

MICROBIAL PHYSIOLOGY AND BIOCHEMISTRY

Growth and Transport

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Microbial Nutrition

All living microorganisms require nutrients for maintenance, growth and reproduction. These nutrients provide raw materials and energy which are used to build new cellular components for replication. In order to replicate, a cell must carry out many different chemical reactions and synthesize a number of molecules for specific cellular structures. The sum total of these reactions is referred to as metabolism which consists of catabolism and anabolism. Catabolic reactions give rise to smaller molecules and release energy, whereas anabolic reactions synthesize more complex molecules and generally require energy. Both catabolic and anabolic reactions take place inside the cell at the same time. A typical cell consists of macromolecules, small molecules, inorganic ions and water. Various macromolecules (such as carbohydrates, proteins etc.) which are present in the cell are made from smaller units known as monomers (such as glucose, amino acids etc.). Microbial nutrition is that aspect of microbial physiology that deals with the supply of monomers or the precursors of the monomers that a cell needs for growth and reproduction. All these molecules are collectively known as nutrients. The requirement of nutrients depends on the type of microorganism. All the nutrients known as macronutrients (which supply carbon, hydrogen, oxygen and nitrogen) are required in large amounts, while others known as micronutrients (which supply inorganic ions such as potassium, magnesium, cobalt etc.) are required in very low and even in trace amounts. The requirement of the major nutrients and the classifications of microorganisms based on their nutritional requirements are described below:

Carbon and energy requirements

All cells require carbon which constitutes as much as 50% of the total dry cell weight. The cellular compounds such as polysaccharides, proteins, nucleic acids and lipids (which make up the bulk of the cell's organic material) contain carbon. The microorganisms which require organic form of carbon (such as sugars, organic acid etc.) to meet their carbon needs are known as heterotrophs. Some microorganism, on the other hand, can derive their carbon requirement from carbon dioxide (the most oxidized form of carbon), present in the atmosphere and are accordingly known as autotrophs. To meet their energy requirements, the microorganisms either depend on the oxidation of organic or inorganic compounds, or on the energy contained in the sunlight. Microbes that obtain their energy from sunlight are called phototrophs. The phototrophic organisms that use organic compounds for their carbon requirements are accordingly called photoheterotrophs and those that use carbon dioxide for their carbon requirements are known as photoautotrophs. On the other hand, microorganisms which derive energy from chemical compounds are known as chemotrophs. While many of them derive energy by oxidizing organic compounds (organotrophs), a few of them derive energy by oxidizing inorganic compounds (lithotrophs). Table 1 gives the summary of nutritional types of microorganisms based on their carbon and energy sources.

Nitrogen requirements

After carbon, the next most abundant element in the cell is nitrogen. A typical bacterial cell contains about 10 % nitrogen by dry cell weight. Nitrogen is necessary for synthesis of amino acids, nucleotides, vitamins and some carbohydrates. In nature, nitrogen is available in both organic and inorganic forms. Inorganic nitrogen compounds such as nitrate, ammonia and

nitrogen are most easily available. While most bacteria use nitrate and ammonia as the nitrogen source, a few bacteria are able to utilize nitrogen gas as a source of nitrogen. This latter group of organisms is accordingly known as nitrogen-fixing bacteria.

Table 1: Nutritional Categories of Microorganisms

Nutritional category	Energy source	Carbon source
a. Photoautotrophs	Light	Carbon dioxide
b. Photoheterotrophs	Light	Organic carbon
c. Chemoautotrophs	Organic or inorganic Compounds	Carbon dioxide
d. Chemoheterotrophs	Organic or inorganic compounds	Organic carbon
(i) Chemolithotrophs	Inorganic compounds	Organic carbon
(ii) Chemoorganotrophs	Organic compounds	Organic carbon

Oxygen requirements

Oxygen is a common atom found in many biological molecules and is present in amino acids, nucleotides, sugars, fatty acids etc. In addition, oxygen in the form of molecular oxygen is required by most microorganisms to generate energy in aerobic respiration process. The requirement of oxygen by different organisms is considered later in the chapter.

Sulfur requirements

Sulfur is present in sulfur containing amino acids, cysteine and methionine, as well as in some polysaccharides such as agar, and in some coenzymes. Microorganisms can meet their sulfur requirement from inorganic compounds of sulfate, sulfur granules, hydrogen sulfide and thiosulfate, as well as from organic sulfur compounds such as cysteine and methionine.

Phosphorus requirements

Phosphorus is present in the cell in nucleic acids, phospholipids (component of cell membrane), coenzymes and several intermediates of metabolic pathways. The most common forms of phosphorus are the phosphate salts of sodium and potassium which almost all microorganisms can utilize.

Trace element requirements

Many minerals such as potassium, magnesium, iron, cobalt, calcium and manganese are required by almost all microorganisms in minute quantities. Most of these trace elements are required for the activity of an enzyme which catalyses a particular reaction inside the cell. For example, iron is an essential component of cytochromes which are present in electron transport system of all aerobic organisms. Similarly, potassium is required by all organisms. A number of enzymes such as those involved in protein synthesis specifically require potassium. Magnesium is an integral component of chlorophyll present in all photosynthetic microorganisms. It stabilizes chromosomes, cell membranes and nucleic acids, and is also required for the activity of many

enzymes. Since the trace elements are required in very low concentration by microorganisms, their requirement is usually met from major nutrients in which these are present as contaminants. Table 2 lists some common elements of microorganisms and their role in cellular processes.

