VIVEKANANDA COLLEGE
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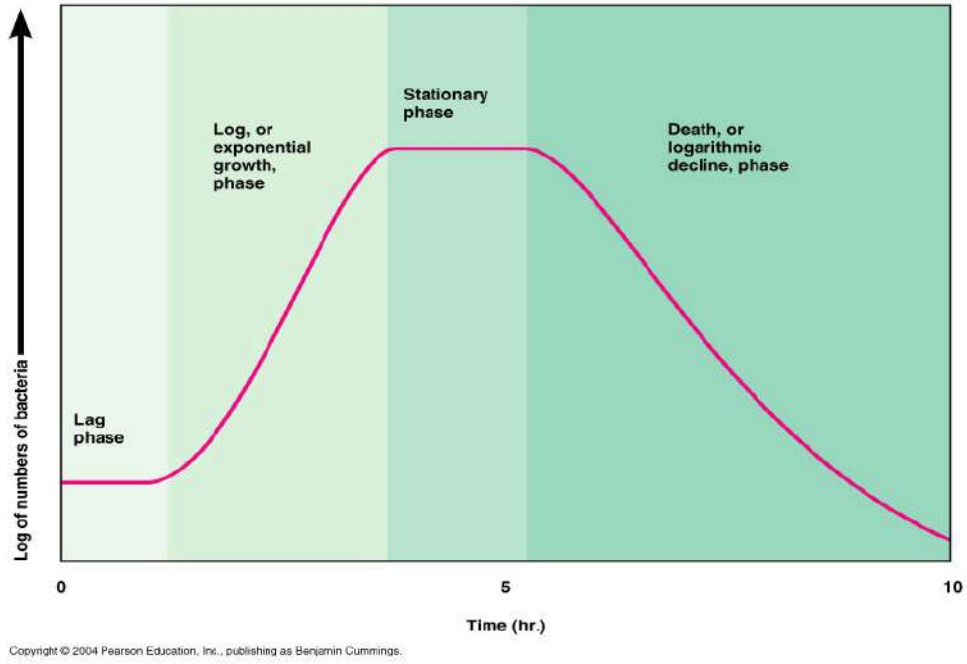
NAAC ACCREDITED ‘A’ GRADE

Topic	: Bacterial Growth
Course Title	: Basic Microbiology and Microbial Genetics
Paper	: CC 10
Unit	: II
Semester	: 4
Name of the Teacher	: Dr. Kakali Roy
Name of the Department	: Biochemistry

Bacterial Growth phases

- Bacteria replicate by the asexual process of **binary fission**.
- These microbes reproduce rapidly at an exponential rate under favourable conditions.
- When grown in culture, a predictable pattern of growth in a bacterial population occurs. This pattern can be graphically represented as the **number of living cells in a population over time** and is known as a **bacterial growth curve**.



- Four distinct phases of the growth curve : lag, exponential (log), stationary and death phase.

Lag Phase

- Initial phase ----- metabolically active --- but not dividing
- Prepare for reproduction, synthesizing DNA and various inducible enzymes needed for cell division

- Increase in size, but no increase in cell number
- May last for an hour or more
- Generally longer if the cells taken from an old or refrigerated culture
- Lag phase may be short when cells taken from young culture and inoculated to a fresh medium

Exponential (Log) Phase

- Cells are dividing by binary fission and doubling in numbers after each generation time.
- High metabolic activity as DNA, RNA, cell wall components, and other substances necessary for growth are generated for division.
- Usually used in biochemical and physiological studies of culture.
- Generation time constant --- logarithmic plot of growth almost straight line
- No. of cells increases as an exponential function of 2^n.

Stationary Phase

- Population growth begins to decline as the available nutrients become depleted and waste products start to accumulate.
- Bacterial cell growth reaches a plateau, or stationary phase, where the number of dividing cells equal the number of dying cells.
- Population level of around 10^9 cell per ml
- No overall population growth -----number of viable cells remain constant
- Growth rate is exactly equal to the death rate

Death Phase

- Number of dying cells begin to exceed the number of new born cells

- Nutrients become less available ---- waste products increase ---number of dying cells continues to rise.
- Number of living cells decreases exponentially ---- population growth experiences a sharp decline.

