MICROBIOLOGY COURSE MATERIAL

Semester - V

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CC12: UNIT–3: PART-A: TYPES OF FERMENTATION PROCESSES

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
SEMESTER – V
CC-12: UNIT – 3
FERMENTATION

❖ Definition:

Fermentation is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. For example, yeast performs fermentation to obtain energy by converting sugar into alcohol. Bacteria perform fermentation, converting carbohydrates into lactic acid. Example: Fermentation involves reaction of NADH with an endogenous, organic electron acceptor. Usually this is pyruvate formed from sugar through glycolysis. The reaction produces NAD+ and an organic product, typical examples being ethanol, lactic acid, carbon dioxide, and hydrogen gas (H_2).

❖ What Are the 3 Different Types of Fermentation?

- Lactic acid fermentation- Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation.
- Ethanol fermentation/alcohol fermentation.
- Acetic acid fermentation.

❖ Purpose

- The purpose of fermentation is to regenerate the electron carriers used in glycolysis and produce a small amount of ATP.
- Human muscle cells also use fermentation. This occurs when muscle cells cannot get oxygen fast enough to meet their energy needs through aerobic respiration. But the most important is that it is a step in muscle cell respiration where no oxygen is used. It is the buildup of lactic acid in the muscles from fermentation that makes our muscles sore from more exercise than usual.

- Fermentation is important to humans because it makes food easier to digest. Fermentation helps break down nutrients in food, making them easier to digest than their unfermented counterparts. For example, lactose — the natural sugar in milk — is broken down during fermentation into simpler sugars — glucose and galactose.

- Fermentation is the breakdown of carbs like starch and sugar by bacteria and yeast and an ancient technique of preserving food. People use these organisms to make yogurt, bread, wine, and biofuels. Common fermented foods include kimchi, sauerkraut, kefir, tempeh, kombucha, and yogurt. These foods may reduce heart disease risk and aid digestion, immunity, and weight loss.

❖ Solid State Fermentation (SSF)

Solid State Fermentation (SSF) is a fermentation method used by several industries like the pharmaceuticals, food, textile etc., to produce metabolites of microorganisms using solid support in place of the liquid medium. It is defined as the growth of microbes without free-flowing aqueous phase. The SSF is alternative to submerged fermentation for production of value-added products like antibiotics, single cell protein, PUFA's, enzymes, organic acids, biopesticides, biofuel and aroma production. The support used is especially grain brans, de-oiled oil seed cakes, and other substances alike.

Initially, mostly fungi were used in such fermentation (as these microorganisms were considered to be very optimally active in very low water activity). Later, many bacterial species and yeasts were used to carry out such fermentation also. The microbiological process of SSF has generated great interest in recent years because it can be used for a variety of purposes, supported by some authors who have even indicated numerous advantages over their liquid counterparts (submerged fermentation).

There are many biotechnological processes that involve the growth of organisms on solid substrates in the absence or near absence of free water. Solid state fermentation (SSF) deals with substrates that are solid and contain low moisture levels. The most regularly used solid substrates are cereal grains (rice, wheat, barley, and corn), legume seeds, wheat bran, lignocelluloses such as straws, sawdust or wood shavings, and a wide range of plant and animal materials. Most of these compounds are polymeric molecules insoluble or sparingly soluble in water but most of them are cheap and easily obtainable and represent a concentrated source of nutrients for microbial growth. SSF can be defined in terms of the following properties:

- A solid porous matrix which can be biodegradable or not, but with a large surface area per unit volume, in the range of 103 to 106 m^2/ cm^3, for a ready microbial growth on the solid/gas interface.

