

Microbial culture techniques

Microbial growth: refers to the **increasing number** of cells, **not** to the changes in the **size** of cells. Microbial growth can **be measured by two ways:** **Gravimetical** methods and **Numerical** methods.

Generation Time

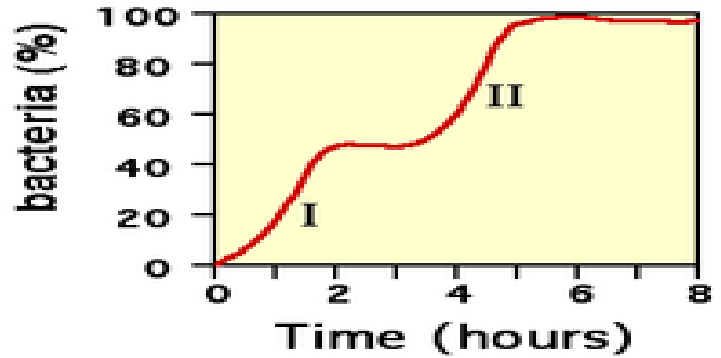
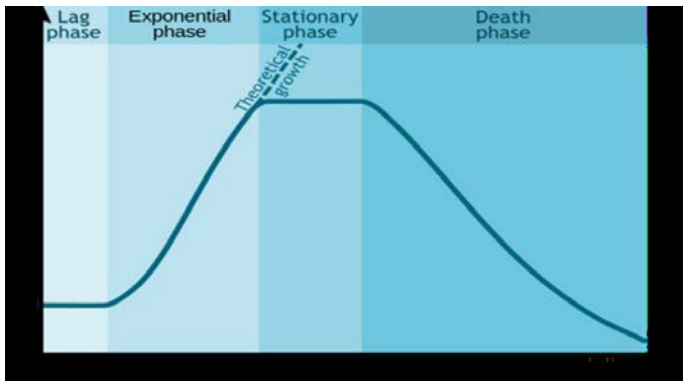
The time required for a cell to **divide** or the population to **double** is called generation (doubling) time.

Four characteristic phases of the growth cycle are recognized:

- 1- **Lag phase:** is the first phase observed. It is characterized by **no increase in cell number**; however, the cells are actively metabolizing, in preparation for cell division.
- 2- **Exponential or log phase:** This is the period in which the **cells grow most rapidly**, doubling at a fairly constant rate. Primary metabolites are produced in this phase.
- 3- **Stationary phase:** the number of **viable cells is equal to the number of dead** cells. The factors that cause cells to enter stationary phase are related to changes in the environment, typically caused by high cell density, depletion of nutrients and accumulation of waste products. Secondary metabolites are produced in this phase.
- 4- **Death phase:** the number of **viable cells decreases** geometrically (exponentially), therefore, cells die quickly.

“Diauxie” or double or biphasic growth:

This phenomenon is characterized by **two growth cycles** —the first one on the **preferred** sugar, followed by a second one on the **less-preferred sugar**. Both are separated by a short period during which the population apparently does not grow. This period is known as the diauxie lag phase.



There are two groups of fermentation processes:

- **Liquid fermentation:** cells are suspended in **aqueous medium**. There are three types of liquid fermentation; Batch, Fed-batch and continuous.
- **Solid fermentation:** The volume of **free liquid** is **minimal** and the cells are adsorbed to a solid and nutrient rich material.

I) Batch Fermentation (also is known as a closed system)

- 1- The culture is inoculated into the sterile medium contained in **a closed vessel**.
- 2- **No additional nutrients** are added once the fermentation process starts (the nutrients of the medium is neither renewed nor metabolic wastes removed).
- 3- Basic controls for pH, temperature, dissolved oxygen, and foam of the fermentation medium are **regulated in** this type of fermentation.
- 4- Exponential growth last only for a **few generations**.
- 5- The growth rate is **not constant** due to lack of stability of the optimal conditions.
- 6- Growth curve with **four phases** is observed.
- 7- The products, be they intra- or extracellular, are **harvested only at the end** of the run.