Generation time

- Most bacteria reproduce by binary fission
- Doubling the number of viable bacterial cells
- At specific time intervals population continuously doubles
- Specific time interval between two subsequent binary fissions ----generation time or doubling time
- Generation times for bacteria vary from about 12 minutes to 24 hours.
- Bacterium *E. coli* ----generation time -----as short as 20 minutes under optimal conditions
- Geometric progression (exponential growth) : $1 \rightarrow 2^1 \rightarrow 2^2 \rightarrow 2^3 \rightarrow 2^4 \rightarrow 2^5 \rightarrow \rightarrow \rightarrow 2^n$ n = number of generations
- **Generation time (G) = t / n = t log 2 / log B_n – log B_0**

  Where t = time elapsed between B_0 and B_n

  n = number of generations

  B_0 = initial population

  B_n = population after time t

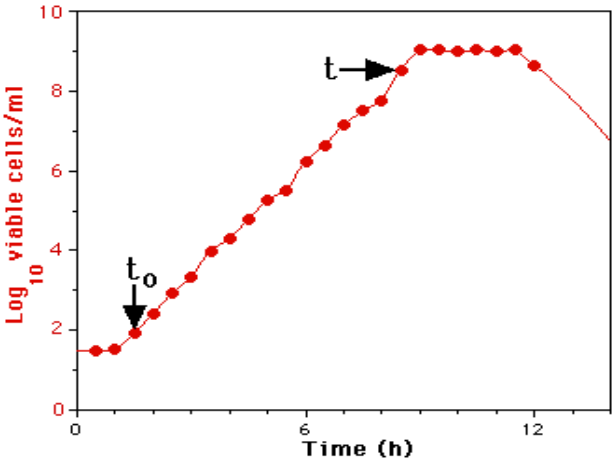
Kinetics of growth

- Growth kinetics is an autocatalytic reaction
- Rate of growth directly proportional to the concentration of cell

- Cell conc. measured by direct method (cell mass conc. and cell number density by its dry weight, turbidity i.e. optical density, plate counts etc.) or by indirect method (Cell density measured by the conc. of proteins, ATP or DNA content).

- Exponential growth rate is the 1st order reaction

- Rate of biomass is correlated with the specific growth rate (μ) and the biomass concentration or cell number, X in time t (hours)

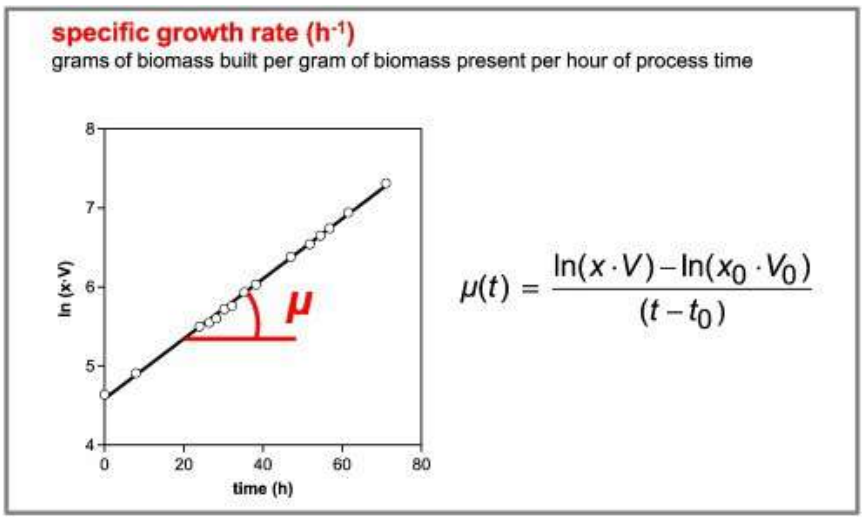


- Rapidity of growth : $dX/dt = \mu X$

  or, $dX / X = \mu\, dt$

- Integrating it from X_0 at $t = 0$ and X_t at $t = t$
- $\ln (X/X_0) = \mu t$ or $X = X_0 e^{\mu t}$
- Taking the neutral log,

  $\ln X = \ln X_0 + \mu t$



V = working volume, V_0 = initial working volume, X.V = mass of biomass, X_0 = initial biomass conc.

