Bacterial Growth (Cultivation of Bacteria)

Bacterial growth is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. Bacterial growth is proliferation of bacterium into two daughter cells, in a process called binary fission.

Bacteria are the most primitive forms of microorganisms but are composed of a great variety of simple and complex molecules and are able to carry out a wide range of chemical transformations. In order to survive and grow, microbial growth requires suitable environmental conditions, a source of energy, and nourishment.

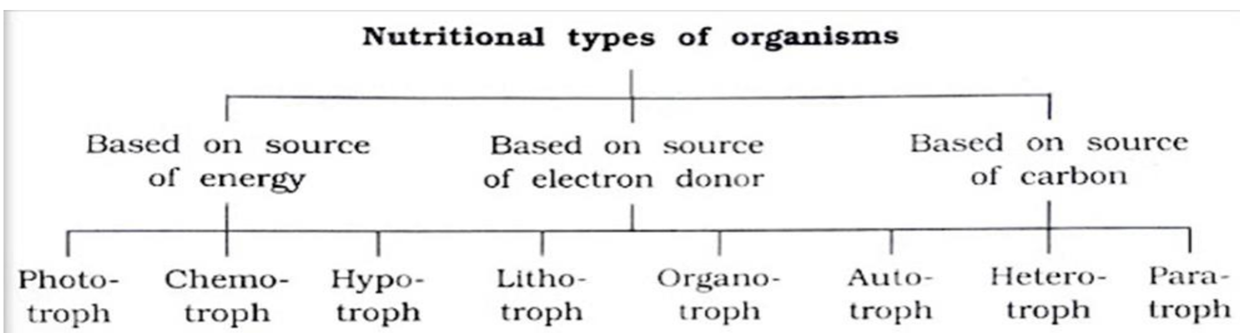
Bacterial cell structure is made up of various components such as carbohydrates, lipids, proteins and nucleic acids. These compounds are made up for basic elements (C, H, N, O), Phosphorus and sulfur are also required for bacterial growth.

The composition of the growth medium is a major factor controlling the growth rate. The growth rate increases up to a maximum when the medium provides a better energy source and more of the biosynthetic intermediates.

Environmental factors affecting growth

Nutritional requirements

Bacteria differ in their nutritional requirements. Amongst various requirements for nutrition, characteristic differences exist for the requirement of sources of energy, electron donor and carbon. Therefore, bacteria are classified in various categories on the basis of these requirements.



Phototrophs bacteria capture light energy from the sun and convert it into chemical energy inside their cells.

Chemotrophs bacteria break down either organic or inorganic molecules to supply energy for the cell.

Hypotrophs which cannot utilize any external source of energy. They require ready-made ATP for growth. It may be obtained from other living host cells. Thus, these organisms grow as parasites. e.g., Viruses

Autotrophs bacteria can synthesize organic molecules from inorganic nutrients (make food).

Heterotrophs bacteria obtain their-ready made food from various organic substances (made food). Most of pathogenic bacteria of human beings, plants and animals are heterotrophs. Some heterotrophs have simple nutritional requirement while some of them require large amount of vitamin and other growth substance. Such organisms are called fastidious heterotrophs (*Neisseria*).

Paratrophs, not able to use non cellular organic or Inorganic carbon compounds. e.g., Viruses

Lithotrophs bacteria use of an inorganic compound as a source of electron donor.

Organotrophs bacteria use of various organic compound as a source of electron donor.

Photolithotrophs bacteria, which obtain energy from light and use inorganic substances as electron donor.

Photo organotrophs bacteria, obtain energy from light and generate reducing power from oxidation of organic compounds.

Photoautotrophic bacteria, which obtain their energy from light and carbon from CO_2

Photoheterotrophs bacteria, obtain their energy from light and carbon from organic substances

Chemolithotrophs bacteria, can use the same inorganic chemical substances as the sources of energy and electron donor.

Chemoorganotrophs bacteria, which obtain their energy and electron donor from the same organic compound.

Chemoautotrophs bacteria, which obtain energy and carbon from inorganic compounds.

Chemoheterotrophs bacteria, which obtain their energy and carbon from organic chemicals.

