pyrolysis vapor, gas, and char.

Once the char is removed, the vapors are cooled and condensed into a liquid “bio-crude” oil. Gasification follows a slightly similar process; however, biomass is exposed to a higher temperature range (>700°C) with some oxygen present to produce synthesis gas (or syngas)—a mixture that consists mostly of carbon monoxide and hydrogen. When working with wet feedstocks like algae, hydrothermal liquefaction is the preferred thermal process. This process uses water under moderate temperatures (200°C–350°C) and elevated pressures to convert biomass into liquid bio-crude oil.

6.14.3Low-Temperature Deconstruction

Low-temperature deconstruction typically makes use of biological catalysts called enzymes or chemicals to breakdown feedstocks into intermediates. First, biomass undergoes a pretreatment step that opens up the physical structure of plant and algae cell walls, making sugar polymers like cellulose and hemicellulose more accessible. These polymers are then broken down enzymatically or chemically into simple sugar building blocks during a process known as hydrolysis.

6.14.4 Upgrading

Following deconstruction, intermediates such as crude bio-oils, syngas, sugars, and other chemical building blocks must be upgraded to produce a finished product. This step can involve either biological or chemical processing. Microorganisms, such as bacteria, yeast, and cyanobacteria, can ferment sugar or gaseous intermediates into fuel blendstocks and chemicals. Alternatively, sugars and other intermediate streams, such as bio-oil and syngas, may be processed using a catalyst to remove any unwanted or reactive compounds in order to improve storage and handling properties. The finished products from upgrading may be fuels or bioproducts ready to sell into the commercial market or stabilized intermediates suitable for finishing in a petroleum refinery or chemical manufacturing plant.

6.15 First-generation biofuels

First-generation biofuels are fuels made from food crops grown on arable land. The crop's sugar, starch, or oil content is converted into biodiesel or ethanol, using transesterification, or yeast fermentation.

6.16 Second-generation biofuels

Second-generation biofuels are fuels made from lignocellulosic or woody biomass, or agricultural residues/waste. The feedstock used to make the fuels either grow on arable land but are byproducts of the main crop, or they are grown on marginal land. Second-generation feedstocks include straw, bagasse, perennial grasses, jatropha, waste vegetable oil, municipal solid waste and so forth.

6.17 Third-generation biofuels

Fig. 7 Biofuel production from microalgae

Microalgae are cultivated by different methods e.g. photoautotrophic, heterotrophic, photoheterotrophic and mixotrophic, then harvested by the bulking method in which microalgae are isolated from suspension through floatation, flocculation or gravity sedimentation. Thickening is the second stage used to concentrate the algal slurry after bulking process.

Algae can be produced in ponds or tanks on land, and out at sea. Algal fuels have high yields, can be grown with minimal impact on fresh water resources, can be produced using saline water and wastewater, have a high ignition point, and are biodegradable and relatively harmless to the environment if spilled. Production requires large amounts of energy and fertilizer, the produced fuel degrades faster than other biofuels, and it does not flow well in cold temperatures. By 2017, due to economic considerations, most efforts to produce fuel from algae have been abandoned or changed to other applications.

6.18 Fourth-generation biofuels

This class of biofuels includes electrofuels and solar fuels. Electrofuels are made by storing electrical energy in the chemical bonds of liquids and gases. The primary targets are butanol, biodiesel, and hydrogen, but include other alcohols and carbon-containing gases such as methane and butane. A solar fuel is a synthetic chemical fuel produced from solar energy. Light is converted to chemical energy, typically by reducing protons to hydrogen, or carbon dioxide to organic compounds.

6.19 Biofuels and the environment

6.20 Carbon neutrality

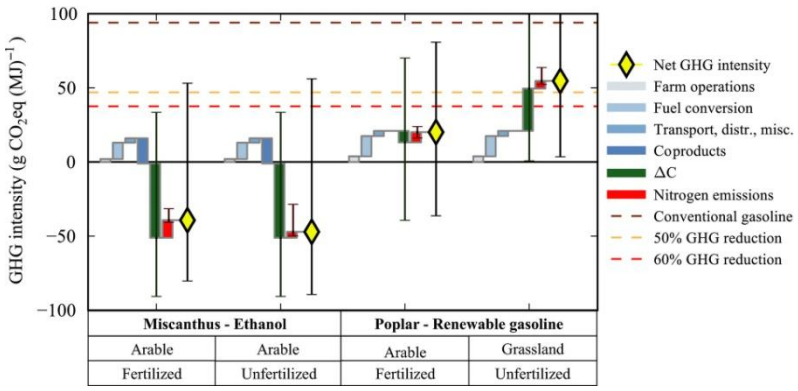

Fig. 8 Carbon negative (miscanthus) and carbon positive (poplar) production pathways.