- **Specific growth rate be a function of three parameters**
  - concentration of growth limiting substrate, S
  - maximum specific growth rate, μ_{max}
  - substrate - specific constant, K_s

➢ Monod equation describes the dependence of the growth rate on the Substrate concentration

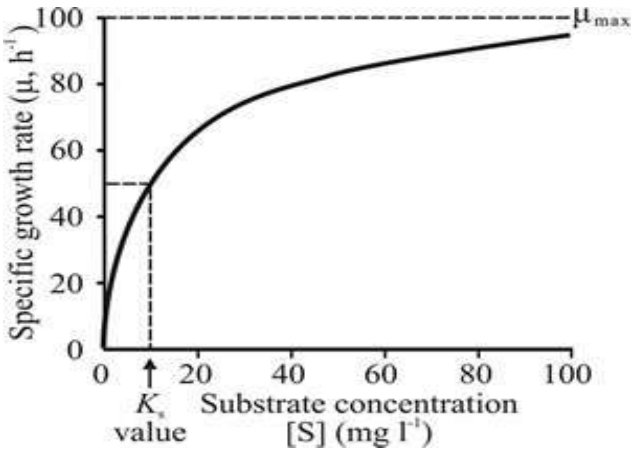
- $\mu = \mu_{max} S / K_s + S$

where, μ = specific growth rate (h^{-1})

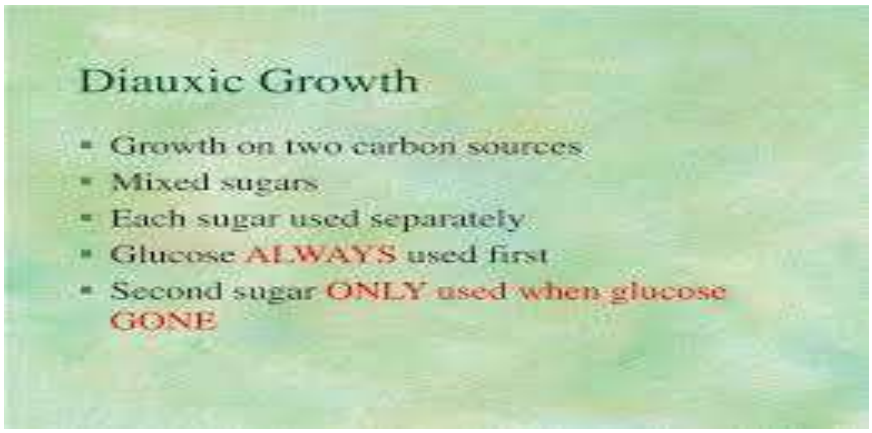
μ_{max} = maximal growth rate (h^{-1})

S = substrate concentration (mg/L)

K_s = half saturation constant (mg/L)



- During log phase growth reaches maximum
- After depletion of substrate, growth rate decreases and finally ceases
- Specific growth rate is independent of [S] as long as excess S is present



Diauxic Growth

Key features of diauxic growth:

- Initially the growth curve is the same as a standard growth curve
- The *stationary phase* is in fact another *lag phase*
- In this second *lag phase* new enzymes are being synthesised by the bacteria to utilise the secondary carbon source

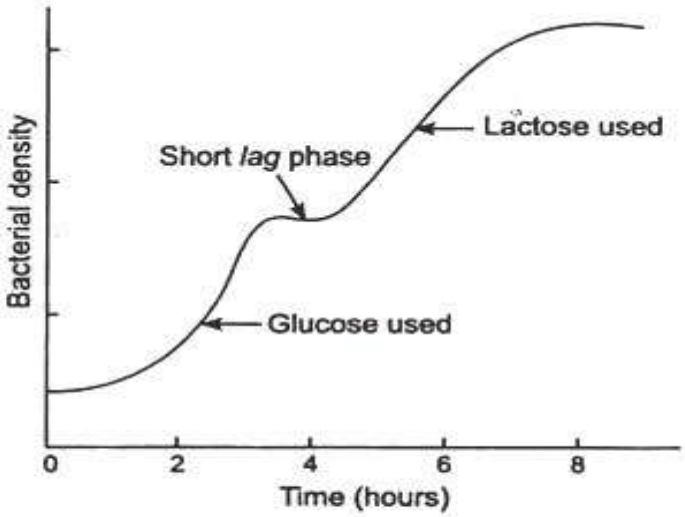


FIG. 19.3. Diauxic or diphasic growth curve of *E. coli* grown with a mixture of glucose and lactose. Glucose is first used, then lactose. A short *lag* phase in diauxic growth is present during which the bacterium synthesizes the enzymes needed for use of lactose.