Table 2: A few trace elements and their role in cellular processes

Element	Cellular function
Cobalt (Co)	Vitamin B ₁₂ ; transcarboxylase (propionic acid bacteria)
Iron (Fe)	Cytochromes, catalases, peroxidases, iron-sulfur proteins (ferredoxin), oxygenases, all nitrogenases
Magnesium (Mg)	Cofactor of many enzymes such as kinases, component of chlorophyll
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme of photosystem II in oxygenic phototrophs
Molybdenum (Mo)	Various flavin-containing enzymes; also in molybdenum nitrogenase, nitrate reductase, sulfite oxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

Growth factor requirements

Many microorganisms are unable to synthesize several amino acids, vitamins etc. and, therefore, require them in preformed state in their growth medium. Although these growth factors are organic compounds, these are not used by the cell in catabolic reactions and, therefore, not used for generation of energy. These are normally utilized in anabolic reactions and are incorporated in various macromolecules of the cell. Compounds such as amino acids, vitamins, purine and pyrimidine bases and nucleosides are generally the growth factors for one or the other type of microorganism. Vitamins, the most commonly needed growth factors, are the components of coenzymes which are required by the cell for various enzymatic reactions. Table 3 contains a list of few vitamins and their functions in the cell.

Microbial Growth

Microbial growth can be defined as an orderly increase of the cellular constituents and may result in an increase in a microbe's size, microbial numbers or both. For many microorganisms, it leads to a rise in cell numbers when they reproduce by binary fission (as in most bacteria) or by budding (as in yeasts). In the case of cells where growth leads to an increase in cell numbers, the individual cells enlarge and divide to give rise to two progeny of approximately the same size. However, for microorganisms like fungi and algae, the growth results in an increase in cell size but not in cell number. Since it is not easy to follow the growth and reproduction of individual organisms because of their small size, the microbiologists normally study the growth by following changes in total cell population.

Table 3: Vitamins and their functions

Vitamin	Functions
p-Aminobenzoic acid	Precursor of folic acid
Biotin	Fatty acid biosynthesis; β -decarboxylations
Folic acid	One-carbon metabolism; methyl group transfer
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α -ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD; oxidation-reduction reactions
Pantothenic acid	Precursor of FMN and FAD; oxidation-reduction reactions
Thiamine (B ₁)	Decarboxylations; transketolase
Vitamins B ₆ (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations

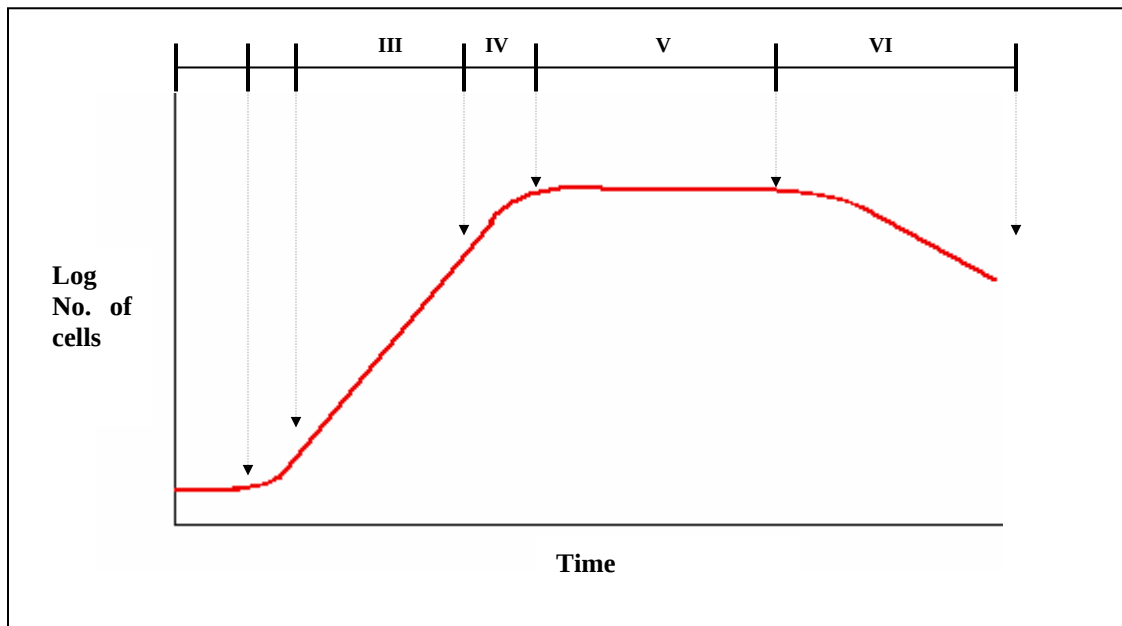
The time taken for the formation of two cells from one cell is called the generation time and one generation is said to have elapsed in this time. Therefore, the generation time, also known as the doubling time, is the time required for a cell population to double (besides the cell number, the cell mass also doubles during this period). The generation times are different for different microorganisms and depend on the availability of nutrients, pH, temperature, moisture content etc. In general, most bacteria have shorter generation times than that of most fungi and algae. A few very rapidly growing bacteria are known to have doubling times of about 10 minutes whereas the slow growing ones could have doubling times of several days. When microorganisms are cultivated in a liquid medium, they are usually grown in a batch mode. During the growth of microorganisms in a batch culture, the nutrients are taken up and the cell constituents are synthesized which result in increase in cell population (or cell mass) and the concentration of microbial metabolites (which are normally the waste products of metabolism) increase.

