FERMENTATION TECHNOLOGY

Dr. Chandan Das

Department Pharmacognosy, School of Pharmacy and Life Science, Centurion University of Technology and Management.

Bhubaneswar, Odisha, India

discoverchandan@gmail.com

Dr. Debajyoti Das

Department of pharmacognosy, School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan, Deemed to be University

Khandagiri Square, Bhubaneswar 751030, Odisha, India

debajyotids@gmail.com

Mr. Ashutosh Meher

Department of Pharmacology, The Pharmaceutical College, Barpali, Bargarh, Odisha, India

mashumeher@gmail.com

Miss. Kumudini Sahoo

Department of Pharmacology, School of Pharmacy and Life Science, Centurion University of Technology and Management.

Bhubaneswar, Odisha, India

kumudini.sahoo@cutm.ac.in



**ABSTRACT**

The term "fermentation" refers to the process by which microorganisms can grow and produce products in environments with either aerobic, microaerobic, or anaerobic conditions. The efficiency of the fermenter is determined by the organism that is able to develop inside of it given the appropriate pH, temperature, oxygen levels, and any other environmental conditions. The process of fermentation can be broken down into three distinct stages. Various parts of fermentation technology are used, with modern applications in the manufacture of staple foods, drinks, the processing of meat and fish, the creation of organic solvents, pharmaceutical, etc. industries, among others. The present chapter provides a comprehensive overview of several varieties of fermentation technology, as well as its scope, history, advantages and limitations, and modern applications in the fields of biotechnology and healthcare.

**History**

Humans have been using fermentation technology to create food and beverages since the Neolithic period, with records of it being found in China (7000–6600 BCE), Georgia (6000 BCE), Egypt (3150 BCE), Babylon (3000 BCE), Mexico (2000 BCE), and Sudan (1500 BCE). The study was initiated by Louis Pasteur (1822–1895) through a series of experimentation with the involvement of living microorganisms. In 1857, Pasteur stated that a living microorganism is responsible factor for the production of lactic acid. His research resulted in the invention of pasteurisation when he discovered in 1860 how bacteria contribute to food degradation. Milk sourness is caused by bacteria, despite earlier theories that it was only a chemical transformation. A certain kind of microorganism induces a particular kind of fermentation and generates a particular kind of finished product. Despite the fact that living microorganisms are the cause of fermentation, this finding neither explains how it occurs nor establishes that microorganisms are the cause of fermentation. Scientists have made numerous unsuccessful attempts to extract the fermentation enzyme from yeast, including Pasteur's. German chemist Eduard Buechner (1897) found that ground yeast could ferment sugar solutions in the same way as living yeast, resulting in the production of alcohol and carbon dioxide. The term "enzyme" has since been applied to all ferments. Buechner was awarded the Nobel Prize in chemistry in 1907 for his discovery of the enzymes that microbes create and accountable for fermentation [1-3].

When sugar is fermented, alcohol or acids are created. Yeast and bacterial cells that lack oxygen undergo lactic acid fermentation. As part of fermentation, microorganisms are also grown on a suitable medium, frequently for a specific use such the production of enzymes, vaccines, antibiotics, or food additives. Since an electron transport chain needs oxygen to function, fermentation takes over as the main method of generating ATP. During fermentation, the NADH and pyruvate produced during glycolysis are converted into NAD+ and a number of other small molecules. Using NADH and pyruvate in the presence of oxygen, respiration produces ATP. Oxidative phosphorylation produces a lot more ATP than glycolysis alone does. Consequently, cells prevent fermentation when oxygen is present. Inorganic phosphate is pyruvate (CH3COCOO, Pi). Two ADP molecules and two Pi molecules are converted by substrate-level phosphorylation to create ATP and water. Additionally, two NAD+ molecules are converted to NADH [2.3].

**Fermentation**

The process of microbial growth and product creation in aerobic, microaerobic, or anaerobic settings is referred to as "fermentation." Aerobic refers to the intentional mixing of the medium with air. The air that was initially present in the microaerobic environment is consumed or replaced when microbial growth takes place. Anaerobic fermentation keeps oxygen out of the fermentation medium since it is poisonous to cells. This fermentation is the method used to make alcohol. Grain and fruit fermentation is the process used to make beer and wine. A soured food could be referred as fermented. Fermentation technology can be defined by several ways as any method that results in alcoholic beverages or acidic dairy products; or any significant microbiological process that takes place with or without the presence of air; or any metabolic process that releases energy only when anaerobic conditions exist; or any metabolic process that uses an organic molecule as the final electron acceptor, releases energy from a sugar or other organic molecules, and does not require oxygen or an electron [1-4].

