Growth and Nutrition of Bacteria

CHAPTER 3

Microorganisms use nutrients to obtain energy and synthesize new cellular components. Nutrients are substances used in biosynthesis and energy production and are requires for microbial growth. The most important nutrients are carbon, hydrogen, nitrogen and oxygen. Microorganisms get their nutrients from sources in their environment. When these microorganisms obtain their nutrients by living on or in other organisms, they can cause disease in that organism by interfering with their host's nutrition, metabolism and disrupt host's homeostasis.

NUTRITION, GROWTH AND MULTIPLICATION OF BACTERIA

NUTRITION

Microbial cell composition shows that over 95% of cell dry weight is made up of a few macro elements and microelements.

Macro elements or Macronutrients

Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.

C, O, H, S and P are components of carbohydrates, lipids, proteins and nucleic acid.

Potassium, calcium, magnesium and iron are exists in the cell as cations and play a variety of roles. For example: potassium (K+) is required for activity of enzymes. Calcium (Ca^{2}+) contributes to the heat resistance of bacterial endospores. Magnesium (Mg^{2}+) serves as cofactor for enzymes. Iron (Fe^{2}+ and Fe^{3}+) is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

Micro elements or trace elements

Micro elements are manganese, zinc, cobalt, molybdenum, nickel and copper are needed by most bacterial cells.

Micro elements are normally a part of enzymes and cofactors and helps in the catalysis of reactions and maintenance of protein structure, for example, zinc (Zn^{2}+) is present at the active site of enzymes. Cobalt (Co^{2}+) is a component of vitamin B_{12}.

Microorganisms require a balanced mixture of nutrients. When essential nutrients are in short supply, microbial growth will be limited.

Carbon is the central component of the biological macromolecules. Carbon is needed for the skeleton or backbone of all organic molecules. When organic nutrients are reduced, they can be serve as energy sources.

Organisms use carbon dioxide and incorporate it into organic molecules. Thus, microorganisms use carbon dioxide as their sole or principal source of carbon to make organic molecules called ***autotrophs.***

Many microorganisms cannot use carbon dioxide as their sole carbon source but rely on the reduced complex molecules such as glucose for the supply of carbon. Organisms that use reduced carbon sources are called ***heterotrophs.*** (these carbon sources come from other organisms). Apart from carbon sources, all organisms require sources of energy and electrons for growth. Microorganisms are classified into groups based on these different sources.

There are two sources of energy –

1. Light energy
2. The energy derived from oxidizing organic or inorganic molecules. When organisms utilize light as their energy source are called ***phototrophs.***

Chemotrophs are organisms obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for electrons.

Organisms use reduced inorganic substances as their electron source are called ***lithotrophs.***

Organotrophs are organism that obtain their electron source from organic compounds. **(Table - 3.1)**

Table - 3.1 : Classification of Microorganisms Based on Different Sources

Carbon Sources	
Autotrophs	Use CO2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Light
Chemotrophs	Oxidation of organic or inorganic compounds
Electron Sources	
Lithotrophs	Reduced inorganic molecules
Organotrophs	Organic molecules

BACTERIAL GROWTH

Growth is defined as an increase in cellular constituents. Growth leads to a rise in cell number when microorganisms reproduce by budding or binary fission.

Growth is a complex process involving different stages.

1. Entry of basic nutrients into the cell.
2. Conversion of these nutrients into energy and vital cell constituents.
3. Replication of the chromosome.
4. Increase in size and mass of the cell.
5. Division of the cell into two daughter cells, each containing a copy of the genome and other vital components.

In binary fission, sequential events are

The cell elongates and chromosomal DNA is replicated.

The cell wall and cell membrane move inward and begin to divide.

The cell wall forming a cross wall completely around the divided DNA.

The cells separate into two individual cells.

In budding, a small outgrowth or bud emerges from bacterium and enlarges until it reaches the size of the daughter cell. Cells separates, forming two identical cells. Some bacteria called filamentous bacteria, reproduce by producing chains of spores located at the tips of the filaments. The filaments fragment and these fragments initiate the growth of new cells.

Generation Time

The generation time is the amount of time needed for a cell to divide. Generation time varies among organisms and depends upon the environment and temperature of their environment. Some bacteria have a generation time of 24 hours, others 1 to 3 hours, but few bacteria divide at an enormous rate. For example: In binary fission, bacteria divides every 20 minutes.