Relationship between above-ground yield (diagonal lines), soil organic carbon (X axis), and soil's potential for successful/unsuccessful carbon sequestration (Y axis). Basically, the higher the yield, the more land is usable as a GHG mitigation tool (including relatively carbon rich land.) A biofuel project is said to be carbon-neutral if the CO_2 absorbed by the crop compensate for the greenhouse gas (GHG) emissions related to the project. CO_2 is the most important of the greenhouse gases, and there is approximately 27% carbon in CO_2 (12/44). This includes any emissions caused by direct or indirect land use change. Many first generation biofuel projects are not carbon neutral given this definition. Some have even higher emissions than some fossil based alternatives.

It is the total amount of absorption and emissions that together determines if the GHG life cycle cost of a biofuel project is positive, neutral or negative. If emissions during production,

processing, transport and combustion are higher than what is absorbed, both above and below ground during crop growth, the GHG life cycle cost is positive. Likewise, if total absorption is higher than total emissions, the life cycle cost is negative.

Whitaker et al. argue that a miscanthus crop with a yield of 10 tonnes per hectare per year sequesters so much carbon that the crop more than compensates for both farm operations emissions and transport emissions. (The emissions originating from *combustion* are fully absorbed by next seasons' above-ground plant growth.) The top chart on the right displays two CO_2 negative miscanthus production pathways, and two CO_2 positive poplar production pathways, represented in gram CO_2-equivalents per megajoule. The bars are sequential and move up and down as atmospheric CO_2 is estimated to increase and decrease. The grey/blue bars represent agriculture, processing and transport related emissions, the green bars represents soil carbon change, and the yellow diamonds represent total final emissions.

Successful sequestration is dependent on planting sites, as the best soils for sequestration are those that are currently low in carbon. The varied results displayed in the graph highlights this fact. For the UK, successful sequestration is expected for arable land over most of England and Wales, with unsuccessful sequestration expected in parts of Scotland, due to already carbon rich soils (existing woodland) plus lower yields. Soils already rich in carbon includes peatland and mature forest. Grassland can also be carbon rich, however Milner et al. argue that the most successful carbon sequestration in the UK takes place below improved grasslands. The bottom chart displays the estimated yield necessary to achieve CO_2 negativity for different levels of existing soil carbon saturation. The higher the yield, the more likely CO_2 negativity becomes.

6.21 Air pollution

In general, substance or energy is considered pollution when released into the environment at a rate faster than the environment can disperse, dilute, decompose, recycle, or store it in some harmless form. Based on this definition, both fossil fuels and some traditional biofuels are polluting the environment. For instance, the IPCC argues that the traditional use of wood in cook stoves and open fires produces pollutants, which can lead to severe health and environmental consequences. However, a shift to modern bioenergy contribute to improved livelihoods and can reduce land degradation and impacts on ecosystem services. According to

the IPCC, there is strong evidence that modern bioenergy has "large positive impacts" on air quality. When combusted in industrial facilities, most of the pollutants originating from woody biomass reduce by 97-99%, compared to open burning. A study of the giant brown haze that periodically covers large areas in South Asia determined that two thirds of it had been principally produced by residential cooking and agricultural burning, and one third by fossil-fuel burning.

6.22 Power production compared to other renewables

To calculate land use requirements for different kinds of power production, it is essential to know the relevant area-specific power densities. Smil estimates that the average area-specific power densities for biofuels, wind, hydro and solar power production are 0.30 W/m^2, 1 W/m^2, 3 W/m^2 and 5 W/m^2, respectively (power in the form of heat for biofuels, and electricity for wind, hydro and solar). The average human power consumption on ice-free land is 0.125 W/m^2 (heat and electricity combined), although rising to 20 W/m^2 in urban and industrial areas. The reason for the low area-specific power density for biofuels is a combination of low yields and only partial utilization of the plant when making liquid fuels (for instance, ethanol is typically made from sugarcane's sugar content or corn's starch content, while biodiesel is often made from rapeseed and soybean's oil content.

6.23 Petroleum microbiology

Petroleum microbiology is a branch of microbiology that deals with the study of microorganisms that can metabolize or alter crude or refined petroleum products. These microorganisms, also called hydrocarbonoclastic microorganisms, can degrade hydrocarbons and, include a wide distribution of bacteria, methanogenic archaea, and some fungi. Not all hydrocarbonoclasic microbes depend on hydrocarbons to survive, but instead may use petroleum products as alternative carbon and energy sources. Interest in this field is growing due to the increasing role of bioremediation in oil spill cleanup.

Recent advances in molecular biology have extended our understanding of the metabolic processes related to microbial transformation of petroleum hydrocarbons. The physiological responses of microorganisms to the presence of hydrocarbons, including cell surface alterations and adaptive mechanisms for uptake and efflux of these substrates, have been

characterized. New molecular techniques have enhanced our ability to investigate the dynamics of microbial communities in petroleum-impacted ecosystems. By establishing conditions which maximize rates and extents of microbial growth, hydrocarbon access, and transformation, highly accelerated and bioreactor-based petroleum waste degradation processes have been implemented. Biofilters capable of removing and biodegrading volatile petroleum contaminants in air streams with short substrate-microbe contact times (<60 s) are being used effectively. Microbes are being injected into partially spent petroleum reservoirs to enhance oil recovery.

