FERMENTATION TECHNOLOGY

Dr. Mahenthiran R
Department of Microbiology, Dr N.G.P. Arts and Science College,
Corresponding author:
Assistant Professor, Department of Microbiology, Dr N.G.P. Arts and Science College,
Coimbatore- 641048, Tamilnadu, India.
e- mail: mahenthiran@drngpasc.ac.in

Ms. Arunavarsini K
Department of Microbiology, Dr N.G.P. Arts and Science College,
Ph.D Scholar, Department of Microbiology, Dr N.G.P. Arts and Science College,
Coimbatore- 641048, Tamilnadu, India.
e- mail:arunvarsini@gmail.com

Mrs. Durgadevi L
Department of Microbiology, Dr N.G.P. Arts and Science College,
Ph.D Scholar, Department of Microbiology, Dr N.G.P. Arts and Science College,
Coimbatore- 641048, Tamilnadu, India.
e- mail: deeptisuren@gmail.com

ABSTRACT

The chapter describes the importance, history, uses, stages, process and equipments used for fermentation. Fermentation which was previously an art has gained importance as a science and its horizons are expanding over the years. A substrate is inoculated with the desired microorganisms to start a fermentation. Because there are so many different substrates, microbes, and possible products. Fermentation is inevitable since it has a vital role to play in the food industry, production of life saving vaccines and drugs, in the agricultural sector, de-composting of biomass aiding in compost making, sewage treatment, environmental management etc. The knowledge of the history, processes, types and stages of fermentation are important to expand and explore options where this technology can be used accordingly and precisely in future. The study about the fermenters, the concept of working, parts of the fermenter and design facilitates in selecting the appropriate fermenter based on the inoculum used and end products obtained.

Key words- Fermentation, Microorganisms, Substrate, Inoculom, Fermentor.

1.INTRODUCTION

The metabolic process of fermentation transforms sugar into compounds like acid, gas, and alcohol. The Latin word for "boil" **(fervere)** is where the word "ferment" first appeared. The boiling look is caused by the anaerobic catalysis of carbohydrates, which generates carbon dioxide bubbles. Yeasts, molds, and bacteria—beneficial microorganisms that receive their energy throughout the process—as well as anaerobic circumstances are ideal for fermentation to occur. There are many different substrates, bacteria, and products that can be used in the countless different fermentation processes.

By inoculating a substrate with the necessary microbes under optimum environmental conditions, fermentation is started and helps produce the intended product. The produced crude product can either be used directly or processed to separate out specific molecular components. Fermentation is used in many aspects of daily life, including the production of industrial goods, the treatment of sewage, and environmental management. Bread, cheese, wine, beer, coffee, industrial and medicinal enzymes, vaccines, therapeutic proteins, bio polymers, and other goods are all products of fermentation. Energy sustainability may be facilitated by the fermentation-based production of fuels like bio-ethanol. Additionally, it helps with digestion and immune improvement, among other health advantages. Probiotics, which are good to the immune system, are abundant in fermented meals.

II. History

About 12000 years ago, the history of fermentation began, although at that time, no one was aware of the underlying science. They were unaware of either the connection between fermentation and microorganisms or the causes of fermentation. Simple oral tradition and practices were used to transmit the rules and guidelines. Louis Pasteur, a French microbiologist, was the first scientist to research fermentation in 1854, and he showed that living organisms, particularly yeasts, start fermentation.

The German scientist Eduard Buchner won the Nobel prize in 1907 for demonstrating that fermentation is caused by yeast cell enzymes. In 1929, Arthorn Harden and Hans Euler-Chelpin, two scientists, received the

Noble Prize for their work in which they precisely defined how enzymes are produced during ferme--ntation. Antibiotics were created utilizing this method by the 1940s.

III. STAGES OF FERMENTATION

Generally two stages are involved in fermentation for converting raw material / untreated product into a final product. They are Upstream Processing and Downstream Processing.