When microorganisms are grown in a batch culture, typically a growth curve as shown in Fig.1 is obtained. The growth curve describes an entire growth cycle which has distinct phases: lag phase, exponential phase (or logarithmic phase, also simply known as log phase), stationary phase and death phase.

Lag phase

When microorganisms are inoculated into a new fresh medium and allowed to grow under suitable culture conditions, there is no immediate increase in cell number or cell mass. Thus, initially the cells adjust themselves to the new environment and synthesize various compounds which are needed by them to grow in the new medium. This initial adjustment period prior to

start of cell division, known as lag phase, takes place for various reasons. If the cells are old (taken from a stationary or death phase of a previous culture), they are normally depleted with ATP, essential cofactors and ribosomes, which need to be synthesized before growth can take place. If the medium is different from the one in which the microorganisms were growing previously, they need to synthesize new enzymes to utilize the new nutrients. Therefore, the lag phase may be short or long depending upon the previous history of the culture and the growth conditions. This phase could be almost eliminated if an exponentially growing culture is transferred to the same medium under the same conditions of growth or if a culture is transferred from a poor medium to a rich medium (the one containing growth factors etc.).



I – Lag phase; II- Accelerating log phase; III – Log phase; IV – Decelerating log phase; V – Stationary phase; VI- Death phase

Fig. 1: A typical growth curve of a bacterial culture. The different phases of growth shown in the figure are (I) lag phase; (II) accelerating log phase; (III) log phase; (IV) decelerating log phase; (V) stationary phase and (VI) death phase

Exponential (or log) phase

After the lag phase, the culture enters the log phase via a short accelerating log phase. During the log phase, each cell divides to form two cells, each of which also divides to form two cells and so on for a period which depends upon the availability of nutrients and growth conditions. During this phase the microorganisms grow at maximum rate possible given their genetic potential and the growth conditions. Their rate of growth is exponential during this phase i.e. they divide and double in numbers at regular time intervals. Since the cells during this log phase are in their healthiest state, they are often taken from the mid log phase for study of enzymes and other cell components. However, because each individual cell in the population divides at a slightly different moment, the growth curve rises smoothly (Figure 1) and is the characteristic

feature of asynchronous culture. On the other hand, a culture in which all the cells divide at the same time is known as synchronous culture and accordingly the growth curve rises in discrete steps. The mathematical treatment for calculation of specific growth rate of asynchronous culture during log phase (which are common) is described later in the chapter.

Stationary phase

In a batch culture, the exponential growth phase is limited. This is because (i) an essential nutrient of the culture medium has been used up and, (ii) a few waste products (which could be toxic also) have been produced by the microorganisms inhibiting their further growth. Therefore, as exponential growth ceases, the microbial population reaches the stationary phase via a short decelerating log phase. Why the exponential growth is limited can be realized from the fact that if a single bacterium with a generation time of 20 minutes only is allowed to grow for 48 hours in an exponential manner, then it will produce microbial mass that will be approximately 4,000 times the mass of the earth! It may be remembered that the mass of a single bacterium is about 1 pg (10^{-12} grams) only. Obviously, it does not happen due to reason mentioned above. As shown in Figure 1, there is no net increase or decrease in cell number during the stationary phase. Although growth usually does not take place during this phase, many cell functions such as energy metabolism and some metabolic processes do continue. It has been found that some organisms continue to grow at a slow rate during this phase but there is no net increase in cell number because while some cells in the population grow, others die, and the two opposite processes balance each other out. This growth phenomenon is known as cryptic growth.

Death phase

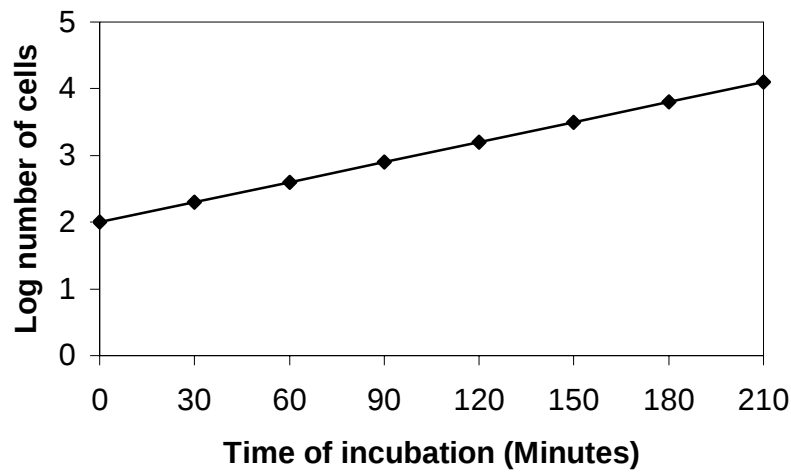
If the cells are allowed to grow beyond stationary phase, then while some cells may remain alive and continue to metabolize, they will eventually die. During this death phase, the cell population continuously decreases with time and the population is said to have entered into death phase of the growth cycle. In some cases, the death of microorganisms is accompanied by cell lysis, a phenomenon in which the internal contents of the cell are leached out of the cell. Fig. 1 indicates that this phase of growth cycle is also exponential and in most cases the death rate during the death phase is much lower than the growth rate during the log phase.

