Cultivation of microorganisms

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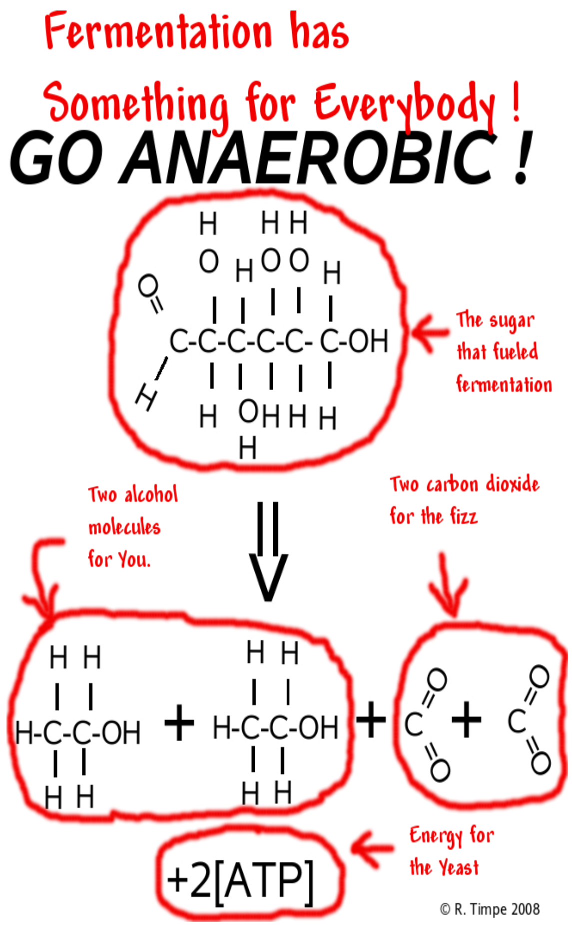
Cultivation is the process of propagating organisms by providing the proper environmental conditions. Organisms require metabolic energy to synthesize macromolecules and to maintain essential chemical gradients across their membranes. Additionally, nutrients must be provided in metabolically accessible form. Microorganisms vary widely in their nutritional demands and their source of metabolic energy. Factors that must be controlled during growth include the nutrients, PH, temperature, aeration, salt concentration, & ionic strength of the medium. The three major mechanisms for generating metabolic energy are **fermentation, respiration & photosynthesis**. At least one of these mechanisms must be employed if an organism is to grow.

Source of metabolic energy

1.Fermentation:

Fermentation is characterized by substrate phosphorylation (an enzymatic process in which a pyrophosphate bond is donated directly to ADP by a phosphorylated metabolic intermediate (such as glucose, lactose or arginine). The formation of ATP in fermentation is not coupled to the transfer of electrons. For example the fermentation of glucose ($C_6H_{12}O_6$) yields a net gain of two pyrophosphate bonds in ATP & produces two molecules of lactic acid ($C_3H_6O_3$).

Without oxygen, pyruvate (pyruvic acid) is not metabolized by cellular respiration but undergoes a process of fermentation. The pyruvate is not transported into the mitochondrion, but remains in the cytoplasm, where it is converted to [waste products]() that may be removed from the cell. This serves the purpose of oxidizing the electron carriers so that they can perform glycolysis again and removing the excess pyruvate.