A. Upstream Processing :

In fermentation, the upstream processing phase is where the microbes or cells are cultivated and the target product gets produced. The amount and quality of the product produced during the fermentation process are determined by upstream processing, which is a crucial step in the process. To guarantee that the required product is generated in a secure and effective manner, it is important to carefully supervise every step of upstream processing. Upstream processing includes three main areas. They are

- **Producing Microorganisms**: It involves choosing an appropriate microbe, enhancing the strain's productivity and yield, and maintaining the purity and sterility of the strain. A suitable inoculum is created by allowing chosen strains to develop repeatedly.
- **The Fermentation Medium**: By removing particulates and inhibiting compounds from the media, the desired medium is created. It is critical to choose a reliable, affordable source of carbon and energy as well as vital nutrients. For maximum production and profit, media optimization is essential. The media can also be categorized as a technical, specified, or sophisticated medium. Chemically determined substrates make up the defined medium. Substrates for complex media can be inexpensive waste product extracts or hydrolysates with an undetermined composition. For complicated medium, expensive substrates including yeast extract, brain-heart infusion, peptone, and tryptone are frequently utilized. Technical media are more widely utilized and less expensive in industry.
- **The Fermentation Process**: Microorganisms are multiplied during the fermentation process, which results in the creation of the desired product. To maximize the growth of microorganisms, it is typically done in controlled environments. It can either be anaerobic fermentation, which occurs when there is no oxygen present, or aerobic fermentation, which occurs when there is oxygen present.While many industrial fermentations take place in aerobic environments, some processes, like yeast-based ethanol generation, are absolutely anaerobic-environment-required.

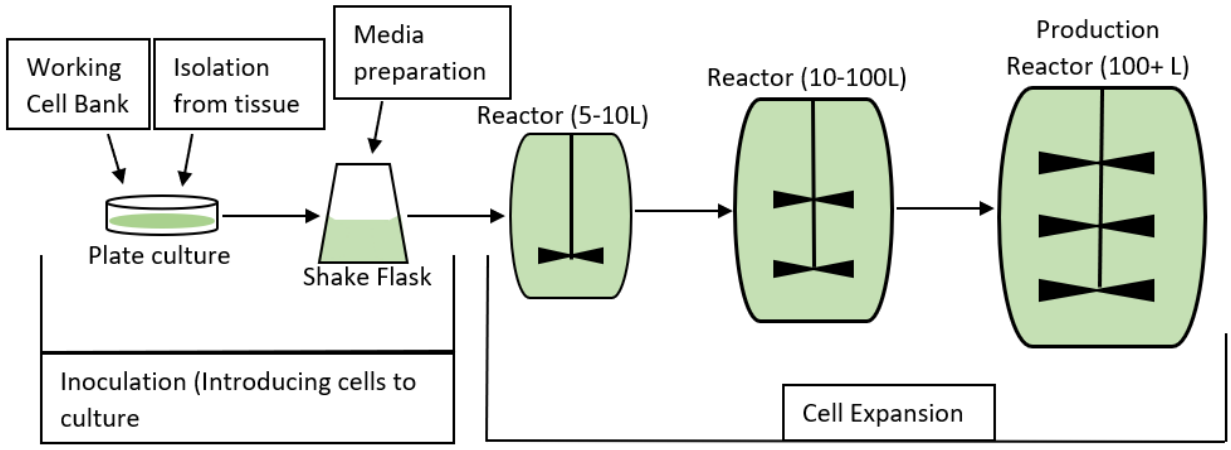

Figure 1: Upstream processing

Depending on how much free water is present in the medium, the process can also be classified as Solid State Fermentation (SSF) or Submerged Fermentation (SmF). Because there is no free-flowing water in SSF, organisms are developed on wet solid substrate. In submerged fermentation, the bacteria develop immersed in a liquid media that contains a lot of free water. Large containers called fermenters, frequently with capacities of several thousand litres, are used for industrial fermentations. Depending on how the culture and media are fed into the fermenter, the fermentation process can be batch, fed-batch, or continuous. The methods used to scale up the process include aeration or system agitation when necessary.

B. Downstream Processing:

It includes all actions taken after fermentation. harvesting, purification, and final processing of fermentation products into dosage forms suitable for the use for which they were intended. Along with increasing product recovery, downstream processing's primary goal is to cut production costs. To turn the final product created or processed in a fermenter into a useful product, it must be used in the downstream stage. Downstream processing includes procedures such as physical separation, which includes adsorption, liquid-liquid extraction, solid-liquid separation, distillation, drying, etc. The downstream processing process consists of three phases.