It is to be emphasized here that the microbial growth shown in Fig. 1 reflects the events in a population of cells and not in individual cells. The terms lag, log, stationary and death phases, therefore, apply only to cell population and not to individual cells.

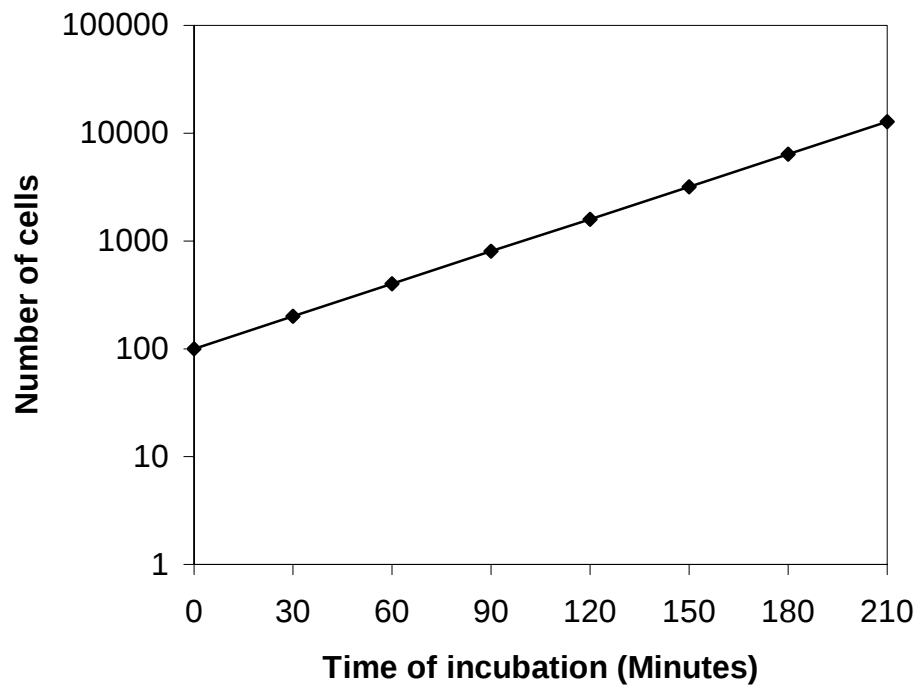
Exponential Growth Phase: Calculation of Growth Rate and Generation Time

As mentioned above, when the number of cells double during a fixed time interval, the cells are said to be growing in an exponential or log phase. If the number of cells is plotted on a log scale versus time on an arithmetic scale to observe the changes in the cell population, a straight line is obtained. Such plots are known as semi-logarithmic plots. A straight line on such a plot represents logarithmic growth rate (Figs. 2a and b). On the other hand, if number of cells and time are plotted on arithmetic plot, then whether the culture is growing in log phase or not are not properly discernible (Fig. 3). The semi-log plots are desirable because the slope of the

straight line can be used to obtain the specific growth rate of the cells; the steeper the slope, higher is the specific growth rate. The calculation of specific growth rate and doubling time of an exponentially growing culture can be illustrated by the following example.



(a)



(b)

Fig. 2: Growth of a microbial culture during log phase as obtained on semi-log plot. The data shown in Table 1 for seven generations of growth have been plotted.

- (a) The data on cell population after converting them to their log values and time of incubation have been shown on arithmetic plot. (Note that the ordinate of the graph has arithmetic scale)
- (b) The data on cell population and the time of incubation have been shown on semi-log plot. (Note that the ordinate of the graph has log scale)