Synchronous growth

- A synochronous or synochronized culture is a microbial culture or cell culture that are all in the same growth stage.
- Thus, the entire population is kept uniform with respect to growth & division.
- But practically it is not possible to determine a single bacterial cell to obtain the information about growth behavior.
- Synchronous culture provides the entire cell crop in the same stage.
- Synchronized culture provides information on measurement made on such culture are equivalent to the measurement made on individual cells.

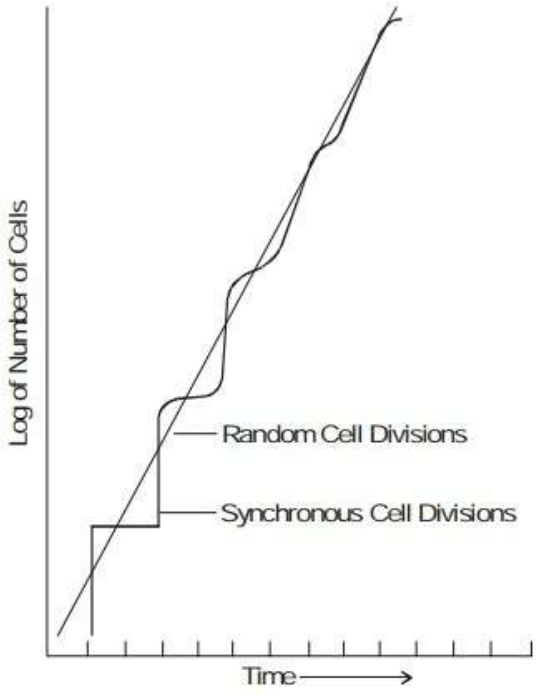


Fig. 5.3. The Synchronous Growth of Microorganism

Batch Culture

Definition

- A large-scale closed system culture in which cells are grown in a fixed volume of nutrient culture medium under specific environmental conditions (e.g. nutrient type, temperature, pressure, aeration, etc.) up to a certain density in a tank or airlift fermenter, harvested and processed as a batch, especially before all nutrients are used up.

Description

- In a batch operation, all necessary medium components and the inoculum are added at the beginning and not during period of fermentation.

- Their concentrations are not controlled but are allowed to vary as the living cells take them up.
- The products, be they intra or extracellular, are harvested only at the end of the run.
- Basic controls for pH, temperature, dissolved oxygen are applied during the course of batch culture.
- The pH, dissolved oxygen and temperature are normally held constant during the course of batch reactor operation.
- The only optimization parameter is the initial medium composition.

Batch Culture

- Closed culture system – initially contains limited amount of nutrient
- No growth – **lag** phase – **time of adaptation**
- Growth rate increases – grow constantly at **maximum** rate – **Log** or **exponential** phase

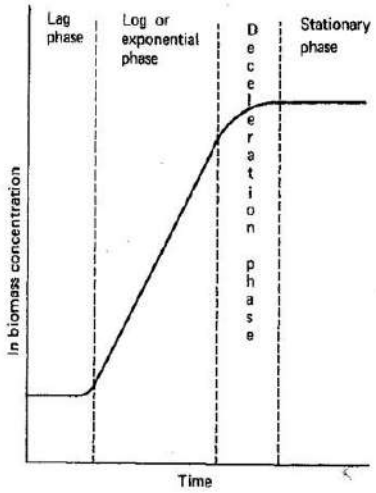


FIG. 2.1. Growth of a typical microbial culture in batch conditions.