- **Harvesting of Cells**: Utilizing techniques like flotation, sedimentation, filteration, centrifugation, coagulation/flocculation, etc., the solids/cells and insoluble products are harvested and separated from the culture broth. When gas is added to a liquid, bubbles form in the process of flotation. On the gas bubbles that ascend to the foam layer, where they are gathered, the cells and other solid particles are adsorbed. By introducing flocculating agents such inorganic salt, organic polyelectrolyte, mineral hydrocolloid, etc., flocculation is accomplished. To make it easier to remove the cells, they aggregate into enormous masses. The most popular technique for separating culture filtrate from biomass is filtering. Density differences between the particles to be separated on the medium are what allow for the separation of particles by centrifugation. It is employed to separate liquid from solid particles.
- **Disruption of cells:** Because of their small size, tough cell walls, and high internal osmotic pressure, microbial cell s are typically challenging to disrupt. Cells can be disturbed by specific physical treatments to release intercellular products. They include high pressure homogenization, ultrasonication, thermal shocks, osmotic shocks, chemical extraction, and enzymatic extraction.
- **Purification of cells:** The product is to be recovered with a high level of purity. After concentration, the crude product is purified using techniques like precipitation, liquid extraction, chromatography (adsorption, ion exchange, gel affinity), and electrophoresis. Membrane techniques like ultra filtration and reverse osmosis are also used. The product is finally dried and crystallized.

IV. TYPES OF FERMENTATION

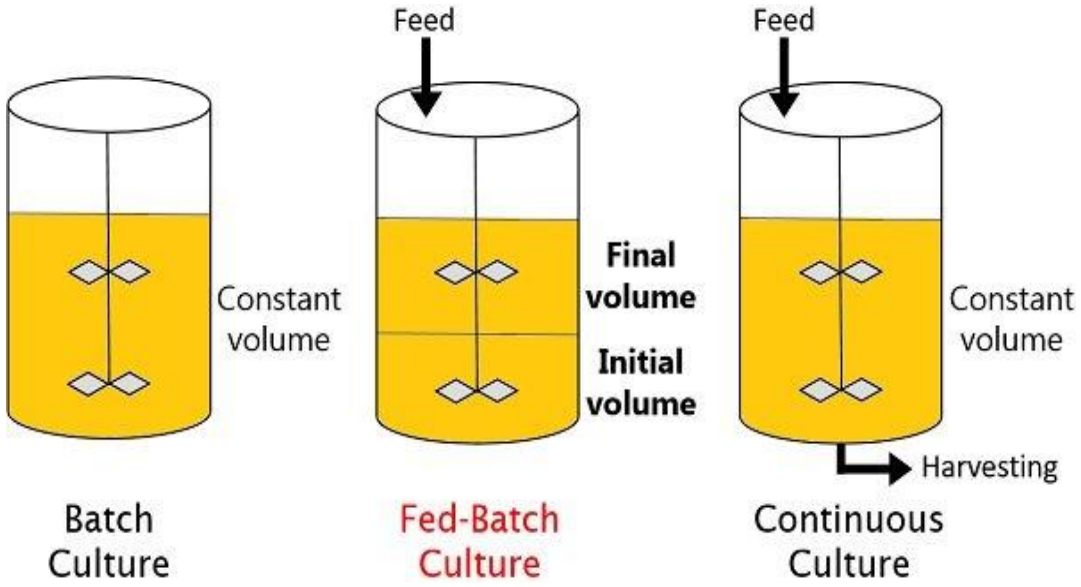

Figure 2: Types of fermentation

A. Batch fermentation:

In batch fermentation, all of the substrate and nutrients are provided at the initial stage of the fermentation process, and the fermentation continue until the required product concentration has been attained. Contrast this with continuous fermentation, where nutrients are continuously added and products are continuously eliminated.Alcoholic beverages, prescription antibiotics, digestive enzymes, and biopolymers can all be produced using a straightforward and adaptable batch fermentation technique. It is appealing for small-

scale production because it is also a relatively affordable method. Batch fermentation involves four stages, which are:

- **Lag phase:** It describes the first stage of fermentation, when cells are adjusting to their new surroundings. During this stage, the cells are not dividing or expanding.
- **Exponential phase:** The exponential phase, in which cells divide quickly, is the stage of growth that is at a rapid pace. During this phase, both the cell concentration and the product concentration exponentially grow.
- **Stationary phase:** Cell growth and cell death rates are identical during the stationary phase. During this phase, both the cell concentration and the product concentration are constant.
- **Death phase:** Death phase: During this stage, cells dies and the product concentration falls.