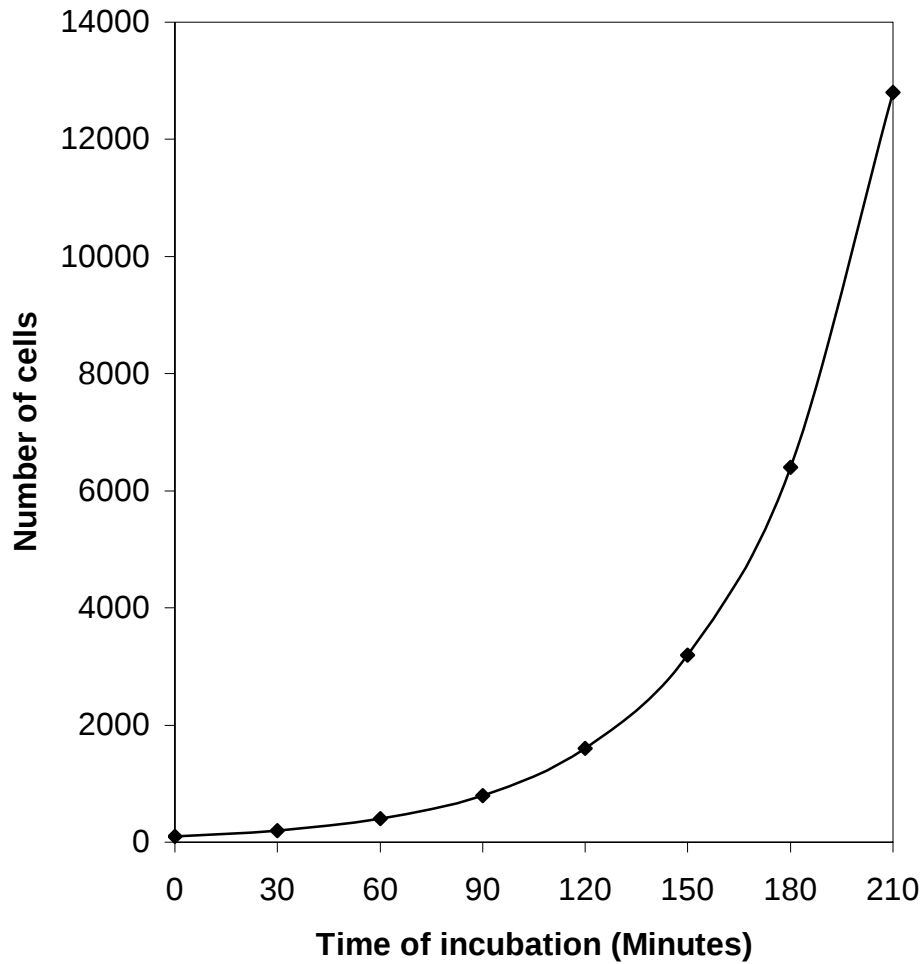


Fig. 3: Growth of a microbial culture during log phase as obtained on arithmetic plot.
(Note that the ordinate of the graph has arithmetic scale)

Consider that a medium is inoculated with 100 cells that divide after every 30 minutes. The cell population will be 200 after 30 minutes, 400 after 60 minutes and so on. Since the population doubles after every generation, the final population is always 2^n where n is the number of generations (Table 4). Thus, the resulting population increase is logarithmic. The rate of growth during log phase is proportional to the cell concentration and can be described by the following equation:

$$\frac{dx}{dt} \propto x$$

or, $\frac{dx}{dt} = \mu x$ (1)

where μ is the proportionality constant, known as specific growth rate. Eq. (1) can be written as:

$$\frac{dx}{x} = \mu dt \quad (2)$$

where x can represent either the cell number (N) or the cell mass (x).

Table 4: An example of exponential growth of a bacterial culture with generation time of 30 minutes

Time (minutes)	Number of Generations	2 ⁿ	Final Population (N _t = 2 ⁿ N ₀)	Log ₁₀ N _t
0	0	2 ⁰ = 1	100	2.0
30	1	2 ¹ = 2	200	2.3
60	2	2 ² = 4	400	2.6
90	3	2 ³ = 8	800	2.9
120	4	2 ⁴ = 16	1,600	3.2
150	5	2 ⁵ = 32	3,200	3.5
180	6	2 ⁶ = 64	6,400	3.8
210	7	2 ⁷ = 128	12,800	4.1

* The initial cell population N₀ is taken as 100

Assuming x = x₀ at t = 0 and x = x_t at time t, the integration of Eq. (2) gives:

$$\ln \frac{x_t}{x_0} = \mu t \quad (3)$$

$$\text{or, } x_t = x_0 e^{\mu t} \quad (4)$$

where e is the base of natural logarithm.

Eq. (4) shows that the cell population increases exponentially during the log phase. Eq. (3) can be written as:

$$\begin{aligned} \log x_t - \log x_0 &= \mu t \\ \text{or, } \log x_t &= \log x_0 + \mu t \end{aligned} \quad (5)$$

After taking natural logarithms, eq. (5) can be written as

$$\begin{aligned} 2.303 \log x_t &= 2.303 \log x_0 + \mu t \\ \text{or, } \log x_t &= \log x_0 + \frac{\mu}{2.303} t \end{aligned} \quad (6)$$

Thus if log x_t is plotted against t, then a straight line is obtained whose slope is equal to μ / 2.303, from which μ can be easily calculated. In a batch culture where the nutrients are in excess, the cells grow at their maximum specific growth rate (μ_{max}) during the log phase. The values of μ_{max} for a few microorganisms are given in Table 5. It is to be emphasized that the

value of specific growth rate is not constant but depends on the composition of the medium and the environmental conditions under which the microorganism is growing.

Table 5: Maximum specific growth rate ($\mu_{\max}$) of a few organisms

Organism	$\mu_{\max} (\text{h}^{-1})$
Animal cells in suspension culture	0.04
<i>Aspergillus nodulans</i>	0.36
<i>Bacillus subtilis</i>	0.80
<i>Escherichia coli</i>	0.88
<i>Fusarium graminearum</i>	0.28
<i>Lactobacillus rhamnosus</i>	0.30
<i>Methylomonas methanolytica</i>	0.53
<i>Penicillium chrysogenum</i>	0.12
Plant cells in suspension culture	0.04
<i>Vibrio natriegens</i>	4.24

Note: The values given in this table are not constant, but vary depending on culture and environmental conditions.