However, these microbial processes have not exhibited consistent and effective performance, primarily because of our inability to control conditions in the subsurface environment. Microbes may be exploited to break stable oilfield emulsions to produce pipeline quality oil. There is interest in replacing physical oil desulfurization processes with biodesulfurization methods through promotion of selective sulfur removal without degradation of associated carbon moieties. However, since microbes require an environment containing some water, a two-phase oil-water system must be established to optimize contact between the microbes and the hydrocarbon, and such an emulsion is not easily created with viscous crude oil. This challenge may be circumvented by application of the technology to more refined gasoline and diesel substrates, where aqueous-hydrocarbon emulsions are more easily generated.

Molecular approaches are being used to broaden the substrate specificity and increase the rates and extents of desulfurization. Bacterial processes are being commercialized for removal of H(2)S and sulfoxides from petrochemical waste streams. Microbes also have potential for use in removal of nitrogen from crude oil leading to reduced nitric oxide emissions provided that technical problems similar to those experienced in biodesulfurization can be solved. Enzymes are being exploited to produce added-value products from petroleum substrates, and bacterial biosensors are being used to analyze petroleum-contaminated environments.

Petroleum is a complex mixture of hydrocarbons and other organic compounds, including some organometallo constituents, most notably complexing vanadium and nickel. Petroleum recovered from different reservoirs varies widely in compositional and physical properties. Long recognized as substrates supporting microbial growth, these hydrocarbons are both a target and a product of microbial metabolism. Biodegradation by microorganisms modifies waxy crude oils in beneficial ways, but conditions for down-hole applications require the use

of thermophiles, resistant to organic solvents, with heat-stable enzymes and reduced oxygen requirements.

A wide range of studies have dealt with biotransformation, biodegradation, and bioremediation of petroleum hydrocarbons, and interest in exploiting petroleum-degrading organisms for environmental clean-up has become central to petroleum microbiology. A common theme of early reviews focused on the examination of factors, including nutrients, physical state of the oil, oxygen, temperature, salinity, and pressure, influencing petroleum biodegradation rates, with a view to developing environmental applications. Metabolic studies were implemented on the aerobic pathways for alkane, cycloalkane, and aromatic and polycyclic aromatic hydrocarbon (PAH) biodegradation, for transformations of nitrogen and sulfur compounds, and, more recently, the microbial mechanisms of anaerobic hydrocarbon catabolism.

Most significantly, through the developments and applications of molecular techniques, our understanding of the processes of hydrocarbon catabolism has advanced substantially, and many novel catalytic mechanisms have been characterized. A molecular approach is also contributing to a more detailed characterization of bacterial membrane structure. We are learning a great deal about cellular and other physiological adaptations to the presence of hydrocarbons, as well as the biochemical mechanisms involved in hydrocarbon accession and uptake. The use of genetically engineered microbes for bioremediation has also been considered.

The vast range of substrates and metabolites present in hydrocarbon-impacted soils surely provides an environment for the development of a quite complex microbial community. Culture-based methods and culture-independent methods are being developed and implemented to improve our understanding of these microbial communities. Isolating and identifying microorganisms responsible for hydrocarbon transformations have long been recognized as important from a fundamental and applied viewpoint, and lists of hydrocarbon-degrading organisms (bacteria, yeasts, fungi, and algae) are available. Leahy and Colwell discussed colony hybridization and dot blot assays in their review and cited molecular tools as revolutionary for describing microbial communities. Magot et al. recently reviewed the current state of knowledge of microorganisms from petroleum reservoirs, including

mesophilic and thermophilic sulfate-reducing bacteria, methanogens, mesophilic and thermophilic fermentative bacteria, and iron-reducing bacteria.

6.24 Summary

There are approximately 10,000 named species of microbes. It is estimated that there are between 10,000 and 100,000 more unidentified species for every identified one. Not only are there many types of bacteria, there are a lot of individual bacteria. A single spoonful of soil can have 100 million individual bacteria. A scraping of your gums can yield 1 million bacteria per cm^2 (a cm^2 is about the size of your little fingernail). The bacteria in and on our bodies makes up about 10% of our dry body weight. Most of the currently known species of bacteria have been identified using traditional microbiological techniques such as the gram stain reaction, morphology, and metabolic reactions. Bacteria rarely live alone but in communities with other bacteria. This is true both in the environment and in and on our bodies.

This class focuses on the role of bacteria in disease. Isolating a single bacterium species is the first step in identifying the bacteria possibly responsible for a disease process. Microorganisms occur in natural environment like soil. They are mixed with several other forms of life. Many microbes are pathogenic. They cause a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control the infectious agent.

The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms.

6.24 Terminal questions

Q.1. Describe screening of industrially important microorganism.

Answer:----------

Q.2. Define biofuel. Explain their applications.

Answer:----------

Q.3. Describe biofuels and their types.

Answer:----------

Q.4. Describe biodiesel production.

Answer:----------

Q.5. Describe ethanol production.

Answer:----------

Q. 6 Describe different generations of biofuels.

Answer:----------

Q. 7 Explain carbon neutrality**.**

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.

4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.