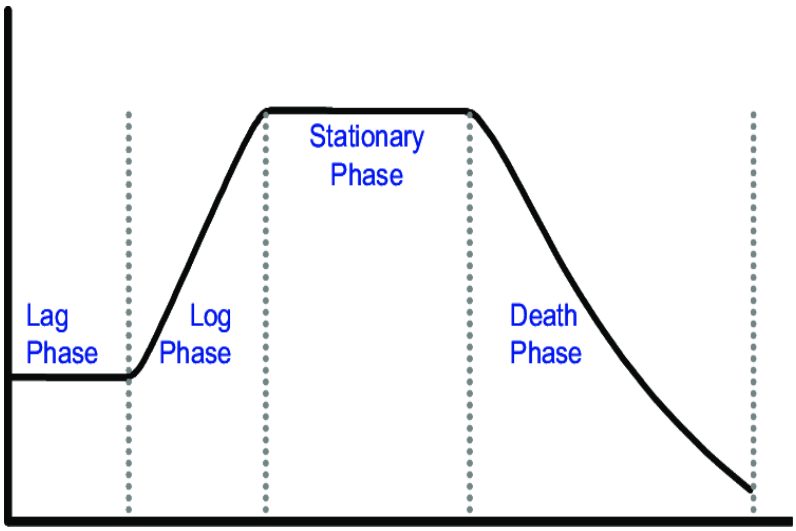

Figure 3: Growth curve

A stirred tank fermentor is primarily used to conduct batch fermentation. A stirrer and a temperature controller are both included in the closed container known as a fermentor, which also has a stirrer to mix the contents of the vessel. To track the fermentation process, the fermentor has pH and dissolved oxygen sensors.

Batch fermentation is a flexible and reasonably priced method that may be used to make a wide range of goods. It is a straightforward procedure that is suited for both small-scale and large-scale production because it is easily scaled up or down.

B. Continous fermentation:

The idea behind continuous fermentation is that new medium is continuously introduced, and both used medium and harvested cells. The frequent supply of nutrients can prolong the bacteria' exponential growth rate. Additionally, the harmful metabolites are eliminated from the culture while the deficient nutrients are replenished. Due to the equal rate of addition and withdrawal, the culture volume is constant.

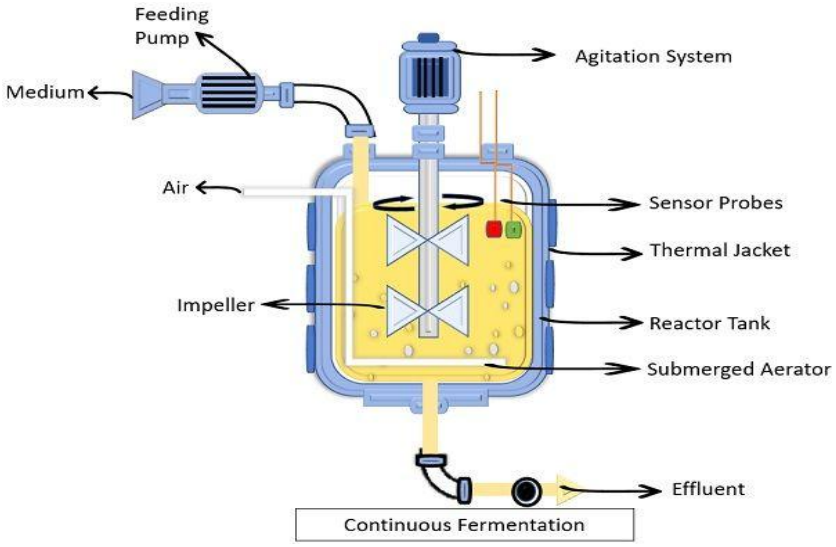

Figure 4: Continuous fermentation

The process involves adding fresh medium or feed solution to the culture, which has no bearing on the fermenter's maximum operating volume. To reach a stable state, the rate of medium exchange can be changed. In a steady state, environmental factors like metabolite concentration and cell growth rate remain constant. Long periods of time can be spent maintaining the culture in its current state. By minimizing downtime, it helps to make this strategy profitable. In this method of fermentation, there are tools to capture the fermenter overflow. Utilizing downstream processing, the fermentation products, including metabolites, are separated from the overflow.

