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4. Feedstock and Solid Substrate Fermentations

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4.1 Information:

The word fermentation is derived from Latin word *fervere which means to boil*. But the conventional definition of fermentation is to break down of larger molecules into smaller and simple molecules using microorganisms.

In biotechnology, fermentation means any process by which microorganisms are grown in large quantities to produce any type of useful materials. Microorganisms used in fermentation include bacteria, fungi, algae and actinomycetes. The commonly used species are as bellow.

a. **Bacteria:** *Acetobacter lacti, Acetobacter woodi, Bacillus subtilis, Bacillus polymyxa, Clostridium etc.*
b. **Algae:** *Spirulina maxima, Chlorella sorokiniana etc.*
c. **Fungi:** *Aspergillus oryzae, Aspergillus Niger, Saccharomyces cervisae, Saccharomyces lipolytica etc.*
d. **Actinomycetes:** *Streptomyces griseus, Streptomyces noursei etc.*

In fermentation some common steps are followed which are stated bellow.

4.2 Preparation of Pure Culture of Desirable Microorganism:

After isolation of microorganisms they are grown and maintain in culture medium as pure culture i.e. a single species of organism without any other undesirable organism i.e. without contamination. Different types of microbial cultures are used for different purposes. Some of the common types of culture are-

a. Batch Culture:

It is the simplest methods of culturing the microorganisms in which the microorganisms are grown on a limited amount of medium. In a batch culture, the microbes pass through a number of stages during their growth such as lag phase, log phase, stationary phase and death phase which are collectively called as growth curve (Figure 4.1).

- **Lag phase:** The growth of microorganisms will not occur immediately after inoculation. They take some time to adjust or adapt to the medium. This time is called Lag phase. The Lag phase can be reduced by using relatively large amount of exponentially growing inoculum which is grown in a medium having similar composition as that used in the fermentation.
- **Log phase or Exponential:** In this phase, the microbes grow in an exponential manner consuming the nutrients present in the medium.
- **Stationary or Deceleration phase:** As soon as the level of nutrients is reduced or exhausted in the medium, the growth of culture gradually slows down. This may also occur due to accumulation of toxic metabolites which inhibits the growth. During this phase, the microorganisms cannot grow and hence their biomass cannot increase.
- **Death or Decline phase:** In this phase the nutrients in the medium exhaust completely and there will be accumulation of toxic materials which leads to death of microbial cells.

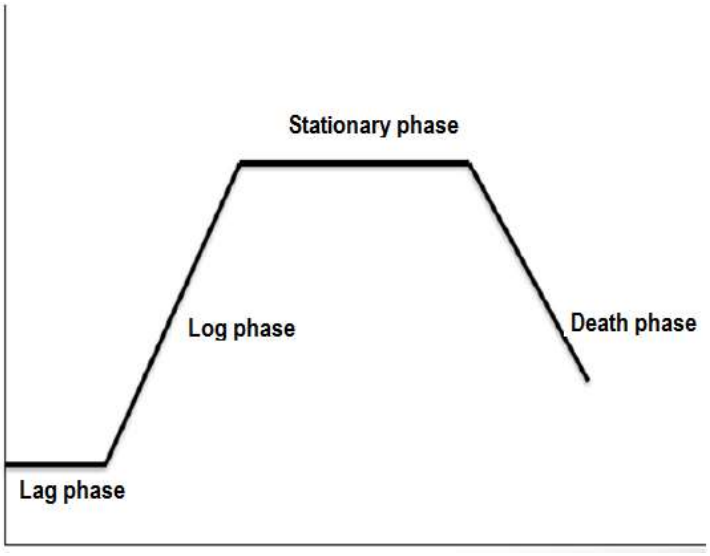

Figure 4.1: Growth Curve of Microorganism

b. Continuous Culture:

The culture medium is designed for cessation of growth due to depletion of nutrients rather than by accumulation of toxins. The exponential growth in the batch culture can be prolonged by the addition of fresh medium to the culture vessel. The addition of fresh medium displaces an equal amount of culture and then continuous production of cells can be achieved. The medium is added continuously to such a system at a suitable rate, the displacement of culture can be balanced by the production of new biomass and a steady state can be achieved as continuous culture.

c. Fed-Batch Culture:

It is also the batch culture which is fed continuously with fresh medium without the removal of original culture from the fermenter. The volume of medium in the fermenter increases continuously.