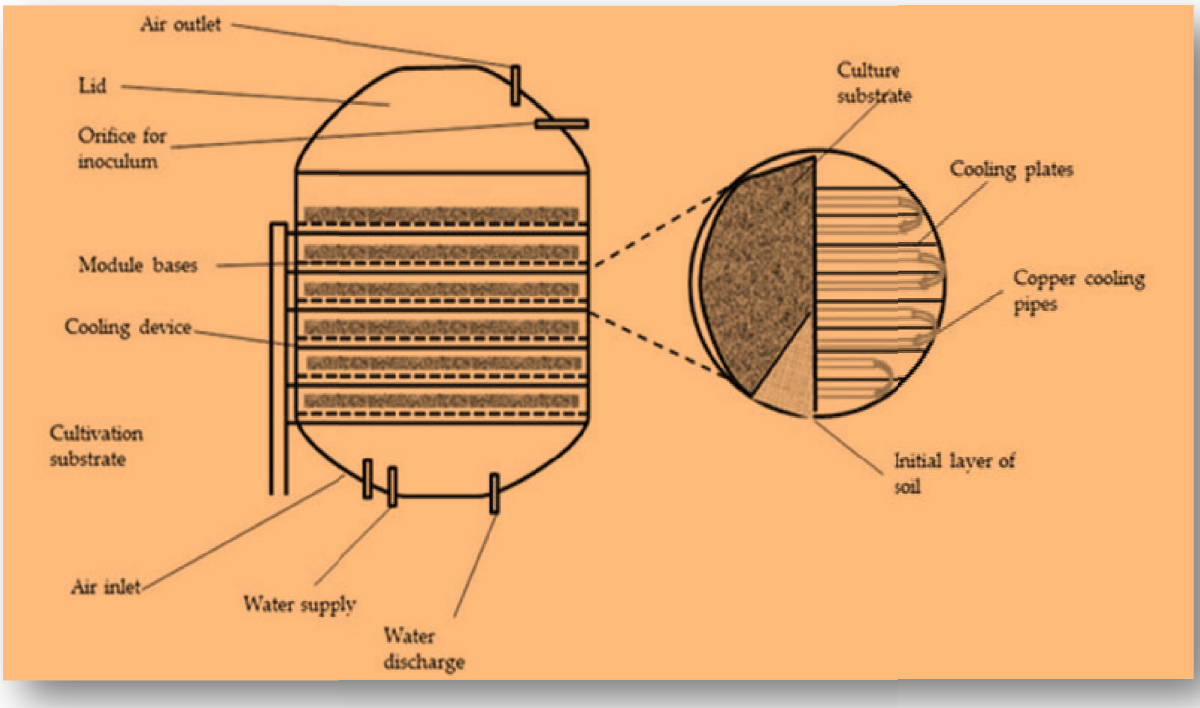
- The matrix should absorb water amounting to one or several time its dry weight with a relatively high water activity on the solid/gas interface in order to allow high rates of biochemical processes.
- Air mixture of oxygen with other gases and aerosols should flow under relatively low pressure and mix the fermenting mash.
- The solid/gas interface should be a good habitat for the fast development of specific cultures of molds, yeasts or bacteria, either in pure or mixed cultures.
- The mechanical properties of the solid matrix should stand compression or gentle stirring, as required for a given fermentation process. This requires small granular or fibrous particles, which do not tend to break or stick to each other.
- The solid matrix should not be contaminated by inhibitors of microbial activities and should be able to absorb or contain available microbial foodstuffs such as carbohydrates (cellulose, starch, sugars) nitrogen sources (ammonia, urea, peptides) and mineral salts.

❖ Organisms used in Solid State Fermentation:

- The microbiological components of SSF can occur as single pure cultures, mixed identifiable cultures or totally mixed indigenous microorganisms.

- Some SSF processes e.g., tempeh and ontjom production, requires selective growth of organisms such as molds that need low moisture levels to carry out fermentation with the help of extracellular enzymes secreted by fermenting microorganisms.

- However, bacteria and yeasts, which require higher moisture content for efficient fermentation, can also be used for SSF, but with a lower yield.



Schematic Representation of Solid State Fermenter

❖ Steps in Solid State Fermentation:

SSF is normally a multistep process involving the following steps:

1) Pre-treatment of substrate raw materials either by mechanical, chemical or biochemical processing to enhance the availability of the bound nutrients and also to reduce the size of the components, e.g., pulverizing straw and shredding vegetable materials to optimize the physical aspects of the process. However, the cost of pre-treatment must be balanced with the eventual product value.
2) Hydrolysis of primarily polymeric substrates, e.g., polysaccharides and proteins.

3) Utilization (fermentation) of hydrolysis products.
4) Separation and purification of end products.

The low moisture content of SSF enables a smaller reactor volume per substrate mass than LSF and also simplifies product recovery. However, serious problems arise with respect to mixing, heat exchange, oxygen transfer, moisture control and gradients of pH, nutrient and product as a consequence of the heterogeneity of the culture. The later characteristic of SSF renders the measurement and control of the above mentioned parameters difficult, laborious and often inaccurate, thereby limiting the industrial potential of this technology. Due to these problems, the microorganisms that have been selected for SSF are more tolerant to a wide range of cultivation conditions.

❖ Applications of Solid State Fermentation:

- Solid-state fermentation has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals, and pharmaceutical products.
- It is widely applied to producing several enzymes, organic acids, flavoring compounds etc., which must be extracted and purified and then used in different products.
- Its application in bioprocesses such as bioleaching, bio-beneficiation, bioremediation, bio-pulping, etc. has offered several advantages.

❖ Advantages of Solid State Fermentation:

- The main advantage of such methods is that it produces a minimum amount of waste and liquid effluent thus not very damaging to the environment.
- Solid substrate fermentation employs simple natural solids as the media.
- Low technology, low energy expenditure and requires less capital investment.
- No need for sterilization, less microbial contamination, and easy downstream processing.

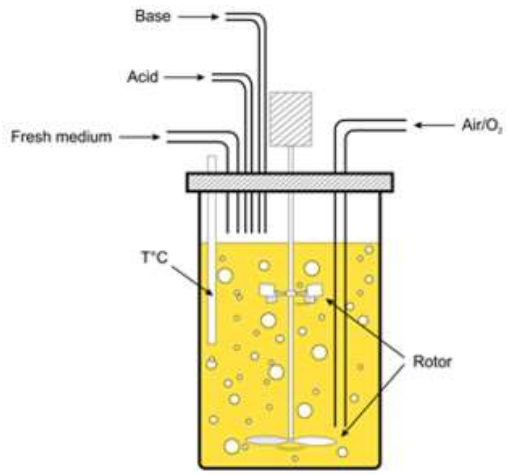
- Utilization of agro-industrial residues as substrates in SSF processes provides an alternative avenue and value addition to these otherwise under or non-utilized residues.
- The yield of the products is reasonably high.
- Bioreactor design, aeration process, and effluent treatment are quite simple.
- Many domestic, industrial and agricultural wastes can be fruitfully used in SSF.