C. Fed-batch fermentation:

A variant of batch fermentation, it involves aseptically adding nutrients. It is a semi-open fermentation system, and as culture is introduced incrementally, the volume of liquid culture in the fermenter grows. For a set amount of time, the inoculated microorganisms are cultured in batch mode, and for the remaining time, nutrients are supplied gradually. At the end of each run, the entire culture suspension is withdrawn. The broth's substrate limitation often determines when to begin feeding, and the feeding profile should be created so that the substrate stays non-excessive while microbial growth is completely supported. The log and stationary phase of the microorganisms in the fermenter are prolonged when the substrate is intermittently introduced. In the exponential growth phase, extra biomass accumulates as a result of the addition of new nutrients.

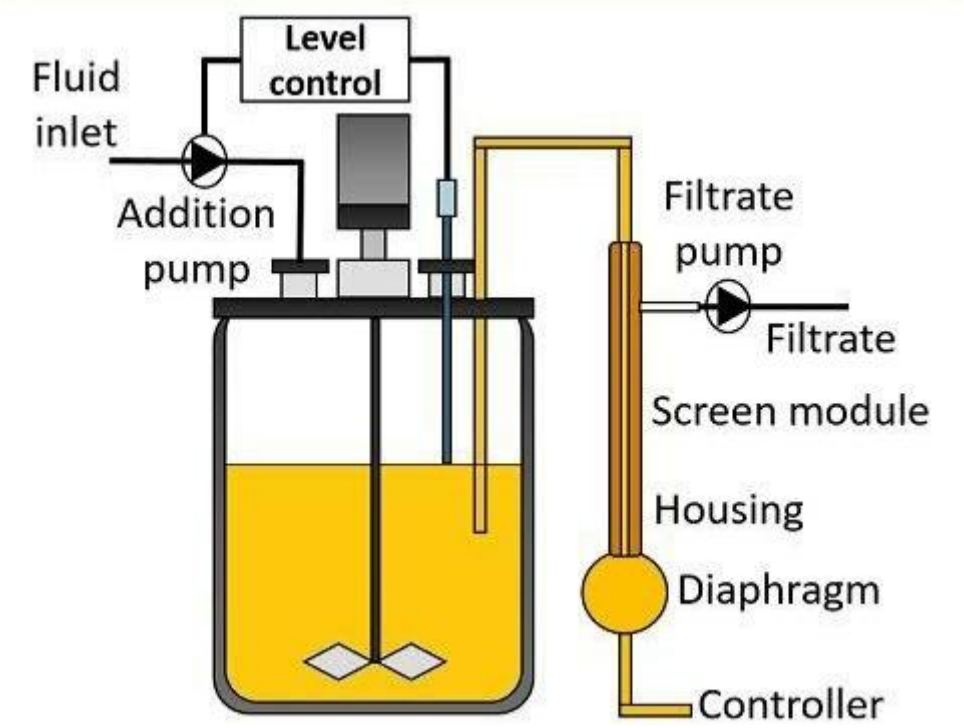

Figure 5: Process of fed- batch fermantation

For bioprocesses aiming for high biomass density or high product output, fed-batch fermentation is highly helpful when the intended product has a favorable correlation with microbial growth. Due to the process's lack of overfeeding the substrate, the accumulation of byproducts is kept to a minimum.The benefits of fed batch culture include increased productivity and greater yields from the addition of nutrients in small amounts. In this approach, the resultant cell densities are likewise very high. When compared to batch fermentation, the product synthesis process can be extended. As a result, it promotes greater output throughout the stationary phase.

V. Fermentor

The fermentation process' fundamental component is the fermenter. A closed vessel with the proper setup for aeration, agitation, pH control, and temperature control is known as a fermenter (bio-reactor). In order to remove the waste biomass of the cultured microorganisms and their products, it is additionally equipped with a drain or overflow vent. It is a containment system that offers bacteria a regulated environment in which to grow. In a liquid culture, the metabolites are created. The device is inevitable to cultivate desirable microbes to produce the suitable product. It also prevents the entry and growth of contaminating microbes from the outside environment. The fermenter is the containment system for cultivation of bacteria and fungi. Bio-reactors are also fermenters which are used to cultivate mammalian or insect cells. A fermenter is used for commercial production where living cells utilize low value substrates to generate high value products. Fermenters are not only used for fermentation but also for food processing and waste treatment.

