FERMENTATION TECHNOLOGY

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.

Keywords: Fermentation, Biotech, pasteurization, phosphorylation, bioreactors, batch culture

Authors

Dr. Chandan Das
Department Pharmacognosy
School of Pharmacy and Life Sciences
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 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

I. HISTORY

Fermentation technology has been used by humans to create food and beverages since the Neolithic period, with records found in China (7000-6600 BCE), Georgia (6000 BCE), Egypt (3150 BCE), Babylon (3000 BCE), Mexico (2000 BCE), and Sudan (1500 BCE). Louis Pasteur (1822-1895) initiated the research through a series of experiments involving living microorganisms. Pasteur stated in 1857 that a living microorganism is responsible for the production of lactic acid. When he discovered how bacteria contribute to food degradation in 1860, his research resulted in the invention of pasteurization.

Despite previous theories, milk sourness is caused by bacteria, not a chemical transformation. A specific microorganism causes a specific type of fermentation and produces a specific type of finished product. Despite the fact that living microorganisms cause fermentation, this discovery neither explains how it occurs nor establishes that microorganisms cause fermentation. Many unsuccessful attempts have been made by scientists, including Pasteur's, to extract the fermentation enzyme from yeast. Eduard Buechner (1897), a German chemist, discovered that ground yeast could ferment sugar solutions in the same way that living yeast could, resulting in the production of alcohol and carbon dioxide. Since then, the term "enzyme" has been applied to all ferments. In 1907 Buechner received the Nobel Prize in Chemistry. [1-3].

Alcohol or acids are produced when sugar is fermented. Lactic acid fermentation occurs in oxygen-depleted yeast and bacterial cells. Microorganisms are grown on a suitable medium as part of fermentation, often for a specific purpose such as the production of enzymes, vaccines, antibiotics, or food additives. Because an electron transport chain requires oxygen to function, fermentation replaces photosynthesis as the primary method of producing ATP. The NADH and pyruvate produced during glycolysis are converted into NAD^+ and a number of other small molecules during fermentation. Respiration generates ATP by using NADH and pyruvate in the presence of oxygen. Oxidative phosphorylation generates far more ATP than glycolysis alone. Furthermore, when oxygen is present, cells stop fermentation. Pyruvate is the inorganic phosphate (CH_3COCOO, Pi). Substrate-level phosphorylation transforms two ADP molecules and two Pi molecules into ATP and water. Two NAD^+ molecules are also changed into NADH. [2-3].

II. FERMENTATION

"Fermentation" is the term used to describe the process of microbial growth and product formation in aerobic, microaerobic, or anaerobic environments. When anything is intentionally mixed with air, it is said to be aerobic. When microbial growth occurs, the air that was initially present in the microaerobic environment is either eaten or replenished. Since oxygen is toxic to cells, anaerobic fermentation keeps it out of the fermentation media. The fermentation process is how alcohol is produced. Beer and wine are produced by the fermentation of grains and fruits. Fermented is another word for a food that has soured. Any technique that produces alcoholic beverages or acidic dairy products is considered fermentation technology. Other definition of fermentation technology is that; any important microbiological process Which occurs with or without alcohol. Any significant microbiological process that occurs in the presence or absence of air; any metabolic process that only releases energy under anaerobic conditions; any physiological pathway that uses an

organic molecule as the final electron acceptor, releases energy from a sugar or other organic molecules, and does not need oxygen or an electron; [1-4].

III. 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.

1. **Ethanol fermentation**: Alcohol fermentation produces the byproducts i.e., methanol and carbon dioxide. The fermentation of one glucose molecule results in the production of two ethanol molecules and two carbon dioxide molecules:

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

2. **Lactic acid fermentation:** It refers 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.
     $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$

     Despite previous theories, milk sourness is caused by bacteria, not a chemical transformation. A specific microorganism causes a specific type of fermentation and produces a specific type of finished product. Despite the fact that living microorganisms cause fermentation, this discovery neither explains how it occurs nor establishes that microorganisms cause fermentation. Many unsuccessful attempts have been made by scientists, including Pasteur's, to extract the fermentation enzyme from yeast. Eduard Buechner (1897), a German chemist, discovered that ground yeast could ferment sugar solutions in the same way that living yeast could, resulting in the production of alcohol and carbon dioxide. Since then, the term "enzyme" has been applied to all ferments. In, Buechner received the Nobel Prize in Chemical reactions.

