WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 3, 260-299. Review Article ISSN 2277– 7105

FERMENTATION TECHNOLOGY

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Article Received on
23 Dec. 2020,

Revised on 13 Jan. 2021,
Accepted on 03 Feb. 2021

DOI: https://doi.org/10.17605/OSF.IO/2JNWG

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INTRODUCTION

1. Fermentation

Definition

Basically fermentation in strict sense is a biological process that occurs under anaerobic condition. Where according to modern biotechnology it chemical change that is brought about in a substance by the action of an enzyme or specially cultivated microorganism to form desired day to day life products to customize pharmaceutical products.

Fermentation is the process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen.

Fermentation results in the production of energy in the form of two ATP molecules, and produces less energy than the aerobic process of cellular respiration. The other end products of fermentation differ depending on the organism. In many bacteria, fungi, protests, and animals cells, fermentation produces lactic acid and lactate, carbon dioxide, and water. In yeast and most plant cells, fermentation produces ethyl alcohol, carbon dioxide, and water.

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound, as opposed to Respiration where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption. Sugars are the most common substrate of fermentation, and typical examples of fermentation products are

ethanol, lactic acid, and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone.

Yeast carries out fermentation in the production of ethanol in beers, wines and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

Fermentation may refer to

Fermentation (biochemistry), is the process of energy production in a cell under anaerobic conditions (without oxygen) eethanol fermentation, a form of anaerobic respiration used primarily by yeasts when oxygen is not present in sufficient quantity for normal cellular respiration

Fermentative hydrogen production

Industrial fermentation, and the breakdown and re-assembly of biochemical for industry, often in aerobic growth conditions.

In food science, fermentation may refer to

Fermentation (food), the conversion of carbohydrates into alcohols or acids under anaerobic conditions used for making certain foods. Fermentation (wine), the process of fermentation commonly used in winemaking. Fermentation (beer), the process of fermentation commonly used in brewing beer. Fermentation (tea), the term used in the tea industry for the aerobic treatment of tea leaves to break down certain unwanted chemicals and modify others to develop the flavor of the tea.

Energy source in anaerobic conditions

Fermentation products contain chemical energy but are considered waste products, since they cannot be metabolized further without the use of oxygen.

A consequence is that the production of adenosine tri-phosphate (ATP) by fermentation is less efficient than oxidative phosphorylation, whereby pyruvate is fully oxidized to carbon dioxide.

Fermentation (performed by yeast and some types of bacteria) breaks the Ethanol pyruvate down into ethanol and carbon dioxide. It is important in bread-making, brewing, and wine-

making. Usually only one of the products is desired; in bread-making, the alcohol is baked out, and, in alcohol production, the carbon dioxide is released into the atmosphere or used for carbonating the beverage. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced.

Lactic acid fermentation breaks down the pyruvate into lactic acid. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some kinds of bacteria (such as lactobacilli) and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste.

These lactic acid bacteria can be classed as homo-fermentative, where the end product is mostly lactate, or hetero-fermentative, where some lactate is further metabolized and results in carbon dioxide, acetate or other metabolic products.

Hydrogen gas is produced in many types of fermentation (mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glycosylate fermentation), as a way to regenerate NAD^+ from NADH. Electrons are transferred to feredoxin, which in turn is oxidized by hydrogenase, producing H_2. Hydrogen gas is a substrate for methanogens and sulfate reducers, which keep the concentration of hydrogen sufficiently low to allow the production of such an energy-rich compound.

Some industrial products of fermentation technology are listed in table 1.

Group	Products
Foods	Dairy products (cheese, yogurt) Vitamins (B1, B12) Amino acids (glutamic acid, lysine) Glucose and high Fructose syrup Mushroom products Baker's yeast Food additives (antioxidants, colors, flavors) Beverages (beer, wine, whisky)
Chemicals Organic (bulk) Organic (Fine) Inorganic	Ethanol, butanol, acetone, organic acids (citric acid, gluconic acid, lactic acid) Enzymes, polymers (xanthan, dextran) Bioaccumulation and leaching
Pharmaceuticals (Healthcare)	Antibiotics Vaccines Steroids Diagnostic enzymes Monoclonal antibodies Enzyme inhibitors

Agriculture	Single cell protein Microbial pesticides Composting processes Plant cell and tissue culture

2. OBJECTIVES

The objective of this project is to review scientific information regarding fermentation technology, and also to review different aspects and general scope of fermentation process, and its application in the production of various products.

To understand the needs and factors that influence fermentation process.

For this following systematic approach was adopted.

Types of fermentation

- Bioreactors used in fermentation.
- Fermentation process
- Media (substrate) for fermentation.
- Microorganisms used for fermentation.
- Culture systems.
- General process of fermentation.
- Measurement and control of fermentation parameters.
- Application of fermentation technology in production of various products.

2. Historical perspective

Since fruits ferment naturally, fermentation precedes human history. Since ancient times, however, humans have been controlling the fermentation process. The earliest evidence of winemaking dates from eight thousand years ago, in Georgia, in the Caucasus area Seven-thousand-year-old jars containing the remains of wine have been excavated in the Zagros Mountains in Iran, which are now on display at the University of Pennsylvania.

The word fermentation originates from a Latin verb fever which literally means to boil. There is strong evidence that people were fermenting beverages in Babylon circa 5000 BC ancient Egypt circa 3150 BC Hispanic Mexico circa 2000 BC and Sudan circa 1500 BC.

There is also evidence of leavened bread in ancient Egypt circa 1500 BC and of milk fermentation in Babylon circa 3000 BC. French chemist Louis Pasteur was the first known

zymologist, when in 1854 he connected yeast to fermentation. Pasteur originally defined fermentation as "respiration without air".

Contributions to biochemistry

When studying the fermentation of sugar to alcohol by yeast, Louis Pasteur concluded that the fermentation was catalyzed by a vital force, called "ferments," within the yeast cells. The "ferments" were thought to function only within living organisms. "Alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells".

Nevertheless, it was known that yeast extracts ferment sugar even in the absence of living yeast cells. While studying this process in 1897, Eduard Buchner of Humboldt University of Berlin, Germany, found that sugar was fermented even when there were no living yeast cells in the mixture, by a yeast secretion that he termed *zymase*. In 1907 he received the Nobel Prize in Chemistry for his research and discovery of "cell-free fermentation."

4. Uses of fermentation technology

The primary benefit of fermentation is the conversion of sugars and other carbohydrates, e.g., converting juice into wine, grains into beer, carbohydrates into carbon dioxide to leaven bread, and sugars in vegetables into preservative organic acids.

Food fermentation has been said to serve five main purposes

- Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates.
- Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations.
- Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins.
- Elimination of anti-nutrients.
- A decrease in cooking times and fuel requirements.

5. Types of fermentation process

There are various types of fermentation process based on various criteria's. A brief account on various types of fermentation process is given below.

Based on the types of process

- Batch Fermentation Process
- Continuous Fermentation Process

Based on oxygen demand:-

- Aerobic Fermentation
- Anaerobic Fermentations

Based on culture method:-

- Surface Culture Method
- Submerged Culture Method
- Semisolid or Solid State Method

Based on types of products form:-

- Gluconic Acid Fermentation
- Citric Acid Fermentation
- Acetone Butanol Fermentation
- Gibberellic Acid Production
- Lactic Acid Fermentation

Batch fermentation process

A tank of fermenter is filled with the prepared mash of raw materials to be fermented. The temperature and pH for microbial fermentation is properly adjusted, and occasionally nutritive supplements are added to the prepared mash. The mash is steam sterilized in a pure culture process. The inoculum of a pure culture is added to the fermenter, from a separate pure culture vessel. Fermentation proceeds, and after the proper time the contents of the fermenter, are taken out for further processing. The fermenter is cleaned and the process is repeated. Thus each fermentation is a discontinuous process divided into batches.

Fermentation process

Growth of microorganisms during batch fermentation confirms to the characteristic growth curve, with a lag phase followed by a logarithmic phase. This, in turn, is terminated by progressive decrements I in the rate of growth until the stationary phase is reached. This is because of limitation of one or more of the essential nutrients. Growth of microorganisms during batch fermentation confirms to the characteristic growth curve, with a lag phase followed by a logarithmic phase.

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Aerobic fermentation process

A number of industrial processes, although called 'fermentations', are carried on by microorganisms under aerobic conditions. In older aerobic processes it was necessary to furnish a large surface area by exposing fermentation media to air. In modern fermentation processes aerobic conditions are maintained in a closed fermenter with submerged cultures. The contents of the fermenter are agitated with an impeller and aerated by forcing sterilized air.