4.3 Fermentor:

Fermentor is also called as bio-reactor. It is a closed container with regulated accessories for operation where target microorganism is applied with substrate to achieve the desire product. A typical fermentor has different features (Figure 4.2). A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors are extensively used for food processing, fermentation, waste treatment, etc. On the basis of the agent used, bioreactors are grouped into the following two broad classes: - (i) those based on living cells and, (ii) those employing enzymes. But in terms of process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state and (iv) immobilized cell bioreactors. All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors. But, in general, theoretical explanation usually lags behind technical realization.

A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (supply oxygen), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc. The first truly large-scale aseptic anaerobic fermentation vessels were developed in the wake of the process developed (during the First World War, 1914-1918) by Weizmann and co-workers of U.K. to produce acetone by a deep liquid fermentation using *Clostridium acetobutylicum*. For this, large cylindrical vessels of mild steel that permitted sterilization with steam under pressure were constructed, and piping, joints and valves were specially designed to maintain aseptic conditions, which were the major problem; mixing was achieved by the large volumes of gas produced during fermentation. The large-scale aerobic fermentation vessels were first used in Central Europe during 1930s for the production of compressed yeast; these fermenters had large cylindrical tanks in which air was introduced at the base via a network of perforated pipes. In later modifications, mechanical impellers were used to improve mixing of broth and dispersal of air bubbles. Fermenter design was considerably improved during 1940s to accommodate the requirements of strict aseptic conditions, and good agitation and aeration for penicillin production from submerged cultures; for this, steel fermenters with working volumes of 54,000 1 were built in U.S.A.

4.3.1 Functions of a Fermenter:

- It should provide a controlled environment for optimum biomass/product yields.
- It should permit aseptic fermentation for a number of days reliably and dependably, and meet the requirements of containment regulations. Containment involves prevention of escape of viable cells from a fermenter or downstream processing equipment into the environment.
- It should provide adequate mixing and aeration for optimum growth and production, without damaging the microorganisms/cells. The above two points (items 2 and 3) are perhaps the most important of all.
- The power consumption should be minimum.
- It should provide easy and dependable temperature control.

- Facility for sampling should be provided.
- It should have a system for monitoring and regulating pH of the fermentation broth.
- Evaporation losses should be as low as possible.
- It should require a minimum of labour in maintenance, cleaning, operating and harvesting operations.
- It should be suitable for a range of fermentation processes. But this range may often be restricted by the containment regulations.
- It should have smooth internal surfaces, and joints should be welded wherever possible.
- The pilot scale and production stage fermenters should have similar geometry to facilitate scale-up.
- It should be contrasted using the cheapest materials that afford satisfactory results.
- There should be adequate service provisions for individual plants.