Saprophytic bacteria, chemoheterotrophs which obtain their food from the dead organic decaying matter.

Basic group	Subgroup	Energy source	e– donor	Carbon source
Phototroph	Photolithotroph	Light	Inorganic	CO_2 or organic
	Photoorganotroph	Light	Organic	Organic
	Photoautotroph	Light	Inorganic/ organic	CO_2
	Photoheterotroph	Light	Organic	Organic
Chemotroph	Chemolithotroph	Chemical	Inorganic	CO_2 or organic
	Chemoorganotroph	Chemical	Organic	Organic
	Chemoautotroph	Chemical	Inorganic or organic	CO_2
	Chemoheterotroph	Chemical	Organic	Organic

Nitrogen source

Nitrogen is needed for the synthesis of amino acids and nucleic acids. Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source.

Hydrogen and oxygen supplied from water added to the culture media

Growth factors (bacterial vitamins)

Growth factors are organic compounds such as amino acids, purines, pyrimidines, and vitamins that a cell must have for growth but cannot synthesize itself.

Minerals

1. Sulfur

Sulfur is needed to synthesizes sulfur-containing amino acids and certain vitamins.

2. Phosphorus

Phosphorus is needed to synthesize phospholipids, nucleic acids and coenzymes.

3. Trace elements

Trace elements are elements required in very minute amounts, like potassium, magnesium, calcium, iron, sodium, zinc, copper, molybdenum, manganese, and cobalt ions. They usually function as cofactors in enzyme reactions, as electron donors or electron acceptors during enzyme reactions.

Hydrogen ion concentration (pH)

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

Neutrophiles grow best at pH range of 6.5 - 7.5 such as pathogenic bacteria

Acidophiles grow best at pH range of 6.0 or below, such as bacteria in yogurt

Alkaliphiles grow best at pH range of 8.2 - 9.0 such as *Vibrio cholerae*

Temperature:

Microorganisms are classified to three groups according to their temperature preferences:

Psychrophiles are cold-loving bacteria. Their optimum growth temperature is between -5 -15 °C or 0 - 20°C

Mesophiles are bacteria that grow best at moderate temperatures. Their optimum growth temperature is between 25- 45 °C or 20 - 40°C (pathogenic bacteria). Most bacteria are mesophilic which grow best at 30-37°C.

Thermophiles are heat-loving bacteria. Their optimum growth temperature is between 45-70 °C or 50-60°C.

Hyperthermophiles are bacteria that grow at very high temperatures. Their optimum growth temperature is between 70C and 110C.

Aeration (oxygen)

Bacteria show a great deal of variation in their requirements for oxygen. Most can be placed in one of the following groups:

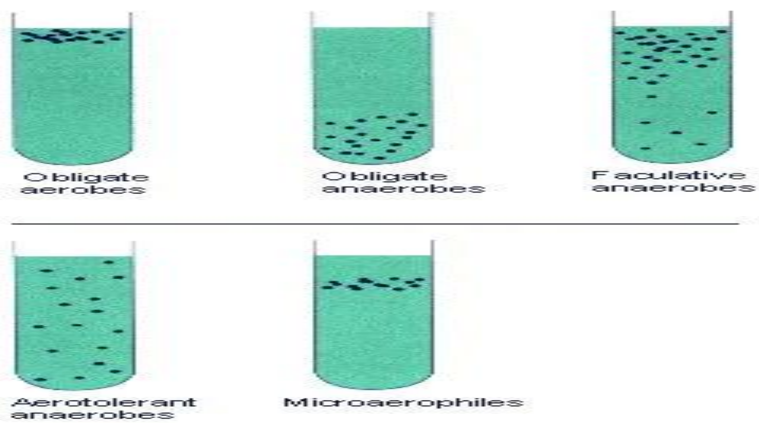
Obligate aerobes are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration (*Mycobacterium tuberculosis)*

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.

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 *(Clostridium)*

Microaerophiles are organisms that require a low concentration of oxygen for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration (*Campylobacter*)

Aerotolerant anaerobes, like obligate anaerobes, but can grow in its presence. They obtain energy only by fermentation.