Anaerobic fermentation process

Basically a fermenter designed to operate under micro acrophilic or anaerobic conditions will be the same as that designed to operate under aerobic conditions, except that arrangements for intense agitation and aeration are unnecessary. Much anaerobic fermentation does, however, require mild aeration for the initial growth phase, and sufficient agitation for mixing and maintenance of temperature.

Surface culture method

In this method the organism is allowed to grow on the surface of a liquid medium without agitation. After an appropriate incubation period the culture filtrate is separated from the cell mass and is processed to recover the desirable product. Sometimes the biomass may be reused. Examples of such fermentations are the alcohol production, the beer production and citric acid production. This method is generally time consuming and needs large, area or space.

Submerged culture method

In this process, the organism is grown in a liquid medium which is vigorously aerated and agitated in large tanks called fermenters. The fermenter could be either an open tank or a closed tank and may be a batch type or a continuous type and are generally made of non-corrosive type of metal or glass lined or of wood. In batch fermentation, the organism is grown in a known amount of culture medium for a defined period of time and then the cell mass is separated from the liquid before further processing while in the continuous culture,

the culture medium is withdrawn depending on the rate of product formation and the inflow of fresh medium.

Gluconic acid fermentations

Gluconic acid used in pharmaceutical industries is produced by the fermentation of glucose either by strains of Aspergillus niger, Penicillium *sp*., or selected bacteria. In the commercial process, a nutrient solution containing 24-38 per cent glucose.

A nitrogen source and salts, with pH4.5 is used to culture a selected strain of fungus in shallow pans or in submerged culture conditions to convert glucose into gluconic acid. The pH of the medium is controlled by the addition of a strong solution of sodium hydroxide. Fermentation is carried out at 33 or 34°C. The medium composition and fermentation conditions determine the production of acids other than gluconic acid and hence it is important to select a mold strain and the fermentation conditions that will avoid the formation of unwanted organic acid.

Citric acid fermentations

Citric acid, which is a key intermediate of the TCA cycle, is produced by fungi, yeast and bacteria as an overflow product due to a faulty operation of the citric acid cycle. The ability of fungi to produce citric acid was first discovered by Wehmer in 1893 and today all the citric acid commercially produced comes from the mold fermentation. Among the organisms used for citric acid production, *A. Niger* has been the mold of choice for several decades. A variety of carbohydrate sources such as beet molasses, cane molasses, sucrose, commercial glucose, starch hydrolysates etc., have been used for citric acid production. Among these, sucrose, cane and beet molasses have been found to be the best. For citric acid production the raw material is diluted to 20-25 per cent sugar concentration and mixed with a nitrogen source and other salts. The pH of the medium is maintained around five when molasses is used and at a lower level (pH 3.0) when sucrose is used.

Lactic acid fermentation

Lactic acid is produced from various carbohydrates such as corn starch, potato starch, molasses, and whey. When starchy materials are used, they are first hydrolyzed to simple sugars. The medium is then supplemented with a nitrogen source and calcium carbonate arid fermentation is carried out by the inoculation with homo-fermentative lactobacilli such as Lactobacillus bulgaricus or Lactobacillus delbruckli. During the fermentation the temperature

is controlled at 43-50°C depending on the organism and the medium is kept in constant agitation to keep the calcium carbonate in suspension.

After the completion of the fermentation (4-6 days), the fermented liquor is heated to 82°C and filtered. The filtrate containing calcium lactate is spray dried after treating with sodium sulfide. To obtain lactic acid, the calcium lactate is treated with sulphuric acid and the lactic acid thus obtained is further purified.

6. Fermentation process

Fermentation process is complex and long term process of formation of fermented products. Starting with all requirements like bioreactors/fermenters, culture media, microorganisms/ inoculums, substrates, followed by actual fermentation process and then formation of the products, and finally end with recovery of subsequent products.

Fermentation process include following components:

6.1. Biorectors/Fermenters

The heart of fermentation technology is fermenter. A bioreactor is basically a devise in which the organism (cells) are cultivated and motivated to form desired products. It is containment system design to give right environment for optimal growth and metabolic activity of the organism.

6.1.1. Types of bioreactors

Based on the designs of the bioreactors, they can be grouped into the following types

1. Continuous stirred tank bioreactors
2. Bubble column bioreactors
3. Airlift bioreactors
4. Fluidized bed bioreactors
5. Packed bed bioreactors
6. Photo bioreactors.

In all types of bioreactors, the ultimate aim is to ensure that all parts of the system are subjected to the same conditions.

1. Continuous stirred tank bioreactors (STRs)

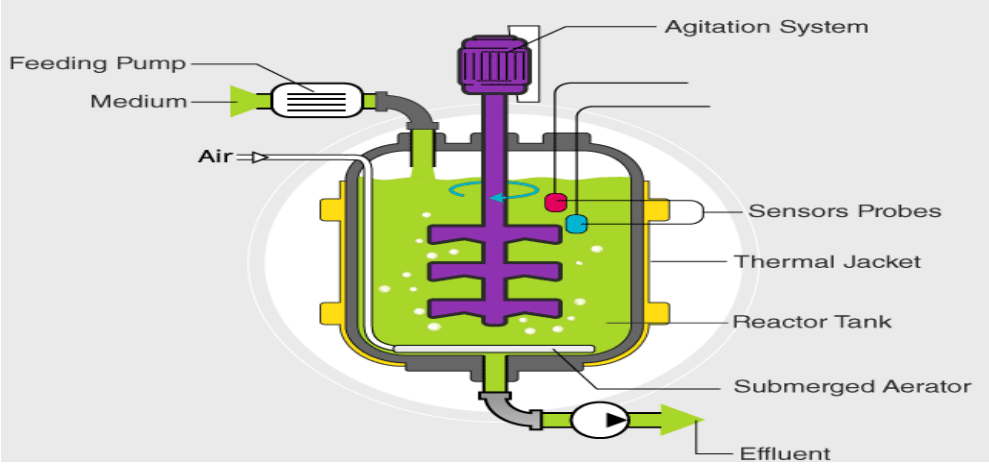


Fig. 1: Continuous stirred tank bioreactors.

A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers). The shaft is filled at the bottom of the bioreactor (Fig.1) The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio. The aspect ratio of a stirred tank bioreactors usually between 3-5. However, for animal culture applications, the aspect ratio is less than 2. The diameter of the impeller is usually 1/3rd of the vessel diameter. The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers are in use.

In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger be a ring with many holes or a tube with a single orifice.

The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel. The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium. This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

Advantages of STRs

There are many advantages of STRs over other types.

These include- The efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

2. Bubble column bioreactors

In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro porous spargers. The flow rate of the air/gas influences the performance factors 0_2 transfer, mixing. The bubble column bioreactors may be fitted with perforated plates to improve performance. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio).

3. Airlift bioreactors

In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube. In one of the two zones referred to ariser. The air/gas is pumped. The other zone that receives no gas is the down comer. The dispersion flows up the rizer zone while the down flow occurs in the down comer.

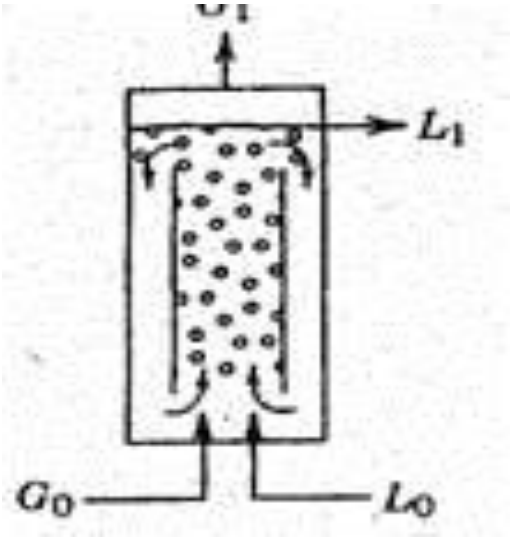


Fig. 2: Airlift bioreactor.

Airlift bioreactors are commonly employed for aerobic Bioprocessing technology. They ensure a controlled liquid flow in a recycle system by pumping.

Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

4. Tower bioreactors

A pressure-cycle fermenter with large dimensions constitutes a tower bioreactor. A high hydrostatic pressure generated at the bottom of the reactors increases the solubility of O_2 in

the medium. At the top of the riser,(with expanded top) reduces pressure and facilities expulsion of CO_2. The medium flow backs in the down comer and completes the cycle. The advantages of tower bioreactor is that it has aeration capacities without having moving parts.