❖ Limitations of Solid State Fermentation:

- The microorganisms that tolerate only low moisture content can be used.
- Precise monitoring of SSF (e.g., O_2 and CO_2 levels, moisture content) is not possible.
- The organisms grow slowly and consequently, there is a limitation in product formation.
- Heat production creates problems, and it is very difficult to regulate the growth environment.

❖ Submerged / Liquid Fermentation

Submerged fermentation is a method of manufacturing biomolecules in which enzymes and other reactive compounds are submerged in a liquid such as alcohol, oil or a nutrient broth. Submerged Fermentation (SmF)/Liquid Fermentation (LF) utilizes free-flowing liquid substrates, such as molasses and broths. The process is used for a variety of purposes, mostly in industrial manufacturing.



Submerged Fermentation

Submerged production began in the 1930's and is the main method used today. Submerged fermentations are traditionally used for the production of microbially derived enzymes. In the submerged process, the substrate used for fermentation is always in liquid state which contains the nutrients needed for growth. The fermentor which contains the substrate is operated and the product biomass is harvested from the fermenter by using different techniques, then the product is filtered or centrifuged and then dried. Submerged fermentation is a method of manufacturing biomolecules in which enzymes and other reactive compounds are submerged in a liquid such as alcohol, oil or a nutrient broth. The process is used for a variety of purposes, mostly in industrial manufacturing. The process can be used to make products such as citric acid, glycerol or lactic acid.

Submerged culture fermentation has been widely used for the production of enzymes because in submerged fermentation unwanted metabolites are not produced and purification of enzymes takes place in an easy way. Submerged fermentation involves submersion of the microorganism in an aqueous solution containing all the nutrients needed for growth. Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic meters. The fermentation media sterilizes nutrients based on renewable raw materials like maize, sugars, and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources.

Batch-fed and continuous fermentation processes are common. In the batch-fed process, sterilized nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilized liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize the fermentation process. Next in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells. This is normally done by centrifugation.

As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed. The enzymes in the remaining broth are then concentrated by evaporation, membrane filtration or crystallization depending on their intended application. If pure enzyme preparations are required, they are usually isolated by gel or ion exchange chromatography. Several types of submerged fermentors are known and they may be grouped in several ways: shape or configuration, whether aerated or anaerobic and whether they are batch or continuous. The most commonly used type of fermentor is the Aerated Stirred Tank Batch Fermentor.

❖ Principles of Submerged Fermentation:

Submerged fermentation involves the growth of the microorganism as a suspension in a liquid medium in which various nutrients are either dissolved or suspended as particulate solids in many commercial media. Submerged fermentation is a process involving the development of microorganisms in a liquid broth. This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products. The process involves taking a specific microorganism such as fungi and placing it in a small closed flask containing the rich nutrient broth. A high volume of oxygen is also required for the process. The production of enzymes then occurs when the microorganisms interact with the nutrients on the broth resulting in them being broken down. The bioactive compounds are secreted into the fermentation broth.

❖ Methods to carry out Submerged Fermentation:

There are two common methods by which submerged fermentation takes place; they are batch-fed fermentation and continuous fermentation. **In batch-fed fermentation** sterilized growth nutrients are added to the culture. It is most common in bioindustries as it occurs during the growth of biomass in the fermenter. It helps raise the cell density in the bioreactor and it is typically highly concentrated to stop dilution. The rate of growth in the culture is maintained by adding nutrients, this also reduces the risk of overflow metabolism.

An open system is constructed for **continuous fermentation.** Then sterilized liquid nutrients are slowly and continuously added to the bioreactor at the same rate at which the converted nutrient solution is being recovered from the system. This results in a steady-rate production of the fermentation broth. In order to maintain a successful fermentation, certain variables must be monitored, for example, temperature, pH, as well as oxygen and carbon dioxide levels.