The generation time or the doubling time of a growing culture can be calculated easily as follows:

If t_d is time taken for a cell population to double in numbers, then the initial cell population N_0 after time t_d will be $2N_0$. Substituting the value of N and t in equation (3), we get the following relation:

$$\ln \frac{2N_0}{N_0} = \mu t_d$$

or, $\mu t_d = \ln 2$

$$\text{or, } t_d = \frac{\ln 2}{\mu} = \frac{0.69}{\mu} \quad (7)$$

Thus, if the value of μ is calculated by the procedure mentioned above, it is very easy to find the doubling or the generation time of the microbial culture. The generation times vary markedly with the species of the microorganisms and can vary from about 10 minutes for a few bacteria to a few days for large bacteria and many eukaryotic organisms. It may be remembered that the generation times are in general much longer in nature than in a cultured environment. Table 6 gives the generation times of a few bacteria, fungi and algae.

Table 6: Generation times of a few selected microorganisms

Microorganism	Generation Time (h)
Bacteria	
<i>Bacillus subtilis</i>	0.43
<i>Clostridium botulinum</i>	0.58
<i>Escherichia coli</i>	0.35
<i>Lactobacillus rhamnosus</i>	2.00
<i>Mycobacterium tuberculosis</i>	12.00
<i>Pseudomonas aeruginosa</i>	0.58
<i>Rhodospirillum rubrum</i>	5.00
<i>Staphylococcus aureus</i>	0.47
Fungi	
<i>Aspergillus terreus</i>	5.50
<i>Monilinia fructicola</i>	30.00
<i>Saccharomyces cerevisiae</i>	2.00
<i>Trichoderma lignorum</i>	24.00
Algae	
<i>Chlorella pyrenoidosa</i>	7.75
<i>Euglena gracilis</i>	10.90

Note: The values given here are not constant, but vary depending on culture and environmental conditions.

Batch Culture

A typical batch culture is a closed system in which a limited amount of nutrients is present initially in the medium. The medium is inoculated with the desired microorganism which is allowed to grow under defined conditions. The culture passes through all the four phases of growth mentioned in the earlier section i.e. lag phase, log phase, stationary phase and death phase. During the growth of the microorganism in this medium, no nutrients are added except acid or alkali for maintaining the pH and air for growth of aerobic microorganisms. Normally the carbon substrate present in the medium serves as the limiting nutrient for growth. The relationship between the specific growth rate and the concentration of the growth limiting substrate can be described by the following equation.

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (8)$$

where S is the residual substrate concentration, $\mu_{\max}$ is the maximum specific growth rate and K_s is the substrate utilization constant.

If $S = K_s$, then eq. (8) become:

$$\mu = \frac{\mu_{\max}}{2} \quad (9)$$

Therefore, K_s is equal to the substrate concentration when μ is half the value of $\mu_{\max}$. It is a measure of the affinity of the organism for the substrate. It is to be noted that the relationship between μ and S (Fig. 4) is asymptotic. The maximum value of $\mu_{\max}$ can be realized only when the substrate concentration is very very large (i.e., $S \gg K_s$). Therefore, when substrate concentration is in excess, the growth of the microorganism takes place at a rate equal to its $\mu_{\max}$ value. This takes place during the exponential phase of growth and is represented by the zone B to C in Fig. 4. The zone A to B in Figure 4 is equivalent to deceleration phase of a batch culture where the growth of the organism has resulted in depletion of the substrate to a growth-limiting concentration. Obviously, when the concentration of a substrate is growth-limiting, it will not support the growth of the organism at its $\mu_{\max}$ value. Whether the deceleration phase of a culture would be long or short depends on the value of K_s . If the value of K_s is low (high affinity for the substrate), then growth rate will not be affected until the substrate concentration has reduced significantly and, therefore, the deceleration phase of such a culture would be short. On the other hand, if the value of K_s is high (low affinity for the substrate), then the growth rate will be deleteriously affected at relatively higher substrate concentration and accordingly the deceleration phase for such a culture would be relatively long. It has been found that normally the values of K_s are quite small, which means that the affinity for the substrate is high. It may be seen from the growth curve (Fig.1) that the cell concentration at the end of the log phase is the highest and, therefore, the decline in substrate concentration is very rapid. The implication of this fact is that the time period during which the substrate concentration is near the value of K_s is very short.

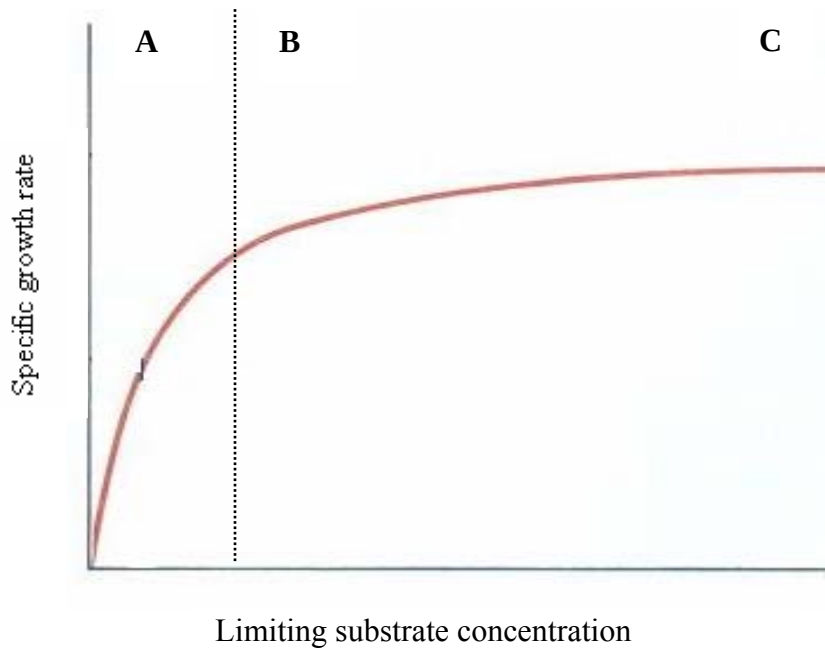


Fig 4: The effect of limiting substrate concentration (S) on specific growth rate (μ) of a bacterial culture