5. Fluidized bed bioreactors

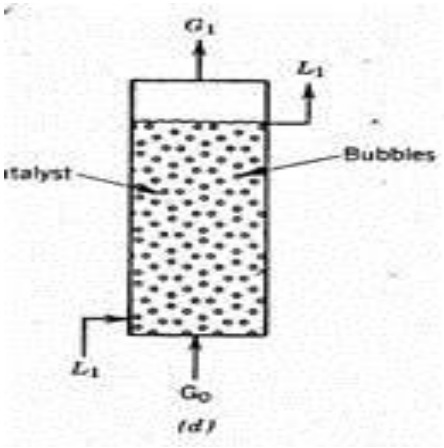


Fig. 3: Fluidized bed bioreactor.

Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out (Fig.3). These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocks.

For an efficient operation of fluidized beds, gas is sparged to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enable good efficiency of bioprocessing.

6. Packed bed bioreactors

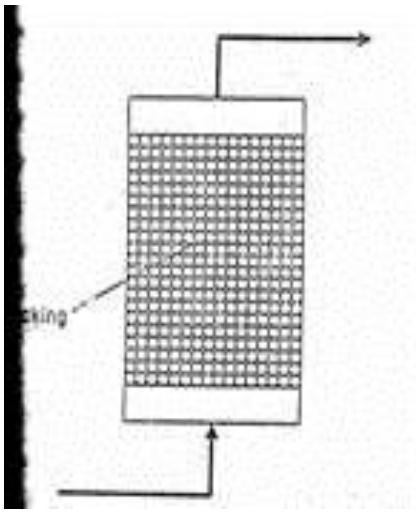


Fig. 4: Packed bed bioreactors.

A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor (fig.4). The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilized biocatalyst. The products obtained in the packed bed bioreactor are released into the fluid and removed. While the flow of the fluid can be upward or downward, down flow under gravity is preferred.

The concentration of the nutrients can be increased by increasing the flow rate of the nutrient broth. Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. However, these bioreactors are preferred for Bio processing technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

7. Photo-bioreactors

These are the bioreactors specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. Since artificial illumination is expensive, only the outdoor Photo bioreactors are preferred. Certain important compounds are produced by employing Photo bioreactors e.g., ß-carotene, asthaxanthin.

The Photobioreactors are made up of glass or more commonly transparent plastic. The array of tubes or flat panels constitutes light receiving systems (solar receivers). The culture can be

circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps.

It is essential that the cells are in continuous circulation without forming sediments. Further adequate penetration of sunlight should be maintained. The tubes should also be cooled to prevent rise in temperature.

Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25—40°C. Microalgae and cyanobacteria are normally used. The organisms grow during day light while the products are produced during night.

- **Tubular photo-bioreactor**

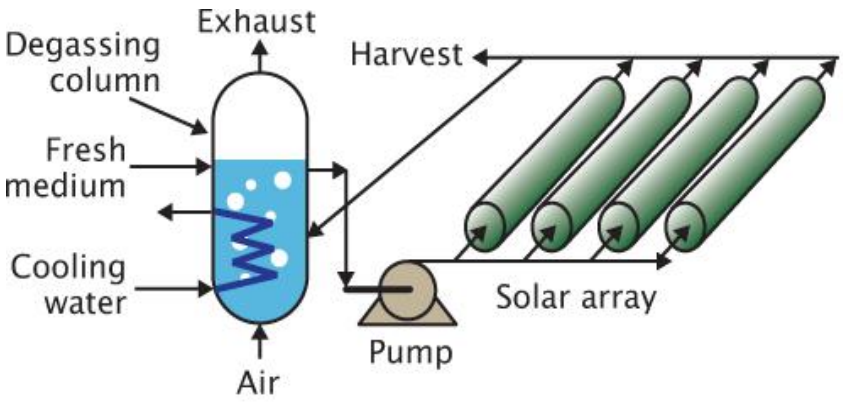


Fig. 5: Schematic diag. of tubular photo-bioreactor.

- **Enclosed photo-bioreactor**

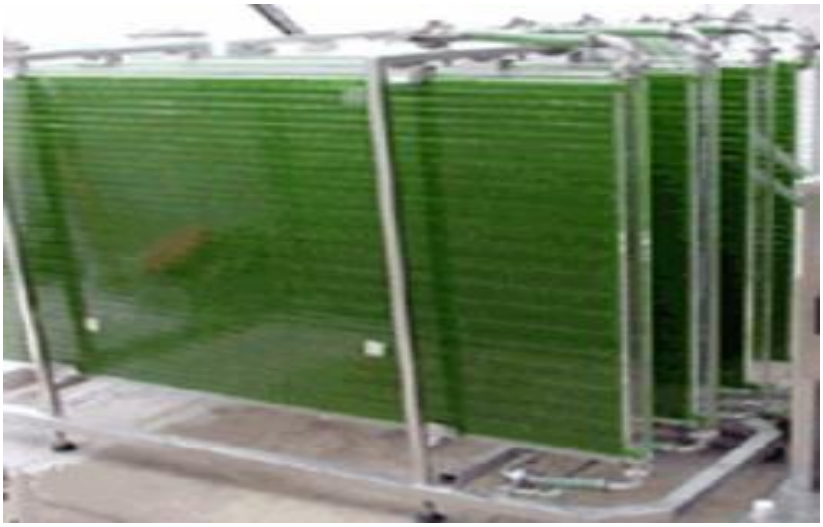


Fig. 6: Enclosed photobioreactor.

Open Pond photo-bioreactor

Open ponds are the oldest and simplest systems for mass cultivation of microalgae. In this system, the shallow pond is usually about one-foot deep, and algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway configuration, in which a paddlewheel circulates and mixes the algal cells and nutrients (fig.7). The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid.

Baffles in the channel guide the flow around the bends in order to minimize space. The system is often operated in a continuous mode, i.e., the fresh feed is added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. Depending on the nutrients required by algal species, several sources of wastewater such as dairy/swine lagoon effluent and municipal wastewater can be used for algal culture. For some marine-type microalgae, seawater or water with high salinity can be used.

Fig. 7: Open pond photobioreactor.

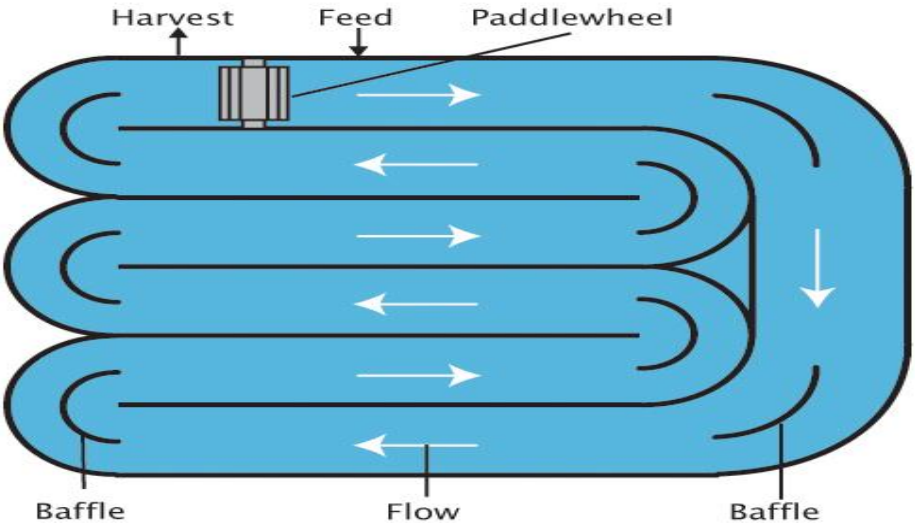


Fig. 8: Schematic diag. of Open pond system.

6.1.2. A conventional bioreactor—common features

The different types and designs of bioreactors are described. The most common features of a typical bioreactor are diagrammatically represented in following Fig.9. and briefly described hereunder.