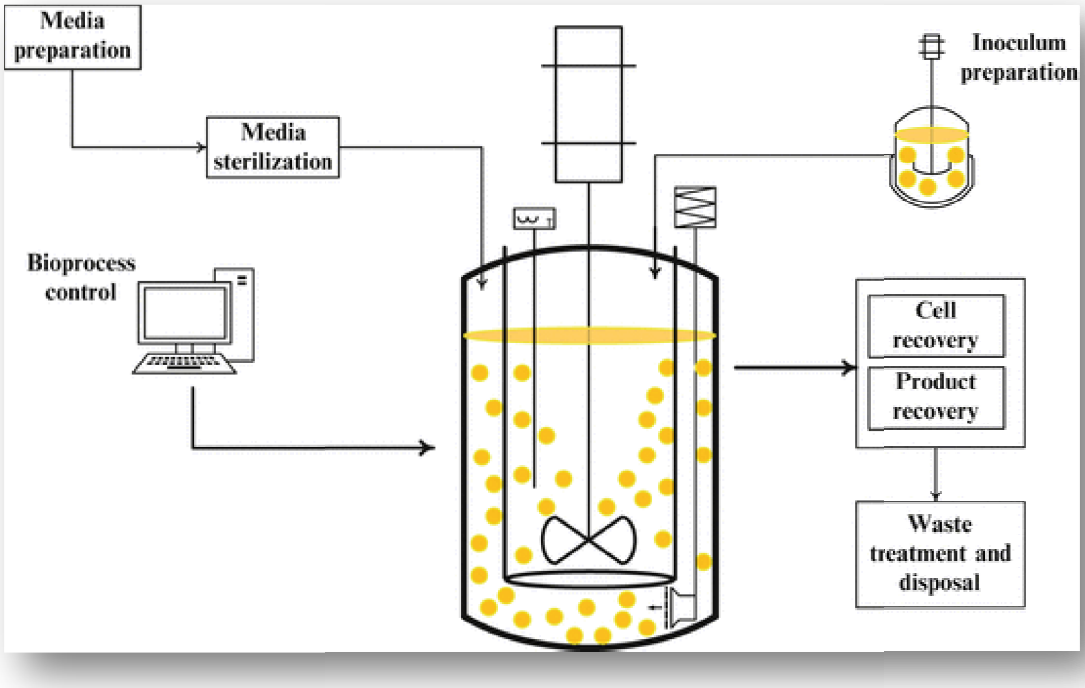
❖ Submerged Fermentation Substrate:

Some common substrates used in submerged fermentation are soluble sugars, molasses, liquid media, fruit and vegetable juices, and sewage/wastewater.

➢ *Applications:*

- Submerged Fermentation/Liquid Fermentation utilizes free-flowing liquid substrates, such as molasses and broths.
- The bioactive compounds are secreted into the fermentation broth.
- The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients.
- This fermentation technique is best suited for microorganisms such as bacteria that require high moisture.
- An additional advantage of this technique is that the purification of products is easier.

- It is primarily used in the extraction of secondary metabolites that need to be used in liquid form.
- Submerged liquid fermentations are traditionally used for the production of microbially derived enzymes.



Schematic Representation of Submerged Fermentation

➢ ***Advantages:***

- ✓ Measure of process parameters is easier than with solid state fermentation.
- ✓ Bacterial and yeast cells are evenly distributed throughout the medium.
- ✓ There is a high water content which is ideal for bacteria.
- ✓ Inoculum ration is usually small.
- ✓ Lower total investment costs.
- ✓ Improved process control.
- ✓ Reduced fermentation time.
- ✓ Reduced floor space requirements.
- ✓ Purification of products is easier.

- ✓ Lower labor costs.
- ✓ Simpler operations.
- ✓ Easier maintenance of aseptic conditions on an industrial scale.
- ✓ Submerged fermentation technology has the advantages of short period, low cost and high yield.
- ✓ In liquid culture the control of the fermentation is simpler and consequently significant reductions in fermentation times can be achieved.
- ✓ In the same way, the use of submerged culture can benefit the production of many secondary metabolites and decrease production costs by reducing the labor involved in solid-state methods.

➢ ***Disadvantages:***

- High costs due to the expensive media.
- Expenses for equipment are higher.
- Consumption of electrical energy is higher.
- The process is very sensitive.
- Agitation is often essential.
- Chances of contamination is higher.
- In recent years, many researchers have demonstrated that SSF has a large impact on productivity, leading to higher yields and improved product characteristics compared to SmF
- Low volumetric productivity.
- Relatively lower concentration of the products.
- More effluent generation.
- Complex fermentation equipments.

❖ What is fermentation?

Fermentation is metabolic process which converts carbohydrates to alcohol, organic acids or gases by the activity of enzymes of microbial origin.

- **Microbes involved in fermentation process:** Bacteria and Fungi

The process of anaerobic respiration in the muscle cells of animals during exercise which produce lactic acid is also a type of fermentation. The technique of fermentation was very ancient in origin. Egyptians and Sumerians had the knowledge of the technique of converting starchy grains to alcohols.

- **For a microbiologist the word fermentation means several processes such as:-**

  - A method of manual cultivation of microbes under aerobic or anaerobic conditions.
  - Any biological process occurs in the absence of oxygen.
  - Spoilage of food by microbial activity.
  - Production of alcoholic beverages, organic acids, antibiotics or biopolymers.
  - Partial oxidation of carbohydrates.

- **What is Industrial fermentation?**

The international use of fermentation technology for the large scale production of microbial biomass or metabolites is called industrial fermentation. Modern industrial fermentation units are genetically engineered microbes for the rapid production of desired metabolites.

- **What is fermenter?**

The heart of industrial fermentation is a fermenter. Fermenter is a type of bioreactor. Fermenter is a system provided with controlled environmental conditions for the growth of microbes in liquid culture and production of specific metabolites. It is a device in which microbes are cultivated and motivated to form the desired products. It is the containment system to provide

the accurate environment for the optimum growth and metabolic activity of the microbes. Fermenter prevents the entry and growth of the contaminating microbes from outside.

- **Fermenter** – Containment system for the cultivation of prokaryotic cells (bacteria) and fungi.
- **Bioreactor** – Containment system for the cultivation of mammalian or insect cells.

