Unit | 12

Applied Biology

Unit Map

12.A Microbial fermentation and production of small and macro molecules

Fermentation is derived from a Latin word 'fermere' which means to boil. In biochemical point of view fermentation is referred to an energy generation process in which organic compounds act as both electron donors and terminal electron acceptors. In general words any process for the production of products by the mass culture of microorganisms is known as Microbial fermentation. Generally there are six components of any fermentation process which are given below

i. Media formulation
ii. Sterilization of the medium, fermenters and other equipments.
iii. Pure culture production for the fermentation process.
iv. Optimization of condition for growth and product formation
v. Product recovery and purification of the product (downstream process)
vi. Effluents treatment and disposal of effluents.

i. Media Formulation The media plays very important role in a fermentation process so it should have all the components needed for supporting cell growth and metabolite production. The media chosen must have following criteria to be fulfilled

- Must produce maximum biomass/growth.
- Maximum yield of product.
- Minimize the yield of undesired products
- Must have consistent quality and quantity
- Least problematic in terms of sterilization, agitation and purification of product.

There are basically two types of media used in fermentation processes

i. Defined and synthetic media It is a type of media whose composition is well defined.
ii. Undefined/Semi-synthetic and complex media It is a type of media whose composition is not defined or its partial defined or having agricultural commodities as substrate.

➢ **General components of media**

Energy sources Energy for growth comes from the oxidation of medium components. Since most of the industrial microbes are chemo-organotrophs therefore for energy the most common sources are: Carbohydrates, proteins, lipids and hydrocarbons.

- **Carbon sources** A variety of sources are used as carbon source such as maize and barley grains, sugar beet, corn steep liquor, soybean meal, sucrose, lactose, hydrocarbons etc. There are various factors which influence the choice of carbon source such as the type of product produced whether it is primary metabolite or secondary metabolite, cost, purity of carbon source which affects choice of substrate, sterilization and government laws and other rules and regulations.
  - **i. Oils and Fats** The various vegetable oil (maize, linseed, soybean etc.) are used as carbon substrates for microbes. These oils are also having anti-foaming properties.
  - **ii. Hydrocarbons and their derivatives** For the production of vitamins, amino acids, nucleic acid, organic acids, commonly n-alkanes, methane and methanol are used as carbon source.
- **Nitrogen sources** There is mainly two types of nitrogen sources; inorganic such as ammonia gas, ammonium salts, nitrate salts and organic sources such as urea, amino acids, proteins, soybean, peanuts etc. The choice of nitrogen sources depends on many factors such as effect of nitrogen source on production of certain product, cost, control mechanisms involved etc.
- **Inorganic Nutrients** Macronutrients such as Mg, P, K, Ca etc. and these are generally supplied from outside into the media. Micronutrients such as cobalt, iron, and manganese are usually present inside the cell only.

- **Chelators** They combine with metal ions and prevent formation of precipitates. EDTA, citric acid and polyphosphates act as chelators.
- **Growth factors** These are preformed components that can be added to the media when it is deficient in the media and stimulates growth. Vitamins, amino acids, fatty acids and sterols are some of the examples of growth factors.
  There are other components also which are not necessary for growth but are essential for yield of product such as Promoters, inhibitors and inducers.

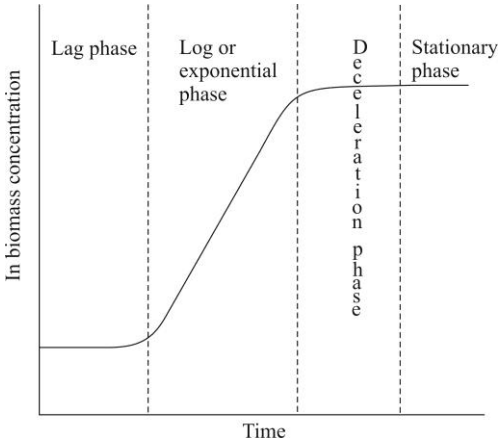
➢ **Types of fermentation**

- **Solid State fermentation** This is a type of fermentation in which microbial growth and product formation occur at the surface of solid substrates such as mushroom cultivation, mold ripened cheese, starter cultures etc. It is also used for the production of extracellular enzymes, valuable chemicals, fungal toxins and fungal spores. Depending upon physical state solid state fermentation can be divided further into two groups
  i. Low moisture solids fermented without agitation
  ii. Suspended solids fermented in packed columns. On large scale, it uses stationary or rotary trays.