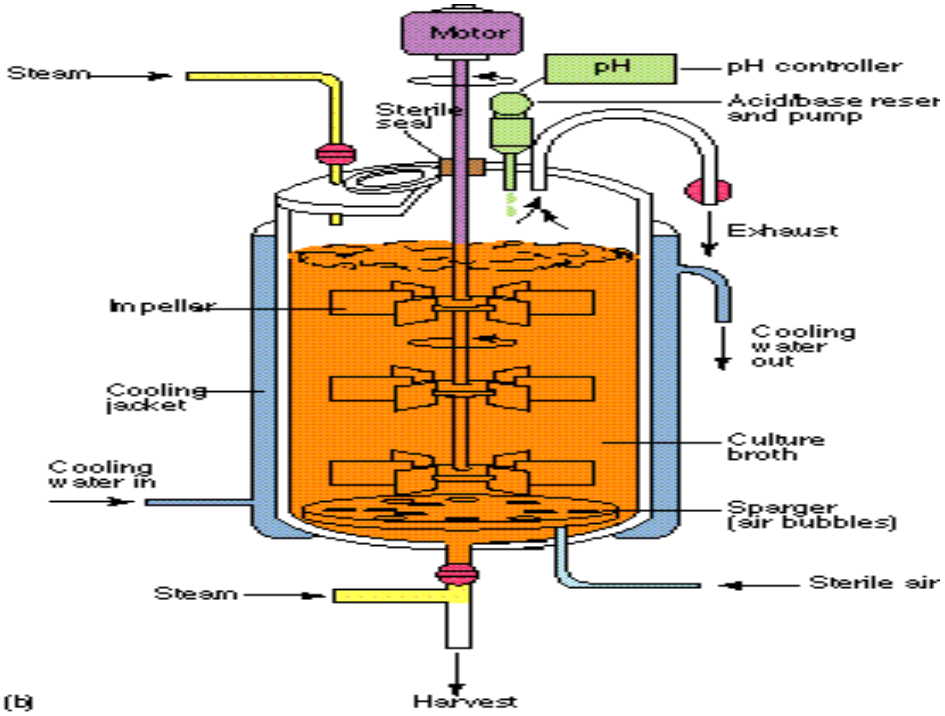


Fig. 9: Typical bioreactor.

Conventional bioreactors are cylindrical vessels with domed top and bottom. The reaction vessel, surrounded by a jacket, is provided with a sparger at the bottom through which air (or other gases such as CO_2 and NH_3 for pH maintenance) can be introduced.

The agitator shaft is connected to a motor at the bottom. The reaction vessel has side ports for pH, temperature and dissolved 0_2 sensors. Above the liquid level of the reaction vessel, connections for acid, alkali, antifoam chemicals and inoculums are located.

The bioreactor is usually designed to work at higher temperature (150-180°C), higher pressure (377-412 kPa). The reaction vessel is also designed to withstand vacuum, or else it may collapse while cooling. The materials used for the construction of bioreactor must be non-toxic and must withstand the repeated sterilization with high pressure steam.

The bioreactor vessel is usually made up of stainless steel. It should be free from crevices and stagnant areas so that no solids/liquids accumulate. Easy to clean channels and welded joints (instead of couplings) are preferred. Transparent material should be used wherever possible it is advantages to inspect medium and culture frequently.

Operation of convential bioreactor

Operation of a bioreactor basically involves the following steps,

1. Sterilization
2. Inoculation and sampling
3. Aeration
4. Control system
5. Cleaning.

6.2. Media and Subsrates used in fermentation

6.2.1. Media

The media used for the growth microorganism in industrial fermentation must contains all elements in a suitable form of cellular substances as well as the metabolic product. While designing a medium several factors must be taken into consideration a most important among them is ultimate product desired in the fermentation. Or the growth link products primary metabolites e.g. ethanol, citric acid the product formation is directly dependent on the growth of organisms hence the medium should be such that it supports good growth. On the other hand for the products which are not directly links to the growth the substrate requirements for

production must also be considered in the laboratory, pure defined chemicals may be use for culturing microorganism.

The media used in fermentation process are of two types.

1. Synthetics media
2. Crude media

1. Synthetics media

Media with all requisite constituents in a pure form and in the desired proportion is called as synthetic media. The actual use of this type media in fermentation is not practicable.

2. Crude media

The non synthetics media with naturally available sources is called as crude media and it is better suited and ideal for good products yield in fermentation.

6.2.2. Substrates used as carbon sources

Carbohydrates constitute the most predominant source of energy in fermentation industry.

Refined and pure carbohydrates such as glucose or sucrose are rarely used economic reasons.

Molasses

Molasses is byproduct of sugar industry and is one of the cheapest source of carbohydrates. Sugarcane molasses (sucrose around 48%) and sugar beat molasses (sucrose around 33%) are commonly used. Besides being rich in sugar, molasses also contains nitrogenous substances. Vitamins and trace elements.

Malt extract

Malt extract, an aqueous extract of malted barley, contains about 80% carbohydrates (glucose, fructose, sucrose, and maltose). Nitrogen compounds constitute around 4.5% (proteins, peptides, amino acids, purines, pyrimidines).

Starch, dextrin and cellulose

The polysaccharides-starch, dextrin and cellulose can be metabolized by microorganism.

They are frequently used for the industrial production of alcohol.

Whey

Whey is a byproduct of dairy industry and is produce worldwide. Most of it is consumed by humans and animals. Whey is a reasonably source of carbon of the production of alcohol, single cell protein, vitamin B_{12}, lactic acid and gebbrallic acid. Storage of whey is a limiting factor for its wide spread used in fermentation industry.

6.2.3. Substrates used as a nitrogen sources

The nitrogen supply to the fermentation microorganism may come from inorganic or organic sources.

1. Inorganic nitrogen sources
2. Organic nitrogen sources
3. Yeast extracts
4. Soy meal
5. Bubble column reactor

6.3. Micro-oganisms used in fermentation

There are over millions of species of microorganism widely distributed in nature. Less than 1% word's microorganisms have been studied. Out of this only a few hundred species are important for the fermentation use.

Table 2: A selected list of organisms along with their products are given in a table.

Microorganisms	Products
Algae	
Chlorella sorokiniana	Single-cell protein
Spirulina maxima	Single-cell protein
Bacteria	
Acitobacteracita	Acetic acid
Acitobacterwoodii	Acetic acid
Bacillus woodii	Bacitracin
B. brevis	Gramicidin
B. thuringiensis	Endotoxin
clostridium aceticum	Acetic acid
pseudomonas denitrificans	Vitamin B_{12}
Actinomycetes	
Streptomices aureofaciens	Tetracycline
S. griseus	Streptomycin
S. tradiae	Neomycin
Nocardiamediterranei	Rifamycin
Micromosporaperpurea	Gentamycin

Fungi	
Aspergillusniger	Citric acid
A. oryzae	Amylase, cellulose, single cell protein
candida lipolytica	Lipase
C.utilis	Single cell protein
Penicilliumcrysogenum	Penicillin
Saccharomyces cerevisiae	Ethanol, wine, single cell protein
S. lipolytica	Citric acid, single cell protein
Rhizopusnigricans	Steroid
Gibberellafujikuroy	Gibberellins

6.4. Principles of micr

Obial growth and culture systems

The growth of microorganisms is a highly complex and coordinated process, ultimately expressed by increase in cell number or cell mass. The process of growth depends on the availability requisite nutrients and their transport into the cells, and the environmental factors such as aeration, O_2 supply, temperature and pH.

In batch fermentation, the growth medium containing the substrates is inoculated with microorganisms, and the fermentation proceeds without the addition of fresh growth medium. In fed-batch fermentation, substrates are added at short time intervals during fermentation. In batch and fed-batch fermentation, the growth of the cells is quite comparable. And in both cases, growth medium is not removed until the end of fermentation process.

6.4.1. Batch culture or batch fermentation

Batch fermentation is regarded as a closed system. The sterile nutrient culture medium in the bioreactor is inoculated with microorganisms. The incubation is carried out under optimal physiological conditions (pH, temperature, O_2 supply, agitation etc.). It may be necessary to add acid or alkali to maintain pH, and anti-foam agents to minimize foam.

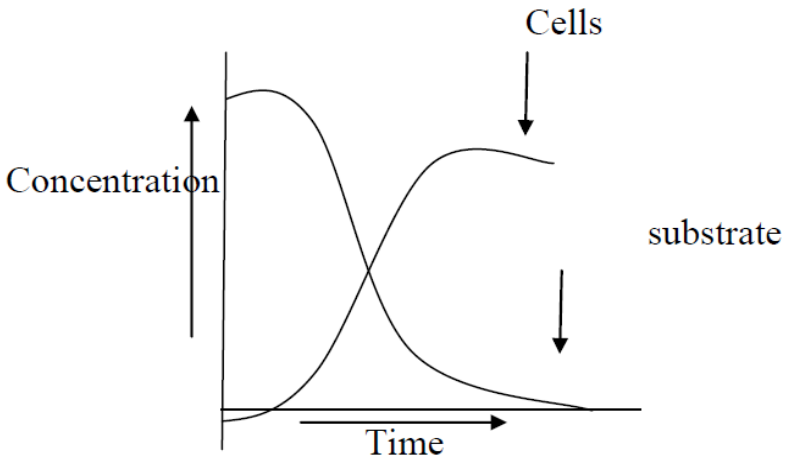


Fig. 10: Representation of microbial growth in relation to substrate in batch fermentation.