- Population growth remains exponential for only a few generation and then enters a stationary phase due to nutrient limitation and waste accumulation

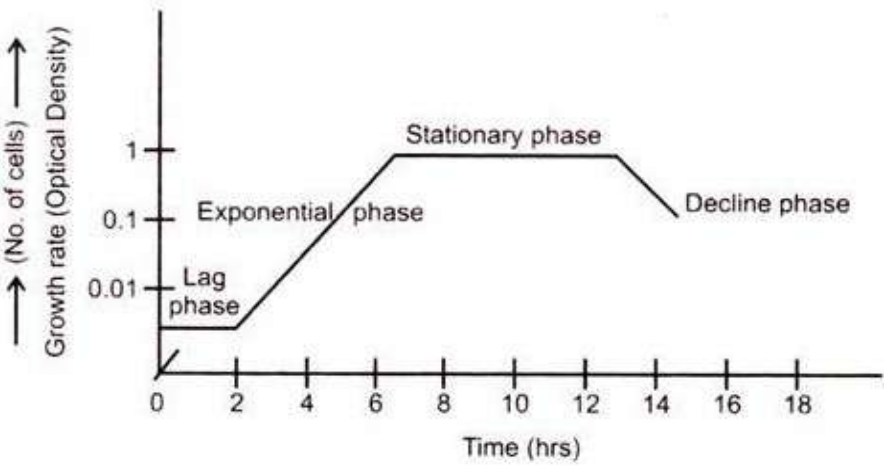


Fig. 3.1: Growth pattern of bacteria in batch culture

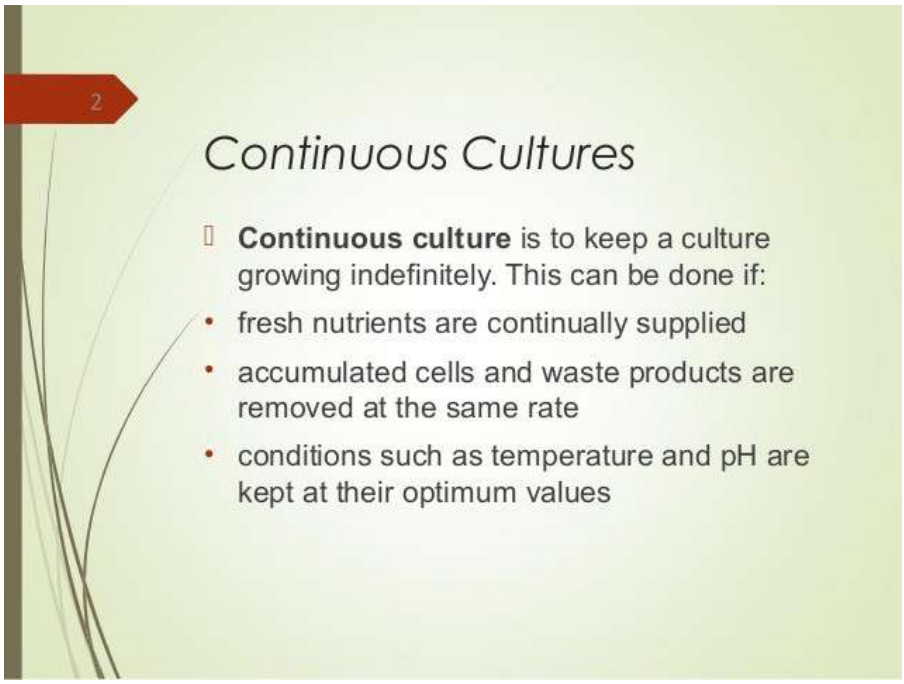
Continuous culture

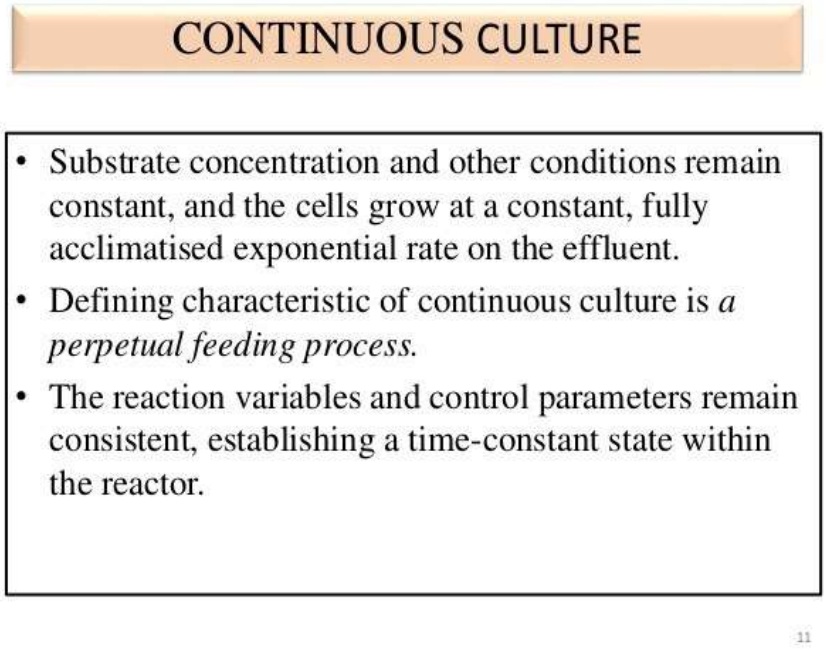
Definition

Continuous culture can define as the culture process, where the bacterial population grows at a constant **cell concentration** and **volume** of the culture vessel or reservoir.

Description

- Population is cultured in an open system
- Continual nutrient addition and waste removal