     $C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$

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].

3. **Aerobic respiration:** The pyruvate produced during glycolysis is completely oxidized by aerobic respiration, resulting in an increase in ATP and NADH via the citric acid cycle and oxidative phosphorylation. However, oxygen is required for this to happen. While obligatory anaerobes do not require oxygen, facultative anaerobes do, and thus oxygen is toxic to them. Lactic acid fermentation is one of the fermentation processes used to regenerate NAD^+ in the absence of oxygen.

4. **Hydrogen gas production in fermentation:** The pyruvate produced during glycolysis is completely oxidised by aerobic respiration, resulting in an increase in ATP and NADH via the citric acid cycle and oxidative phosphorylation. However, oxygen is required for this to happen. While obligatory anaerobes do not require oxygen, facultative anaerobes do, and thus oxygen is toxic to them. Lactic acid fermentation is one of the fermentation processes used to regenerate NAD^+ in the absence of oxygen.

5. **Methane gas production in fermentation:** Methane and carbon dioxide may be produced during the dismutation process of acetic acid. As a consequence of their fermentative metabolism, methangenic archaea catalyse this disproportionation reaction. The methyl group of acetic acid receives an electron from the carboxylic group's carbonyl function, which produces CO_2 and methane gas, respectively.

6. **Industrial fermentation:** In industrial fermentation, fungi and bacteria are purposefully used to produce goods that are beneficial to people. Both the food sector and other businesses can use fermented goods. Fermentation is used to create a variety of common chemicals, including acetic acid, citric acid, and ethanol. For aerobic fermentation, the amount of microorganisms, cells, cellular components, enzymes, temperature, pH, and oxygen all affect how quickly fermentation takes place.

The concentrated solution is typically used throughout the product recovery process. A few of the commercially available enzymes produced through fermentation utilizing genetically engineered bacteria include rennet, invertase, and lipase. In other instances, such as with starter cultures of baker's yeast and lactic acid bacteria, the goal is the generation of biomass itself. [4].

7. **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 bio-ethanol 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 utilized 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**: Nitrogen is necessary for the majority of organisms to make proteins, nucleic acids, and other biological components. Nitrogen can be given as bulk protein, like soy meal, pre-digested polypeptides, like peptone or tryptone, or as ammonia or nitrate salts, depending on the organism's enzyme capacity.

    - Phosphorus: Phosphorus is necessary for the synthesis of both phospholipids, which make up cellular membranes, and nucleic acids. The type of broth, the needs of the organism, and the desired outcome of the fermentation all affect how much phosphate needs to be supplied.

    - **Growth factors** and **trace nutrients**: For organisms that cannot synthesize all the vitamins they need, growth factors and trace nutrients are added to the fermentation broth. Traditional sources of vitamins and minerals for fermentation media include yeast extract. Unrefined carbon and nitrogen sources typically contain inorganic nutrients, such as trace metals including iron, zinc, copper, manganese, molybdenum, and cobalt, even though employing pure carbon and nitrogen sources may be important. Because fermentation broth generally contains a range of proteins, peptides, or starches that can reinforce foam, fermentations that release a lot of gas will typically form a layer of foam. Antifoaming compounds may be used to stop the production or accumulation of this foam. Mineral abrasive salts like carbonates and phosphates can be used to maintain pH values that are close to optimum. When there are significant amounts of metal ions present, a chelating agent may be necessary. [6-8].

IV. TYPES OF FERMENTATIONS

1. Production of biomass (viable cellular material)
2. Production of extracellular metabolites (chemical compounds)
3. Production of intracellular components (enzymes and other proteins)
4. 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].

1. **Production of biomass:** Occasionally bacterial communities or biomass are the predicted result of fermentation. Single cell proteins, baker's yeast, lactobacillus, and E. coli are a few examples. Algae are produced in large open ponds that permit photosynthesis to produce single-cell protein. Mutations must be avoided if the biomass is to be employed as a starting culture for additional fermentations.