A. History of fermentor:

De Beeze and Liebmann (1944) used the first large scale fermenter above 20 litre capacity for production of yeast. The British Scientist, Chain Weizmann (1914-1918) developed a fermenter for production of acetone during the first world war. Large scale aerobic fermenters were used for the first time in Central Europe in 1930’s for the production of compressed yeast.

The fermenter comprised of a large cylindrical tank with air was introduced through a series of pipes at the base. In later stages, mechanical impellers were used to increase the rate of mixing in order to break up and disperse the air bubbles. This process aided in compressed air requirements. Forming of vortex in the liquid was prevented by baffles on the walls of the fermenter. Aeration tubes with water and steam were used for cleaning and sterilization and this system was patented by Strauch and Schmidt in 1934.Pencillin production by submerged culture technique in aseptic conditions with good aeration and agitation was a very crucial factor

paving way for the development of a carefully designed and purpose – built fermentation vessel. In 1950, the first pilot fermenter of India was erected at Hindustan Antibiotic Limited, Pimpri, Pune.

B. Parts of Fermenter:

A sizable container known as a fermenter is constructed of stainless steel or another rust-free material. It has a motor that is controlled automatically. Heaters and a thermostat system can control and maintain the temperature. Pipes are used to put or take water and substrate out of the fermenter. Aeration is delivered by a pipeline system and gas sources. Aeration and pH regulation are both done via sensors.

C. Design of fermenters:

Depending on the type and fermentation it is used for, different fermenters have different designs. All varieties of fermenters work with heterogeneous systems, or ones that have two or more of the three phase states of liquid, gas, and solid. For a fermentation to be successful, there must be an effective transfer of mass, heat, and momentum from one phase to another. The following should be provided by a fermenter.

- **Agitation:** The mixing of cells and medium is a requisite for optimum fermentation.
- **Aeration:**Aeration is required in aerobic fermenters to supply the oxygen for carrying out the fermentation process.
- **Regulation of factors:** The factors like temperature, pH, pressure, aeration, nutrient feeding and level of liquid is to be regulated for effective fermentation.
- **Sterilization:** Sterility is to be maintained which ensures the purity of the product.
- **Withdrawal of cells/medium:** Provision for harvesting of cells and medium is vital.

Typically, 20 to 25 percent of the fermenter's volume is left empty of medium called "head space" to promote aeration, foaming, and splashing. The fermenters are made in a way that they should support industrially significant cultures' optimal development and biosynthesis. Modern fermenters are typically coupled with a computer for effective process monitoring and data collecting.

D. Size of fermentor:

Depending on the requirements, different sized fermenters are available. The sizes range from laboratory fermenters of 1-2 liters to ones of 5,000 liters or even more. The process and mode of operation also influence the fermenter's size. The production of diagnostic enzymes and molecules for molecular biology is done in fermenters ranging in size from 1 to 20,000 liters. Enzymes and antibiotics are produced in fermenters with capacities ranging from 40 to 80,000 liters. Wine, beer, amino acids, amylases, and proteases are produced in fermenters ranging in size from 100 to 1,50,000 liters. Amino acids, wine, and beer are produced in fermenters that range in size from 2,000 to 5,000.

E. Construction of Fermenters:

The two types of fermentation are anaerobic and aerobic, respectively. In anaerobic fermenters, just the removal of heat produced during the fermentation process is necessary. On the other hand, aerobic fermenters need the right tools to guarantee effective mixing and administration of enough aeration. The majority of industrial fermenters are built utilizing the following materials and are aerobic.