- **Submerged Fermentation** In submerged fermentation, a mycelia organism e.g., fungi grows as dispersed hyphal fragments or as pellets under the surface of media. It is much easier to operate and may be applied on large scale.

Either of the fermentation is carried out in Batch and Continuous fermentation form

- **Batch Fermentation** Batch culture is a closed culture system which contains limited amount of nutrient medium. In this process culture has to pass through various stages such as lag phase, log phase, stationary phase and decline phase.



The lag phase is the phase in which organisms do not produce any product and adapt itself to the surroundings. For any fermentation process, this phase has to be reduced to minimum and organisms in log phase should be present to have economically viable product.

i. Lag phase no growth takes place in this phase and its time of adaptation which an organism takes whenever it is exposed to a new environment.

ii. Exponential Phase In this phase, substrate concentration is in excess and growth is at μ_{max}. This is the most important phase of any batch process and maximum products are produced in this phase. The exponential phase is represented as follows

$$dx/dt=\mu x$$

Where μ represents specific growth rate
x represents concentration of microbial biomass

On integration equation is:

$$X_t=X_oe^{\mu t}$$

And on taking log of above equation

$$\ln X_t=\ln X_oe^{\mu t}$$

t=hours
X_o= original biomass concentration
X_t= Biomass concentration after the time interval,
e = base of the natural logarithm
On taking natural logarithm equations becomes
$\ln X_t = \ln X_o + \mu t$.

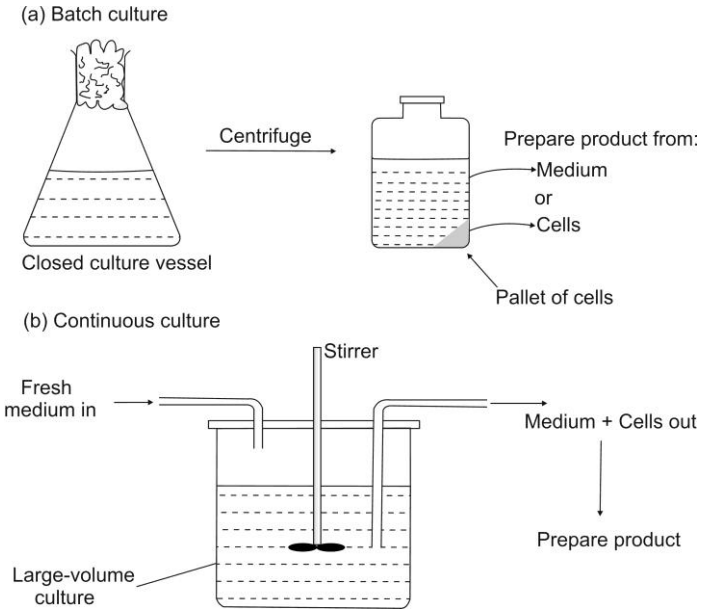
Thus a plot of natural logarithm of biomass concentration against time should give a straight line and the slope of which would equal μ.

i. **Stationary phase** In this phasee growth rate declines to zero or growth rate is equal to the death rate due to depletion of nutrients and accumulation of toxic substances in the medium. The cells are still metabolically active.
ii. **Decline phase** This phase follows the stationary phase. The nutrient depletion or the toxic product accumulation leads to death of the cells and thus decline phase approaches.

- **Continuous fermentation** It is a type of fermentation in which nutrients are supplied and end products are continuously removed. It maintains a steady state over a long period of time.

Comparison of Batch and Continuous culture

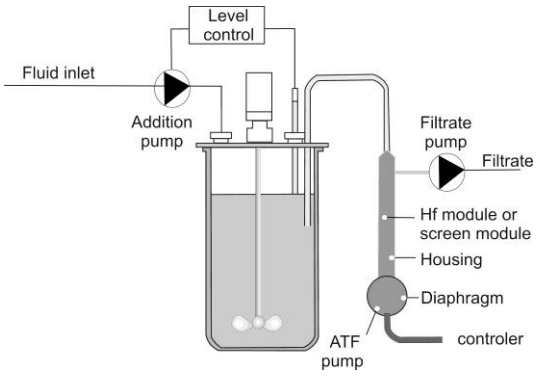
- In batch fermentation, productivity is maximum only at the end of process whereas in continuous culture, the productivity will be constant as it is being operated at an optimum dilution rate and will always be constant.



- Continuous culture can be operated for a very long time period whereas batch culture may be operated for limited time period or preferably terminated after one cycle is completed.
- Continuous culture is labour intensive process whereas a batch process requires intensive labour during the initial stages only.
- As the continuous fermentation processes usually runs for longer time than a batch fermentation so there is greater probability of contaminating organisms entering the system and also there is greater probability of equipment failure.