Aerobes can survive in the presence of oxygen only by virtue of an elaborate system of defenses. Without these defenses key enzyme systems in the organisms fail to function and the organisms die. Obligate anaerobes, which live only in the absence of oxygen, do not possess the defenses that make aerobic life possible and therefore cannot survive in air. The tolerance to oxygen is related to the ability of the bacterium to detoxify superoxide and Hydrogen peroxide, produced as byproduct of aerobic respiration

The assimilation of glucose in aerobic condition results in the terminal generation of free radical superoxide (O2-). The superoxide is reduced by the enzyme superoxide dismutase to oxygen gas and Hydrogen peroxide (H2O2). Subsequently, the toxic hydrogen peroxide generated in this reaction is converted to water and oxygen by the enzyme catalase, which is found in aerobic and facultative anaerobic bacteria, or by various peroxidases which are found in several aerotolerant ana

Ions and salt

All bacteria require metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++, Mn++ etc. to synthesize enzymes and proteins.

Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt (halotolerant)

There is some halophilic that require high concentration of salt in media (0.2 to 5.2%). Halophiles use to survive in high concentrations of salt is the synthesis of compatible solutes. These work by balancing the internal osmotic pressure with the external osmotic pressure, making the two solutions isotonic (*Staphylococcus* in 5% NaCl).

Normally, the salt concentration of microbial cytoplasm (osmotic pressure) is equal the external environment salt concentration, in this case the osmotic pressure is optimum (isotonic).

-The external salt concentration rise, water will flow out of the cytoplasm through the cell membrane into the environment, thereby causing the microorganisms to shrink and die (hypertonic).

-By comparison, if exterior water is free of salt, it will flow through the cell membrane into the cytoplasm of the cell, causing the organism to swell and burst (hypotonic).

Bacterial concentrations can be measured by in terms of:

1. **Cell concentration or turbidity** (the number of cells per unit volume of culture)
2. **Biomass concentration** (dry weight of cells per unit volume of culture).

Growth curve

The bacterial growth curve represents the number of live cells in a bacterial population over a period of time.

Multiplication takes place in geometric progression. Population occurs in exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth. The time taken by the bacteria to double in number during a specified time period is known as the **generation time**.

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.

1.Lag Phase:

Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Time is required for adaptation to new environment. Although there is no apparent cell division occurring (no multiplication), the cells may be growing in volume or mass, synthesizing enzymes and intermediates are formed and accumulate increasing in metabolic activity until they are present in concentration that permits growth to start.

The length of the lag phase is apparently dependent on a wide variety of factors including:

1.Size of the inoculum: Lag phase was reduced with the increase of inoculum size

2. Time necessary to recover from physical damage or shock in the transfer;

3. Time required for synthesis of essential coenzymes and new enzymes that are necessary to metabolize the substrates present in the medium.

2. Exponential Phase or Logarithmic (Log) Phase.

The cells multiply at the maximum rate in this exponential phase, i.e. there is linear relationship between time and logarithm of the number of cells. Mass increases in an exponential manner. This continues until one of two things happens:

1. One or more nutrients in the medium become exhausted
2. Toxic metabolic products accumulate and inhibit growth.
3. Nutrient oxygen becomes limited for aerobic organisms.