- Exponential phase maintained for long periods

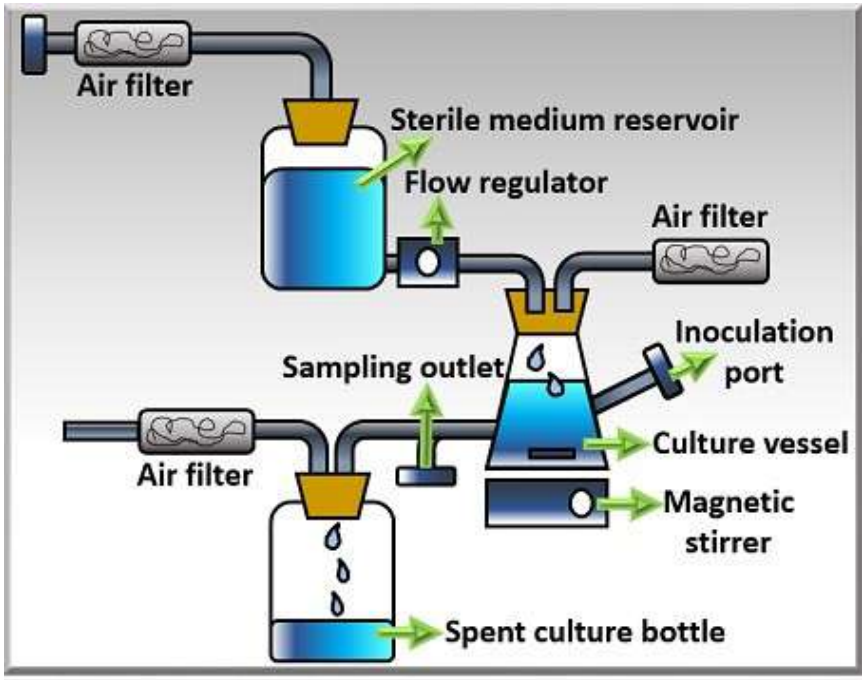




- **Chemostat** and **turbidostat** are the two special types of equipment which are most commonly used in the process of continuous culture.

Chemostat

- Sterile nutrient medium is added into the reservoir along with the microbial cells.
- Cell density is kept constant by keeping the dilution rate and flow rate of nutrient medium constant.
- Concentration of essential nutrient within the culture vessel control the growth rate of the cells
- Conc. of substrate within the culture vessel is controlled by the dilution rate