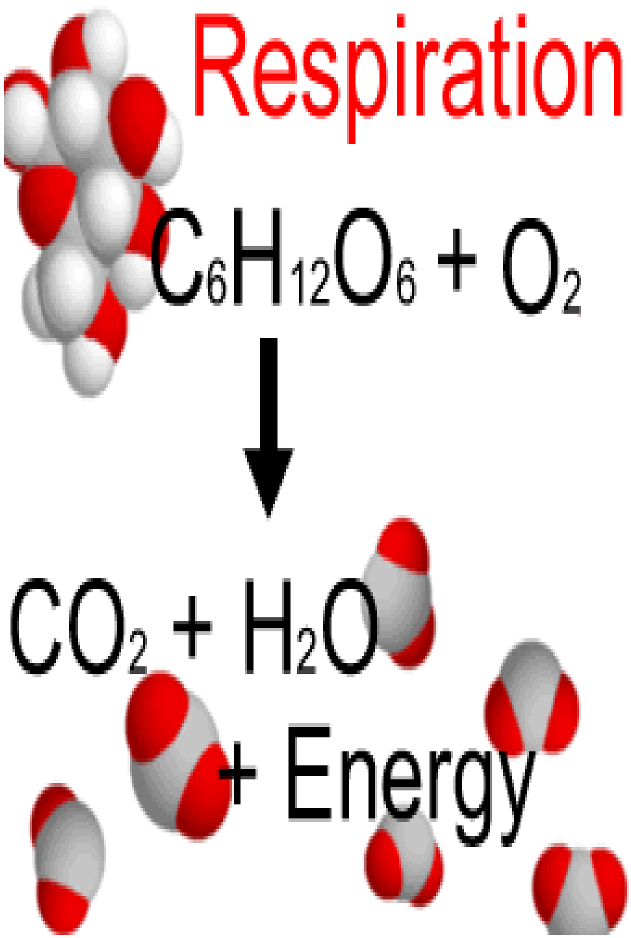


This waste product varies depending on the organism. In skeletal muscles, the waste product is lactic acid. This type of fermentation is called lactic acid fermentation. In yeast, the waste products are ethanol and carbon dioxide. This type of fermentation is known as alcoholic or ethanol fermentation. The ATP generated in this process is made by substrate-level phosphorylation, which does not require oxygen.

Source of metabolic energy

2.Respiration:

Is a chemical reduction of an oxidant (electron acceptor)by a reductant (electron donor) establishes the proton motive force across the bacterial membrane. The reductant may be organic or inorganic. Lactic acid serves as a reductant in some microorganisms & hydrogen is a reductant for others. Oxygen (O2) often employed as an oxidant, other oxidant that are employed by some organisms include CO_2, sulfate (SO_4) or nitrate (NO_3).



Respiration

36 ADP + $36P_i$ → 36 ATP

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

Glucose + Oxygen → Carbon dioxide + Water

Respiration

In physiology, respiration (often mistaken with breathing) is defined as the transport of oxygen from the outside air to the cells within tissues, and the transport of carbon dioxide in the opposite direction. This is in contrast to the biochemical definition of respiration, which refers to cellular respiration: the metabolic process by which an organism obtains energy by reacting oxygen with glucose to give water, carbon dioxide and ATP (energy).

Although physiologic respiration is necessary to sustain cellular respiration and thus life in animals, the processes are distinct: cellular respiration takes place in individual cells of the animal, while physiologic respiration concerns the bulk flow and transport of metabolites between the organism and the external environment.

In unicellular organisms, simple diffusion is sufficient for gas exchange: every cell is constantly bathed in the external environment, with only a short distance for gases to flow across. In contrast, complex multicellular animals such as humans have a much greater distance between the environment and their innermost cells, thus, a respiratory system is needed for effective gas exchange. The respiratory system works in concert with a circulatory system to carry gases to and from the tissues.

Respiration versus Fermentation

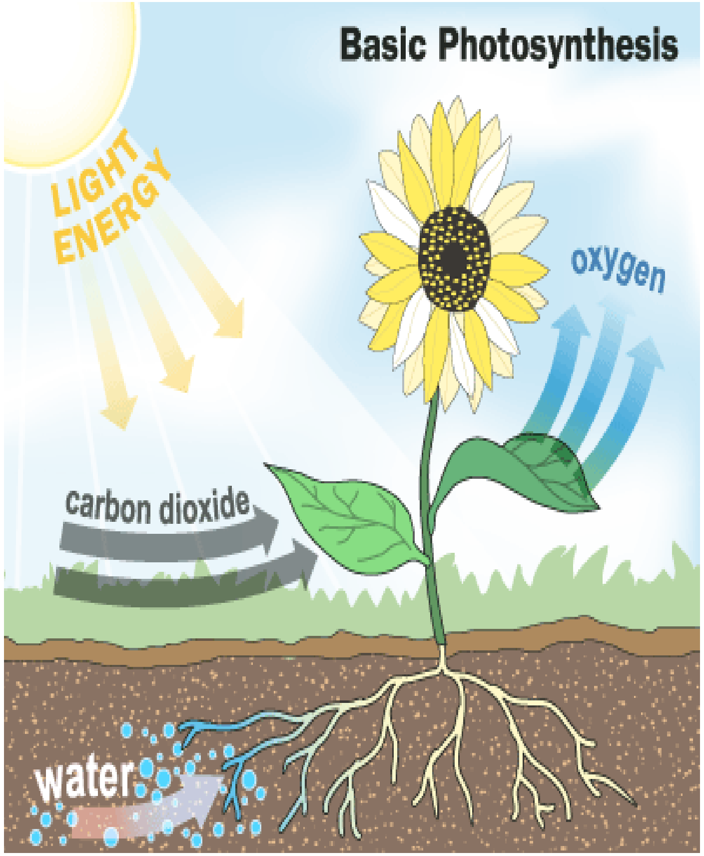
The energy released in respiration is used to synthesize ATP to store this energy. The energy stored in ATP can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell **membranes**.

