Bacterial Growth, Nutritional Requirements and Physical Growth Factors

Bacterial Growth

Growth of Bacteria
Bacterial growth involves both an increase in the size of organisms and an increase in their number. Whatever the balance between these two processes, the net effect is an increase in the total mass i.e., *biomass* of the culture. The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, 4 cells, 8 cells, 16, 32, and so forth.

Reproduction
Bacteria reproduce by binary fission. Multiplication takes place in geometric progression. The division of nucleus (chromosome) undergoes prior to cell division. When the cell grows about twice its size, the nuclear material divides initially, and a transverse septum originates from mesosome /plasma membrane and cell wall and divides the cell into two parts. The two daughter cells receive an identical set of chromosomes. The daughter cells separate or remain attached and may be arranged singly, in pairs, clumps, or chains.

Growth Curve
The growth curve indicates multiplication and death of bacteria. When a bacterium is inoculated in a medium, it passes through four growth phases which will be evident in a growth curve drawn by plotting the logarithm of the number of bacteria against time. Number of bacteria in the culture at different periods may be : (1) Total count- includes both living and dead bacteria, or (2) Viable count- includes only the living bacteria. Microbial concentration can be measured in terms of cell concentration, i.e. the number of viable cells per unit volume of culture, or of biomass concentration, i.e. dry weight of cells per unit volume of culture. Bacterial viability is generally recognized and quantified by detecting growth of single cells into colonies in **colony-forming unit (cfu) counts**.

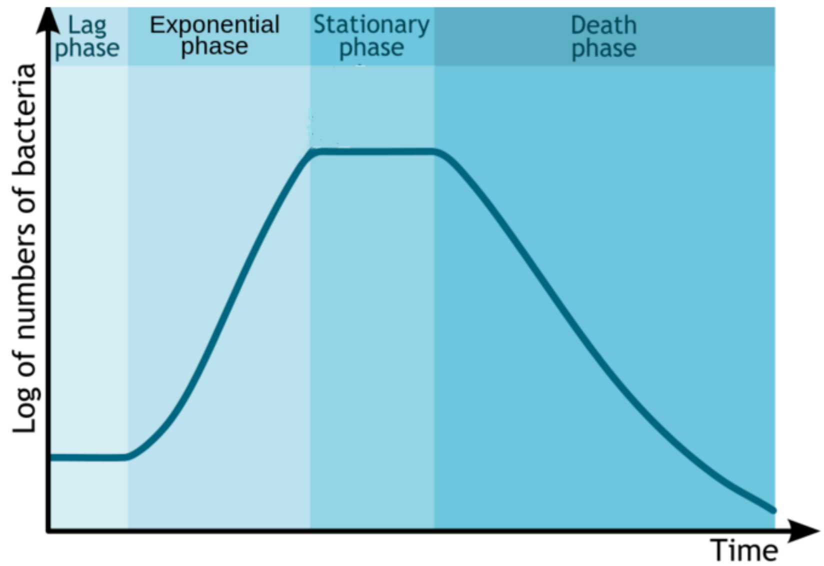
Growth Phases
1. Lag Phase. In this phase, there is increase in size of bacterial cell but no multiplication. Time is required for adaptation (synthesis of new enzymes) to new environment. During this phase, vigorous metabolic activity occurs, but cells do not divide. Enzymes are formed and accumulate until they are present in optimal concentration to permits growth to start.

2. Logarithmic (Log) Phase or Exponential Phase. The bacterial cells multiply at maximum rate in this exponential phase, i.e. there is linear relationship between time and logarithm of the number of cells. Bacterial cell increases in an exponential manner. This continues until one of two things happens: either nutrients in the medium become exhausted, or there is accumulation of toxic metabolic products, and thereby inhibit growth.

Generation Time: The average time required for the population, to double is known as the generation time or doubling time. Linear plots of exponential growth can be produced by plotting the logarithm of biomass concentration as a function of time. Antibiotics act better at this phase. .