4.3.2 Fermenter Design:

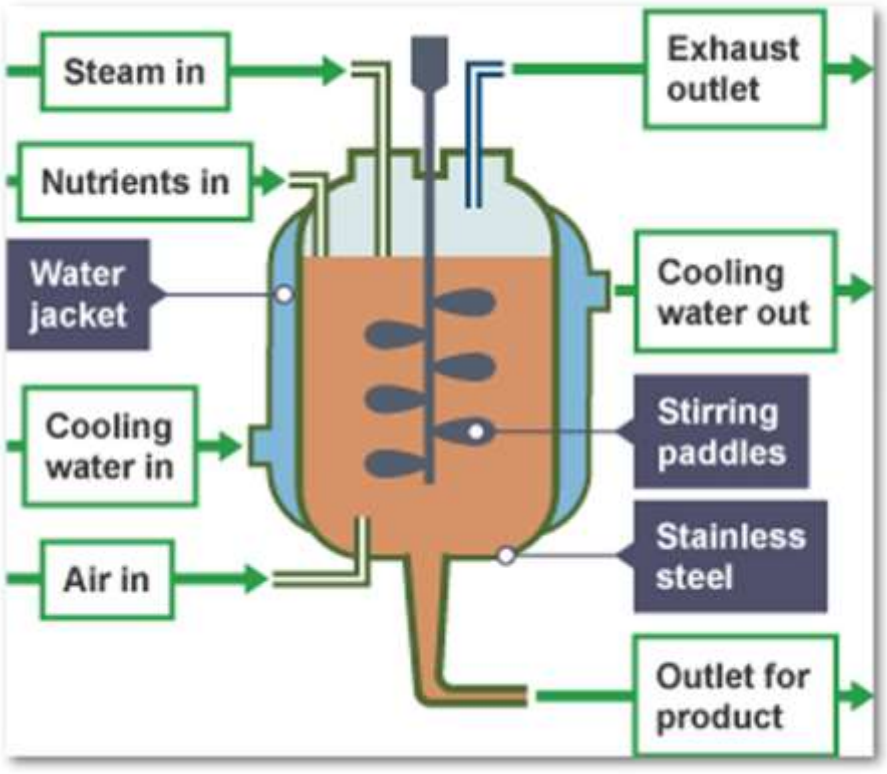

Figure 4.2: A Typical Fermenter

4.3.3 Typical Fermenter Design:

It should be strong enough to withstand the pressure exerted by large volume of the medium. The materials used for the construction of fermenter should not be corroded by the fermentation product and it should not yield toxic ion to the medium.

The fermenter should have provision for the control and prevention of the growth of contaminating microorganisms because industrial fermentation requires pure culture. If aerobic organisms are used in the process, there should be provision for rapid incorporation of sterile air into the medium so that the oxygen is immediately dissolved in the medium and available to the microorganisms. The Carbon dioxide produced by the microorganisms should be removed from the medium. Stirring is necessary to mix the organisms with the medium and to make nutrients and oxygen available to individual microbe. The fermenter should provide provision for the addition of antifoaming agents intermittently depending on the foaming status of the medium. Thermostatic system should be available to maintain constant temperature in the fermenter. There should be provision for aseptic withdrawal of culture during fermentation and also for the aseptic introduction of inoculum at the starting of the fermentation process. A system should be available for detection of pH of the culture medium and also for its adjustment.

4.3.4 Fermentation of Penicillin:

Initially, the strain of microorganism is grown in a sporulation medium and subsequently, the inoculum is transferred into the fermentation medium. After inoculation, incubation is done for 5-7 days to allow spore production. Later, the spores are transferred to the liquid medium in shake flask to allow vegetative growth. Finally, appropriate volume of inoculum is added to final fermenter containing the sterile medium of which consists of lactose, glucose, $NaNO_3$, $ZnSO_4$, $CaCO_3$, phenylacetic acid and vegetable oil etc. The pH is maintained at 7.0 using $CaCO_3$ which is optimum for the production of penicillin. The medium is maintained at a temperature of 22-25^{o}C. After 48-96 hours of cultivation when optimum production of penicillin is obtained, the fermented broth containing about 1% of penicillin is processed. The crude antibiotic is subjected to the extraction procedure.

4.3.5 Solid-State Fermentation Technology:

Solid-state fermentation (SSF) has long been applied to the food industry. SSFs are processes carried out with microbes growing on nutrient impregnated solid substrate with little or no free water. The growth of koji, an enzyme-rich mold grown on shallow trays of steamed rice, is a classic example of SSF. Solid state fermentation (SSF) can be directly carried out with abundant low-cost biomaterials (starch, cellulose, lignin, hemicellulose, chitin, etc.) with minimal or no pretreatment, and thus is relatively simple, uses less energy than submerged fermentation (SmF), and can provide unique micro environments conducive to microbial growth and metabolic activities. Large amounts of excess plant biomass are produced by the agro-industry. It is desirable to use this as a renewable resource for sustainable chemical production via microbial cultivation. If not used to generate a value-added product, the biomass would remain in the waste stream and require expensive disposal or treatments.

a. Advantages:

The medium is cheap. Cereals, wheat bran, and other agricultural products can be used for solid-state fermentation. Special products can be produced. For example, red pigments, whose production is enhanced by solid-state fermentation. The purification, recovery, and disposal of downstream processes in solid-state fermentation are usually simpler than in liquid fermentation.

Solid-state fermentation can produce food with a special flavor and improve nutritional value. For example, tempeh can be used as a substitute for meat and its amino acid and fatty acids can be easily digested. There is no wastewater discharge in solid-state fermentation. Traditional examples of solid state fermentations are the formation of compost, mushroom cultivation, the production of starter cultures or mushroom spawn, leavening of bread dough, and mould ripening of cheeses and production of chocolate and coffee other food products such as salami and soy sauce.

4.4 References:

1. *Liping Wang, Shang-Tian Yang 2007)*. Solid State Fermentation and Its Applications *in Bioprocessing for Value-Added Products from Renewable Resources*