Batch culture or batch fermentation

Batch culture or Batch fermentation process are following types i.e.,

1. Log phase
2. Acceleration phase
3. Logarithmic (log) phase (exponential phase)
4. Deceleration phase
5. Stationary phase
6. Death phase

1. **Lag phase:** the initial brief period of culturing after inoculation is referred to as lag phase. During the lag phase, the microorganisms adapt to the new environment—available nutrients, pH etc. There is no increase in the cell number, although the cellular weight may slightly increase.
2. **Acceleration phase:** this is a brief transient period during which cells start growing slowly. In fact, acceleration phase connects the lag phase and log phase.
3. **Log phase:** the most active growth of microorganisms and multiplication occur during log phase. The cells undergo several doublings and the cell mass increases. When the number of cells biomass is plotted against time on a semi logarithmic graph, a straight line is obtained, hence the term log phase. Growth rate of microbes log phase is independent of substrate concentration as long as excess substrate is present, and there are no growth inhibitors in the medium.

4. **Deceleration phase:** As the growth rate of microorganism during log phase decreases, They enter deceleration phase. This phase wry short-lived and may not be observable.
5. **Stationary phase:** As the substrate in the oath medium gets depleted, and the metabolic end products that are formed inhibit the growth, the cells enter the stationary phase. The microbial growth may either slow down or completely stop. The biomass may remain almost constant during stationary phase.
6. **Death phase:** This phase is associated with cessation of metabolic activity and depletion of energy reserves. The cells die at an exponential rate. In the commercial and industrial fermentations, the growth of the microorganisms is halted at the end of the log phase or just before the death phase begins, and the cells are harvested.

7. General fermentation process

The general fermentation process basically consists of inoculum preservation, inoculum build-up, prefernienter culture and finally production fermentation. A brief account of the four stages of fermentation is given below.

7.1 Inoculum preservation (culture maintenance)

The preservation of high-yielding strains of microorganisms for fermentation is very important for product formation in possible, without cell division. There are different methods of preservation.

Storage at low (2-6°C) temperature

In this method, the microorganisms can be stored in a refrigerator in liquid culture or as stab culture. Although this is the easiest method of preservation, there is a high risk of contamination.

Storage by freezing

The microbial cultures can be frozen and preserved for several years. In the freezers, the preservation can be done at -18°C or, at -80°C. For preservation at -196°C, liquid nitrogen must be used. If proper care is not taken, as many as 95°/s of the cells may be killed by freezing and thawing.

Storage by lyophilization

Preservation of microorganisms by lyophilization (i.e., freeze drying) is the best method, although, it requires special equipment. In fact, Lyophilization is the method of choice by many fermentation biotechnologists.

7.2 Inoculum builds up

The preserved cultures have to be revived for their industrial use. This can be done by growing the cultures in liquid or on solid media. The actual process and the conditions used for inoculums build-up largely depend on the preservation technique used. There are wide variations in the growth times which depend on the type of preservation and the organisms used as given below i.e., Refrigerated cultures (2-6°C), Frozen cultures (18°C, 80°C, 196°C) and Lyophilized cultures.

Refrigerated cultures (2-6°C)

Bacteria 6-24 hours

Actinomycetes 1-3 days

Fungi 1-5 days

Frozen cultures (18°C, 80°C, 196°C)

Bacteria 6-48 hours

Actinomycetes 1-5 days

Fungi 1-7 days

Lyophilized cultures

For all organisms 4-10 days

For proper growth, and to obtain sufficient quantity of inoculums, a series of cultures are prepared. For good fermentation yield, the number of cells and spores, nutrient medium, temperature and age of the inoculums are important.

7.3 Prefermenter culture

Fermenter preculture or prefermenter culture is often required for inoculating large sized bioreactors. Inadequate quantity of inoculum will not only delay the product formation, but also reduce the yield drastically, by culturing the microorganisms (the inoculum build-up) in small fermenters, the size of the inoculum can be increased for large-scale industrial use. Biotechnologists have worked out the requisite noculuni concentrations for optimal

fermentation e.g. for bacterial fermentation, the inoculum concentration should be between 0.2 to 3.0%; for fungal fermentation, it is in the range of 5-10%

7.4 Production fermentation

The general features and the different types of bioreactors are already described The size of the fermenter used mainly depends on the product. For example, a small bioreactor (1-20 liter size) can be used for producing diagnostic enzymes and substances for molecular biology by recombinant microorganisms, while large bioreactors (> 450 liters) are employed for producing single-cell protein and amino acids. for appropriate production by fermentation, several .

8. Measurement and Control of bioprocess parameters

There are a large number of physical, chemical and biological parameters that can be measured during fermentation/ bioprocessing 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.

Important parameters that can be measured during bio processing are listed as follows.

Table 3: Important parameters that can be measured during bio-processing.

Physical parameters	Temperature Pressure Flow rates Viscosity Turbidity Power consumption
Chemical parameters	pH Substrate concentration Product concentration 0_2 concentration (dissolved) Waste gases concentration (e.g. $C0_2$) Ionic strength.
Biological parameters	Activities of specific enzymes Protein concentration Energetics (ATP concentration) DNA / RNA Content

Temperature

The temperature must be so maintained that there occurs maximal growth of microorganisms with optimal product formation, although this is not always possible. In general, there are two temperature ranges to run the fermentations a mesophile range (20—45°C) and a thermopile

range (>45°C). Sometimes, two different temperatures are used for the same fermentation process—a higher temperature is employed for good growth (in trophophase), and then the temperature is decreased for optimizing product formation (in idiophase).

Pressure

Appropriate maintenance of hydrostatic pressure, particularly in large sized bioreactors is very important. This is because pressure influences the solubility of 02 and CO2 in the culture medium. An overpressure in the range 0.2—0.5 bar is generally used.

Aeration

A bioreactor gets aerated by the supply of 02 and therefore, adjustment must be made to furnish required amount of 02 to the microorganisms. Usually, the aeration rate is in the range of 0.25—1.25 vvm (volume of air/volume of liquid/minute).

Stirring

The type and the speed of impellers determine the stirring rate in a fermenter. In general, the impeller speed decreases as the size of the fermenter increases. Thus, for a small bioreactor (size 1—20 liters), the impeller speed is in the range of 250—350 rpm, while for a large bioreactor (size around 450 liters, the impeller speed is 60—1 20 rpm.

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. Electrodes are also available for measuring several other inorganic ions.

O_2 and CO_2 measurement

Oxygen electrodes and CO_2 electrodes can be used to measure 0_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.

9. Application of fermentation technology

Industrial production of selected antibiotics is briefly describedas below.

1. Penicillins production

Penicillin's are a group of ß-Lactum containing bactericidal antibiotics. Being the first among the antibiotics to be discovered, penicillin is historically important. The basic structure of all the penicillin consists of a ß-Lactum ring and a thizolidine ring fused together to form 6-aminopenicillanic acid.

Organism for penicillin production

In the early days, penicilliun notatum was used for the large scale production of penicillin. Currently, Penicillium chrysogenum and its improved mutant strains are preferred. Previously, the penicillin production used to be less than 2 units/ml and with the new strains, the production runs into several thousands of Units/ml. One of the high yielding strains was Q176 is preferred by several penicillin manufacturers.

Genetic engineering for improved penicillin production

Some of the genes involved in penicillin biosynthesis by *P.*chrysogenum have been identified. Genetic manipulations were carried out so as to substantially increase the penicillin production.

Production process of penicillin

An outline of the flow chart for the industrial production of penicillin is depicted in Fig.14 the lyophilized culture of spores is cultivated for inoculum development which is transferred to prefermenter, and then fermenter. Penicillin production is an aerobic process and therefore, a continuous supply of O_2 to the growing culture is very essential. The required aeration rate is 0.5-1.0 vvm. The pH is maintained around 6.5, and the optimal temperature is in the range of 25—27°C. Penicillin production is usually carried out by submerged processes.

The medium used for fermentation consists of corn steep liquor (4-5% dry weight) and carbon source. An addition of yeast extract, soy meal or whey is done for a good supply of nitrogen. Sometimes, ammonium sulfate is added for the supply of nitrogen. Phenyl acetic acid which serves as a precursor for penicillin biosynthesis is continuously fed; Further, continuous feeding of sugar is advantageous for a good yield of penicillin.