**Examples of Fermentation**

It is not required to do fermentation in an anaerobic environment. When carbohydrates are readily accessible for consumption, for example, yeast cells significantly prefer fermentation to aerobic respiration, even in the presence of adequate oxygen. During fermentation, NADH interacts with an organic, endogenous electron acceptor. The pyruvate, produced during the glycolysis process from the sugar is transformed into different compounds during fermentation by a number of methods.

**Ethanol fermentation**

Alcohol fermentation produces the byproducts ethanol and carbon dioxide. The fermentation of one glucose molecule results in the production of two ethanol molecules and two carbon dioxide molecules:

C6H12O6 → 2 C2H5OH + 2 CO2

**Lactic acid fermentation:** Itrefers production of lactic acid in two ways i.e.,

*Homolactic fermentation***:** Here, only lactic acid is produced in this process. Pyruvate from glycolysis is converted to lactic acid by a simple redox process. One glucose molecule yields two lactic acid molecules in total.

C6H12O6→2CH3CHOHCOOH

When animal muscles need oxygen and energy more quickly, then the blood can supply it. Additionally, it is demonstrated by some bacteria (such *Lactobacilli*) and certain fungi. During transformation of lactose into lactic acid, a specific kind of bacteria gives sour flavor in yoghurt. In homolactic fermentation where these lactic acid bacteria are capable of producing lactic acid as their principal by product.

*Heterolactic fermentation***:** It involves the production of lactic acid in addition to other acids, alcohols, carbon dioxide, acetate, and other metabolic byproducts. Heterolactic fermentation occurs between lactic acid fermentation and other types, such alcoholic fermentation. The fermentation of lactose forms glucose and galactose:

C12H22O11 + H2O → 2C6H12O6

The advantages of changing lactic acid into another substance are:

* The biological processes that lactic acid inhibits may be beneficial to the organism that is fermenting, since they drive out competitors that cannot tolerate the acidity, extending the food's shelf life. However, after a certain point is reached, the acidity starts to harm the microorganisms that produce it.
* The imbalance is upset by the high lactic acid content, which slows development and lowers the pace at which fermentation can occur.
* The simple transformation of lactic acid into ethanol makes reactions simple.
* Even while acetic acid is more acidic and less volatile than ethanol, it nevertheless releases a lot more energy when oxygen is in short supply. It is more volatile than lactic acid because it is a lighter molecule and generates fewer hydrogen bonds with its surroundings. This increases its volatility and quickens the reaction.
* Longer monocarboxylic acids such as propionic, butyric, and butyric can also develop, which speeds up growth and lowers the amount of acidity produced per unit of glucose ingested.

Sugar is the most common starting material for fermentation, and the process produces ethanol, lactic acid, carbon dioxide, and hydrogen gas as its byproducts. Butyric acid and acetone are two unique chemicals that can be produced through fermentation. The ethanol in beer, wine, and other alcoholic drinks is fermented by yeast, which also generates a sizable amount of carbon dioxide [1-3].

**Aerobic respiration**

Aerobic respiration completely oxidises the pyruvate produced during glycolysis, resulting in an increase in ATP and NADH via the citric acid cycle and oxidative phosphorylation. However, for this to occur, oxygen is needed. While obligatory anaerobes do not need oxygen, facultative anaerobes do, making oxygen toxic to them. Lactic acid fermentation is one of the fermentation processes that takes place in the absence of oxygen to regenerate NAD+.

**Hydrogen gas production in fermentation**

Several fermentation techniques, such as mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, and glyoxylate fermentation, are used to produce hydrogen gas in order to regenerate NAD+ from NADH. After receiving electrons from ferredoxin, hydrogenase uses them to oxidise it and release H2. Hydrogen gas is used as a substrate by methanogens and sulphate reducers, which lowers the hydrogen concentration and encourages the synthesis of this chemical with high energy content. However, at quite high quantities, such as in flatus, hydrogen gas can still occur.

