A study material for M.Sc. Biochemistry (Semester: IV) Students
on the topic (EC-1; Unit I)

Microbial Growth

The Fastest Growing Cells

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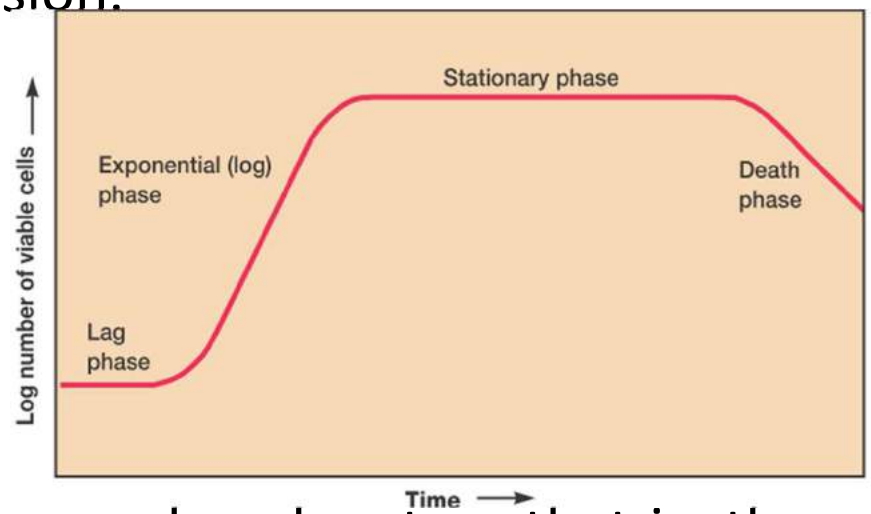
Microbial Growth

Growth may be defined as an increase in cellular constituents. It leads to a rise in cell number when microorganisms reproduce by processes like budding or binary fission.

Growth Curve:



- Population growth is studied by analyzing the growth curve of a microbial culture.
- When microorganisms are cultivated in liquid medium, they usually are grown in a batch culture or closed system that is, they are incubated in a closed culture vessel with a single batch of medium.
- Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase.
- The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of then umber of viable cells versus the incubation time. The resulting curve as four distinct phases.

Lag Phase

- When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, and therefore this period is called the lag phase.
- Cell division does not take place right way and there is no net increase in mass.
- A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cells may be old and depleted of ATP, essential cofactors, and ribosome; these must be synthesized before growth can begin.
- The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase.
- On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short.

