Techniques of microbial cultures

Principles of microbial growth

Growth: is an increase in the cell material, which is expressed in biomass or number of cells, It is a biological sequential steps stimulate by enzymes, ideal growth depends on:
1) The existence of the necessary nutrients for growth and the method of distribution.
2) Ideal environmental conditions such as (temperature, pH, aeration, etc.).

• **Doubling time**: is the period of time necessary to double the weight of the biomass.

• **Generation time**: is the time required for doubling the number of cells.

Generation time equals to doubling time through balanced growth (logarithmic phase).

Microorganisms growth curve

The growth curve of microorganisms has divided to:

1- **Lag phase**: there is no growth, but chemical analysis explained the occurrence of no appeared metabolic activities that helps cells to adapt with new cultural conditions. It is fast transition phase starts after the inoculation and ends at the beginning of the second phase.

2 - **Logarithmic phase**: This phase in which the growth is unlimited, the nutrients found in abundance, there is no inhibitor materials, maximum growth rate and increasing the number of cells , and then begins a gradual downward slowly, and the production of the primary metabolites in this phase , such as vitamins , proteins and nucleic acids and others which are important for the growth of the cell.

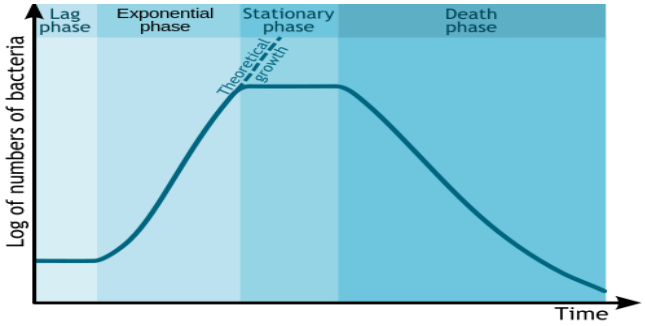
3- Stationary phase: where growth ceases because of the entry into force of nutrients and accumulation inhibitor materials and shows a balance of biomass where the growth rate is equal to the rate of death, termed stationary phase is a misnomer because the cells are metabolically active and produce secondary metabolites in this phase such as antibiotics and growth regulators.

4- Death phase: in which the growth rate becomes negative and stop all metabolism operations and gets the dissolution of the cells.

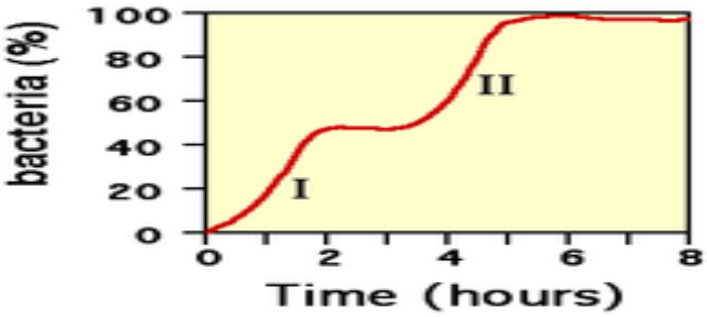
Biphasic growth:

This happens when there is tow carbon sources in the medium such as lactose and glucose, the bacteria begins by consuming easiest carbon source (glucose) ,when the bacteria

arrives the stationary phase starts with consumption the second sugar(lactose) and also passes through the other phases of growth.



Growth curve of bacteria



Biphasic growth of bacteria

The microorganisms used in biotechnology are cultivated in solid or liquid media and by using many systems, it divided into two types depending on the material used mainly:

1-Liquid state fermentations

Liquid state fermentations are including three systems:

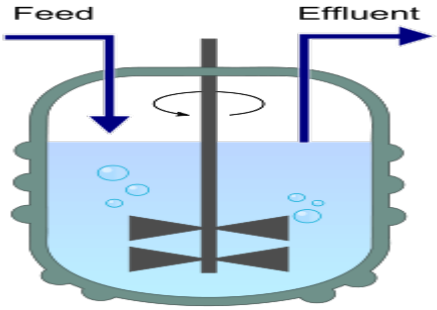
1-Batch culture system

This is a closed system where an inoculum (starter) is adding to the nutritional medium and provide ideal conditions for growth and rarely have the growth rate constant in these cultures due to lack of stability of the conditions of nutrition, which is characteristic of this system. Observed curve growth in this system with four phases. .