➢ **What are the main parts of a fermenter and their uses?**

A fermenter possesses the following mechanical parts:

- A large vessel made of stainless or rust free materials.
- Motors provided with an automatic control system.
- Heaters with thermostat system for providing and manipulating temperature.
- Pups for the addition or removal of the substances and water to the fermenter.
- Gas source and pipeline system for aeration.
- Sensors for pH and aeration.
- Peripheral manual or automatic controlling facilities.

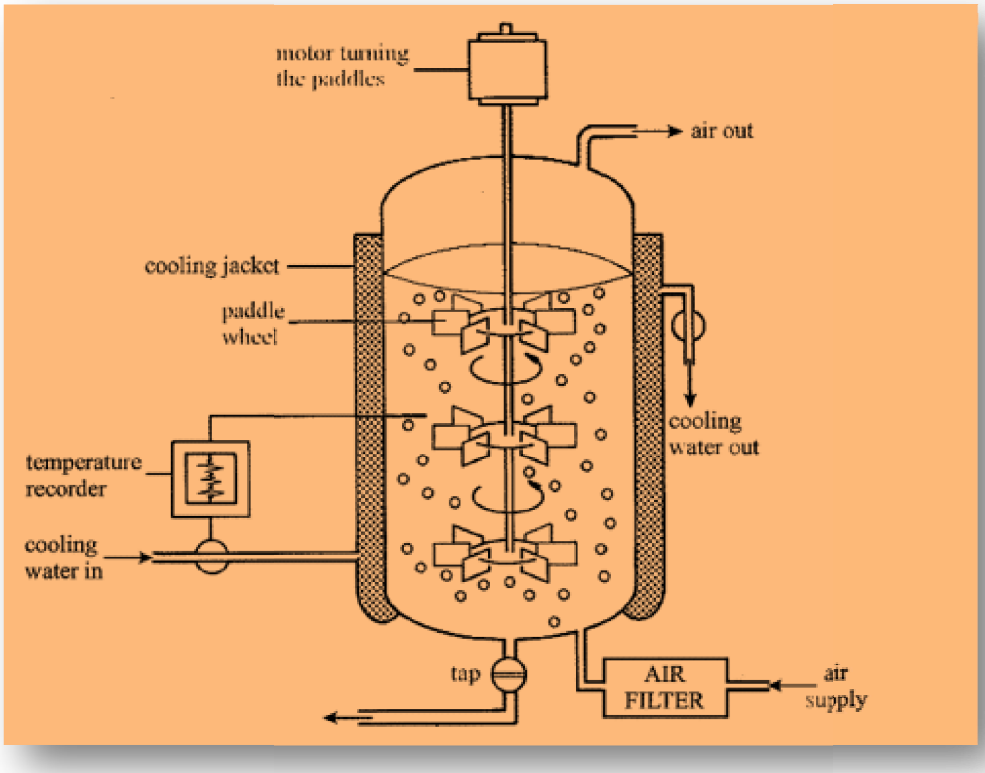


DIAGRAM OF A FERMENTER

The mechanical components of the fermenter prove:

- A space for taking raw materials (culture media/ carbon sources).
- Provide a contamination free environment for the growth of microbes.
- Maintain optimum temperature in the system.
- Provide adequate mixing and agitation in the medium.
- Provide ample aeration for aerobic fermentation.
- Control and maintain optimum pH condition in the fermenter.
- Monitor the concentration of dissolved oxygen in the system.
- Allow the addition of nutrients in between the fermentation process (in continuous fermentation).
- Facility for maintaining a wide range of organisms.

- Provision for collecting over-flow from the fermenter (in continuous fermentation).

What are the different types of fermentation processes/ methods?

There are three types of industrial fermentation processes based on the methods of fermentation and types of fermenters:

1. **Batch Fermentation**
2. **Continuous Fermentation**
3. **Fed – Batch Fermentation**

1. Batch Fermentation:

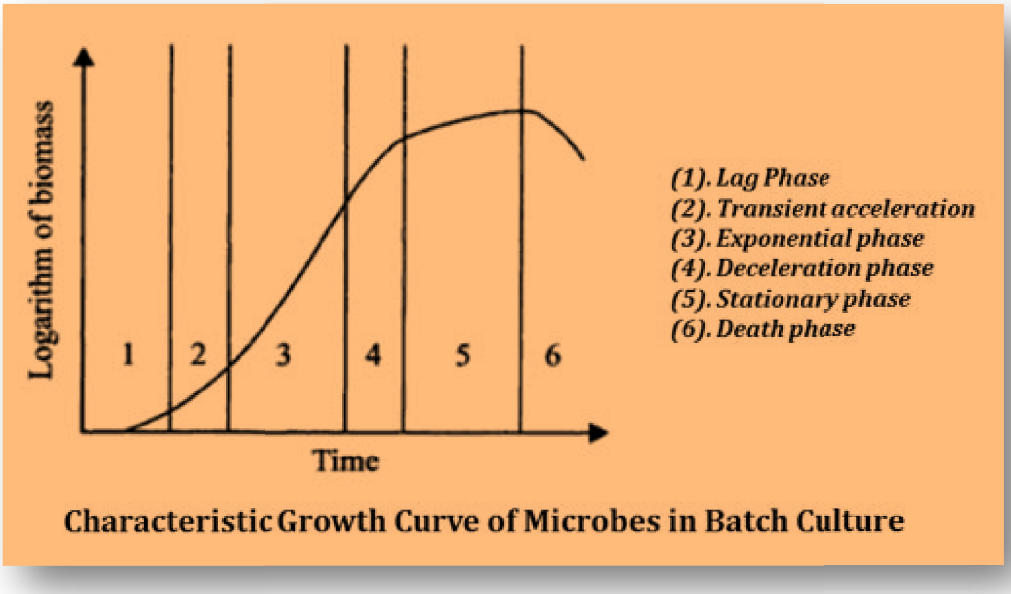
Microorganisms are inoculated into a fixed volume of medium. As the growth takes place, the nutrients are consumed and the product of growth accumulates in the fermenter. Product of growth may be of two types: -