II) Fed Batch fermentation (also known as semi-continuous or semi- batch system)

- 1- It is **quite similar to Batch** fermentation **except** that the **nutrients** or one or two components of nutrients are **added periodically** in the fermentation medium, therefore, the culture volume increases during the course of operation until the volume is full.

- 2- This type of fermentation **lengthens the log and stationary phase** of the cells thereby causing **increased amount** of bioproduct.
- 3- The growth rate is **semi constant**.
- 4- The products, be they intra- or extracellular, are harvested only at the end of the run.

Reasons for Fed-Batch Cultures

- To remove repressive effects of rapidly utilized carbon sources.
- To reduce the toxic effect of some medium components.

Applications of Fed-Batch Cultures

1- The yeast cell production

The yeast cell production, in which sugar (**glucose**) was added **periodically** during the course of fermentation to **maintain a low sugar concentration** to **suppress** alcohol formation.

2- Penicillin production

Penicillin fermentation, in which the **energy source** (e.g., glucose) and **precursors** (e.g., phenyl acetic acid) were added **periodically** during the course of fermentation **to improve** penicillin production.

III) Continuous fermentation (is also known as open system or Continuous flow culture (CFC))

- 1- There is **continuous removal** of culture medium as well as **continuous addition** of sterile nutrient medium.
- 2- Conditions are **predetermined** as to what should be the **flow rate** of incoming nutrient solution so that the **volume** of fermenting medium **remains the same** and also fermenting **microbes remain in same phase** of growth termed as **steady state of growth**.
- 3- Growth rate is **constant**.

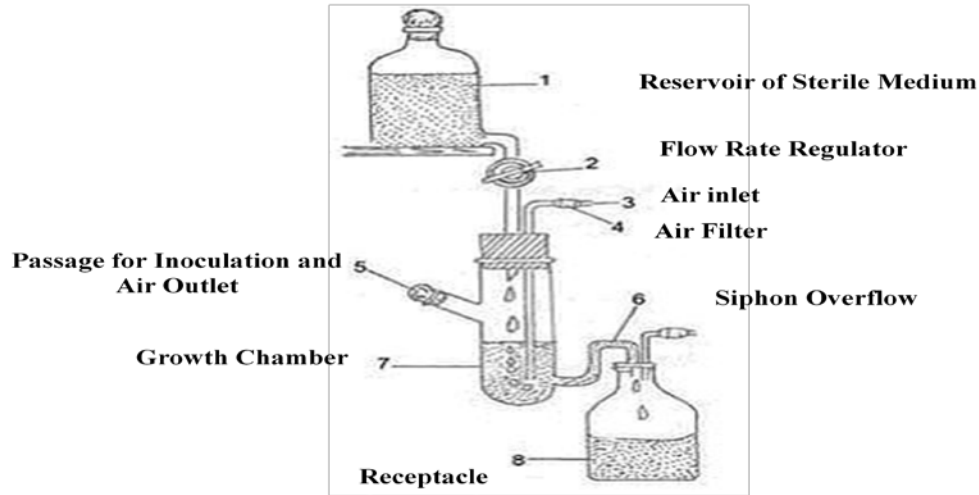
The steady state of growth can be achieved by:

1- Chemostat

The growth factor selected is **one of the components** of the nutritional medium(e.g. carbon source, Nitrogen source, Magnesium, Sulphate or phosphorus) **controls the growth process**, and

adding continuously to the culture when it depleted. The volume of the chemostat can be controlled either by using : a pump system or an overflow system.