3. Maximal Stationary Phase. Due to exhaustion of nutrients or accumulation of toxic products death of bacteria starts and the growth cease completely. The count remains stationary due to balance between multiplication and death rate. Importance: Production of exotoxins, antibiotics, metachromatic granules, and spore formation takes place in this phase.

4. Decline phase or death phase. In this phase, there is progressive death of cells. However, some living bacteria use the breakdown products of dead bacteria as nutrient and remain as persister. Viable count comes down during decline phase.



Bacterial Growth Curve

Factors affecting bacterial Growth:

I. Nutrition
II. Physical factors

I. Nutritional Requirement of Bacteria

All bacteria require two things for growth:

1) A source of energy
2) A source of matter for building additional cells: C, O, H, N, S, P, trace minerals.

Elemental Assay of E. coli (dry weight)

50% carbon	2% sulfur
20% oxygen	2% potassium
14% nitrogen	0.05% calcium, magnesium, chlorine
8% hydrogen	0.2% iron
3% phosphorus	0.3% trace elements

1. Nutritional requirements

All living forms of life does possess nutritional requirements for growth. Bacterial growth requirement, illustrates great diversity. Microorganisms are often grouped according to their energy source, electron source and their source of carbon.

A. **Energy source**

1. **Phototrophs** use radiant energy (light) as their primary energy source.
2. **Chemotrophs** rely on chemical compounds for their energy.

B. **Electron source** (All the organisms require a source of electrons for their metabolism)

1. **Lithotrophs**: Use reduced inorganic compounds (e.g., water) as electron donors.
   Chemolithotrophs : Use chemical compounds as source of energy as well inorganic compounds as electron source.
   Photolithotrophs: Use light as energy source and inorganic compounds as electron source.

2. **Organotrophs**: Use organic compounds as electron donor
   Chemoorganotrophs : Use chemical compounds as source of energy and organic compounds as electron source
   Photoorganotrophs: Use light as energy source and organic compounds as electron source.

C. **Carbon source (For synthesizing cell components)**

Carbon is the structural backbone of the organic compounds that make up a living cell. Virtually all chemical substances in microorganisms contain **carbon** in some form, whether they be proteins, fats, carbohydrates, or lipids. Perhaps 50 percent of a bacterium's dry weight is carbon. Carbon is obtained from organic materials in the environment, or it may be derived from carbon dioxide. Both chemoautotrophic and photoautotrophic microorganisms obtain their energy and produce their nutrients from simple inorganic compounds such as carbon dioxide

Based on their source of carbon bacteria are classified as autotrophs or heterotrophs.

1. **Autotrophs**: utilizes carbon dioxide as a sole source of carbon. An autotroph can synthesize organic molecules from inorganic compounds.
2. **Heterotrophs**: Utilizes organic carbon. A Heterotroph cannot synthesize organic molecules from inorganic compounds.

Based on nutritional requirements, microorganisms can be placed into following nutritional types: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

1. **Photoautotrophs** use light as an energy source and carbon dioxide as their sole carbon source. Include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants. Photoautotrophs transform carbon dioxide and water into carbohydrates and oxygen gas through photosynthesis .

   Cyanobacteria, as well as algae and green plants, use H atoms from water to reduce CO_2 to form CHO, and during this process oxygen gas is given off (an oxygenic process). Other photosynthetic bacteria (the green sulfur bacteria and purple sulfur bacteria) carry out an anoxygenic process, using sulfur, sulfur compounds or hydrogen gas to reduce carbon dioxide and form organic compounds.