- Biomass
- Metabolites

The nutrient environment in the fermenter is continuously changed. This change in the environment in fermenter will enforce change in the metabolism of cells. This also results in the cessation of the cell multiplication. Cessation of growth is due to the scarcity of nutrients and accumulation of metabolites. Once the microbes reached the stationary phase they start to accumulate the metabolites. Metabolites are extracted from the fermenter by the downstream processes. After the fermentation is over, the residues are taken from the fermentation tank and the vessel is then cleaned and sterilized before next batch of fermentation. Thus in batch fermentation, the large scale production is done as separate

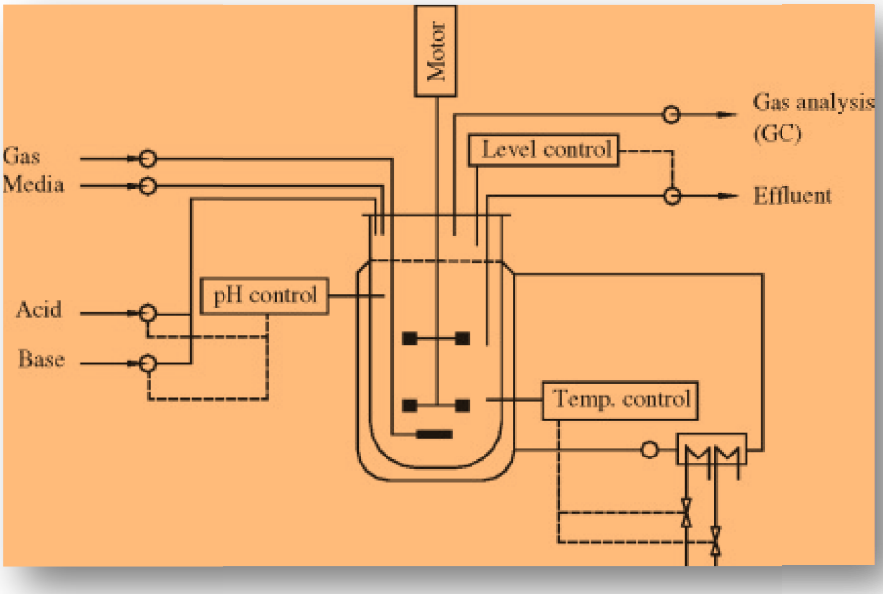
batches. Microbes in batch culture show the following pattern of growth with distinct phases.