It is estimated that approximately 10% of the metabolized carbon contributes to penicillin production, while 65% is utilized towards energy supply and 25% for growth of the organisms. The efficiency of penicillin production can be optimized by adequate supply of carbon source. Thus, by adding glucose and acetic acid, the yield can be increased by about 25%. For efficient synthesis of penicillin, the growth of the organism from spores must be in a loose form and not as pellets. The growth phase is around 40 hours with a doubling time of 6-8 hours. After the growth phase is stabilized; the penicillin production exponentially increases with appropriate culture conditions. The penicillin production phase can be extended to 150—180 hours.|

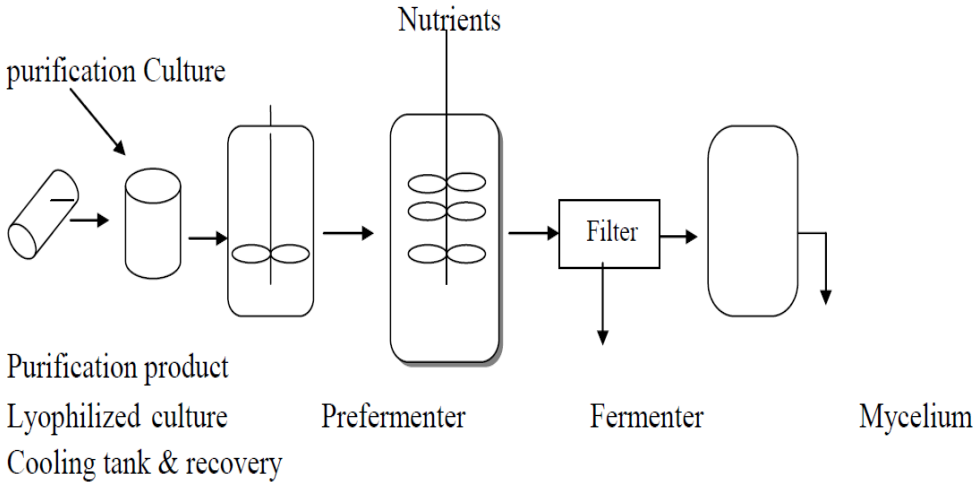


Fig. 11: An outline of the flow chart for penicillin fermentation.

Recovery of penicillin

As the fermentation is complete, the broth containing about 1% penicillin is processed for extraction. The mycelium is removed by filtration Penicillin is recovered by solvent (n-butyl acetate or methyl ketone) extraction at low temperature (<10°C) and acidic pH (<3.0). By this way, the chemical and enzymatic (bacterial penicillinase) degradations of penicillin can be minimized. The penicillin containing solvent is treated with activated carbon to remove impurities and pigments. Penicillin can be recovered by adding potassium or sodium acetate. The potassium or sodium salts of penicillin can be further processed (in dry solvents such as n-butanol or isopropanol) to remove impurities.

2. Tetracyclines production

Tetracyclines are broad spectrum antibiotics with widespread medical use. They are effective against Gram-positive and Gram-negative bacteria, besides other organisms (mycoplasmas,

chlamydia, and rickettsias). Tetracyclines are used to combat stomach ulcers (against Helicobacter pylori.)

They are the most commonly used antibiotics, next to cephalosporin's and penicillin's. Tetracyclines inhibit protein biosynthesis by blocking the binding of aminoacyltRNA to ribosomes (A site).

Organisms for tetracycline production

The first tetracycline antibiotic that was isolated was chlortetracycline from the cultures of Streptomyces aureofaciens (in1945). There are at least 20 Streptomyces's identified now that usually produces a mixture of tetracycline's.

Production process of chlortetracycline

The fermentation medium consists of corn steep liquor, soy flour or peanut meal for the supply of nitrogen and carbon sources. Continuous feeding of carbohydrate is desirable for good growth of the organism and production of the antibiotic.

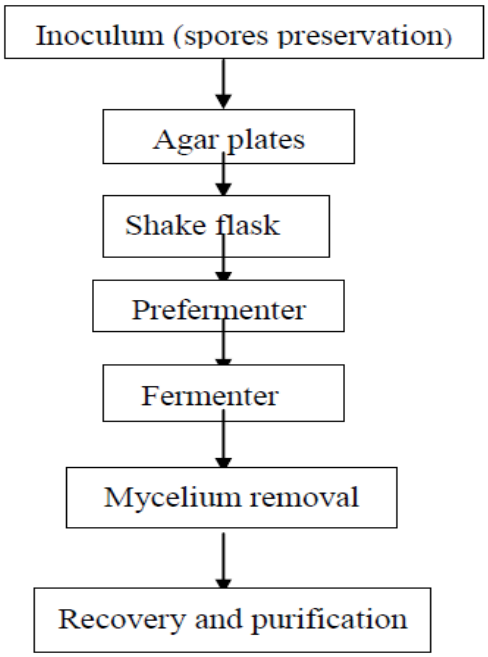


Fig. 12: An outline of production chart for chlortetracycline Recovery of chlortetracycline.

This can be done either by addition of crude carbon sources or by supplying glucose or starch. For more efficient production of chlortetracycline, the supply of ammonium and

phosphate has to be maintained at a low concentration. An outline of die production process for chlortetracycline is depicted in Fig.15.

The ideal fermentation conditions are temperature 27-30°C, pH - 6.5-7.5, aeration 0.8-1.0 vvm. The duration of fermentation is around 4 days.

At the end of the fermentation, the culture broth is filtered to remove the mycelium. The filtrate is treated with n-butanol or methyl isobutyl Ketone in acidic or alkaline condition for extracting the antibiotic. It is then absorbed to activated charcoal to remove other impurities. Chlortetracycline is eluted and crystallized.

3. Microbial production of amino acids

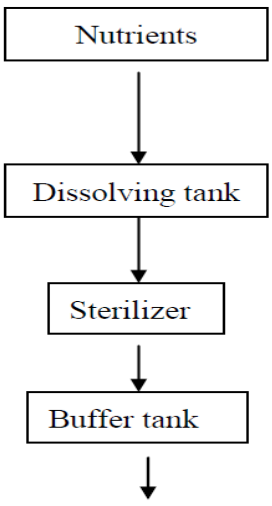
3.1. L-glutamic acid production

L- glutamic acid was the first amino acid to be produced by microorganism.

Organism for glutamic acid production

The original bacterium, Corynebacterium glutamicum, that was first used for large scale manufacture of glutamic acid continues to be successfully used even today. The other important organisms employed for glutamic acid production belong to genera Mycobacterium, Brevibacterium and Arthrobacter.

All these organisms have certain morphological and physiological characters comparable to C.glutamicum. Biochemically, glutamic acid producing bacteria have a high activity of glutamate dehydrogenase and a low activity of α-ketoglutarate dehydrogenase. They also require the vitamin biotin.



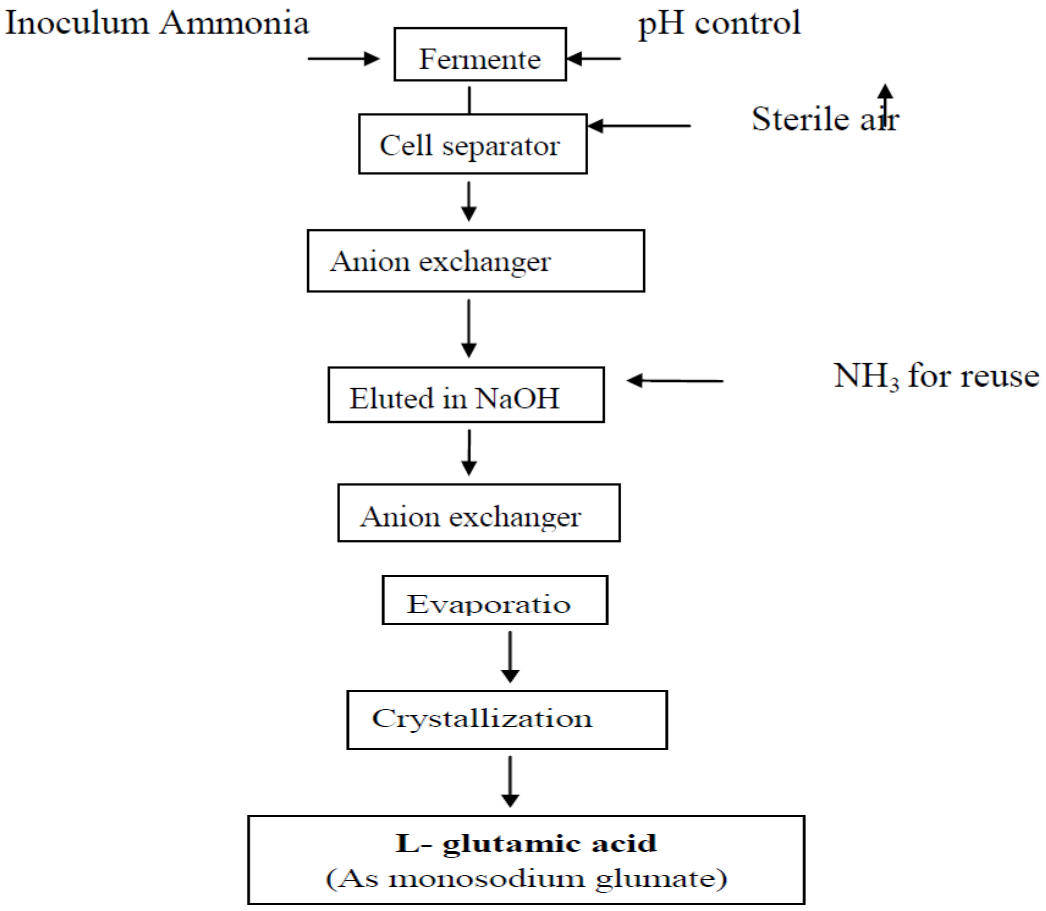


Fig.13: Diagrammatic representation of glutamic acid production plant.