2. **Photoheterotrophs** use light as an energy source but cannot convert carbon dioxide into energy. Instead, they use organic compounds as a carbon source. E.g., green non-sulfur bacteria, purple non-sulfur bacteria.
3. **Chemolithoautotrophs** use inorganic compounds such as hydrogen sulfide, sulfur, ammonia, nitrites, hydrogen gas, or iron as an energy source and carbon dioxide as their main carbon source.
4. **Chemoorganoheterotrophs** use organic compounds as both an energy source and a carbon source. Saprophytes live on dead organic matter while parasites get their nutrients from a living host. Most bacteria, and all protozoans, fungi, and animals are chemoorganoheterotrophs.

D. Nitrogen source

Nitrogen is used for the synthesis of proteins, amino acids, DNA, RNA and ATP. Bacteria that obtain nitrogen directly from the atmosphere are called nitrogen-fixing bacteria. e.g., *Rhizobium* and *Azotobacter*, both found in the soil. Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source

E. Minerals

1. **Sulfur**: Sulfur is needed to synthesizes sulfur-containing amino acids and certain vitamins. Depending on the organism, sulfates, hydrogen sulfide, or sulfur-containing amino acids may be used as a sulfur source.
2. **Phosphorus:** Phosphorus is an essential element for nucleic acid synthesis and for the construction of phospholipids, teichoic acid, DNA, RNA, and ATP. Phosphate ions are the primary source of phosphorus.
3. **Trace elements** to support bacterial growth requires metal ions such as Potassium, magnesium, calcium and Iron-Usually functions as cofactors in enzyme reactions.

Trace elements (Micro nutrients) : Elements required in very low concentrations i.e., **Trace elements such** as copper, molybdenum, manganese, and cobalt ions. Cofactors usually function as electron donors or electron acceptors during enzyme reactions.

Most bacteria does not require sodium, but certain bacteria marine, cyanobacteria, photosynthetic bacteria, halophiles require 12-15% NaCl, for maintenance of cell wall integrity, stability and activity of enzyme.

Some Important cofactors and examples of their functions:

K^+	Principle cellular counterion
Mg^{++}	DNA polymerase
Ca^{++}	Intracellular signalling, wall structure
Fe^{++}	Cytochromes
Mn^{++}	PsII, photosynthesis
Co^{++}	Vitamin B12 constituent (methylations)
Cu^{++}	Superoxide dimutase
Zn^{++}	Some DNA binding proteins

F. Water : Water is an essential cell component.

G. **Growth factors**

Growth factors are organic compounds such as amino acids , purines , pyrimidines , and vitamins that a cell must have for growth but cannot synthesize itself. *Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious* .

Other requirements for microbial growth include such as: Vitamins-certain bacteria may also require organic growth factors such as vitamins, amino acids, purines, and pyrimidines.

Vitamins functions either as co-enzymes for several enzymes or as building blocks for co-enzymes. Some bacteria are capable of synthesizing their requirement of vitamins from other compounds in the media. Whereas some bacteria cannot synthesize the vitamins required for their growth. It is required to supplement the media with vitamins required for their growth of such bacteria.

Bacteria	Requirement of vitamins
Brucella abortus	Niacin (Nicotinic acid)
Bacillus anthracis	Thiamine
Clostridium tetani	Riboflavin
Lactobacillus species	Pyridoxine, Cobalamin

Role of ingredients in media

Ingredients	Characteristics	Nutritional role
Yeast extract	An aqueous extract of yeast cells	Rich source of B Vitamins, Source of Carbon and Source of organic Nitrogen.
Peptone	Product obtained by partial digestion of proteinaceous animal or plant materials (e.g., Meat, casein) with acids or enzymes	Source of organic Nitrogen, Source of Carbon and contains some vitamins.
Beef extract	An aqueous extract of lean beef, Tissues are extracted by boiling and then dried.	Source of organic nitrogen, High content of vitamins, amino acids and contains CHO.
Agar	Complex CHO obtained from sea weed/marine algae	Solidifying agent. Not used for nutritional value

II. Physical conditions required for growth:

Certain physical conditions affect the type and amount of microbial growth.

a. Temperature

Enzyme activity depends on the **temperature** of the environment, and microorganisms are classified in three groups according to their temperature preferences:

psychrophilic organisms prefer cold temperatures of about 0°C to 20°C;

mesophilic organisms prefer temperatures at 20°C to 40°C; Most pathogenic bacteria grows best at 37^0 C.

thermophilic organisms (thermophiles) prefer temperatures higher than 40°C.