❖ CHARACTERISTIC GROWTH CURVE OF MICROBES IN BATCH FERMENTATION



Characteristic Growth Curve of Microbes in Batch Culture

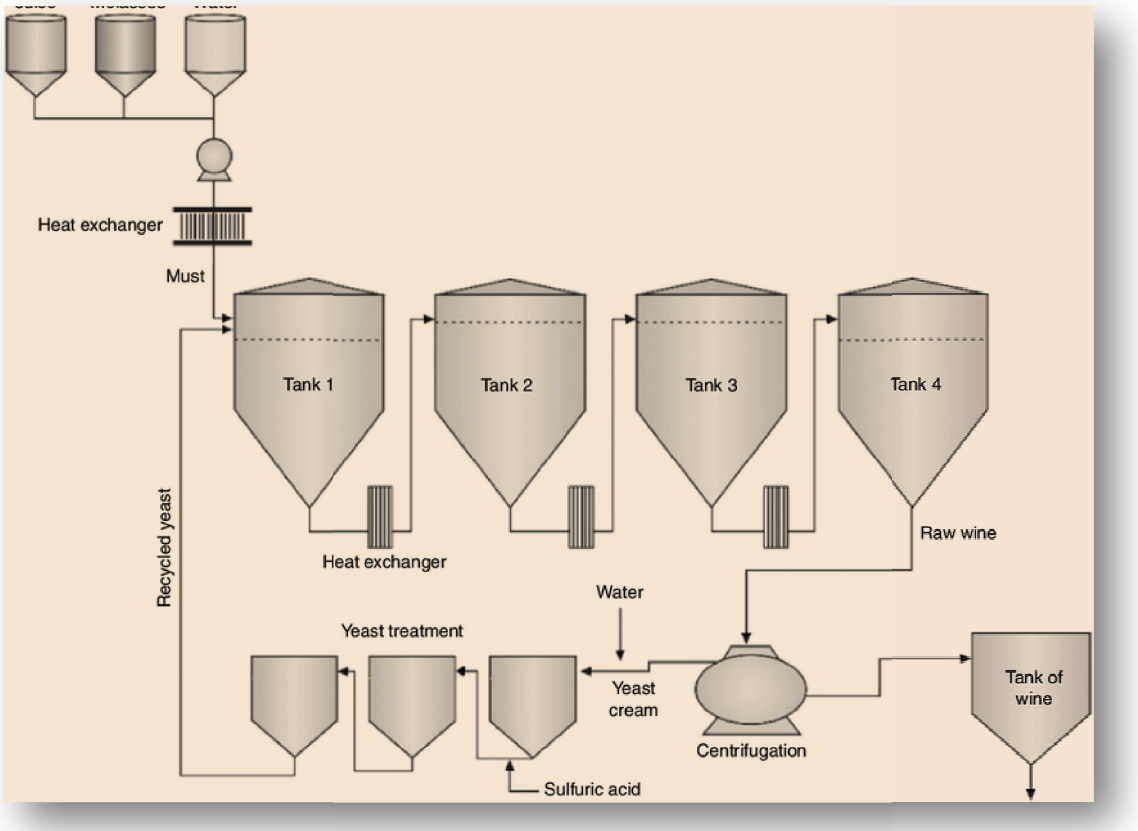
1. **LOG PHASE:** Initial phase, no apparent growth of microbes, they adapt to the environmental condition.
2. **TRASIENT ACCELERATION:** The inoculum begins to grow slowly.
3. **EXPONENTIAL PHASE:** Microbial growth proceeds at maximum possible rate.
4. **DECELERATION PHASE:** Decline in the growth rate of microbes.
5. **STATIONARY PHASE:** No overall growth rate (death of the cells equals to the division of cells). Most of the secondary metabolites are produced in this phase.
6. **DEATH PHASE:** No growth at all, cells start to die and the population size is decreased. Usually fermentation stops before the death phase.



SCHEMATIC DIAGRAM OF BATCH FERMENTER

2. Continuous Fermentation:

Here the exponential growth rate of microbes is maintained in the fermenter for prolonged periods of time by the addition of fresh media at regular intervals. Microbes reach the exponential growth rate and continue as such due to the availability of the nutrients. The exponential growth rate of microbes continues till the vessel becomes completely filled in the cells. Continuous fermenter processes device for the collection of overflow from the vessel. The metabolites or the product of fermentation is extracted for the overflow by downstream processing. Thus unlike batch fermentation, in continuous fermentation, the fermentation process never stops in between and it continues to run for a longer period of time with the addition of nutrients and harvesting the metabolites at regular intervals.

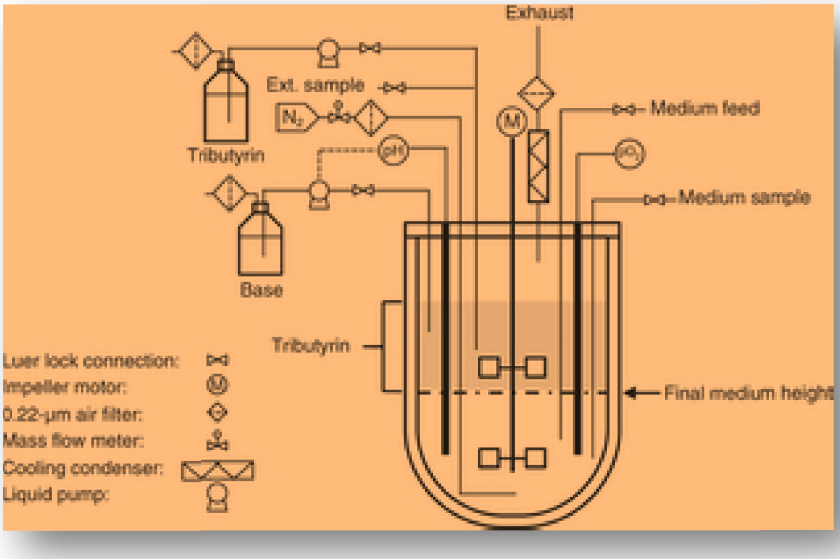


SCHEMATIC DIAGRAM OF A CONTINUOUS FERMENTER

3. Fed – Batch Fermentation:

It is a modified version of batch fermentation. Here the substrate is added in increments at different times throughout the course of fermentation. Periodical addition of substrate keeps the prolonged log and stationary phase of the microbes in the fermentation. This result in the rapid increase of biomass consequently increased production of metabolites can be achieved in the stationary phase. Thus Fed – Batch technique is an improved version of fermentation by avoiding disadvantages of batch and continuous fermentation techniques.

Fed-batch culture is, in the broadest sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run. An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion". It is also a type of **semi-batch culture**. In some cases, all the



SCHEMATIC DIAGRAM OF FED-BATCH FERMENTER

nutrients are fed into the bioreactor. The advantage of the fed-batch culture is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels). Generally speaking, fed-batch culture is superior to conventional batch culture when controlling concentrations of a nutrient (or nutrients) affects the yield or productivity of the desired metabolite. The types of bioprocesses for which fed-batch culture is effective can be summarized as follows:

1. Substrate inhibition

Nutrients such as methanol, ethanol, acetic acid, and aromatic compounds inhibit the growth of microorganisms even at relatively low concentrations. By

adding such substrates properly lag-time can be shortened and the inhibition of the cell growth markedly reduced.