Fermentation is less efficient at using the energy from glucose since 2 ATP are produced per glucose, compared to the 38 ATP per glucose produced by aerobic respiration.

3. Photosynthesis:

Photosynthesis is similar to respiration in that the reduction of an oxidant via a specific series of electron carriers establishes the proton motive force. The difference is that in photosynthesis the reductant & oxidant are created photochemically by light energy absorbed by pigments in the membrane. Plants & some bacteria employ a substantial amount of light energy in making water & CO_2. Oxygen is evolved in this process & organic matter is produced.

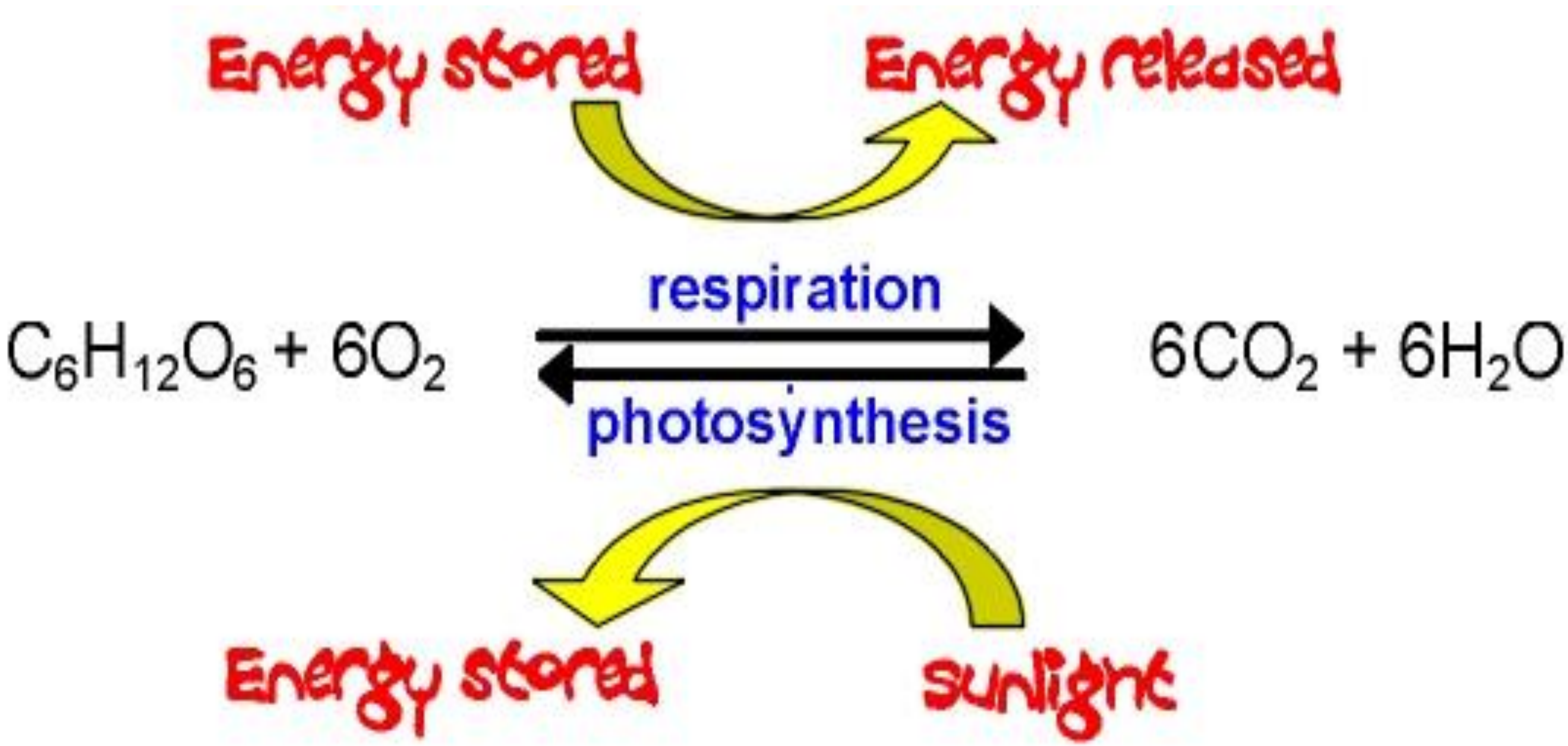
Source of metabolic energy



Photosynthesis

Photosynthetic organisms are photoautotrophs, which means that they are able to synthesize food directly from carbon dioxide using energy from light. However, not all organisms that use light as a source of energy carry out photosynthesis, since *photoheterotrophs* use organic compounds, rather than carbon dioxide, as a source of carbon. In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called *oxygenic photosynthesis*. Although there are some differences between oxygenic photosynthesis in plants, algae and cyanobacteria, the overall process is quite similar in these organisms. However, there are some types of bacteria that carry out anoxygenic photosynthesis, which consumes carbon dioxide but does not release oxygen.