Process of production and recovery

Some important information on the production of glutamic acid by *Brevibacterium divaricatum* is given below.

Carbon source	-------	Glucose (12°/o)
Nitrogen source (0.5%)	-------	Ammonium acetate
pH	-------	7.8
Temperature	-------	38^0 c
Period for fermentation	-------	30-35 hours

A schematic representation of glutamic acid production plant is shown in Fig.16. As the fermentation is complete, the cells are separated; the culture broth is passed through anion exchanger. The glutamic acid bound to the resins is eluted in NaOH, while the ammonia released can be reused. With NaOH, glutamic acid forms monosodium glutamate (MSG) which can be purified by passing through anion exchanger. MSG can be subjected to evaporation and crystallization.

3.2. Lysine production

Lysine is present at a low concentration in most of the plant proteins. Being an essential amino acid, supplementation of plant foods with lysine increases their nutritional quality. L-Lysine is predominantly produced by Corynebacterium glutamicum and to some extent by Brevibacteriurnufavum or B. facto fermentum.

Production process of L-lysine

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

An alkaline solution containing about 50% L-lysine can be obtained after biomass separation, evaporation and filtration. A crystalline preparation with 98-99% L-lysine can be obtained by subjecting the culture broth to ion-exchange chromatography, evaporation and crystallization. Both the above grades of lysine are suitable for supplementation of feeds.

4. Microbial production of vitamins

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These vitamins cannot be synthesized by the higher organisms, including man, and therefore they have to be supplied in small amounts in the diet. Microorganisms are capable of synthesizing the vitamins. In fact, the bacteria in the gut of humans can produce some of the vitamins, which if appropriately absorbed can partially meet the body's requirements. It is an accepted fact that after administration of strong antibiotics to humans (which kill bacteria in gut), additional consumption of vitamins is recommended.

Microorganisms can be successfully used for the commercial production of many of the vitamins e.g. thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamin B_{12}, ascorbic acid, 3-carotene (pro-vitamin A), ergosterol (pro-vitamin D).

4.1. Vitamin b_{12} (cynocobalamine)

The disease, *pernicious anemia*, characterized by low levels of hemoglobin, decreased number of erythrocytes and neurological manifestations, has been known for several decades. It was in 1926 some workers reported the liver extracts could cure pernicious anemia. The active principle was later identified as vitamin B_{12}, a water soluble B-complex vitamin.

Commercial production of vitamin B_{12}

Vitamin B12 is commercially produced by fermentation. It was first obtained as a byproduct of Streptomyces fermentation in the production of certain antibiotics (streptomycin, chloramphenicol, or neomycin). But the yield was very low. Later, high-yielding strains were developed- And at present, vitamin B12 is entirely produced by fermentation, It is estimated that the world's annual production of vitamin B12 is around 15,000 kg.

High concentrations of vitamin B12 are detected in sewage-sludge solids. This is produced by microorganisms. Recovery of vitamin B12 from sewage-sludge was carried out in some parts of United States.

Microorganisms and yields of vitamin B_{12}

Several microorganisms can be employed for the production of vitamin B_{12}, with varying yields. Glucose is the most commonly used carbon source. Some examples of microbes and their corresponding yields are given in Table. The most commonly used microorganisms are Propionibacterium freudenreichii, Pseudomonas denitrificans, Bacillus megaterium and Streptomyces olivaceus.

Table 4: Microorganismswithcorresponding yield of vit.B_{12}.

Microorganism	Yield (mg/I)
Bacillus megaterium	0.51
Streptomyces olivaceus	3.31
Butyribacteriumrettgeri	5.0
Micromonosporasp	11.5
Propionibacteriumfreudenreichil	19.0
Propionibacteriumshermanll	35.0
Pseudornonasdenitrificans	60.0
Hybrid strain	
Rhodopseudomonasprotamicus	135.0

Genetically engineered strains for vitamin B_{12} production

By employing modern techniques of genetic engineering, vitamin B_{12} production can be enhanced. A protoplast fusion technique between Protamino bacterrubber and Rhodopseurlomonas spheroidés resulted in a hybrid strain called Rhodopseudomonas protamicus. This new strain can produce as high as 135 mg/I of vitamin B_{12} utilizing carbon source.

Production of vitamin B12 using propionibacteriumsp

Propionibacterium freudenrenchii and P.shermarili, and their mutant strains are commonly used for vitamin B_{12} production. The process is carried out by adding cobalt in two phases.

Anaerobic phase this is a preliminary phase that may take 2-4 days. In the anaerobic phase 5'-deoxyadenosylcobinamide is predominantly produced.

Aerobic phase: In this phase, 5,6-dimethyl- benzimidazole is produced from riboflavin which gets incorporated to finally form coenzyme of vitamin B_{12} namely: 5-deoxyadenosyl cobalamin. The bulk production of vitamin B_{12} is mostly done by submerged bacterial fermentation with beet molasses medium supplemented with cobalt chloride.

Recovery of vitamin B_{12}

The cobalamins produced by fermentation are mostly bound to the cells. They can be solubilized by heat treatment at 80-120°C for about 30 minutes at pH 6.5-8.5. The solids and mycelium are filtered or centrifuged and the fermentation broth collected.

The coalmines can be converted to more stable cyanocobalamines. This vitamin B_{12} is around 80% purity and can be directly used as a feed additive. However, for medical use (particularly for treatment of pernicious anemia), vitamin B_{12} should be further purified (95—98% purity).

Production of vitamin B_{12} using pseudomonas sp

Pseudomonas denitrificans is also used for large scale production of vitamin 12 in a cost-effective manner. Starting with a low yield (0.6 mg/I) two decades ago, several improvements have been made in the strains of P.denitrificans for a tremendous improvement in the yield (60 mg/l). Addition of cobalt and 5, 6 L-dimethyl benzimidazole to the medium is essential. The yield of vitamin B_{12} increases when the medium is supplemented with betaine.

Carbon sources for vitamin B_{12} production

Glucose is the most commonly used carbon source for large scale manufacture of vitamin B_{12}. Other carbon sources like alcohols (methanol, ethanol, isopropanol) and hydrocarbons (alkanes, decane, hexadecane) with varying yields can also be used.

5. Microbial production of foods and beverages

5.1. Yoghurt

Yoghurt is produced by fermenting whole milk by employing a mixed culture of Lactobacillus bulgaricus and Streptococcus thermophilus. While L.bulgaricus produces acetaldehyde that imparts a characteristic taste, S.thermophilus results in the formation of lactic acid to give acid flavor In addition, both these bacteria produce extra cellular polymers that increase the viscosity of the fermented milk. Yoghurt is very delicious and in fact frozen yoghurt is becoming popular as an alternative to ice cream.

5.2. Bread

Bread is a fermented product of cereal flours such as wheat and rye. The cereal flour mixed with water, salt, sugar, fat and other ingredients is subjected to fermentation by yeast, *Saccharomyces cerevisiae* (top fermenting strain). The main reaction that occurs during bread formation is fermentation of hexoses to CO_2 and ethanol.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The ethanol produced either gets evaporated or forms esters. The CO_2 gets entrapped in the dough resulting in its expansion.

The expansion and stretching of the dough, particularly with wheat is due to the unique elastic protein namely gluten. Gluten is mainly responsible for retaining the shape of bread.

Besides yeast enzymes, the enzymes (e.g. amylases) of other microorganisms also help in fermentation and baking of bread. The texture of bread is influenced by fats and emulsifiers added to the dough. The bread making is carried out with three objectives

- Good leavening due to CO_2 formation.
- Flavor development.
- Good texture.

The yeast fermented bread has the above characteristics. This is in contrast to the bread produced with baking powder which also produces CO_2. But this does not have the same

flavor and texture as that produced by yeast. Thus the yeast, which is appropriately referred to as baker's yeast is a package of enzymes to give a desired product.