2. High cell density (High cell concentration)

In a batch culture, to achieve very high cell concentrations, *e.g.* 50-100 g of dry cells/L, high initial concentrations of the nutrients in the medium are needed. At such high concentrations, the nutrients become inhibitory, even though they have no such effect at the normal concentrations used in batch cultures.

3. Glucose effect (Crabtree effect)

In the production of baker's yeast from malt wort or molasses it has been recognized since early 1900s that ethanol is produced even in the presence of sufficient dissolved oxygen (DO) if an excess of sugar is present in the culture liquid. Ethanol is a main cause of low cell yield. Aerobic ethanol formation in the presence of glucose concentration is known as glucose effect or Crabtree effect. To reduce this effect, a fed-batch process is generally employed for baker's yeast production. In aerobic cultures of *Escherichia coli* and *Bacillus subtilis*, organic acids such as acetic acid, (and in lesser amounts, lactic acid and formic acid), are produced as byproducts when sugar concentration is high, and these acids inhibit cell growth as well as show deteriorating effect on the metabolic activities. The formation of these acids are called bacterial Crabtree effects.

4. Catabolite repression

When a microorganism is provided with a rapidly metabolizable carbon-energy source such as glucose, the resulting increase in the intracellular concentration of ATP leads to the repression of enzyme(s) biosynthesis, thus causing a slower metabolization of the energy source. This phenomenon is known as catabolite repression. Many enzymes, especially those involved in catabolic pathways, are subject to this repressive regulation. A powerful method of overcoming the catabolite repression in the enzyme biosynthesis is a

fed-batch culture in which glucose concentration in the culture liquid is kept low, where growth is restricted, and the enzyme biosynthesis is derepressed. Slow feeding of glucose in *Penicillin* fermentation by *Penicillium chrysogenum* is a classical example in the category.

5. Auxotrophic mutants

In a microbial process employing an auxotrophic mutant (nutritionally requiring mutant), excess supply of the required nutrient results in abundant cell growth with very little accumulation of the desired metabolite due to feedback inhibition and /or end-product repression. Starvation of the required nutrient, however, lowers cell growth as well as the overall production of the desired metabolite, as the production rate is usually proportional to the cell concentration. In such a bioprocess, the accumulation of the desired metabolite can be maximized by growing the mutant on a limited amount of the required nutrient. To cultivate the mutant on a low concentration of the required nutrient, it is fed to the batch culture at a controlled rate. This technique is often used in industrial amino acid productions with the auxotrophic mutants. An example is lysine production with homoserine- or threonine/methionine requiring mutant of *Corynebacterium glutamicum* being lacking for homoserine dehydrogenase gene.

6. Expression control of a gene with a repressible promoter

Transcription of a gene having a repressible promoter upstream of the open reading frame is repressed by a combination of the so-called holo-repressor with the operator region on the DNA. When a specified chemical compound exists in the culture liquid, the compound (or its metabolite) in the cells combines as co-repressor with an apo-repressor (a kind of transcription factor) to form the holo-repressor. Keeping the concentration of this compound as low as possible (while still allowing for sufficient cell growth) permits continued expression of the regulated gene. Fed-batch culture is a powerful technique to

do so. Examples of the repressible promoter are *trp* promoter and *phoA* promoter.

7. Extension of operation time, supplement of water lost by evaporation, and decreasing viscosity of culture broth.

❖ Types of Culturing Strategies:

High Cell Density Culture

The fed-batch strategy is typically used in bioindustrial processes to reach a high cell density in the bioreactor. Mostly the feed solution is highly concentrated to avoid dilution of the bioreactor. Productions of heterologous proteins by fed-batch cultures of recombinant microorganisms have been extensively studied. The controlled addition of the nutrient directly affects the growth rate of the culture and helps to avoid overflow metabolism (formation of side metabolites, such as acetate for *Escherichia coli*, lactic acid in mammalian cell cultures, ethanol in *Saccharomyces cerevisiae*), oxygen limitation (anaerobiosis).

Constantly-fed-batch culture

The simplest fed-batch culture is the one in which the feed rate of a growth-limiting substrate is constant, *i.e.* the feed rate is invariant during the culture. This type of the fed-batch culture is named constantly-fed-batch culture (CFBC), and is well established mathematically and experimentally.

Exponential-fed-batch culture

Under ideal condition, cells grow exponentially. If the feed rate of the growth-limiting substrate is increased in proportion to the exponential growth rate of the cells, it is possible to maintain the cells' specific growth rate for a long time while keeping the substrate concentration in the culture liquid at a constant

level. The required feed rate (volumetric or mass) must be increased exponentially with time so that this mode of fed-batch culture is called exponentially-fed-batch culture (EFBC).

Substrate limitation offers the possibility to control the reaction rates to avoid technological limitations connected to the cooling of the reactor and oxygen transfer. Substrate limitation also allows the metabolic control, to avoid osmotic effects, catabolite repression and overflow metabolism of side products.