Respiration & photosynthesis



Nutrition

Nutrients in the growth media must contain all the elements necessary for the biologic synthesis of new organisms; these include a carbon source, nitrogen source, sulfur source, phosphorus source & mineral sources.

In general the following nutrients must be provided:

Hydrogen donors & acceptors

Carbon source

Nitrogen source

Minerals: sulfur & phosphorus

Growth factors: amino acids, purines & pyrimidines.

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, & such factors as pH, temperature, & aeration must be carefully controlled.

Agar is a jelly-like substance, obtained from algae. is a polysaccharide extract of a marine alga, which is uniquely suitable for microbial cultivation because it is resistant to microbial action & because it is dissolved at 100 C, but not gel until cooled to 45 C.

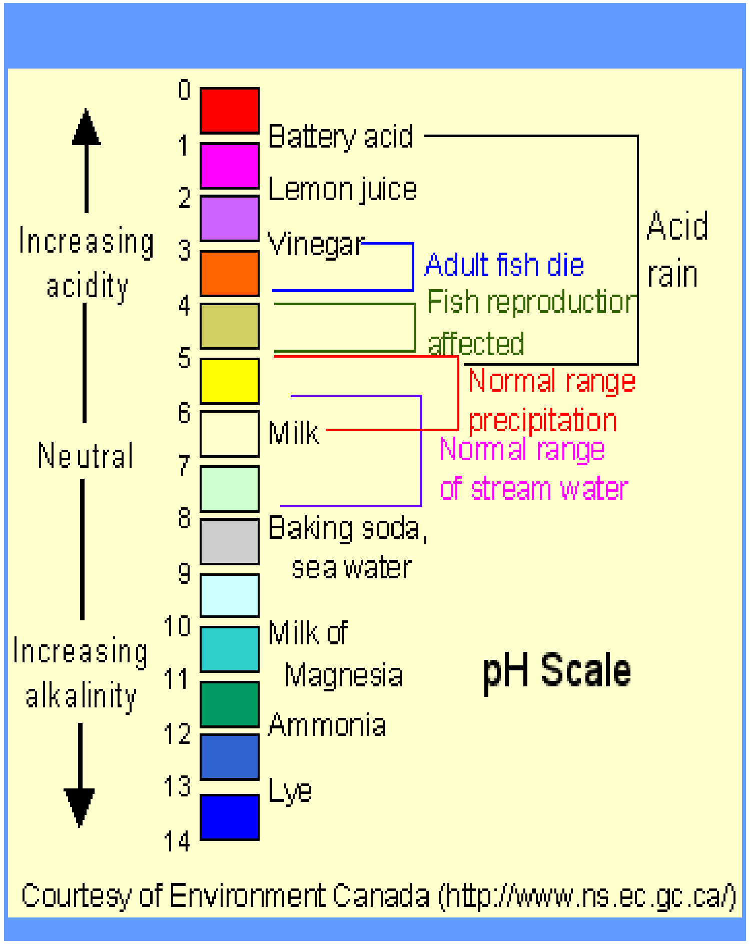
Hydrogen ion concentration (pH)

Most organisms have a fairly narrow optimal pH range. The optimum pH must be determined for each species. Most organisms grow at a pH of 6.0-8.0, although some forms (**acidophils**) have optimal pH as low as 3.0, & others (**alkaliphiles**) have optimal pH as high as 10.5.

Microorganisms regulate their internal pH over a wide range of external pH values by a set of proton transport systems in the cytoplasmic membrane.

Temperature:

Different microbial species vary widely in their optimal temperature range for growth. Psychrophilic forms grow best at low temperature (15-20 C), mesophilic forms grow best at 30-37 C, & most thermophilic forms grow best at 50-60 C. Most organisms are mesophlic.



Aeration:

The role of oxygen as hydrogen acceptor. Many organisms are obligate aerobes (e.g. Pseudomonas), specifically require oxygen as hydrogen acceptor; some are facultative (e.g. Streptococci), able to live aerobically or anaerobically [It grow better at 5-10% CO_2 condition (Candle jar)]; & others are obligate anaerobes [e.g. Clostridia (anaerobic jar)] , requiring a substance other than oxygen as hydrogen acceptors & being sensitive to oxygen inhibition.

Ionic strength & osmotic pressure:

Organisms require high salt concentrations are called **halophilic**; those require high osmotic pressures are called **osmophilic**. Most bacteria are able to tolerate a wide range of external osmotic pressures & ionic strength because of their ability to regulate internal osmolality & ion concentration.