2. **Production of extracellular metabolites:** In contrast to secondary metabolites, which are created during an organism's stationary phase of its life cycle, primary metabolites are produced during an organism's growth phase. Ethanol, citric acid, glutamic acid, lysine, vitamins, and polysaccharides are a few examples of primary metabolites. The drugs penicillin, cyclosporin A, gibberellin, and lovastatin are examples of secondary metabolites. [4, 5].

   Primary metabolites: During an organism's growing period, its regular metabolism produces a variety of chemicals known as primary metabolites. The examples are the glycolysis byproducts lactic acid and ethanol. Some Aspergillus niger strains create citric acid as a byproduct of the citric acid cycle in order to acidify their surroundings and keep out rivals. A few Micrococcus species can create glutamate, while a few Coryne bacteria species can produce lysine, threonine, tryptophan, and other amino acids. All of these substances are created by the normal "operations" of the cell and subsequently discharged into the environment. Therefore, bursting the cells is not necessary to extract the substance. [4-7].

3. **Secondary metabolites:** Secondary metabolites are substances created during the stationary phase. For instance, penicillin prevents the development of bacteria that would compete with penicillium moulds for resources. Bacteriocins, which some bacteria can produce and which also prevent the growth of rival bacteria, are produced by Lactobacillus species among others. These compounds, whether used as antibiotics or antiseptics, provide evident advantages for those wishing to prevent the spread of microorganisms (such as gramicidin S). Additionally, secondary metabolites, some of which are fungicides like griseofulvin, are formed. Similar to primary metabolites, secondary metabolites are frequently not produced when glucose or other carbon sources are present since they would promote growth. Additionally, they are discharged into the environment without causing cell membrane damage. [4-6].

4. **Production of intracellular components:** Amongst some of the cellular contents are microbial enzymes including catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, and many others. This method is also used to produce recombinant proteins, such as insulin, the hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase, and others. The requirement to rupture (lyse) the cells at the end of fermentation and to control the environment to enhance the amount of the product is the key difference between this approach and the others. [6-8].

5. **Transformation of substrate:** Steroids and phenylacetylcarbinol undergo biotransformation, which includes changing one chemical into another. In the situations of food fermentations and sewage treatment, it entails the conversion of a raw material into a finished product.

6. **Food fermentation:** More than 7,000 years of history can be attributed to the creation of ancient fermented foods like bread, wine, cheese, curds, idli, and dosa. Before man even learned about germs, they were already in existence. Beer is produced as a byproduct of the fermentation process, as are some foods like Marmite. [5,6].

7. **Ethanol fuel:** The primary step in producing ethanol for ethanol fuel is fermentation. Yeast ferments common crops including corn, potatoes, cassava, and sugar cane to create ethanol, which is then transformed into fuel.

8. **Sewage treatment:** Enzymes produced by microorganisms during the sewage treatment process break down sewage. Carbon dioxide and safe soluble chemicals are produced during the breakdown of solid organic molecules. The resulting liquids can either be cleaned to remove any bacteria before being dumped into the sea or rivers, or they can be utilized as liquid fertilizers. Digested solids, often known as sludge, are dried and utilized as fertilizer. Biogas can be created from gaseous waste products, such methane, and utilized to run electric generators. Bacterial digestion provides the benefit of decreasing the volume and smell of sewage, which reduces the need for a dumping area. [5-7].

V. Agricultural Feed

Fermented byproducts from the agroindustrial sector can be used to feed animals, notably ruminants. The protein content and digestibility of cellulosic wastes have both risen as a result of fungi's breakdown of the wastes.

1. **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.