A minimum and a maximum growth temperature range exist for each species.

The temperature at which best growth occurs is the **optimum growth temperature.**

b. Gaseous requirements

Bacteria show a great deal of variation in their gaseous requirements.

(i) Oxygen. Bacteria may be classified into four groups on oxygen requirement :

Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor.

Most can be placed in one of the following groups:

1. **Obligate aerobes** are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration .
2. **Microaerophils** are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration.
3. **Obligate anaerobes** are organisms that grow only in the absence of oxygen and, in fact, are often inhibited or killed by its presence. They obtain their energy through anaerobic respiration or fermentation .
4. **Aerotolerant anaerobes**, like obligate anaerobes, cannot use oxygen to transform energy but can grow in its presence. They obtain energy only by fermentation and are known as obligate fermenters.
5. **Facultative anaerobes** are organisms that grow with or without oxygen, but generally better with oxygen. They obtain their energy through aerobic respiration if oxygen is present, but use fermentation or anaerobic respiration if it is absent. Most bacteria are facultative anaerobes.

Anaerobic bacteria are killed by oxygen or toxic oxygen radicals. Multiple mechanisms play role for oxygen toxicity : (1) do not have cytochrome systems for oxygen metabolism, (2) may have low levels of superoxide dismutase, and (3) may not have catalase.

(ii) Carbon dioxide: All bacteria require CO_2 for their growth. Most bacteria produce CO_2. Certain bacteria does require more/additional supplementation of carbon dioxide for their growth, such organisms are said to be **capnophilic. e.g.,** *Brucella abortus* grow better in presence of 5 per cent CO_2.

c. pH

Microorganisms can be placed in one of the following groups based on their optimum pH requirements:

1. **Neutrophiles** grow best at a pH range of 5 to 8. *Most pathogenic bacteria are neutrophiles*.
2. **Acidophiles** grow best at a pH below 5.5.
3. **Alkaliphiles** grow best at a pH above 8.5.

d. Osmosis

Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). Osmosis is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy. While water molecules are small enough to pass between the phospholipids in the cytoplasmic membrane, their transport can be enhanced by water transporting transport proteins known as aquaporins. The aquaporins form channels that span the cytoplasmic membrane and transport water in and out of the cytoplasm.

To understand osmosis, one must understand what is meant by a solution. A solution consists of a solute dissolved in a solvent. In terms of osmosis, solute refers to all the molecules or ions dissolved in the water (the solvent). When a solute such as sugar dissolves in water, it forms weak hydrogen bonds with water molecules. While free, unbound water molecules are small enough to pass through membrane pores, water molecules bound to solute are not. Therefore, the higher the solute concentration, the lower the concentration of free water molecules capable of passing through the membrane.

A cell can find itself in one of three environments: isotonic, hypertonic , or hypotonic . (The prefixes iso-, hyper-, and hypo- refer to the solute concentration).

- In an isotonic environment , both the water and solute concentration are the same inside and outside the cell and water goes into and out of the cell at an equal rate.

- If the environment is hypertonic), the water concentration is greater inside the cell while the solute concentration is higher outside (the interior of the cell is hypotonic to the surrounding hypertonic environment). Water goes out of the cell.

- In an environment that is hypotonic, the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.

Most bacteria require an isotonic environment or a hypotonic environment for optimum growth. Organisms that can grow at relatively high salt concentration (up to 10%) are said to be osmotolerant . Those that require relatively high salt concentrations for growth, like some of the Archaea that require sodium chloride concentrations of 20 % or higher **halophiles**.

*****