Temperature & Aeration:

Ionic strength & osmotic pressure

Osmolality is regulated by the active transport of K+ ions into the cell; internal ionic strength is kept constant by a compensating excretion of the positively charges organic compounds.

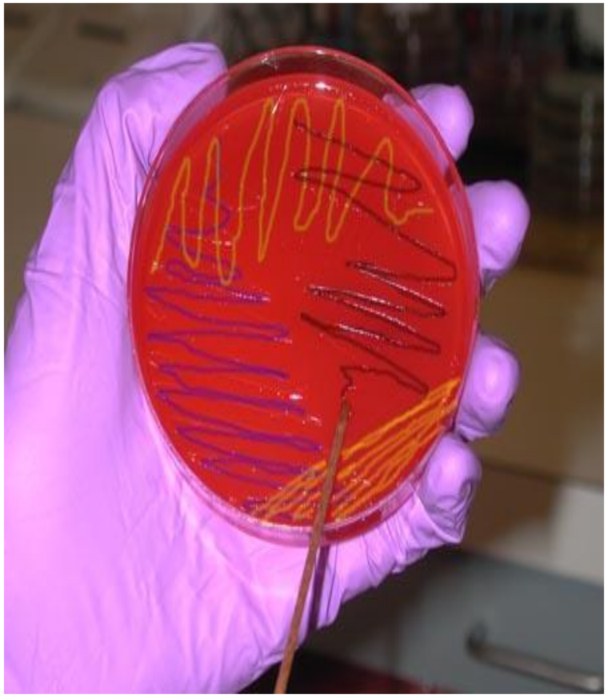
Cultivation methods:

For cultivation of microorganisms two things must be considered; the choice of a suitable medium & the isolation of a bacteria in pure culture.

The medium:

The technique used & the type of medium selected depend upon the nature of investigation. Generally, three situations may be encountered:

1. To raise a crop of cells of a particular species.
2. To determine the number & type of organism present in a given material.
3. To isolate a particular type of organism from a natural source.



Isolation of microorganisms in pure culture

Isolation of microorganisms in pure culture:

To do this, a single cell must be isolated from all other cells & cultivated in such a manner that its collective progeny also remain isolated. Several methods are available

Plating method:

If few enough cells are placed in or on a gelled medium, each cell will grow into an isolated colony. The ideal gelling agent for most microbal media is agar, an acidic polysaccharide extracted from certain red algae.

Dilution method:

The suspension is serially diluted & samples of each dilution are plated or streaked on solid media.

The growth, survival and death of microorganisms

The growth

Survival of microorganisms in the natural environment:

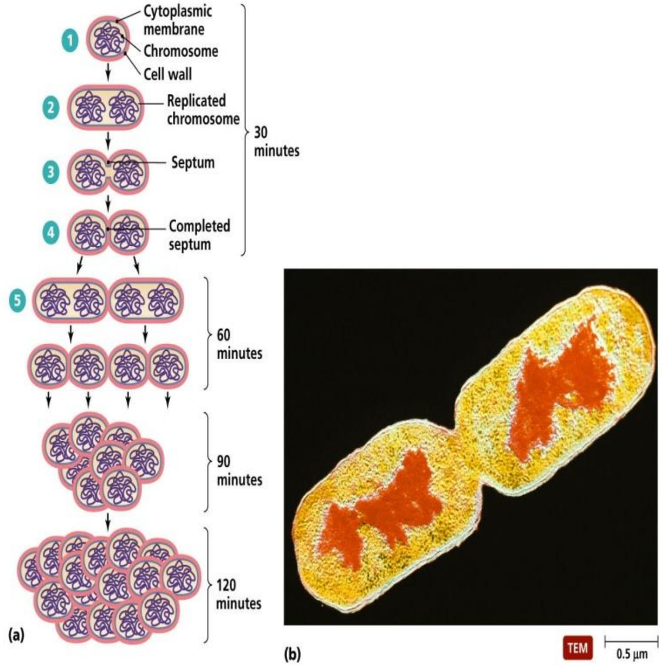
The population of microorganisms in the biosphere is roughly constant; growth is counterbalanced by death. The survival of any microbial group within its environment is determined by maintenance of a pool of living nutritional deprivation & by successful competition for nutrients.

The meaning of growth:

Growth is the orderly increase in the sum of all the components of an organism. Cell multiplication is a consequence of growth. In unicellular organisms, growth leads to an increase in the number of individuals making up a population or culture.