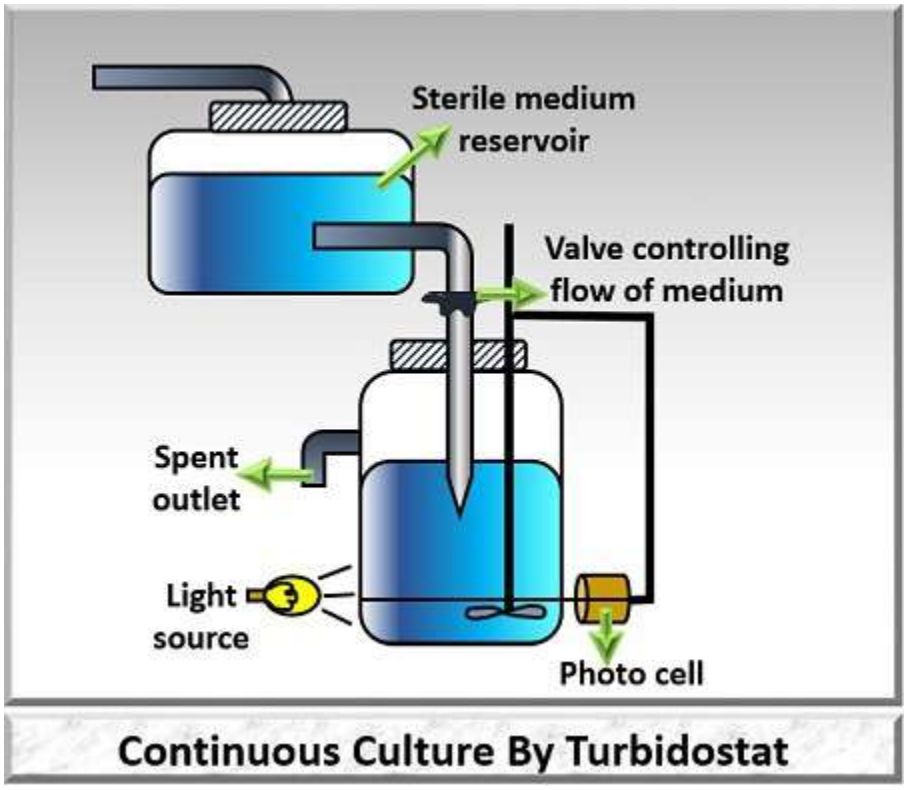


Continuous Culture By Chemostat

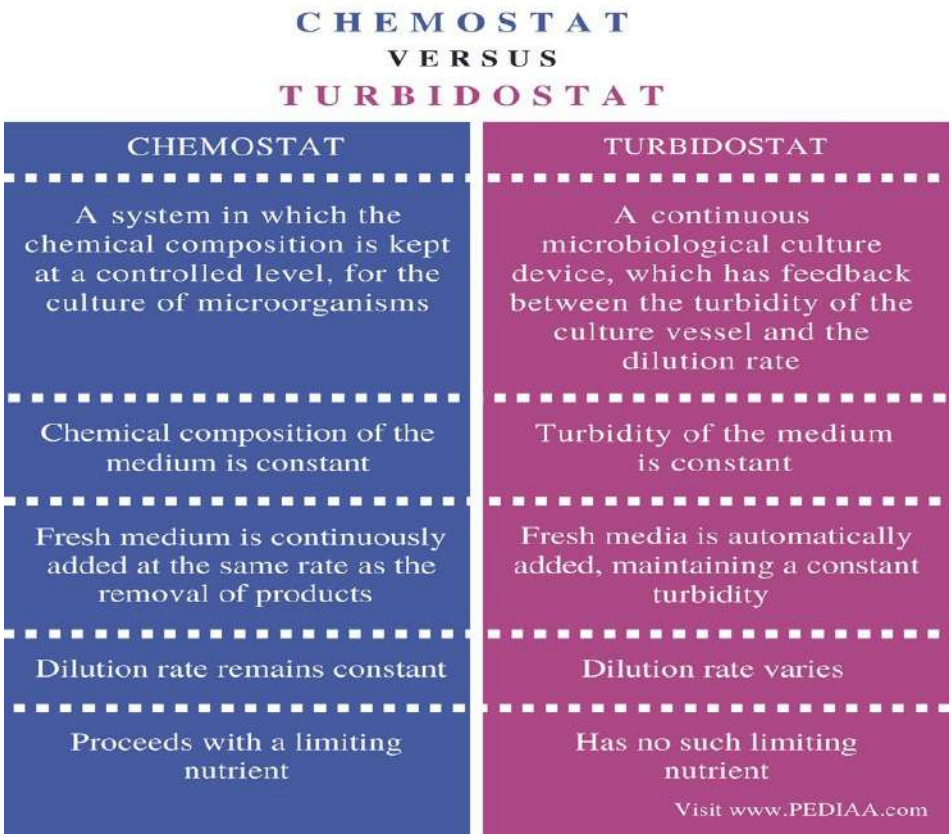
Turbidostat

- Maintains the constant cell density by controlling the flow rate of the fresh medium.
- Fresh nutrient medium is automatically regulated which maintains predetermined turbidity.
- The fresh nutrient medium is added to the culture vessel through a valve which controls the flow of the medium.
- The fresh nutrient medium is added when the optical density increases.

- The photoelectric device measures the turbidity of the medium by absorbing the light source.



Continuous Culture By Turbidostat



Synchronous Culture

Definition

A **synchronous culture** is a microbiological culture or a cell culture that contains cells that are all in the same growth stage.

Description

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.
- Since numerous factors influence the cell cycle, some of them stochastic (random), 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.
- Since cells are too small for certain research techniques, a synchronous culture can be treated as a single cell; the number of cells in the culture can be easily estimated, and quantitative experimental results can simply be divided in the number of cells to obtain values that apply to a single cell.
- Synchronous cultures have been extensively used to address questions regarding cell cycle and growth, and the effects of various factors on these.

- Synchronous cultures can be obtained in several ways:

➢ **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.

