MICROBIAL CULTURE TECHNIQUES

Microbial cultures should be provided with the required chemical and physical environment for proper multiplication and physiological state, so that the cells can carry out the required bioconversion satisfactorily. The chemical environment of a microbial cell is its nutritional conditions in which it is growing. It also includes the correct pH and temperature.

Nutrients for Microbial Culture

- Like any other living system, microorganisms also require a source of energy, carbon, nitrogen, oxygen, iron and other minerals, micronutrients, and water for growth, and multiplication. All these nutrients that are essential for the growth and multiplication of microbial organisms are supplied in the form of nutrient media.
- For laboratory-scale cultivation we may use certain costly media components, but for industrial purposes they should be economical and readily available.
- The media that we use for growing microbes may be **synthetic**, **semi-synthetic**, or **completely natural**. If the nutritive components of the media are not of natural origin, such nutritive media are known as synthetic media, which we can synthesize in the laboratory following certain recipes, mixing the required salts, minerals, and carbon source.
- There are a large number of commercially-available nutrient media, which contain both salts and minerals. Such nutrient media are known as semi-synthetic media. For example, commercially available nutrient broth, trypticase soya broth (TSB), brain-heart infusion (BHI) broth, yeast extract, potato dextrose agar, casein digest, etc., are some examples of semi-synthetic media.

- For laboratory-scale cultivation of bacteria and other microorganisms, these synthetic or semi-synthetic media are preferred, but for industrial-scale cultivation these media are not recommended from an economical point of view.
- For commercial purposes, the recommended media should be cheap and available year round.

The following are the minimum components required in a microbial medium for cultivation of microbes in a laboratory:

Carbon source. A simple carbon source, which is simple to use and easily available, can be used. Sugars such as glucose, lactose, sucrose, and complex polysaccharides such as starch, glycogen cellulose, a mixture of various carbohydrates, and other compounds such as cereal grain powders, cane molasses, etc., are usually used as carbon sources in microbial culture media. The main purpose of the carbon source is to provide energy and carbon skeleton for the synthesis of various other biological compounds.

Nitrogen sources. The major types of nitrogen sources used in culture media are ammonium salts, urea, animal tissue extracts, amino acid mixtures, and plant-tissue extracts.

Micro elements or trace elements. Elements required in small amounts or in traces are to be added into the medium as salts in required amounts. The elements such as copper, cobalt, iron, zinc, manganese, magnesium, etc., are the microelements.

Growth factors. Growth factors are certain organic compounds that are essential for the growth and multiplication of cells, but cannot be synthesized

by the cells. Such compounds should be supplemented in the medium. Certain amino acids and vitamins are also included in this category.

Anti-foams. This is not a nutritive component of the media. Media rich in nutritive components such as starch, protein, and other organic material and also the proteins and other products secreted by the growing cells can result in excessive foaming while the culture media is agitated for aeration. To prevent the formation of foam some anti-foaming agents are included in the media. Certain types of fatty acids such as olive oil and sunflower oil and silicones are commonly used in cell cultures as anti-foam agents.

Energy sources. The carbon sources used in culture media such as carbohydrates, sugars, proteins, lipids, etc., can work as energy sources for the growth and metabolism of the microbial cells.

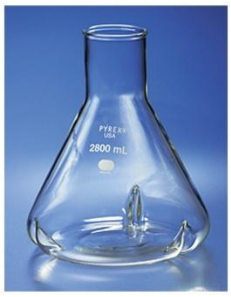
Water. Water is the base of any culture media, whether it is liquid or solid. In solid culture media such as the media of solid state fermentation or agar media the quantity of water is comparatively less than liquid media. In laboratory experiments, single-distilled water or double-distilled water is usually used. But in large-scale microbial cultivation for industrial purposes, the pH and the dissolved salts present should be considered when formulating the media requirements and its concentration. Water is also required for a large number of other services in the laboratory such as cooling, heating, steaming, etc. Therefore, any laboratory should be provided with a source of clean water of consistent quality.

Microbial Culture Equipment

In the laboratory, microbial cells can be grown in tubes and vials, when the volume is five to ten ml, and in Erlenmeyer flasks when the volume is 100 to 1,000 ml. Improvements in the culturing of microbes can be done by making improvements in the design of the flasks and also by using shakers.