The growth curve:

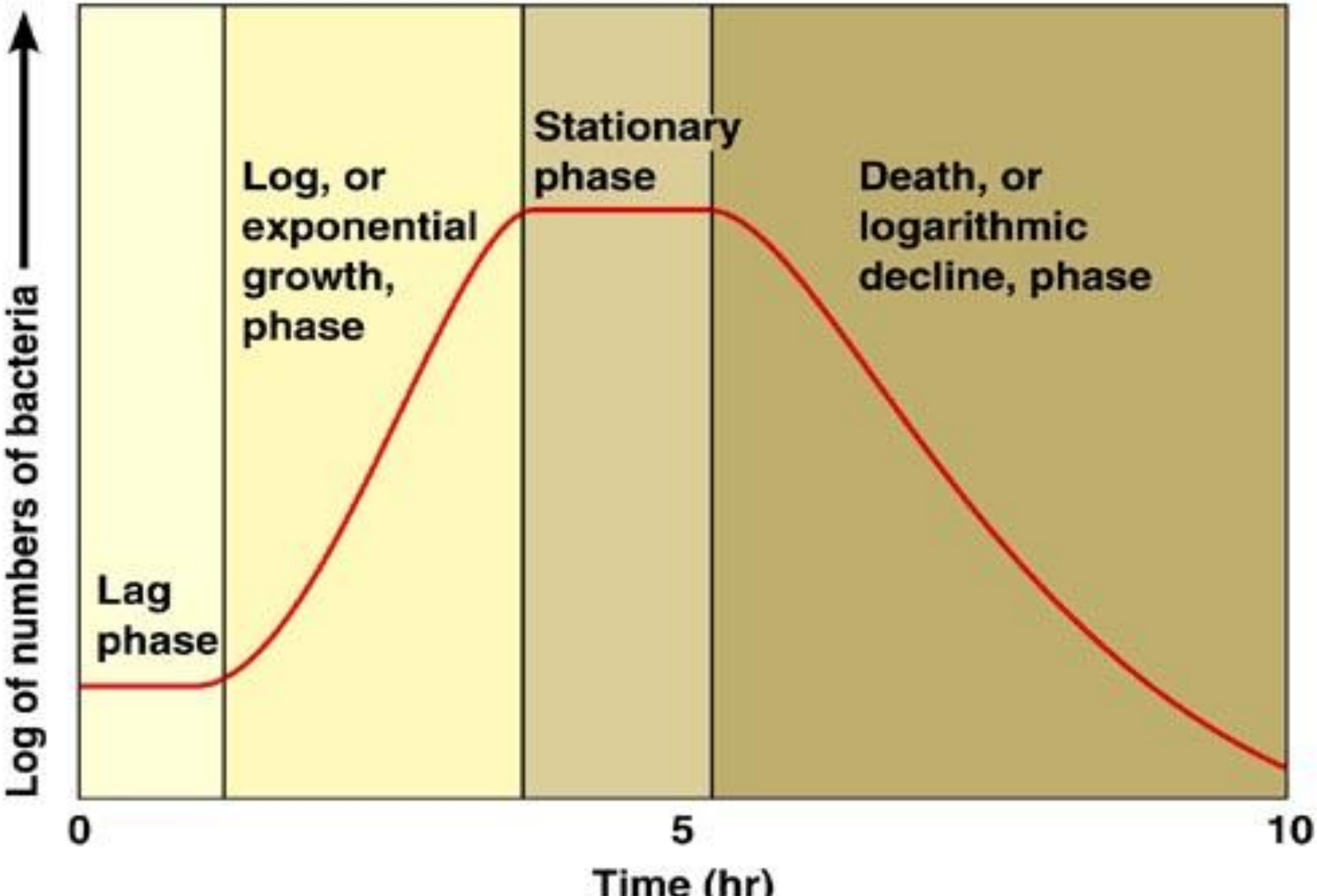
If a liquid medium is inoculated with microbial cells taken from a culture & the number of viable cell/ml determined periodically & plotted, a curve of 6 phases is usually obtained represented by letters A-F.



Phases of growth curve

Section of curve	phase	Growth rate
A	Lag phase	Zero
B	Acceleration phase	Increased
C	Exponential phase	Constant
D	Retardation phase	Decreasing
E	Maximum stationary phase	Zero
F	Decline phase	Death

The bacterial growth curve



The lag phase:

