Bacteriology

Lec 7 Growth and multiplication

Growth means an increase in size, number, weight, and mass. It is a group of reactions and events led to an increase the macromolecules number and then cell division and reproduction.

Cell cycle

A group of steadily successive events are interrupted with periods which depending on environmental conditions. The required time from the beginning to the end of division known as generation time and the resulting growth called growth rate.

Growth rate and generation time

Generation time: The time for a single cell to undergo fission.

1- Species of M.O. **2-** Nutrients.

3- Environmental conditions: PH, and temperature. **4-** Growth phase.

Prokaryotic cell cycle:

Most of studies on prokaryotic cell cycle were done on *E.coli* because of it is easy to handle. Prokaryotic cell cycle includes:

1- **First stage:**

This period is still under speculating. Mostly, under the optimal condition it disappears due to the shortage of generation time, also the environmental conditions greatly affect the cell.

2- **Second stage:**

A- stage of DNA synthesis abbreviated as C instead of S, it means

chromosome replication.

B- It required most of cycle time.

C- It controls the continuity of the cycle, since when the DNA synthesis is interrupted the cell will not divide.

D- It is affected, a little, by the environmental conditions.

3-Third and fourth stages:

A-After the DNA synthesis stage, there is a gap before the cell is dividing into tow daughter cells.

B- It represents both third and fourth stages, G2 and M.

C- It referred as to D.

D- It is affected, a little bit, by the environmental conditions.

Growth curve of bacteria:

When bacteria are inoculated into a new culture media, it shows a characteristic growth curve which has four phases:

1- Lag phase:

During this phase, bacteria exhibit growth in size but no increase in cell number and the bacteria are preparing for synthesis of DNA, various enzymes, and other components, which are for cell division .The lag phase varies in length with the conditions of the M.O and the nature of the media, this mean that the phase may be long if the inoculum is from an old culture or if the culture is refrigerated.

2- Logarithmic(exponential) phase:

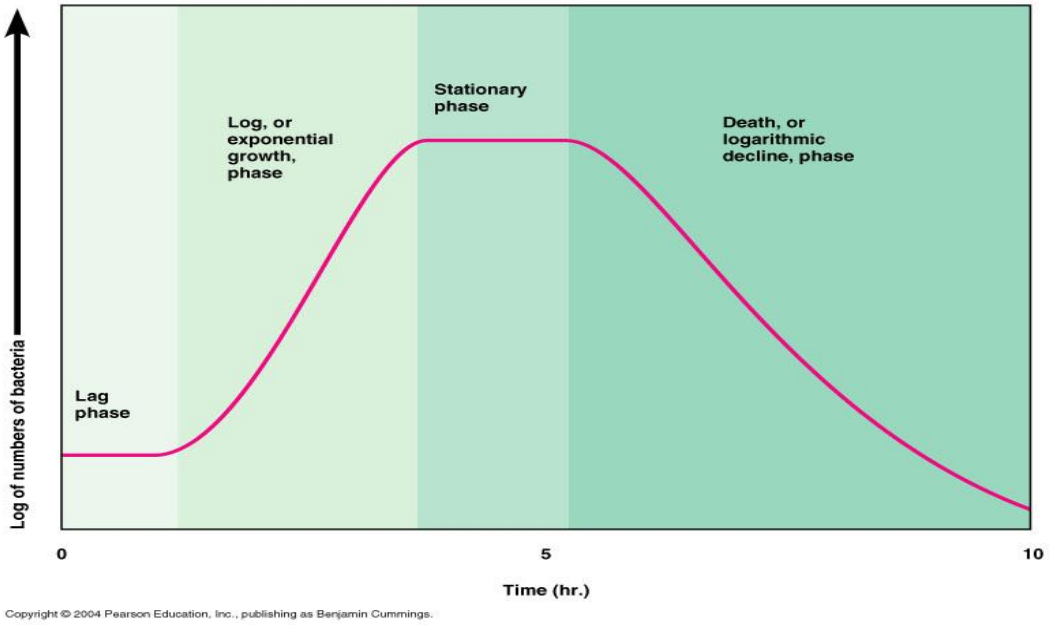
During this period the cells divide steadily at a constant rate. The log of the number of cells is plotted against time results in a straight line. Under appropriate conditions, the growth rate is maximal during this phase, and the population is most nearly uniform in terms of chemical compositions of cells.