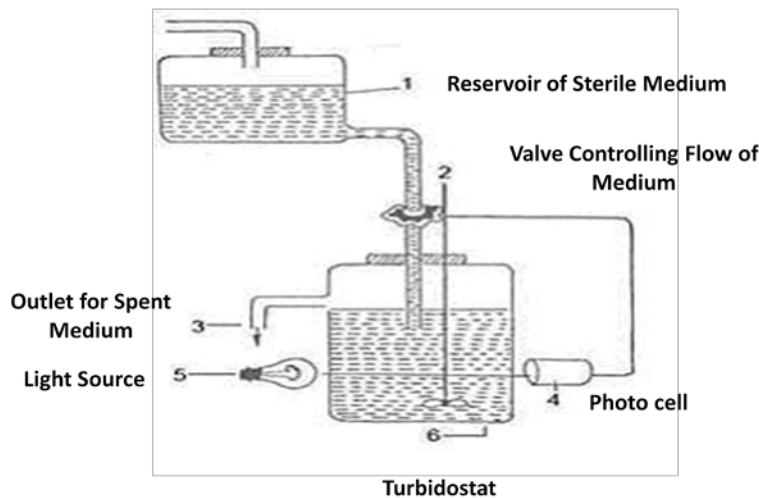
Chemostat, a continuous culture system



2- Turbidostat

The cell density in turbidostat is measured by photo electric device, which sends signal to the turbidostat to increase or decrease the flow rate of the medium to the fermentor vessel. The pump attached to the fermentor for controlling the flow rate will turn on or off depending on the increase or decrease in the level of biomass beyond set point.

Turbidostat



3- Biostat

Regulated by measure biomass indirectly by measuring the amount of gas produced by organisms or change in pH due to certain biochemical activity.

Applications of continuous culture:

1- Industry: Used in the production of **therapeutic Pharmaceuticals**, antibiotics, ethanol, and fermented foods such as cheese.

2- Research: Used **to collect data** to be used in the creation of a mathematical model of growth for specific cells or organisms, **analysis of biological processes** in microorganisms, and study **biofilm formation** in *Pseudomonas aeruginosa*.

3- Biological waste treatment.

4- Cell propagation.

Solid State Fermentation (SSF)

Solid-state fermentation (SSF) is defined as the **fermentation process** in which microorganisms **grow on solid materials** without the presence of **free water**. The growth of microorganisms in these cultures depends on **Water activity**(A_w).

1- **Bacteria** grow at $0.9(A_w)$

2- **Fungi** grow at $0.6-0.7(A_w)$, the **molds** are largely used because they can tolerate the low levels of A_w .

The **source of solid material** as well as culture is cereal grains of wheat, rice, maize etc. SSFs are used for production of food, enzymes, organic acids, SCP...etc.

Steps of SSF:

1- The grains **are moistened** with water and **ground** to form a paste. Additional supplements like salts etc. may be added to the solids prior to sterilization.

2- The solid material is then **transferred to shallow** metallic containers and is **steam sterilized**.

3- This is followed by the **spraying of culture inoculum** on to the **surface** of sterilized medium and **incubation** is carried out under controlled conditions of temperature, air and humidity.

SSF processes can be classified based on the seed culture for fermentation into:

1- **Pure culture**, such as lactic acid production from wheat bran using *Lactobacillus amylophilus*.

2- **Mixed culture**, such as cellulase production using *Trichoderma reesei* with *Aspergillus* spp.

Dialysis fermentation

Dialysis is a membrane process where solutes ($MW \sim < 100$ Da) **diffuse** from **one side** of the membrane (feed side) **to the other** (dialysate side) according to their **concentration gradient**.

In Dialysis fermentation, a **selectively permeable** membrane separates a **culture chamber**, in which fermentation takes place, from a **medium reservoir**. **Nutrients** in the reservoir **diffuse to** the **culture chamber** while **metabolite** products **diffuse** to the medium reservoir. **Low product concentrations** are maintained in the culture chamber, **minimizing** the effects of metabolic **inhibition**.



Fig. 17.5. The Dialysis technique