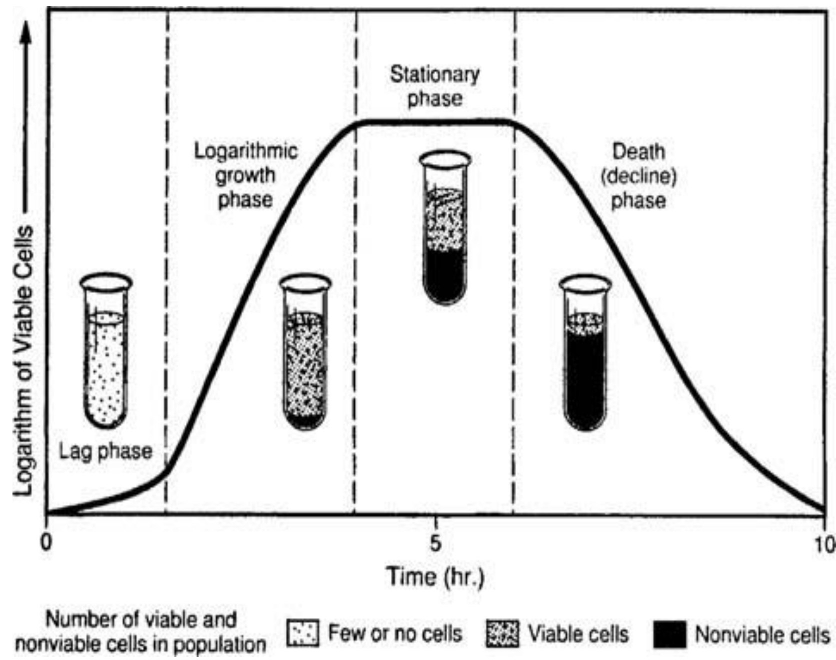
The lag phase represent a period during which the cells, depleted of metabolites of enzymes as a result of unfavorable conditions that existed at the end of their previous growth, adapt to their new environment. Enzymes & metabolites are formed & accumulate until they are present in concentrations that permit growth to resume. The duration of lag phase is depending on the similarity between the previous & new culture media.

The Exponential phase:

During this phase, the cells are in a steady state. New cells materials are being synthesized at a constant rate & catalytic & the masses increase in exponential manner. This continues until one of two things happens:

1. One or more nutrients in the medium become exhausted.
2. 2. Accumulation of toxic metabolic products & inhibit growth.

The lag phase



For aerobic organisms, the nutrients in the medium become limited are usually oxygen. When the cell concentration exceeds about 1x 10^7/ ml the growth rate will decease unless oxygen is forced in the medium. When the bacterial concentration reaches 4-5x 10^9/ml, the rate of oxygen diffusion cannot meet the demand even in the aerated medium, and thus growth is slowed.

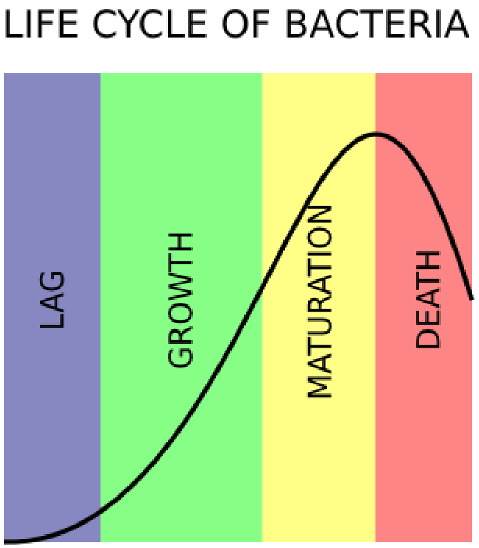
The maximum stationary phase:

The exhaustion of nutrients or the accumulation of toxic products finally causes growth to stop completely. When this occurs, the total cell count slowly increases although the viable count stays constant, i.e. the death rate is balanced by the formation of new cells through growth & division.

The decline or death phase:

After a period of time in the stationary phase, which varies with the organism & with the culture conditions, the death rate increases until reaches a steady level? A small number of survivors may persist for months. This persistence may reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die & lyses.

maximum stationary phase
and
decline or death phase



Maintenance of cells in the exponential phase:

Cells can be maintained in exponential phase by transferring them repeatedly into fresh medium of identical composition while they are still growing exponentially.

The meaning of death:

For microbial cells, death means the irreversible loss of the ability to reproduce (growth & division). The test of death is the culture of cells on solid media; a cell is considered dead if it fails to give rise to a colony on any medium. Then the reliability of the test depends upon choice of medium & conditions.