**Methane gas production in fermentation**

The dismutation reaction of acetic acid can result in the production of methane and carbon dioxide. This disproportionation reaction is catalysed by methanogenic archaea as a byproduct of their fermentative metabolism. The carbonyl function of the carboxylic group transfers an electron to the methyl group of acetic acid, resulting in the generation of CO2 and methane gas, respectively.

**Industrial fermentation**

Industrial fermentation include deliberate use of bacteria and fungi to ferment products that are helpful for people. Fermented products can be employed in both the food industry and other industries. A number of common substances, such as acetic acid, citric acid, and ethanol, are produced through fermentation. The quantity of microorganisms, cells, cellular components, enzymes, temperature, pH, and oxygen for aerobic fermentation all have an impact on the rate of fermentation. The product recovery procedure frequently includes concentration of the diluted solution. Rennet, invertase, and lipase are just a few of the commercially produced enzymes that are produced through fermentation using genetically modified bacteria. The production of biomass itself is the aim in some situations, such as with starter cultures of baker's yeast and lactic acid bacteria [4].

**Composition of fermentation medium**

The bacteria used in fermentation grow on (or in) a specially designed growth medium that supplies the nutrients to the organisms require to live. Despite the fact that there are many distinct kinds of media, they all contain a source of carbon, a source of nitrogen, water, salts, and micronutrients.

**Carbon:** A large portion of the medium used to manufacture bioethanol might be made up of any readily available, inexpensive carbon source. Usually, carbon sources are sugars or other carbohydrates, but in substrate conversions, they can also be alcohols or other compounds (such the creation of vinegar). In large-scale fermentations to make ethanol, low-cost sources of carbohydrates like molasses, corn steep liquor, sugar cane juice, or sugar beet juice are utilised to keep prices down. In more delicate fermentations, purified glucose, sucrose, glycerol, or other sugars may be substituted to reduce fluctuation and help maintain the purity of the final product. In order to select the organisms that express the enzymes in a considerable number, starch may be fed to those organisms to create enzymes such beta galactosidase, invertase, or other amylases [4-6].

**Nitrogen**: Most organisms require supplies of nitrogen in order to produce proteins, nucleic acids, and other biological elements. Depending on the organism's enzyme capacity, nitrogen can be provided as bulk protein, such as soy meal, pre-digested polypeptides, such as peptone or tryptone, or as ammonia or nitrate salts.

**Phosphorus:** Phosphorus is required for the synthesis of nucleic acids as well as the phospholipids found in cellular membranes. The quantity of phosphate that must be added is determined by the nature of the broth, the requirements of the organism, and the intended outcome of the fermentation.

**Growth factors** and **trace nutrients**: Growth factors and trace nutrients are given to the fermentation broth for organisms that cannot synthesise all the vitamins they require. Yeast extract is a traditional source of minerals and vitamins for fermentation media. While using purified carbon and nitrogen sources may be necessary, unrefined carbon and nitrogen sources typically contain inorganic nutrients, such as trace metals including iron, zinc, copper, manganese, molybdenum, and cobalt. Fermentations that produce a lot of gas will typically form a layer of foam because fermentation broth frequently contains a variety of proteins, peptides, or starches that can reinforce foam. To prevent the formation or accumulation of this foam, antifoaming agents may be used. To keep pH levels close to ideal, mineral abrasive salts like carbonates and phosphates can be utilized. A chelating agent may be required when metal ions are present in large quantities [6-8].

**Types of fermentations:**

* Production of biomass (viable cellular material)
* Production of extracellular metabolites (chemical compounds)
* Production of intracellular components (enzymes and other proteins)
* Transformation of substrate (in which the transformed substrate is itself the product)

The organisms can be made from bacteria, yeast, mould, animal cells, or plant cells. The specific organisms used in the fermentation need to take into account factors like temperature, nutrition, and dissolved oxygen levels [1-3].

**Production of biomass**

Sometimes the expected outcome of fermentation is microbial cells or biomass. Examples include single cell proteins, baker's yeast, lactobacillus, and *E. coli*. For the production of single-cell protein, algae are grown in huge open ponds that allow for photosynthesis. If the biomass is to be used as a starter culture for additional fermentations, care must be taken to prevent mutations.

**Production of extracellular metabolites**

Primary metabolites are produced during the organism's growth phase, whereas secondary metabolites are produced during the stationary phase of an organism's life cycle. Some examples of primary metabolites are ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides. Examples of secondary metabolites include the medications penicillin, cyclosporin A, gibberellin, and lovastatin [4, 5].