5.3. Wines

Wines are originally Middle Fast and European drinks, although almost every country now produces them. Large scale production of wines is carried out by using grapes of species zinfandel. Louis Pasteur often used to state 1wine is the most healthy and most hygienic of beverages'

Fermentation of fruit juice into wine

Production of wines

Quality of the grapes is very important for the production of wines.

The grapes are crushed and the juice extracted. This grape juice ready for fermentation process is technically referred to as must.

It is a practice to add sulfur dioxide to must to inhibit the growth of non-wine yeast and contaminating bacteria. Sulfur dioxide which can kill other organisms can be tolerated by wine-fermenting yeasts.

Sometimes, they must may also be subjected to partial or complete sterilization.

The must in suitable bioreactors is inoculated, with desired strains of the yeast Saccharomyces cerevisiae.

The fermentation conditions (temperature, time etc.) are actually dependent on the type of wine produced. At the end of fermentation, wines are transferred to storage tanks (or vats) and allowed to age, which may take some months or years. Ageing of wine is very important for the development of characteristic flavor and aroma.

The alcohol content of wines is in the range of 10-16%.

Fermentation is one of the most ancient of human's technologies and is now one of the most Wine commercially prosperous biotechnological processes. The technique of winemaking is known since the dawn of civilization and has followed human and agricultural progress. The earliest biomolecular archaeological evidence for plant additives in fermented beverages dates from the early Neolithic period in China and the Middle East when the first plants and

animals were domesticated and provided the basis for a complex society and permanent settlements.

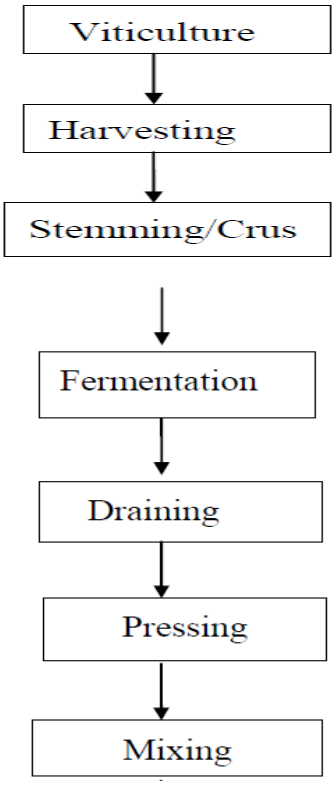
In ancient China, fermented beverages were routinely produced from rice, millet, and fruits. However, in earlier years in Egypt, a range of natural products, specifically herbs and tree resins, were served with grape wine to prepare herbal medicinal wines.

Home-made wine production has been practised with various fruits such as apple, pear and strawberry, cherries, plum, banana, pineapple, oranges, cucumber, watermelon, and guava. Using species of S. cerevisiae which converts the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters, and other chemical compounds which also help to preserve the wine.

Yeasts from other sources such as palm wine have also been used[30] in the production of fruit wine.

Production of wine

Wine Production: Main Steps



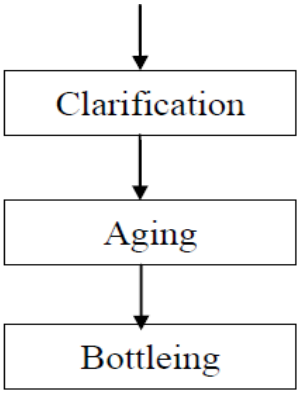


Fig. 14: Wine cultivatation product.

Example

- Red Wine
- White Wine
- Dry Wine
- Sweet Wine
- Fortified Win
- Fermentative production of wine and other distilled bevarages

CONCLUSION

Fermentation is biotechnological process practiced by mankind since ancient times. Understanding the scientific principle of fermentation by Louis Pasteur. Today a large number of chemicals are produced by fermentation technology and is playing measure role industrial production. Various parameters like physical(temperature, pressure, flow rate, viscosity, power consumption),chemical (pH, substrate concentration, product concentration, O_2 concentration (dissolved),biological (activities of specific enzymes, protein concentration, DNA/RNA content) and environmental are required to be controlled and maintained throughout the process of fermentationPhysiological criteria of fermentation which maintain physical, chemical, environmental condition is called fermenter.

The fermenters are classified on the volume of capacity as follows

- Lab scale
- Pilot scale
- Industrial fermenter

On the basis of type of fermenters, they are classified into

- Submerged fermenters
- Surface fermenters

While industrial fermenters are classified on the basis of

- Mechanically stirred fermenters
- Forced convection fermenters
- Pneumatic reactors fermenters

There are 'n' numbers of species of microorganisms widely distributed in nature. In which less than 1% microorganisms have been studied. Out of which 100 species are important for the use of fermentation.

The final processing of the product is done by

- Drying
- Lyophillisation
- Blending along with the adjuvants

The yields and quality is improved by various development and strain development progress. Amino acids and antibiotics like penicillin's, tetracycline, etc. Vitamins like B2 and B12 Foods and beverages like bread, yogurt, bear and wines respectively are produced by fermentation technology.

REFERENCES

1. Vyas, S.P and Dixit V.K. Pharmaceutical biotechnology, CBS publishers and distributors New Delhi, 1998; 1: 160-185.
2. U. Satyanarayana, Biotechnology, books & allied (P) Ltd, 2005; 1: 239-280, 311-370.
3. Dr. John I. Disouza and Suresh G. Killedar, Biotechnology and Fermentation process, Niraliprakashan, fifth edition, 2010; 3: 1-3.89.
4. L. Brennan, P. Owende, Biofuels from microalgaeda review of technologies for production, pro-cessing, and extractions of biofuels and co-products, Renewable and Sustainable Energy Reviews.
5. D.-T. Tran, C.-L. Chen, J.-S. Chang, Effect of solvents and oil content on direct transesterification of wet oil-bearing microalgal biomass of Chlorella vulgaris ESP-31 for

biodiesel synthesis using immobilized lipase as the biocatalyst, Bioresource Technology, 2013; 135: 213e221.

6. S.-H. Ho, P.-J. Li, C.-C. Liu, J.-S. Chang, Bioprocess development on microalgae-based CO2fixation and bioethanol production using Scenedesmus obliquus CNW-N, Bioresource Technology, 2013; 145: 142e14.
7. L. Brennan, P. Owende, Biofuels from microalgaeda review of technologies for production, pro- cessing, and extractions of biofuels and co-products, Renewable and Sustainable Energy Reviews, 2010; 14: 557e577.
8. D.-T. Tran, C.-L. Chen, J.-S. Chang, Effect of solvents and oil content on direct transesterification wet oil-bearing microalgal biomass of Chlorella vulgaris ESP-31 for biodiesel synthesis using immobilized lipase as the biocatalyst, Bioresource Technology, 2013; 135: 213e221.
9. S.-H. Ho, P.-J. Li, C.-C. Liu, J.-S. Chang, Bioprocess development on microalgae-based CO2fixationand bioethanol production using Scenedesmus obliquus CNW-N, Bioresource Technology, 2013; 145: 142e14.
10. L. Brennan, P. Owende, Biofuels from microalgaeda review of technologies for production, pro-cessing, and extractions of biofuels and co-products, Renewable and Sustainable Energy Reviews, 2010; 14: 557-577.
11. D.-T. Tran, C.-L. Chen, J.-S. Chang, Effect of solvents and oil content on direct transester ification ofwet oil-bearing microalgal biomass of Chlorella vulgaris ESP-31 for biodiesel synthesis using immobilized lipase as the biocatalyst, Bioresource Technology, 2013; 135 213e221.
12. S.-H. Ho, P.-J. Li, C.-C. Liu, J.-S. Chang, Bioprocess development on microalgae-based CO2fixationand bioethanol production using Scenedesmus obliquus CNW-N, Bioresource Technology, 2013; 145: 142-149.
13. The Microbiology of Wine and Vinifications. John Wiley and Sons Ltd, England, pp. 358-405. Sandhu DK and Joshi VK (1994). Comparative fermentation behaviour and chemical characteristics of Saccharomyces and Zymomonas fermented culled apple juice. Indian Journal of Experimental Biology, 32: 873. Sevda SB, Khartmol P and Rodrigues L (2010). Studies in preparation of bananawine DOA, 2014; 30: 06.
14. Shanmugasundaram S, John Kennedy Z, Thirupathi V and Narayanan L. Qualitative changes in banana pulp and juice during wine making process, 2005.
15. The Indian Journal of Nutrition and Dietetics, Spreer W, Ongprasert S, Hegele M, Wunsche JN and Muller J, 2009; 42: 560-570.

16. Yield and fruit development in mango (Mangifera indica L. cv. Chok Anan) under different irrigation regimes. Agricultural Water Management, 96: 574-584.