2. **Types of Fermentation Processes**

   - **Submerged Cultivation:** Microbial cells are immersed in bioreactors to maximize productivity and production and produce high-quality end products. By cultivating microorganisms in industrial bioreactors, a range of products can be made during batch, fed-batch, or continuous processes.
   - **Batch Cultivation:** In an aseptic setting, the medium, nutrients, and inoculum are added to the bioreactor during a batch culture. The bioreactor's culture broth volume

should remain consistent throughout cultivation. Small changes in culture volume are caused by measuring or adding air or gas, as well as by feeding acid/base solutions at a low rate to keep the pH at a specified level. These modifications are typically disregarded because of their modest size in comparison to the bioreactor's operating capability.

The preparation of the medium, filling the bioreactor, sterilizing it in place, inoculating, cultivating, harvesting the product, and cleaning the bioreactor are all phases in a batch process. A high rate of product synthesis, productivity optimization, and maximum end-product yield are necessary to carry out batch operations as efficiently as possible. [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 bio-catalytic 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].

3. **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 a cidification process used to produce vinegar [7,8].

4. **Continuous Cultivation:** The continuous culture continuously and aseptically delivers nutrients to the bioreactor while removing the culture broth, which contains cells and metabolites. The volume of the culture broth remains constant due to the constant feed-in and feed-out rates.

Chemostat is characterized in continuous culture by a steady specific growth rate of cells equal to the dilution rate and is controlled by the availability of the limiting nutrient. However, if a constant volume is maintained during the exponential phase of growth, the microbial density or turbidity can be kept constant by ensuring that the rate of broth outflow equals the rate of fresh medium input. The nutristat type may be used (a constant parameter associated with cell growth controlled by the dilution rate). The chemostat is widely used in practice and 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 characterized 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:

- Increased 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].

5. **Solid Substrate Fermentation**

1. Microscopic organisms develop on the surface of concentrated, water-insoluble substrates (containing polysaccharides as a carbon and energy source) with little or no

free water in processes known as solid substrate fermentation and water-insoluble substrate fermentation. This method originated in Eastern countries, where it has been used for centuries to produce traditional delicacies such as soy sauce, miso, and sake. Because of the very low water activity associated with solid substrate fermentation, the key characteristics of this system differ significantly from those of traditional submerged cultivation [9.10]. The use of a concentrated medium, which reduces reactor size and saves investment costs, is one of the many advantages of solid substrate fermentation over traditional submerged technology. There is minimal risk of yeast and bacterial contamination because of the substrate's complexity and low moisture content.

2. Higher product yield and easier product recovery, and
3. Agricultural wastes are utilized in some applications as substrates.

REFERENCES

[1] Y. Chisti, Solid substrate fermentations, enzyme production, food enrichment, in *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, vol. v, M.C. Flickinger and S.W. Drew, Eds. New York: John Wiley & Sons, Inc., 1999, pp. 2446-2462.
[2] N.M. Fish, and M.D. Lilly, The interactions between fermentation and protein recovery, Biotechnology, vol.2, pp. 623-627, 1984 .
[3] Y. Chisti, and M. Moo-Young, Clean-in-place systems for industrial bioreactors: design, validation and operation, J. Ind. Microbiol, vol. 13, pp. 201-207, 1994.
[4] Y. Chisti, Shear sensitivity , in *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, vol. v, M.C. Flickinger and S.W. Drew, Eds. New York: John Wiley & Sons, Inc., 1999, pp. 2379-2406.
[5] H. B. Reisman, Economic analysis of fermentation process, Ann. Rep. Fern. Process, vol. 1, pp. 49-71, 1971.
[6] G.T. Banks, Scale-up of fermentation process, Topic in Enzyme and Fermentation Biotechnology, vol. 3, pp. 170-267, 1979.
[7] W.H. Bartholomen, Scale-up of submerge fermentation, Adv. App. Micro, vol. 2, pp. 289-300, 1960.
[8] G.H. Bell and M. Gallo, Effect of impurities on oxygen transfer, Process Biochem, vol.6, pp. 33-35, 1971.
[9] N.Y. Chen, The design of airlift fermentors for use in biotechnology, Biotechnol. Gen. Eng. Rev, vol. 8, pp. 379-396, 1990.
[10] D.W. Hubbard, L.R. Harris and M.K. Wierenga, Scale-up of polysaccharide fermentation, Chem. Eng. Prog, vol. 84, pp. 55-61, 1988.