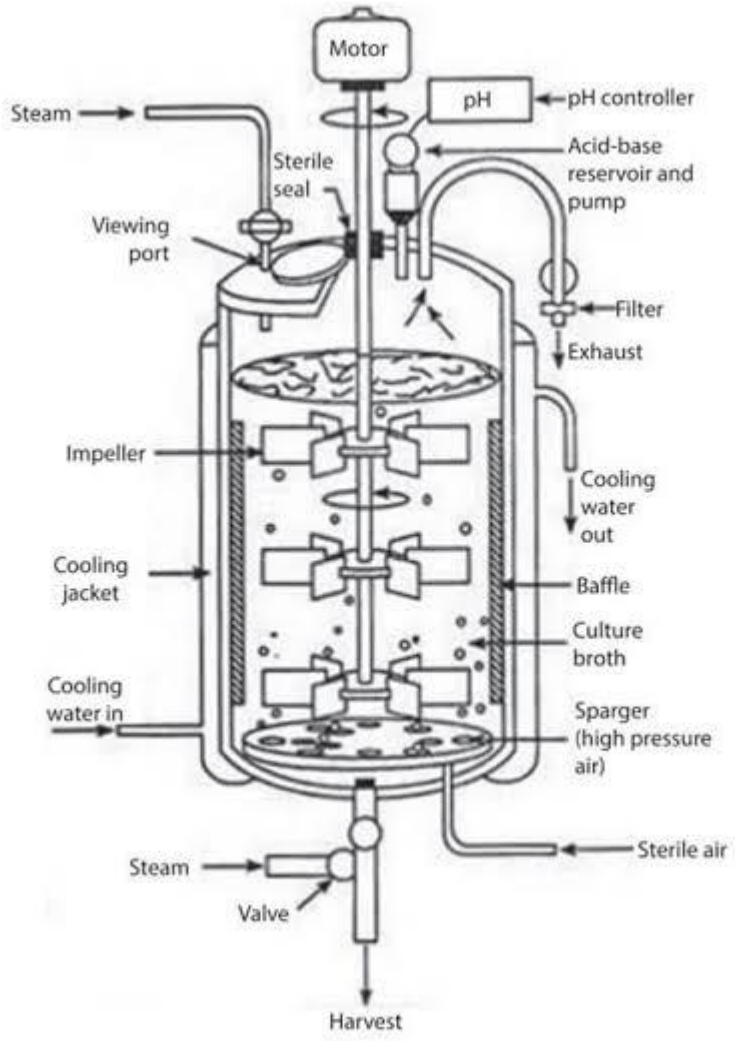

Figure 6: Structure of a fermentor

- **Cooling Jacket:** Large-scale manufacturing fermenters are often made of stainless steel. The fermenter has a cylindrical shape, is closed at the top and bottom, and is equipped with various pipelines and valves. Fermentation in a fermenter must be successfully completed by heating the nutrient medium and removing the heat that is produced. As a result, a cooling jacket is installed externally in a fermenter to facilitate the passage of steam for sterilizing or cooling water. Large fermenters with inadequate heat transfer through the jacket may also include additional internal coils for running cooling water or steam.
- **Aeration system:** The aeration system is a fermenter's most crucial component.Due to oxygen's poor solubility in water and slow rate of transfer into the growing medium, the bacteria in the fermenter have a greater need for oxygen, which increases the demand for oxygen in the culture. To provide adequate oxygen availability and aeration during the cultivation process, a good aeration system must be provided. A fermenter's adequate aeration is ensured by the employment of two aeration tools: the impeller and sparger.
- **Sparger:** High pressure air that has been filtered, sterilized, or oxygenated is forced into the fermenter through a series of holes in a metal ring or nozzle. Air enters the fermenter as microscopic bubbles, and oxygen diffuses into the culture media as a result.
- **Impeller:** Effective fermentation requires stirring or agitating the contents of the fermenter. The culture medium is agitated with the aid of the impeller, also known as an agitator. Stirring makes it possible to perform the following. Both the microbial cells and the gas bubbles are disseminated throughout the liquid culture media, ensuring that the microbial cells have uniform access to the nutrients. The size of the fermenter determines the size and placement of the impeller. Tall fermenters have multiple impellers installed for sufficient aeration and agitation. The impeller must be installed above the fermenter's base and should be 1/3 the diameter of the fermenter.

- **Baffles:** Metal strips called baffles are radially affixed to the fermenter walls. They are used in all kinds of fermenters to increase aeration effectiveness and prevent vortices. Baffles are typically used in fermenters with agitators to enhance aeration effectiveness and reduce vortex formation. Four baffles are typically utilized, however bigger fermenters could include six or eight baffles. To increase cooling, additional cooling coils can be added to baffles. Additionally, the baffles may be set up so that there is a space between them and the fermenter wall. By scouring the area around and behind the baffles, this would reduce the growth of microorganisms on the baffles and the fermenter wall.
- **Temperature Control:** The fermenter requires to have suitable temperature control features. Heat will be produced by both microbial activity and agitation. It might not be necessary to remove or add heat if this heat creates a temperature that is ideal for the fermentation process.
- **Foam regulator:** Most microbial fermentations result in foam. Both a medium component, such as protein present in the medium, or a chemical generated by the microbe can cause foaming. At the air-broth interface, the proteins may denature and form a protein film that is difficult to rupture. The elimination of cells by foaming will result in those cells going through autolysis and releasing additional proteins into the solution. This will further stabilize the foam as a result.