Batch culture is used for the production of biomass and primary and secondary metabolites.

2-Continuous culture system

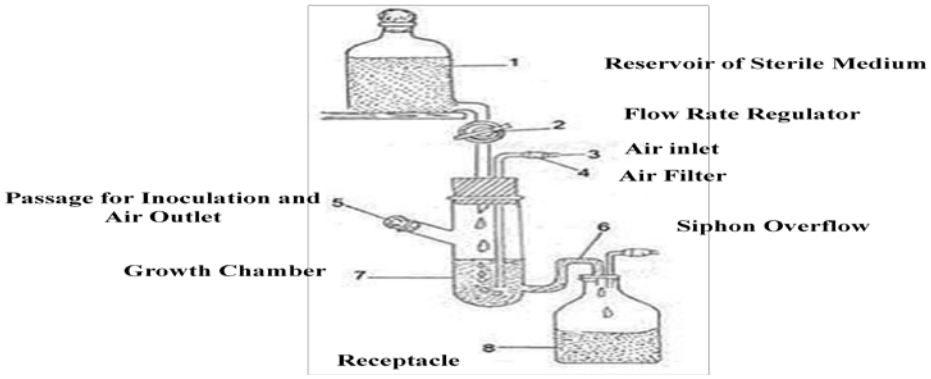
The nutritional medium is adding and pulls continuously part of an equivalent of the size of the culture, where the growth of the microorganism under conditions of stable and consistent, and can control the conditions of the culture continuously by measuring each determining factor for the growth alone. The control of this system with the following:



a-Chemostat

The growth factor selected is one of the components of the nutritional medium controls the growth process, and adding continuously to the culture when implements within a fermenter, and pull at the same time smaller equivalent of the culture, for example, be the glucose is the limiting factor for growth in the medium when it available the growth in the maximum and when it consumes the growth is less and thus less productivity.

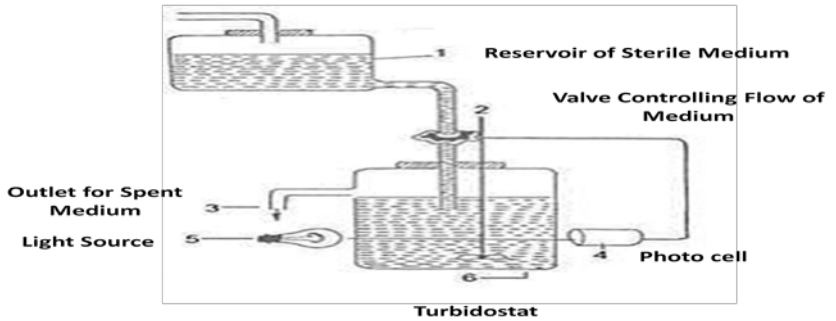
Chemostat, a continuous culture system



Turbidotat

the concentration of cells remains fixed in the culture by controlling of the flow of the nutritional medium to the fermenter, so that the turbidity remains within certain limits. This done by using a photoelectric cell that gives a signal to the pump that proves the nutritional medium to open if the biomass exceeded limit installer and shut down if less of the constant term.

Turbidostat



Turbidostat

Biostat

Here biomass measured by carbon dioxide output, and based on that they made up the maximum amount of biomass, this means a higher amount of CO_2, and this point is to withdraw the product and the addition of new nutritional medium. The method of chemostat most common because it is not needed for complex controlling devices.

Features of continuous culture system:

Although the advantages of this system, but it is little used compared to the batch system because it is susceptible to contamination (also occurs from the inside because of a problem

of strain degeneration) and because of the long experience in dealing with the batch system, and its advantages:

A- Productivity: The productivity very high compared to the batch system because of maintaining optimal conditions for production during the fermentation process and the lack of non-productive period (the process of preparation and sterilization), and the cost of construction, equipment and maintenance are less.

B- The homogeneity of the process and ease: The organization of these cultures automatically by maintaining a concentration of cells, nutritional medium and product consistently reverse batch system, in addition to the need for a few of the hands of the workforce.