3- Stationary phase:

During this phase the growth rate is equal to the death rate. Food begins to run out, poisonous waste products accumulate, PH changes, hydrogen acceptors are used up, and energy transfers are diminished. The rate of fission begins to decline, and the organisms die in increasing numbers.

4- Death (decline) phase:

Eventually the number of viable bacterial cells begins to decline, signaling the onset of the death phase .The kinetics of bacterial death, like those of growth, are exponential.



Growth curve of bacteria:

When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase).

Batch culture:

When liquid media is inoculated with bacteria the nutrients are expended and metabolic products accumulate in the closed environment, so that the normal bacterial growth curve is a characteristic of the batch culture.

Continuous culture:

Bacteria may also be grown in continuous culture where nutrients are supplied and end products removed continuously, so that the logarithmic growth phase is maintained and the bacteria never reach stationary phase because liquid medium is continuously fed into the bacterial culture and this can be done by using:

1- Chemostat
2- Turbidostat
3- Dialysis technique.

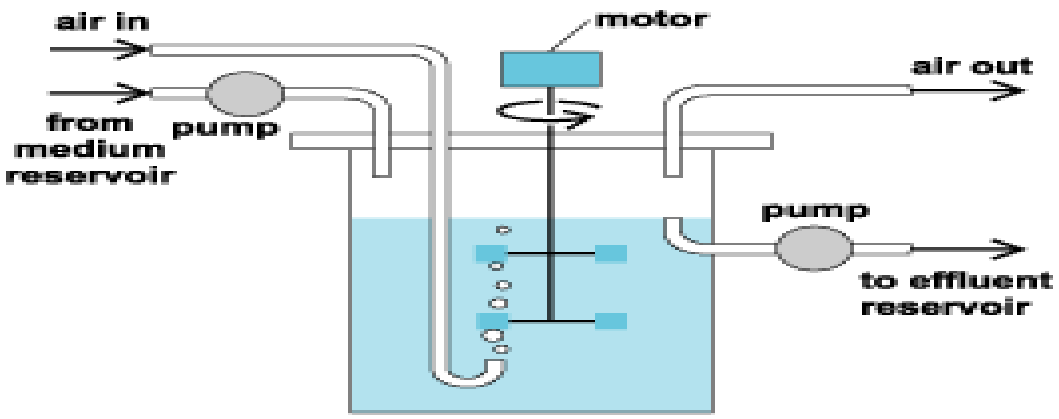
Differences between batch culture and continuous culture

Characteristics	Batch culture	Fed-batch culture	Continuous culture
Cultivation system	Closed type	Semi-closed type	Open type
Addition of fresh nutrition	No	Yes	Yes
Volume of culture	Constant	Increases	Constant
Removal of wastes	No	No	Yes
Chance of contamination	minimum	Intermediate	Maximum
Growth phase	Lag, log, stationary and decline phase	Lag, log , stationary and decline phase	Lag and log phase
Log phase	Shorter	longer	Longest and Continuous
Density of bacteria	Change with time	Change with time	Remain same
Product yield	Low	Medium	High