3. Maximal Stationary Phase.

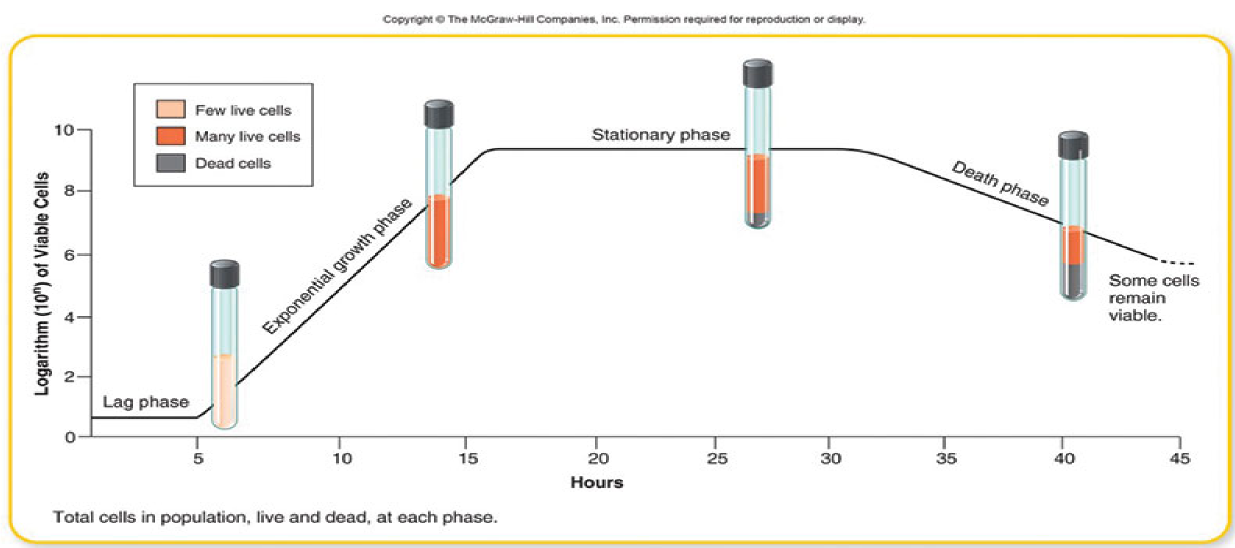
Death of bacteria starts and the growth cease completely. The count remains stationary due to balance between multiplication and death rate.

Production of exotoxins, antibiotics (Secondary metabolites), 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 persisted.

Death of bacteria is the irreversible loss of ability to reproduce.



The growth of bacteria can be calculated from the following formula:

$$G = \frac{t}{n} = \frac{t}{3.3 \log b/B}.$$

- In the formula, **G** (generation time) = **t** (time, in minutes or hours) /**n** (number of generations)
- **B** = number of bacteria at the beginning of a time
- **b** number of bacteria at the end of a time

Example 1: What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in 4 hours of growth?

$$G = \frac{t}{3.3 \log b/B}$$

$$G = \frac{240\text{minutes}}{3.3 \log 10^7/10^4}$$

$$G = \frac{240\text{minutes}}{3.3 \times 3}$$

$$G = \frac{240\text{minutes}}{9.9}$$

$$G = 24 \text{ minutes}$$

Example 2: What is the generation time of a bacterial population that increases from 1000 cells to 100,000,000 cells in 6 hours of growth?