VI. Growth kinetics

The autocatalytic reaction of growth kinetics indicates that the rate of growth is inversely proportional to the cell concentration. Both direct and indirect methods are used to determine the cell concentration. The dry weight of the cells, turbidity (optical density), plate counts, and other direct methods are used to measure the cell number density and mass concentration. The relationship between a microbe's particular growth rate and its substrate concentration is explained by the concept of microbial growth kinetics. The laboratory culture conditions have a significant impact on the kinetics of microbial growth. Because of cost and local availability of specific growth components, it is important to optimize microorganism growth in a given medium. Even Nevertheless, some bacteria have unique needs and only grow in certain growth conditions.

REFERENCES

[1] Kuila. A, Sharma. V (2018). In Principles and Applications of Fermentation Technology.

[2]Avili, M. G., Fazaelipoor, M. H., Jafari, A., & Ataei, S. A. (2012). Comparison between batch and fed-batch production of rhamnolipid by Pseudomonas aeruginosa.

[3]Blaby, I. K. (2011). Modes of Culture / Microbial. In Comprehensive Biotechnology (Second Edition, Vol. 1 Elsevier B.V.

[4] Chisti , Y. and Moo - Young , M. (1999) Fermentation technology, bioprocessing, scale - up and manufacture , in Biotechnology: The Science and the Business , 2nd edn (eds V.Moses , R.E. Cape , and D.G. Springham), Harwood Academic Publishers , New York , pp. 177 – 222 .

[5] Krishna Prasad, Nooralabettu (2010). Downstream Processing- A New Horizone in Biotechnology. Prentice Hall of India Pvt. Ltd, New Delhi. ISBN 978-81-203-4040-4.

[6] M. Rahman, "Medical applications of fermentation technology," Adv. Mater. Res., 2013,
doi: 10.4028/www.scientific.net/AMR.810.127.

[7] Microorganisms and Fermentation of Traditional Foods (Food Biology Series)" 4th Edition
by Ramesh C Ray and Montet Didier.

[8] FAO/WHO, 1996. Fermentation: assessment and research. Report of a Joint FAO/WHO Workshop on Fermentation as a House-hold Technology to Improve Food Safety, 11 – 15 December

[9] Betts JI and Baganz F (2006) Miniature bioreactors: current practices and future opportunities. Microbial Cell Factories, 43(3): 113-119.

[10] Chisti Y (1999) Fermenttaion (industrial): Basic considerations, In: Robinson R, Batt C and Patel P (eds) Encyclopedis of Food Microbiology,Academic press, London, pp. 663-674.

[11] Jagani H, Hebbar K, Gang SS, Raj PV, Chandrashekhar R and Rao JV (2010) An overview of fermenter and the design considerations to enhance its productivity. Pharmacology online, 1: 261-301.

[12] Shuler ML and Kargi F (2004) Bioprocess Engineering, Basic concepts 2nd ed., Pearson education (Singapore) Pvt. Ltd. pp. 327-333.
Srivastava, A. K. (2011). 2.38 – Fed-Batch Fermentation – Design Strategies. Comprehensive Biotechnology

[13] Stanbury, P.F., Whitaker, A. and Hall, S.J. (2001) Principles of Fermentation technology
2nd ed., Pergamon Press, oxford.

[14] Najafpour, G., 2015. Biochemical engineering and biotechnology. Elsevier.
Yang, Y., & Sha, M. (2017). A Beginner's Guide to Bioprocess Modes-Batch, Fed-Batch, and Continuous Fermentation.

[15] Crueger, W., Crueger, A., & Aneja, K. R. (2017). Crueger's biotechnology: A textbook of Industrial microbiology. MedTech.

[16] Ghosh, B., Bhattacharya, D., & Mukhopadhyay, M. (2018). Use of Fermentation Technology for Value-Added Industrial Research. Principles and Applications of Fermentation Technology, August, 141–161.