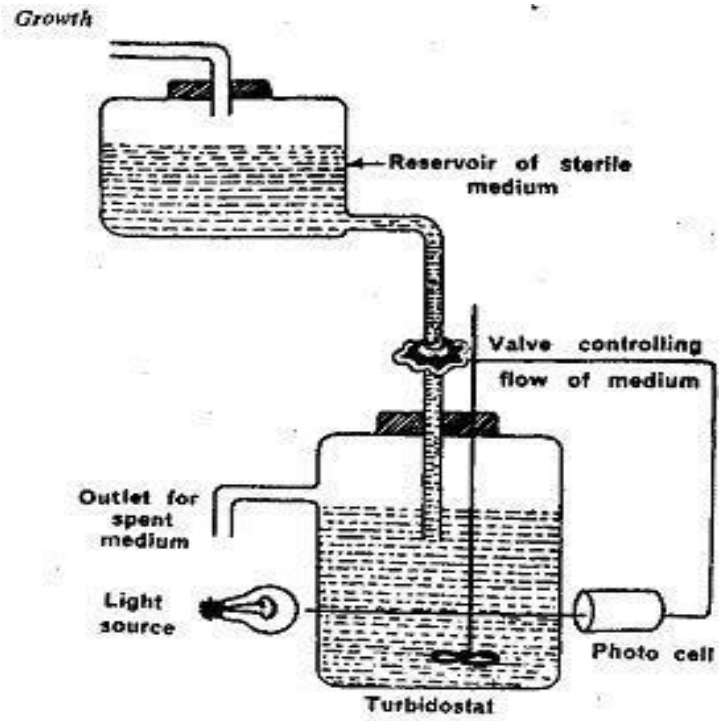
Continuous culture

To study the metabolism of an organism for experimental research or industrial processes, it is often desirable to maintain a microbial population in the logarithmic phase of growth in a constant environment. This is accomplished by a technique called continuous culture of M.O. A variety of different pieces of apparatus has been developed to grow
M.O in continuous culture:

1- **Chemo stat:** A **chemo stat** (from *chem*ical environment is *stat*ic) is a **bioreactor** to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms are continuously removed at the same rate to keep the culture volume constant. By changing the rate with which medium is added to the bioreactor the **specific growth rate** of the **microorganism** can be easily controlled within limits.



2- **Turbido stat:** is another continuous culture apparatus, the system include an optical device which measures the absorbency of the culture density (turbidity) in the growth vessel .Changes in turbidity retard (or increase) passage of light through the culture. These changes activate mechanisms that control the flow of nutrient into, and the flow of waste out of, the main culture vessel.



Advantages of Continuous culture system:

They provide a constant source of cells in the logarithmic phase of growth for the study of physiology and genetics of the organisms. Secondly, these systems allow the cells to be grown continuously in limiting concentration of the nutrient. Such growth gives valuable information on the catabolism of the limiting substrate.

Methods of cell cycle study:

For a study, it is preferable that all culture cells being at one stage, a synchronous culture should be chosen, and this would be bone by:

1- Induction methods

2- Choosing methods.

Synchronous culture:

A synchronous or synchronized culture is a microbiological culture or a cell culture that contains cells that are all in the same growth stage. Normal, non-synchronous cultures have cells in all stages of the cell cycle. Obtaining a culture with a unified cell cycle stage is very useful for biological research. A synchronous culture can be treated as a single cell; the number of cell in the culture can be easily estimated.

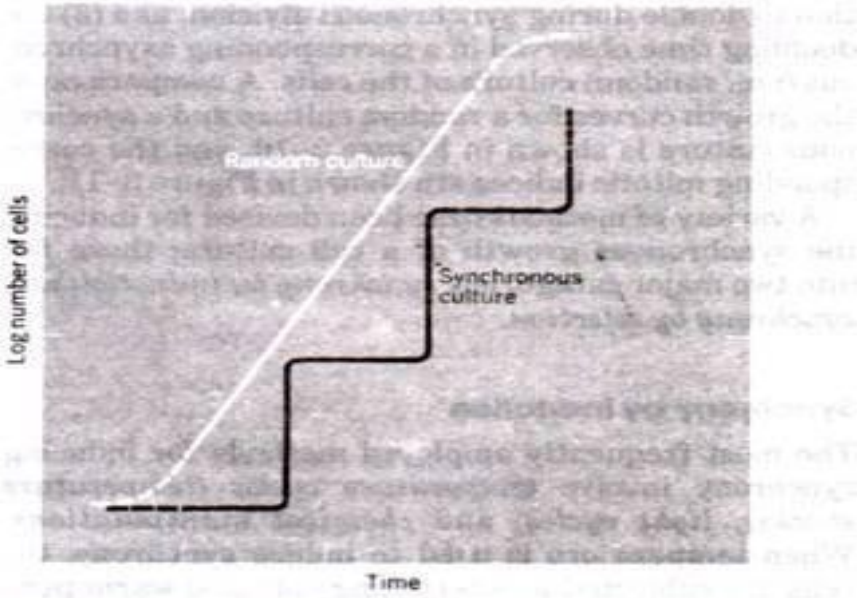


FIGURE 2–10 A comparison of the growth curves for idealized *random* and *synchronous* cell cultures.