$$G = \frac{t}{3.3 \log b/B}$$

$$G = \frac{360\text{minutes}}{3.3 \log 10^8/10^3}$$

$$G = \frac{360\text{minutes}}{3.3 \times 5}$$

$$G = \frac{360\text{minutes}}{16.5}$$

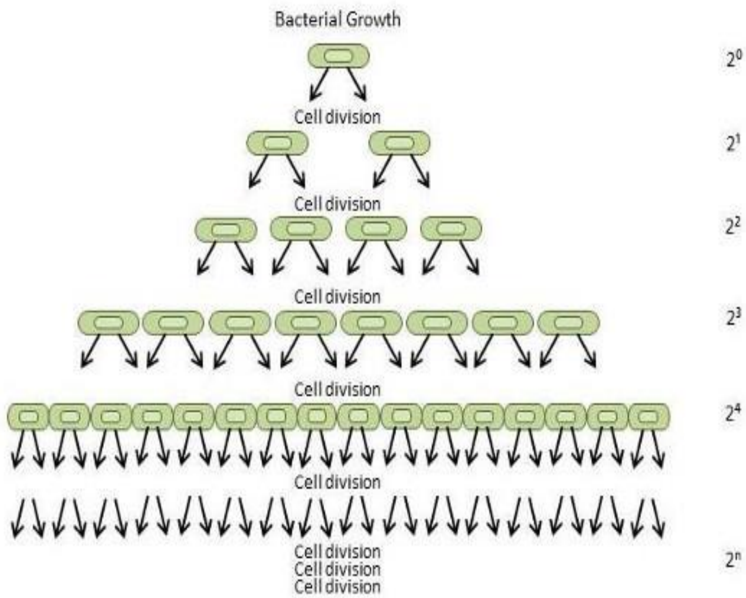
G = 22 minutes

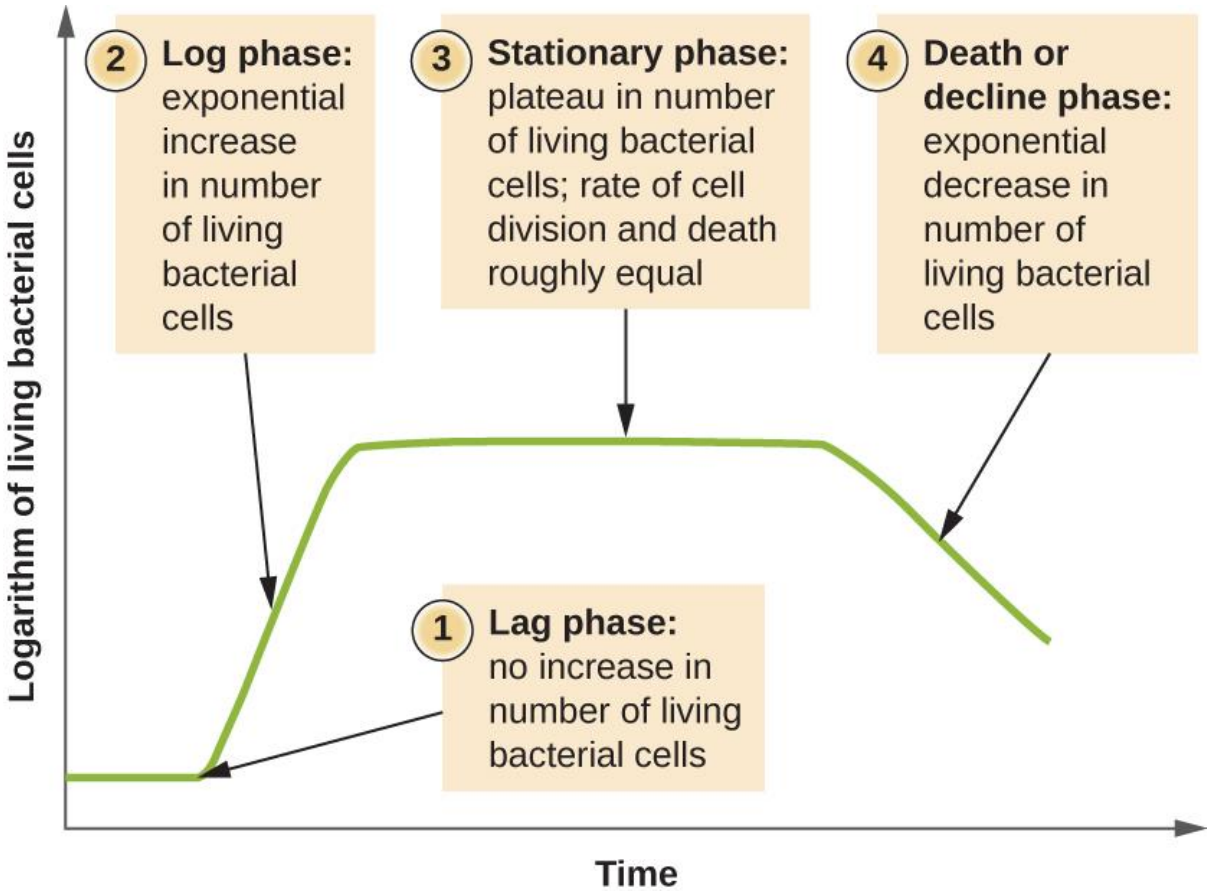
The generation time, which varies among bacteria, is controlled by many environmental conditions and by the nature of the bacterial species.

Clostridium perfringens, fastest-growing bacteria, optimum generation time of about 10 minutes

Escherichia coli generation time 15-20 minutes

Mycobacterium tuberculosis slow-growing bacteria, generation time 12-16 hours.





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