Log or Exponential Phase

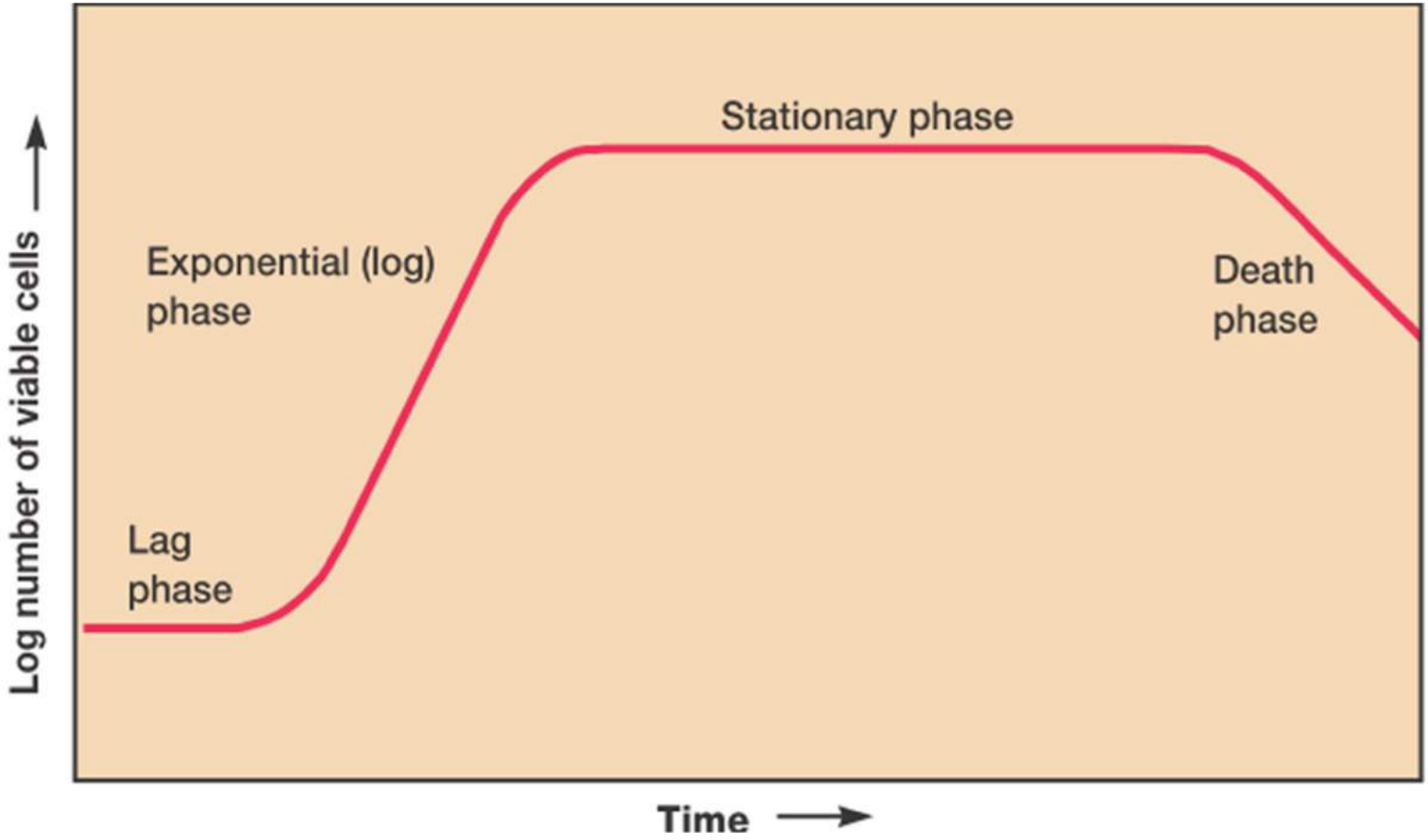
- Microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing.
- Growth rate is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. The growth curve rises smoothly rather than in discrete jumps.
- The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies.
- Exponential growth is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other.

Stationary Phase

- Population growth ceases and the growth curve becomes horizontal.
- Stationary phase usually is attained by bacteria at a population level of around 10^9 cells per ml.
- Other microorganisms normally do not reach such high population densities, protozoan and algal cultures often having maximum concentrations of about 10^6 cells per ml.
- In this phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease to divide.
- Microbial populations enter the stationary phase for several reasons.
  - One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow.
  - Aerobic organisms often are limited by O_2 availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O_2 concentration adequate for growth.
  - Population growth also may cease due to the accumulation of toxic waste products. For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited. Streptococcal cultures also can enter the stationary phase due to depletion of their sugar supply.
  - growth may cease when a critical population level is reached.

Death Phase

- Detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells characteristic of the death phase.



The Mathematics of Growth

- During the exponential phase each microorganism is dividing at constant intervals.
- The population will double in number during a specific length of time called the generation time or doubling time. Suppose that a culture tube is inoculated with one cell that divides every 20 minutes. The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2^n where n is the number of generations. The resulting population increase is exponential or logarithmic.

Table 6.1 An Example of Exponential Growth

Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[a]The hypothetical culture begins with one cell having a 20-minute generation time.

These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

Then inspection of the results in table 6.1 will show that

$$N_t = N_0 \times 2^n.$$

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant *(k)*.** This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (g), can now be calculated. If the population doubles ($t = g$), then

$$N_t = 2\,N_0.$$

Substitute $2N_0$ into the mean growth rate equation and solve for k.

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (g) can be determined directly from a semilogarithmic plot of the growth data (**figure 6.4**) and the growth rate constant calculated from the g value. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 10^3 cells to 10^9 cells in 10 hours.

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10\ \text{hr})} = \frac{9 - 3}{3.01\ \text{hr}} = 2.0\ \text{generations/hr}$$

$$g = \frac{1}{2.0\ \text{gen./hr}} = 0.5\ \text{hr/gen. or } 30\ \text{min/gen.}$$