Continuous Culture (Chemostat and Turbidostat)

Chemostat

Our discussion of microbial growth has so far been confined to batch cultures which are essentially closed systems. In batch cultures, during the early stages of logarithmic growth phase, the conditions remain relatively constant but during the later stages when the cell growth is quite large, drastic changes take place in the chemical composition of the culture medium. For many studies such as those on physiological processes involving synthesis of an enzyme, exponentially growing cells are needed. It is, therefore, necessary to maintain the cultures in constant environment for long periods. Such systems are possible with a continuous culture which is essentially an open system in which the culture volume is maintained constant by adding fresh medium continuously and removing the spent culture medium continuously at the same rate. When such a system is in equilibrium, various parameters of the system such as culture volume, cell number and concentration of nutrients remain constant. Under such a situation, the system is said to be in steady state.

The continuous culture can be operated either as a chemostat or as a turbidostat. In a chemostat the sterile medium is fed into the culture vessel at the same rate as the culture medium containing microorganisms is removed (Fig. 5). The culture medium contains a limiting nutrient (which could be a carbon source such as glucose, nitrogen source, amino acid or oxygen). Because of the presence of a limiting nutrient in the culture medium, the growth rate of the microorganism depends on the rate at which new medium is fed into the culture vessel. Further, the final cell population also depends on the concentration of the limiting nutrient. The flow of the medium into the vessel is related to the volume of the vessel by the term D , known as dilution rate. Thus, dilution rate essentially expresses the rate of nutrient exchange in the culture vessel. It is defined as:

$$D = \frac{F}{V} \quad (10)$$

where F is the flow rate and V is the culture volume.

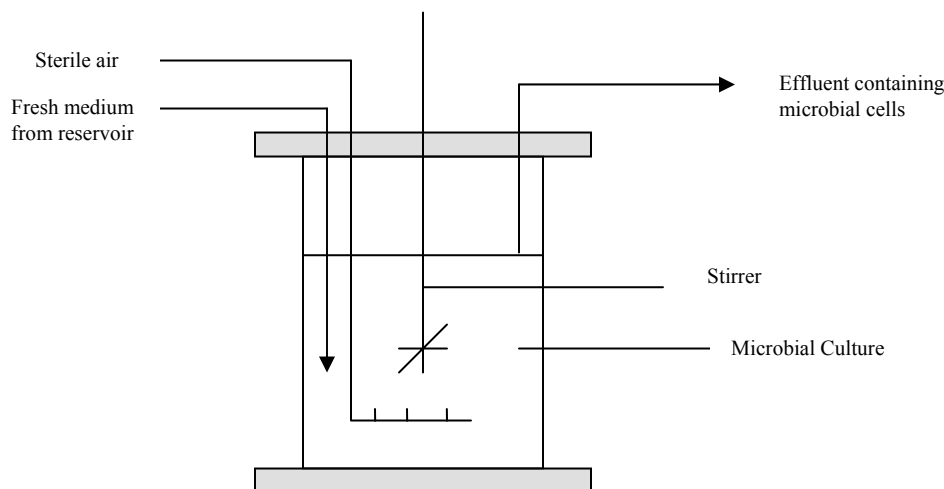


Fig. 5: Schematic diagram of a culture vessel used as chemostat for continuous culture of microorganisms

For example, if the flow rate is 60 ml/h and V is 300 ml, the dilution rate will be 0.2 h^{-1} . The net change in cell concentration (x) over a period of time can be expressed as:

$$dx/dt = \text{growth} - \text{output}$$

$$\text{or, } dx/dt = \mu x - Dx \quad (11)$$

Under steady state conditions, the cell concentration (x) becomes constant and, therefore, $dx/dt = 0$. Equation (11), therefore, becomes:

$$0 = \mu x - Dx$$

$$\text{or, } \mu = D$$

Thus under steady state conditions, the specific growth rate of a microbial culture is equal to the dilution rate. It, therefore, means that the dilution rate controls the value of specific growth rate at steady state conditions. Since the dilution rate D is a variable which can be experimentally controlled, it is possible to achieve a particular specific growth rate by changing the dilution rate. It may be noted that under batch culture conditions, a microorganism grows at its maximum specific growth rate ($\mu_{\max}$) because the medium contains excess of the limiting substrate. It is, therefore, obvious that a chemostat can be operated only at dilution rates which are less than the value of $\mu_{\max}$. Thus, within certain limits, the dilution rate can be used to control the growth rate of a microbial culture. The fact that a chemostat cannot be run at values of D approaching the value of $\mu_{\max}$ will become clear from the following mathematical treatment.

The mechanism underlying the controlling effect of the dilution rate is described by the Monod equation, which is:

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (12)$$

Since at steady state $\mu = D$, equation (12) becomes

$$D = \frac{\mu_{\max} S}{K_s + S} \quad (13)$$

where S is the steady state concentration of the limiting nutrient.