Baffle Flasks

Baffle flasks are the modified flasks for microbial cultivation, in which there are v-shaped notches or indentations in the sides of the flasks. The presence of baffles improves the efficiency of oxygen transfer and thereby the growth of microbes because the baffles increase the turbulence while the media is agitated on a shaker.



Shakers

Shakers are the special equipment designed for rotating a platform orbitaly, so that the culture flasks with media kept on the platform of the shaker will be continuously agitated. This agitation helps the medium to be homogeneous in cell-mass distribution, media components, and efficient oxygen transfer.

Fermentors

These are bioreactors used for the cultivation of microbial cells on large scale under controlled conditions for industrial purposes. This closed metallic or

glass vessel has the adequate arrangement for aeration, mixing of media by agitation, temperature control, pH control, anti-foaming, control of overflow, sterilization of media and vessel, cooling, and sampling (removal of sample, while the fermentor is on). Agitation of the media in the bioreactor may be through stirring or aeration or both. This equipment is convenient for operation continuously for a number of days. The essential parts of a laboratory fermentor are given in Figure below.

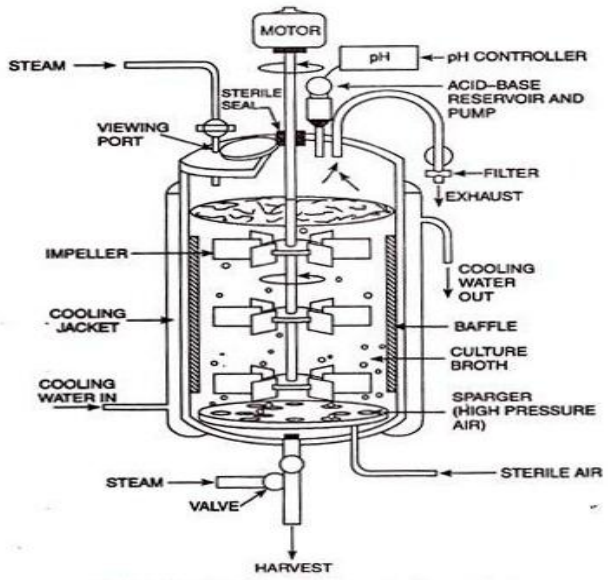


FIG. 39.1. An industrial aerobic fermentor (internal view)

As indicated in the figure, the bioreactors are provided with controls for monitoring and adjusting the many physical and chemical parameters such as temperature, pH, nutrient composition, foaming, etc. Maximum cell growth and product formation can be achieved by controlling these parameters that assist cell growth and metabolism leading to high output of the product. A stirred tank bioreactor is the most commonly used bioreactor for microbial cultivation, in which the microbial medium is stirred with an impeller.

Types of Microbial Cultures

The culturing of the microbial system can be achieved in different ways.

1. Batch culture.

- This is a small-scale laboratory experiment in which a microbial culture is growing in a small volume flask.
- It consists of a limited volume of broth culture in a flask inoculated with the bacterial or microbial inoculum and follows a normal growth phase.
- It is a closed-culture system because the medium contains a limited amount of nutrients and will be consumed by the growing microorganisms for their growth and multiplication with the excretion of certain metabolites as products.
- In batch cultures, the nutrients are not renewed and the exponential growth of cells is limited to a few generations.

2. Fed-batch culture.

- The batch culture can be made into a semi-continuous culture or fed-batch culture by feeding it with fresh media sequentially at the end of the log phase or in the beginning of the stationary phase without removing cells.

3. Continuous culture.

- Bacterial cultures can be maintained in a state of exponential growth over long periods of time using a system of continuous culture, designed to relieve the conditions that stop exponential growth in batch cultures.
- Continuous culture, in a device called a chemostat, can be used to maintain a bacterial population at a constant density. This is a very convenient method to get continuous cell growth and product formation over a long period of time.

- In continuous culture, the nutrient medium including the raw material is supplied at a rate that is equal to the volume of media with cells and product displaced or removed from the culture. The volume removed and the volume added is the same.