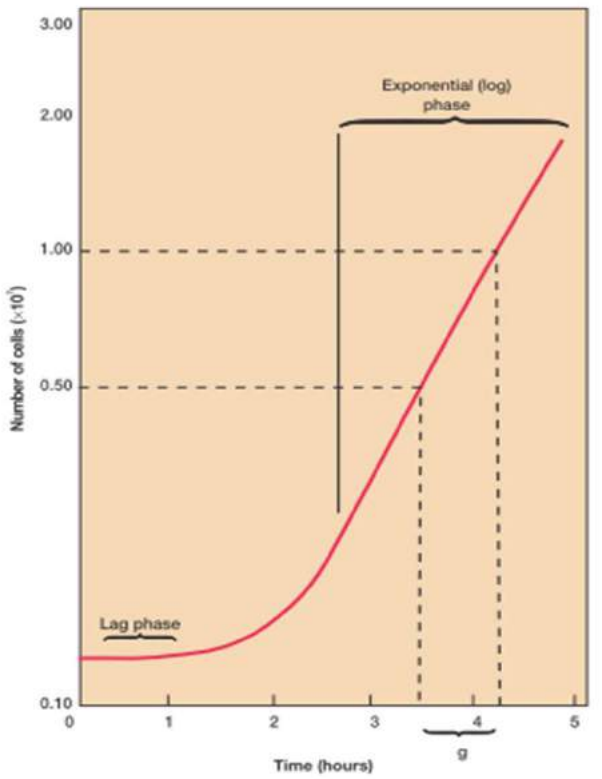


Figure 6.4 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

Generation times vary markedly with the species of microorganism and environmental conditions. They range from less than 10 minutes (0.17 hours) for a few bacteria to several days with some eukaryotic microorganisms. Generation times in nature are usually much longer than in culture.

Measurement of Microbial Growth

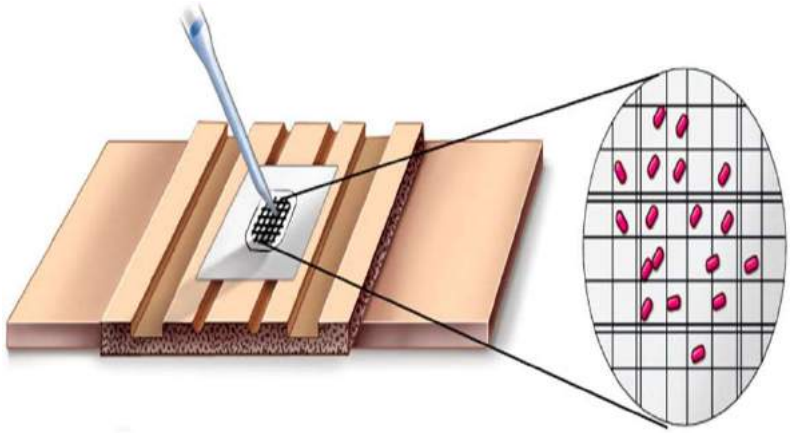
- There are many ways to measure microbial growth to determine growth rates and generation times.
- No single technique is always best; the most appropriate approach will depend on the experimental situation.

Measurement of Cell Numbers

- The most obvious way to determine microbial numbers is through direct counting.
- No single technique is always best; the most appropriate approach will depend on the experimental situation.