- **Fed batch fermentation** Culture nutrients are continuously and semi-continuously fed while effluent is removed discontinuously. It is also known as semi-continuous system or variable volume continuous culture. There are two types of fed batch cultures

i. **Fixed volume fed batch culture** It refers to the periodic withdrawal of a portion of the culture and use of the residual culture as the starting point for further fed batch process.



ii. **Variable volume fed batch culture** It refers to the culture in which volume changes with fermentation time due to feeding of substrate. This change in volume depends on the requirements, limitations and objectives of the operator.
E.g. of fed batch is antibiotic fermentation process where glucose solution is intermittently added for the production of secondary metabolites.

➢ **Scale Up**

Scale up means increasing the scale of fermentation from laboratory scale to pilot scale followed by commercial scale. Fermentation technologist's aim is to increase scale without decrease in yield at pilot and commercial scale. The factors, which play significant role in scale up are:

- **Inoculums development** The culture used to initiate the fermentation must be healthy in active state, free of contamination (pure culture) and with product formation capabilities. The process adopted to produce optimum inoculums for scale up purpose is known as inoculums development purpose.
- **Sterilization** Sterilization is very important in any experiment as it can lead to destruction of any experiment. With the increase in scale number of contaminants will also increase proportionally as sterilization is scale dependent factor so the sterilization procedure should be adjusted according to the scale up process.
  Sterilization involved for scale up of any fermentation process requires
  i. Sterilizing the medium to be employed
  ii. Sterilizing the fermenter vessel
  iii. Sterilizing all materials including air to be added to the fermentation during the process
  iv. Maintaining aseptic condition during the fermentation

  i. Sterilization of Media Media can be sterilized by
    - **Batch Sterilization** of medium is achieved either in the fermentation vessel or in mash cooker
    - **Continuous Sterilization** a time period during which the medium is heated to the sterilization temperature a holding time at the required temperature and a cooling period to restore the medium to the fermentation temperature. The length of the holding period depends upon the length coil and flow rate of the medium.
      Media for animal cell culture cannot be sterilized by steam because they contain a lots of heat labile components therefore they are sterilized by filtration method which include membrane filters which with variable pore sizes (0.1-0.4 µm) for removal of bacterial, fungal, micro plasma and viral contaminants.

  ii. Sterilizing the fermenter vessel The fermentation vessel can be sterilized in a separate batch or it can be sterilize with the steam through pipes. The fermenter if it is steamed sterilized under pressure the medium is preferably sterilized in separate vessel and subsequently added aseptically. This is known as Ex-situ sterilization whereas in In-situ sterilization the temperature is raised prior to the injection of live steam. This is achieved by introducing the steam into the fermenter coil and jacket.

  iii. Air Sterilization Aerobic fermentation requires a continuous addition of sterile air. It can be achieved either by heat treatment or by passing the air from fixed pore filters. Most common filters are PTFE which are hydrophobic and resistant to wetting and heat.

- **Environment factors** Scale up process result in change in environmental parameters such as availability of nutrients, pH, temperature, dissolved oxygen concentration, shear conditions, dissolved CO_2 concentration, foam production. All the above parameters are affected by agitation and aeration either in term of bulk mixing or the provision of O_2.Nutrient availability, pH, temperature and shear conditions are more related to bulk mixing whereas latter ones are related to air flow and oxygen transfer. Thus, aeration and agitation tends to dominate scale up process.
- **Fermenters/Bioreactor** A Fermenter/bioreactor is a vessel used to provide optimum conditions for the controlled growth of microorganisms/ cells by regulating agitation, temperature and aeration.
  The main components of fermenters are agitators, spargers, baffles, cooling jacket, pH probes, temperature probes, acid probes, dissolved oxygen probes (DO probes) etc. Bioreactors can be operated in a batch, fed batch or semi continuous or continuous bases. The design of the fermenters must be fulfilling the following criteria:
  i. It must support optimum cell biomass and higher product yield by providing optimal and controlled conditions.
  ii. It must sustain aseptic environment for the growth of microorganisms for number of days
  iii. It must be having proper arrangements of mixing, aeration, regulating pH and temperature etc.
  iv. It must have proper sampling, inlet and outlet ports.
  v. It must support a wide range of fermentation processes.
  vi. It must require minimum use of labour in operation, harvesting, cleaning and maintenance.