Uses of continuous system

A-The production of biomass: This system is ideal for the production of single cell protein compared to the batch system as the strain degeneration does not affect negatively on production, for example by producing SCP from bacteria that grow on methanol.

B- Isolation and improvement the strains: This is done because of the high electoral nature of the chemical organizer which is used in the continuous system compared to batch system, for example the election of the strains of high productivity of enzymes.

C- Get the industrial microorganisms: because of the advantages of this system which provides good information on the microorganisms and their uses in physiology, biochemistry and others.

Fed-batch system

It is described as a batch system fed continuously with nutritional medium without removing any amount of the culture and thus increasing culture size with time. The amount of nutritional medium inputted is equal to the amount that is consumed by the cells, therefore; the concentration of cells remains constant in the culture and being semi-stable in contrast to the continuous system, where the growth rate is decreasing in the fed- batch system and remains constant in the continuous system. The applications of these cultures:

A-Removing the catabolic repression:

This is done to carbon sources quick representation as to prevent the phenomenon of braking catabolism that inhibit the synthesis of enzymes, and so that the lactose was used as a source of slow -degradable instead of glucose in the production of penicillin.

B- Prevent the toxic effect of some of the components in the nutritional medium:

This system is used when there is a toxic ingredient to microorganisms during the production process as in the use of Na-phenyl acetate as the raw material for the production of penicillin and toxic at the same time to *Penicillium chrysogenum* so that this controlled by adding them in small batches in a row.

Culture of microorganisms by dialysis

This technique consists mainly of two parts separated from each by a membrane, and in one of the two sections microorganisms suspended in the liquid medium, and the other part which is larger than the first is used for the storage of the new nutritional medium. The nutrients pass through the membrane from the reservoir to the culture and pass some metabolites from the last to the tank. Organization of the work depends on the properties of the membrane and the volume ratio of the two sections to each other.

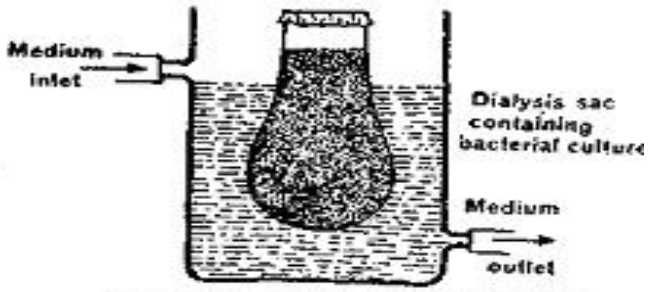


Fig. 17.5. The Dialysis technique

Types of membranes

1- The special dialysis membranes:

It consists of cellophane or different plastics and pores ranging between (0.3 - 10) nm, which holding large molecules, such as enzymes and toxins and allow the passage of small molecules such as sugars and mineral salts.

2- Cellulose acetate membranes or porcelain:

It is actually a membrane filter openings ranging between (25-200) nm and so they obscure the microbial cells leaving large molecules to pass.

3- Transport solution membranes:

It is made of teflon, which allows the passage of molecules of gas only, and are not possessed pores in physical meaning, but works as a solvent pass molecules dissolved in a manner simple diffusion.

Properties of dialysis culture

A- Working to prolong the period of exponential growth due to continuous provision for nutrients and removing toxic metabolites.

B- The apparent increase in biomass.

Solid-state fermentation (SSF)

It means the growth of microorganisms on the solid material in the case of absence or near absence of free water, The nutritional media that commonly used here are cereals, legumes, wheat bran and cellulosic materials, and these substances poorly soluble in water, cheap and contains high concentrations of nutrients, and are used in food production, enzymes, organic acids and SCP.

The growth of microorganisms in these cultures depends on water activity (A_w) where the bacteria grow in 0.9 compared to fungi grow at 0.6-0.7, therefore; the molds are largely used because they can tolerate the low levels of A_w.

Types of solid-state fermentations

1- Solid-state fermentation using pure cultures

This method is used in the fermentation for the production of enzymes, organic acids and SCP, for example *Aspergillus oryzae* used in eastern foods production.

3- Solid-state fermentation using mixed cultures

This type is used for obtaining a maximum production as in turning straw into biomass using the yeast *Candida lipolytica* and fungus *Chaetomium cellulolyticum*, and these fermentations are generally cheap and did not need high-technique.