Primary metabolites**:** Primary metabolites are substances produced by an organism's normal metabolism throughout its growth period. Lactic acid and ethanol, which are both products of glycolysis, are the examples. Citric acid is a byproduct of the citric acid cycle that some *Aspergillus niger* strains produce to acidify their environment and keep out competitors. A few *Coryne* bacteria species can produce lysine, threonine, tryptophan, and other amino acids, whereas a few *Micrococcus* species can produce glutamate. The ordinary "operations" of the cell produce all of these compounds, which are then released into the environment. So, to extract the product, the cells do not need to be burst [4-7].

Secondary metabolites:Compounds produced in the stationary phase are known as secondary metabolites. Penicillin, for example, inhibits the growth of bacteria that may compete with Penicillium moulds for nutrients. Some bacteria, including *Lactobacillus* species, have the ability to create bacteriocins, which also inhibit the growth of competing bacteria. These substances have clear benefits for those who want to stop the spread of bacteria, either as antibiotics or as antiseptics (such as gramicidin S). Secondary metabolites are also produced, some of which are fungicides like griseofulvin. Similar to primary metabolites, secondary metabolites are often not synthesized in the presence of glucose or other carbon sources that would facilitate the growth. They are also released into the surroundings without damaging the cell membrane [4-6].

**Production of intracellular components**

Microbial enzymes such catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, and many others are among the intracellular components. Recombinant proteins including insulin, the hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase, and others are also made using this technique. The main distinction between this method and the others is the requirement to rupture (lyse) the cells at the conclusion of fermentation and to manage the environment to increase the amount of the product [6-8].

**Transformation of substrate**

The biotransformation of phenylacetylcarbinol and steroids involves converting one substance into another. It comprises the transformation of a raw material into a finished product in the cases of food fermentations and sewage treatment.

**Food fermentation**

Making bread, wine, cheese, curds, idli, dosa, and other ancient fermented foods may be traced back more than 7,000 years. They were created long before man even knew the existence of microbes. As result of the fermentation process include brewing of beer and some food like Marmite [5,6].

**Ethanol fuel**

Fermentation is the main process for creating ethanol for ethanol fuel. Common crops including corn, potatoes, cassava, and sugar cane are fermented by yeast to produce ethanol, which is subsequently converted into fuel.

**Sewage treatment**

During the sewage treatment process, enzymes made by microorganisms break down sewage. The breakdown of solid organic molecules results in the production of carbon dioxide and harmless soluble compounds. The produced liquids can either be used as liquid fertilizers or treated to remove any bacteria before being thrown into the sea or rivers. Sludge, additionally called digested solids, is dried and used as fertilizer. Gaseous waste materials, such methane, may be transformed into biogas and used to power electric generators. Bacterial digestion has the advantage of reducing the quantity and odor of sewage, which minimizes the requirement for dumping area [5-7].

**Agricultural Feed**

It is possible to feed animals, especially ruminants with fermented waste products from the agroindustrial sector. The degradation of cellulosic wastes by fungi has increased the protein content and improved the digestibility of the wastes.

**Fermentation process**

The different stages of fermentation includes:.

**Stage I:** Upstream processing, which includes sanitation, air purification, the preparation of a liquid medium, the removal of particulates and inhibitory compounds from the medium?

**Stage II:** In fermentation, biological agents like microbes are used to transform substrates into the desired product.

**Stage III:** The downstream processing of fermentation includes the separation of cells from fermentation broth, the purification and concentration of the desired product, as well as the disposal or recycling of trash.

**Types of Fermentation Processes**

**Submerged Cultivation**

In bioreactors, microbial cells are submerged to achieve optimum productivity and yield and manufacture high-quality end products. A variety of products can be produced during batch, fed-batch, or continuous process by cultivation of microorganisms in industrial bioreactors.

**Batch Cultivation**

A batch culture takes place in an aseptic environment in which the medium, nutrients, and inoculum are added to the bioreactor. Throughout cultivation, the volume of culture broth should remain constant in the bioreactor. The minor variations in culture volume are brought on by sampling or adding air or gas into the culture, as well as by a low feed rate of acid/base solutions to maintain the pH at a desired level. Due to their small size in relation to the bioreactor's working capacity, these alterations are usually ignored. A batch process involves a number of steps, including preparing the medium, filling the bioreactor, sterilizing in place, inoculating, cultivating, harvesting the product, and cleaning the bioreactor. To carry out batch operations as effectively as feasible, a high rate of product synthesis, productivity optimization, and maximum end-product yield are required [7-9].