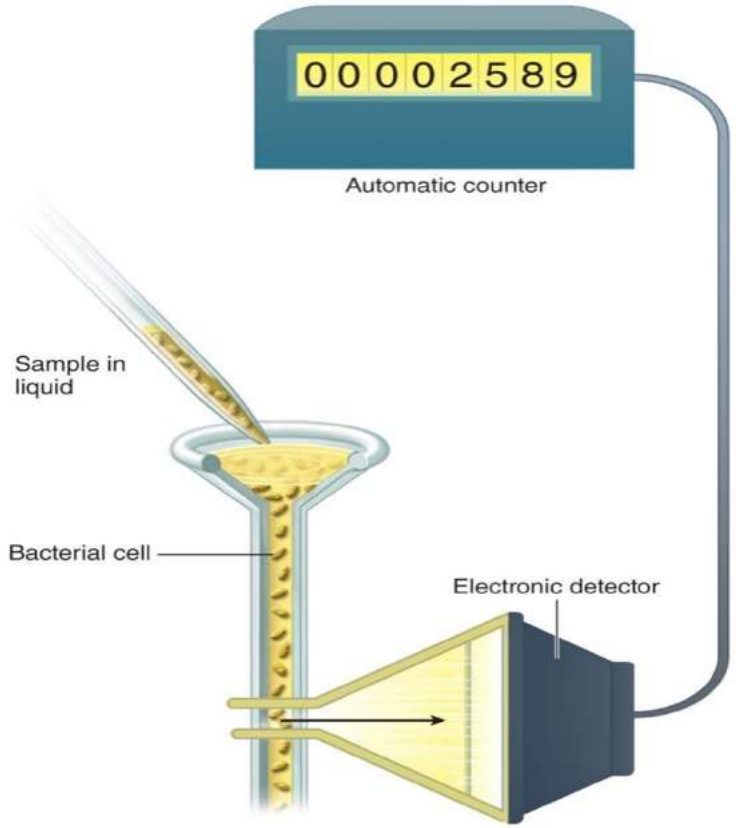
1. Petroff-Hausser Counting Chamber

- Counting cells directly (live and dead)
- Slide with depressed etched grids (25 squares)
- Covered with a coverslip
- 25 squares (area) = $1mm^2$
- Depth = 0.02mm
- Bacteria/mm3 = (bacteria/square)(25 squares)(50)
- Bacteria/ml= (bacteria/square)(25 squares)(50) (10^3)
- Determination of cell numbers:
  (20 bacteria) (25 squares)(50)(10^3) = 2.5 x 10^7 cells/ml



2. Coulter Counter

- Counting cells directly (live and dead)
- A Coulter counter is an apparatus for counting and sizing particles suspended in electrolytes.
- It is used for cells, bacteria, prokaryotic cells and virus particles.



3. Membrane filtration technique

- Counting only live cells
- Membrane traps bacterial on the surface
- Membrane transferred to an agar plate
- Colonies grow → counted

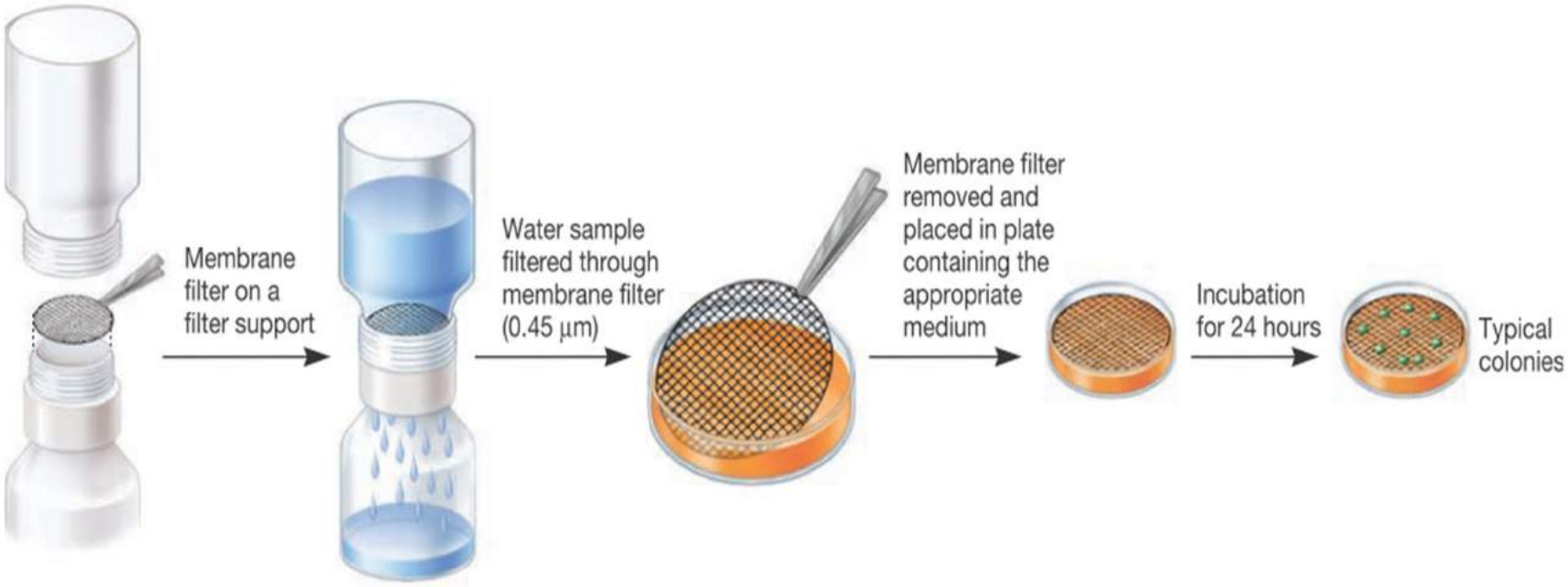


Figure 6.13 The Membrane Filtration Procedure. Membranes with different pore sizes are used to trap different microorganisms. Incubation times for membranes also vary with the medium and microorganism.