THE BACTERIAL GROWTH CURVE

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they are usually grown as batch culture or closed system (incubate in a closed vessel with single time medium given). During incubation, no fresh medium is provided, as a result nutrient concentrations decline and concentration of waste increases. The growth of microorganisms reproduced by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases.

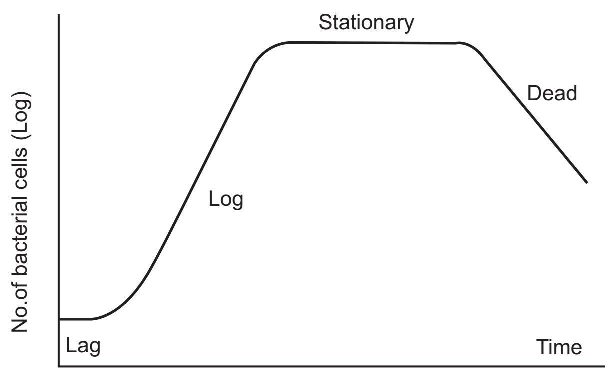

Fig. 3.1 : Bacterial Growth Curve

Bacterial Growth Curve

Lag Phase:

- In lag phase, microorganisms are introduced into fresh culture medium, no increase in cell number.
- Cell division does not takes place quickly and there is no net increase in mass, the cell is synthesizing new components.
- Before cell division, lag phase are essential for a variety of reasons . the cells may be old and depleted of ATP, essential cofactors and ribosomes; they must be synthesized before growth can begin.
- Microorganisms need to adjust to conditions like medium may be different. So, new enzymes would be needed to use different nutrients.

- The cells retool, replicatie their DNA, begin to increase in mass and finally divide.
- The length of lag phase varied with the condition of the microorganisms and the nature of the medium.
- The lag phase may long if the inoculum is from an old culture or inoculation of a culture into a chemically different medium.
- When a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase wil be short or absent.
- The period between inoculation and beginning of multiplication is known as lag phase.

Exponential Phase or Log Phase:

- During the log phase, microorganisms are growing and dividing at the maximal rate based on genetical potential, the nature of the medium and the condition like temperature etc.
- Microorganisms are dividing and doubling in number at regular intervals, their rate of growth is constant.
- The population of cells is most uniform in terms of chemical and physiological properties during this phase; so, this phase cultures are usually used in biochemical and physiological studies.
- Exponential growth is balanced growth. In this growth, all cellular constitutents are manufactured at constant rates, if nutrient level or other environmental conditions change unbalanced growth results.
- To synthesize cellular constituents, the cells construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increase in protein and DNA synthesis. Finally, reproductive rate takes place.
- The time required for one bacterial division during this phase is called **generation time.** The number of organisms present in each generation period, is almost twice than previous period. In graph, bacterial count is plotted against time and a straight line is obtained.
- Log phase is of short duration because of
  1. Exhaustion of nutrients.
  2. Accumulation of toxic metabolic end products.
  3. Rise in cell density.
  4. Change in pH.
  5. Decrease in oxygen tension.

Stationary Phase:

- Stationary phase is the period the growth curve become horizontal due to cease in population growth.
- During this phase, bacteria population level is around 10^9 cells per ml. but other microorganisms do not reach to this densities, protozoan and algal cultures has maximum concentrations of about 10^6 cells per ml.
- In the stationary phase, the total number of viable microorganisms remain constant because of balance between cell division and cell death or cells cease to divide though remaining metabolically active.
- Factor limits log phase period are indirectly responsible to enter the stationary phase.
- Batch culture usually enter stationary phase in response to starvation. As the result of starvation only few bacteria have morphological changes such as endospore formation, remaining only decrease in overall size, protoplast shrinkage and nucleoid condensation.

Death or Phase of Decline:

- Environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells.
- The death of a microbial population is usually logarithmic (a constant proportion of cells dies every hour). Thus rate of death exceeds the rate of reproduction and the number of viable cell declines.
- Finally, all the cells die and culture become sterile.