Advantages of batch culture:

* Due to the quick development, the possibility of contamination or cell mutation was reduced.
* Compared to continuous operations, less capital is required to produce the same volume of bioreactors.
* Greater flexibility to different biological and product systems.
* Many compounds are produced in a single reactor.

**Disadvantages of Batch Culture:**

* The working volume of the nutrient becomes depleted;
* There is the substrate restriction and depletion.
* As the system is closed and there is no stream flow to remove effluent, which leads to accumulation of toxins.
* The substrate depletion may cause the growth pattern to quickly reach the death phase in older cultures.
* Due to the batch system's prolonged operation, vital nutrients become depleted and metabolites accumulate as byproducts.
* Inhibition may prolong biocatalytic processes. By preventing enzyme activities, byproducts of inhibitory products might disrupt the cells.
* The batch process has the conventional production issue of needing a cycle. The product must be delivered for downstream processing, after which the system must be cleaned and loaded with fresh feed, making the process extremely labor-intensive for downtime and cleaning [6-8].

**Fed-Batch Cultivation**

Fed-batch culture is a semi-open system in which the product is preserved inside the bioreactor while one or more nutrients are aseptically added gradually. The bioreactor’s culture broth volume increases throughout this time.

A**dvantages** of fed-batch over batch cultures are:

* There is a potential extension of product synthesis.
* There is ability to boost cell densities and, consequently, product amounts, which are normally inversely correlated with biomass concentration.
* There is the capacity to boost the yield or productivity by carefully and systematically by adding the nutrients.

**Disadvantages:**

* Lower productivity levels as a result of the time required to fill, heat, sterilize, cool, empty, and cleaning of the reactor.
* It has higher labour expenses.

The common food fed-batch fermentations include the mass production of baker's yeast, the production of pure ethanol, which is then combined with other ingredients to create alcoholic beverages, such as liquors, and the submerged acetification process used to produce vinegar [7,8].

**Continuous Cultivation**

The continuous culture provides nutrients to the bioreactor constantly and aseptically while simultaneously removing the culture broth, which contains cells and metabolites. The volume of the culture broth is constant as a result of the constant feed-in and feed-out rates. In Continuous culture, chemostat is characterised by a steady specific growth rate of cells equal to the dilution rate and is controlled by the availability of the limiting nutrient. However, the microbial density or turbidity can be kept constant if a constant volume is kept during the exponential phase of growth by making sure that the rate of broth outflow equals the rate of fresh medium input. The nutristat type can be utilised (a constant parameter associated with cell growth controlled by the dilution rate). The chemostat is extensively used in practise as well as in laboratories [7-9].

A**dvantages** of continuous culture (chemostat) over the batch mode:

* There is the capacity to establish environments that enhance and prolong product synthesis.
* There is capacity to maintain consistently high levels of product quality (the steady state is characterised by a homogeneous cell culture represented by a constant concentration of biomass and metabolites), and
* There is a discernible reduction in the "unprofitable" periods of the bioreactor operation.

Despite these advantages, a number of problems still impede continuous operation from being widely used on a large scale. These include:

* iIncreased risk of contamination due to the pumping in and out of the bioreactor's medium.
* A long-term operation's vulnerability to genetic changes in the producing strain, and
* Technical facilities might demand further investments [8-10].

**Solid Substrate Fermentation**

The terms solid substrate fermentation are used to describe processes in which microbes are grown on the surface of concentrated, water-insoluble substrates (containing polysaccharides as a carbon and energy source) with little or no free water. This process was developed in the Eastern countries, where it has been employed for many centuries to create classic delicacies like soy sauce, miso, and sake. Due to the very low water activity associated with solid substrate fermentation, this system's key characteristics differ greatly from those of traditional submerged cultivation [9.10]. There are several **advantages** of solid substrate fermentation over the conventional submerged technology such as:

* The utilisation of a concentrated medium, which minimises reactor size and saves investment costs.
* There is minimal risk of yeast and bacterial contamination because of the substrate's complexity and low moisture content.
* Higher product yield and easier product recovery, and
* Agricultural wastes are utilized in some applications as substrates.

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