Measurement of Cell Mass

- Microbial Dry Weight
  - Cells growing in liquid medium
  - Collected by centrifugation
  - Washed
  - Dried in an oven
  - Weighed

- Turbidity and Microbial Measurement
  - More rapid and sensitive techniques – microbial cells scatter light that strikes them.
  - B/c microbial cells in a population are constant size, the amount of scattering is directly proportional to the biomass of cells present.
  - 10^7 cells per ml population- medium appears cloudy.
  - The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels.

Continuous culture of microorganisms

- Continual provision of nutrients and removal of wastes.
- A microbial population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods in a continuous culture system.
- Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate.

Two major types of continuous culture systems commonly are used:

Chemostats and Turbidostats

1. **Chemostats:** A **chemostat** (from **Chem**ical environment is **stat**ic) is a bioreactor to which fresh medium is continuously added, while culture liquid is continuously removed to keep the culture volume constant. By changing the rate with which medium is added to the bioreactor the growth rate of the microorganism can be easily controlled.

A chemostat is a device which can be used to cultivate microorganisms at a steady rate.

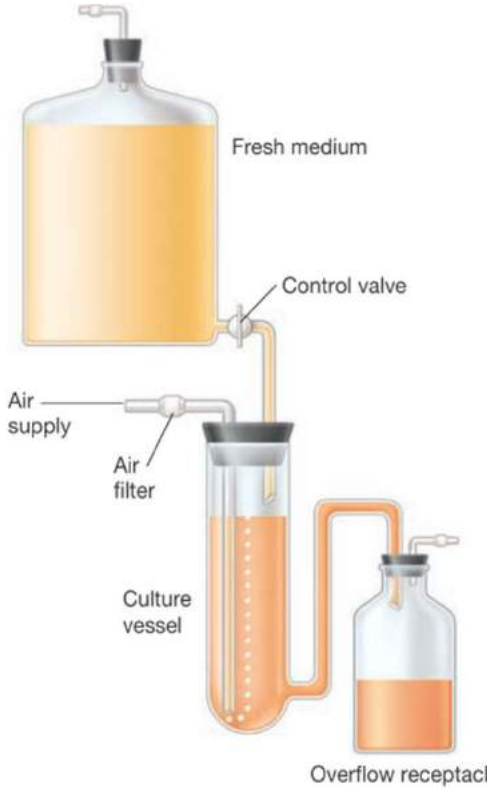


Figure 6.16 **A Continuous Culture System: The Chemostat.** Schematic diagram of the system. The fresh medium contains a limiting amount of an essential nutrient. Growth rate is determined by the rate of flow of medium through the culture vessel.

Two important factors that controls the chemostat

a. Concentration of the limit nutrient:

Medium possess an essential nutrient in limiting quantities. B/c of the limiting nutrient, the growth rate is determined by the rate at which the medium is fed into the growth chamber. The final cell density depends on the concentration of the limiting nutrient.

b. Dilution rate:

- At steady state the specific growth rate (μ) of the micro-organism is equal to the dilution rate (D). The dilution rate is defined as the rate of flow of medium over the volume of culture in the bioreactor

$$D = \frac{\text{Medium flow rate}}{\text{Culture volume}} = \frac{\text{F}}{\text{V}}$$

2. The Turbidostat

- The second type of continuous culture system, the turbidostat, has a photocell that measures the absorbance or turbidity of the culture in the growth vessel.
- The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density.

Table 6.3 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Growth optimum between pH 8.5 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth.	*Micrococcus luteus, Pseudomonas, Mycobacterium;* most algae, fungi, and protozoa
Facultative anaerobe	Does not require O_2 for growth, but grows better in its presence.	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence.	*Clostridium, Bacteroides, Methanobacterium, Trepomonas agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%).	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures.	*Photobacterium profundum, Shewanella benthica, Methanococcus jannaschii*

Acknowledgement and Suggested Readings:

1. Microbiology, An Introduction; Tortora, Funke and Case; Pearson Publication
2. Microbiology; Prescott, Harley and Klein; The MacGraw-Hill Companies
3. Microbiology: Principles and Explorations; Jacquelyn G Black; John Wiley and Sons Inc.
4. Brock Biology of Microorganisms; Madigan, Martinko, Stahl and Clark; Benjamin Cummings (Pearson Publication)

Thanks