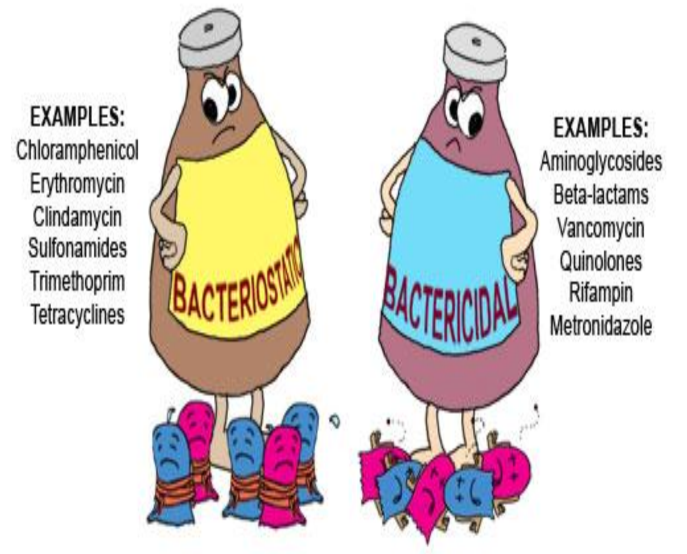
The meaning of death

Antimicrobial agents

Biocide: A general term describing a chemical agent, usually broad-spectrum that inactivates microorganisms.

Bacteriostatic: referring to the property by which a biocide is able to inhibit bacterial multiplication. Multiplication resume upon removal of the agent. The term **fungistatic** & **sporostatic** refer to biocides that inhibit the growth of fungi & spores respectively.

Bacteriocidal: referring to a property of a biocide that able to kill bacteria. Bacteriocidal action differs from bacteriostatic only in being irreversible. The term **fungicidal, sporocidal & virucidal** refer to the property of a biocide able to kill fungi, spores & viruses respectively.



Sterilization and Disinfectants:

Sterilization: A physical or chemical process that completely destroys or removes all microbial life including spores.

<u>Disinfectants:</u> Biocides used to kill microorganisms on intimate objects or surfaces. It may be sporostatic but not necessarily sporocidal.

<u>Septic:</u> The presence of pathogenic microbes in living tissues.

<u>Antiseptic:</u> A biocide or product that destroys or inhibits the growth of microorganisms in or on living tissues.

<u>Antibiotics:</u> Naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentration.

<u>Antibacterial:</u> synthetic derivatives of antibiotics

Antimicrobial agents

Physical agents:

A.Heat: is the simplest means of sterilization. A 100 ^{0}C can kill all, but spores of bacteria within 2-3 minutes. A temperature of 121 ^{0}C for 15 minutes is used to kill spores.

Autoclaving is a steam & heat sterilization by 121^{0}C and 15 lb/sq inch for 20-30 minutes. Autoclaving is very efficient in killing all bacteria & their spores & thus routinely used for sterilization of culture media.

Hot air sterilization: is used to sterilize that must remain dry by using 160-170 ^{0}c for 1 hour.

B. Radiation: Uv light & ionizing radiation have various applications as sterilizing agents.

C. Chemical agents:

1.Alcohols: Ethyl alcohol (ethanol) & isopropyl alcohol are usually used as broad-spectrum antimicrobials against bacteria, viruses & fungi, but not spores. They are usually used in 60-90%.

2. Aldehyde: Gluteraldehyde is commonly used in 2% for sterilization of surgical equipments with sporocidal activity.

Autoclave

Antimicrobial agents

Formaldehyde is bactericidal, sporocidal & virucidal used in 40% (Formaline) for sterilization of surgical & maternity theaters & hospital wards.

3. Biguanides: such as chlorohexidine is widely used as antiseptic.

4. Halogen releasing agents:

The widely used chlorine-releasing agents are sodium hypochlorite, chlorine dioxide & sodium dichloroisocyanurate, which are oxidizing agents. Hydrochlorous acid is the active component responsible for the bactericidal, virucidal effects of these compounds. At higher concentrations, these compounds are sporocidal.

Iodophors, e.g. pavidon- iodine is widely used in sterilization of skin before surgical operations. Iodine is rapidly bactericidal, fungicidal, virucidal & sporocidal.

5. Phenol & phenolic compounds:

6. Peroxygen: such as hydrogen peroxide (H_2O_2) has broad-spectrum activity against viruses, bacteria, yeasts & bacterial spores. Sporocidal activity requires higher concentrations (10-30%) of H_2O_2 & longer contact time.

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Mode of action of antibiotics

1. Damage of DNA by physical or chemical antimicrobial agents.
2. Protein denaturation; a number of physical or chemical agents causing the proteins to become non-functional.
3. Disruption of cell membrane or cell wall.
4. Removal of free sulfhydryl groups which are essential for the activity of cellular enzymes & coenzymes.
5. Chemical antagonism; the interference by a chemical agent with normal reaction between a specific enzyme & its substrate.

Reversal of antibactertial action:

1. Removal of agent
2. Reversal by substrate
3. Inactivation of agent
4. Protection against lysis