Synchronous cultures can be obtained in several ways:

A-Induction method; this can be done by

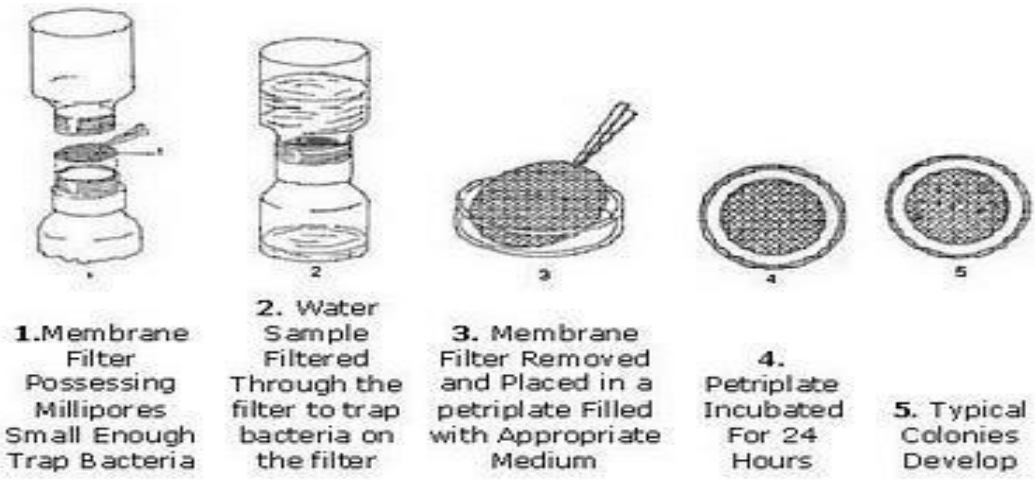
1- External conditions can be changed, so as to arrest growth of all cells in the culture, and then changed again to resume growth .The newly growing cells are now all starting to grow at the same stage, and they are synchronized. For example, for photosynthetic cells light can be eliminated for several hours and then re-

introduced. Another method is to eliminate an essential nutrient from the growth medium and later to re- introduce it.

2- Cell growth can also be arrested using chemical growth inhibitors. After growth has completely stopped for all cells, the inhibitor can be easily removed from the culture and the cells then begin to grow synchronously. Nocodazole , ex: is often used in biological research for this purpose.(chemical factors).

B-Choosing method:

Cells in different growth stages have different physical properties. Cells in a culture can be thus being physically separated based on their density or size, for instance. This can be achieved using centrifugation (for density) or filtration (for size). The most widely used method for obtaining synchronous culture is the, Helmstetter -Cummings technique , a bacterial culture is filtered through a membrane. Most bacteria pass through, but some remain bound to the membrane. Fresh medium is then applied to the membrane and the bound bacteria start to grow. Newborn bacteria that detach from the membrane are now all at the same stage of growth; they are collected in a flask that now harbors Synchronous culture.



Methods that used for measured bacterial growth

1. Methods for measurement of cell mass:

Methods for measurement of the cell mass involve both direct and indirect techniques:

A- Direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation. This is the most direct approach for quantitative measurement of a mass of cell. The sample is centrifugation or filtered and the residue or the pellet is washed a number of times to remove all extraneous matter, the residue is then dried and weight. It can be used only with very dense cell suspension. It is commonly used for measuring growth of moulds in certain phases of industrial work.

B- Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.

C- Indirect measurement of chemical activity such as rate of O2 production or consumption, CO2 production or consumption.

D- Turbidity measurements employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve.

1. Source of Light of a single wave-length (monochromatic)

2. Filter

3. Tube with cell free medium

4. Tube with suspension of microorganisms

5. Photocell or Detector

2. Methods for measurement of cell numbers:

A- Direct microscopic count also called Breed method or Haemocytometer, is possible using special slides known as counting chamber .Dead cells cannot be distinguished from living ones. Only dense suspension can be counted (cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.

B- Electronic counting chamber; count numbers and measure size distribution of cells. Such electronic devices are more often used to count eukaryotic cells such as blood cells.

C- Indirect viable cell count also called plate count. Involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in nontoxic diluents (water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a colony forming unit (CFU). And the number of CFU is related to the viable number of bacteria in the sample.

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted.

Disadvantages are

1- only living cells develop colonies that are counted,

2- clumps or chains of cells develop into a single colony,

3- colonies develop only from those organisms for which the cultural condition are suitable for growth.

3- Determination of cell activity:

Measurement of a specific chemical changes produced on a constituent of the medium, ex: acid production from sugar in the nutrient medium. The amount of

acid produced is proportional to the magnitude of the cell suspension. Also, specific enzyme may be assayed to measurement cell growth.