Microbial numbers are frequently determined from counts of colonies growing on a special membrane filters having pores small enough to trap bacteria. In the membrane filter techniques, a sample is drawn through a special membrane filter. The filter is then placed on an agar medium or on a pad soaked with liquid media and incubated until each cell forms a separate colony. A colony count gives the number of microorganisms in the filtered sample.

FACTORS AFFECTING MICROBIAL GROWTH

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. Understandings of environmental influences help in the control of microbial growth and the study of the ecological distribution of microorganisms.

pH:

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration. Like other factors, pH also affects microbial growth. Each species of microorganisms has a definite pH growth range and growth optimum conditions.

According to pH conditions, microorganisms can be classified into

1. Acidophiles are organisms which grow between pH 0 to 5.5;
2. Neutrophiles are organisms which grow between pH 5.5 to 8.0;
3. Alkalophiles are organisms which grow between pH range of 8.5 to 11.5.

Extreme alkalophiles has growth optimum at pH 10 or higher. Most bacteria and protozoa are neutrophiles. Most fungi prefer slightly acid surroundings, about 4 to 6; algae seem to favor slight acidity. Although, microorganisms will often grow over wide ranges of pH and far from their optimum pH. But complete variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Microorganisms die if the internal pH drops below 5.0 to 5.5. Changes in the external pH also might alter the ionization of nutrient molecules and thus reduce their availability to the organisms.

Temperature:

Environmental temperature affects microorganisms like all other microorganisms. Microorganisms are susceptible because they are usually unicellular and their temperature varies with of the external temperature. High temperatures damage microorganisms by denaturing enzymes, transport carrier and other proteins. Microbial membranes are also disrupted by extreme temperatures; the lipid bilayer simply melts and disintegrates. The microorganisms growth is inhibited because the damage cannot be repaired. At very low temperatures, membranes solidify and enzymes don't work rapidly. When organisms are above or below the optimum temperature, both function and cell structure are affected.

Microorganisms has the minimum and maximum growth temperatures and the range over which growth is possible. Microorganisms can be placed in one of five classes based on their temperature ranges for growth.

1. Psychrophiles
2. Facultative Psychrophiles
3. Mesophiles
4. Thermophiles
5. Hyperthermophiles

Psychrophiles grow well at 0 °C and have an optimum growth temperature of 15 °C or lower; the maximum is around 20°C. Examples for psychrophiles are *Pseudomonas, Vibrio, Bacillus* and *Anthrobacter.* Psychrophiles begin to leak cellular constitutents at temperatures higher than 20 °C because of cell membrane disruption.

Many bacterial species grow at 0 to 7 °C, they have optimum conditions between 20 and 30 °C and maximum at about 35 °C. celcius. These called psychrotrophs of facultative psychrophiles.

Mesophiles are microorganisms with optimum temperatures around 20 to 45 °C; minimum temperature of 15 to 20 °C and maximum is about 45 °C or lower. Almost all human pathogens are mesophiles, they have constant temperature 37 °C.

Thermophiles are microorganisms grow at temperature of 55 °C or higher. Their growth minimum is around 45 °C and optimum temperature between 55 and 65 °C. These organisms found in composts, self- heating hay stacks, hot water lines and hot springs.

A few thermophiles can grow at 90 °C or above and maximum temperature about 100 °C. Organisms have optimum temperature for growth between 80 °C and 113 °C called hyperthermophiles.

Moisture:

Moisture is important factor require for the growth of bacteria. About 80% of the bacterial body weight contain water, so drying microorganisms are affecting their structure and functions.

Oxygen Concentration:

Aerobe is an organism able to grow in the presence of atmospheric oxygen, whereas organisms that can grow in absence of oxygen is called **anaerobe.** Organisms strictly require or completely dependent on atmospheric oxygen is called **obligate aerobes.** Facultative anaerobe are organisms able to act like aerobes in the presence of oxygen but able to survive when conditions become anaerobic. **Aerotolerant anaerobes** are organisms that are basically anaerobic; but they are not inhibited by atmospheric oxygen. E.g. *Enterococcus.*

Strict or obligate anaerobes (e.g. *Bacteriodes, Fusobacterium* and *Clostridium)* do not tolerate oxygen levels below the range of 2 to 10% for growth but that are damaged by the normal atmospheric level of oxygen (20%). **(Table - 3.2)**