➢ Cell growth can also be arrested using chemical growth inhibitors. After growth has completely stopped for all cells, the inhibitor can be removed from the culture and the cells then begin to grow synchronously.

➢ Cells in different growth stages have different physical properties. Cells in a culture can thus be physically separated based on their density (by centrifugation) or size (by filtration).

➢ In 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. New born bacteria that detach from the membrane are now all at the same stage of growth; they are collected in a flask that now harbours a synchronous culture.

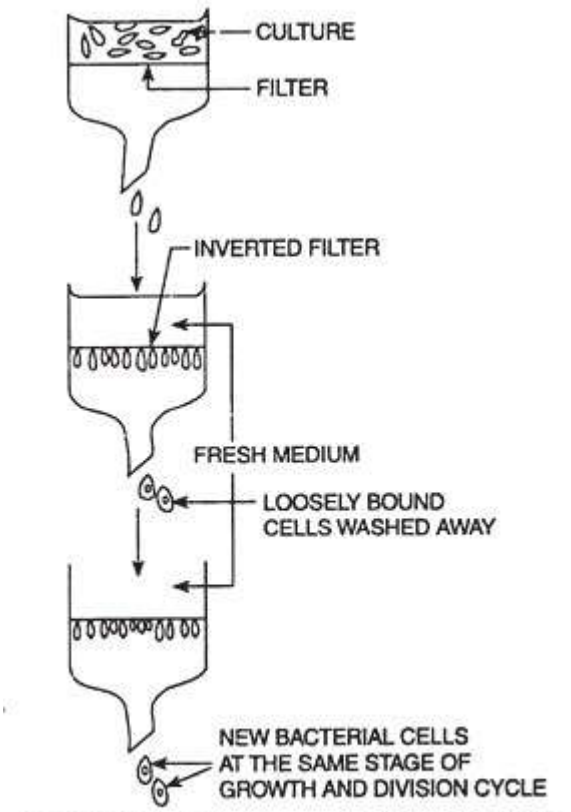


FIG. 19.4. Helmstetter-Cumming technique of obtaining synchronous cultures.

- An excellent and most widely used method to obtain synchronous cultures is the **Helmstetter-Cummings Technique** in which an unsynchronized bacterial culture is filtered through **cellulose nitrate membrane filter**.
- The loosely bound bacterial cells are washed from the filter, leaving some cells tightly associated with the filter.
- The filter is now inverted and fresh medium is allowed to flow through it.
- New bacterial cells, that are produced by cell division and are not tightly associated with the filter, are washed into the effluent.
- Hence, all cells in the effluent are newly formed and are, therefore at the same stage of growth and division cycle.
- The **effluent** thus represents a **synchronous culture**.

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