Rearrangement of equation (13) gives the following expression for calculation of steady state concentration of the limiting nutrient ($\bar{S}$).

$$\bar{S} = \frac{K_s D}{\mu_{\max} - D} \quad (14)$$

From equation (14), it is clear that if $D = \mu_{\max}$, $\bar{S}$ is infinity.

Thus, high dilution rates (where D approaches the value of $\mu_{\max}$) are not able to support the growth and the cells are removed from the system. This phenomenon is known as **washout** phenomenon. As a consequence, the limiting nutrient concentration increases at high dilution rates because fewer microorganisms are present in the culture vessel to use it. The nature of relationship of cell concentration, doubling time and limiting nutrient concentration with dilution rate at steady state is shown in Fig. 6.

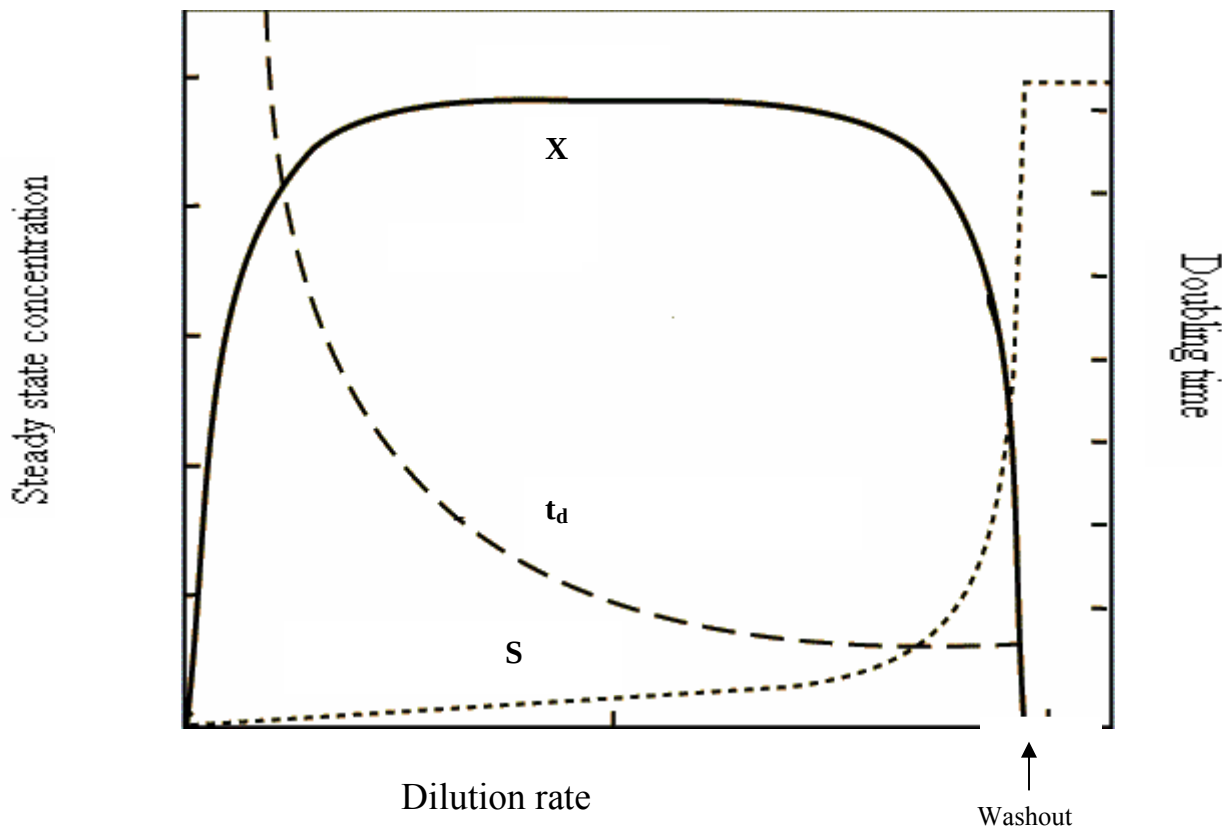


Fig. 6: The effect of dilution rate (D) on steady state concentration of cell mass (X) and substrate (S). t_d is the doubling time of the culture, at steady state

Turbidostat

The turbidostat type of continuous culture system measures the turbidity of a microbial culture in the culture vessel. In this system, a pre-determined value of cell concentration (which is directly related to absorbance) is maintained by controlling the flow rate of the fresh medium to the culture vessel. Hence, as opposed to a chemostat, the dilution rate in a turbidostat varies and the culture medium lacks a limiting nutrient. Thus, whereas a chemostat is most stable and effective at lower dilution rates, a turbidostat is operated best at high dilution rates.

Primary and Secondary Metabolites

As mentioned earlier, the growth of a microbial culture is divided into four distinct phases i.e. lag, log, stationary and death phase. Besides this kinetic description of growth, the behavior of a culture can also be described according to the products it produces during various phases of growth. During the log phase, the products which are produced by a microorganism are normally essential for growth. Such products include carbohydrates, organic acids, amino acids, nucleotides, lipids, protein etc. Since these products are linked to the energy metabolism of the

cell, (i.e. ATP generation and production of these metabolites are intimately linked to each other), they are commonly known as primary metabolites or primary products of metabolism. The phase in which they are produced i.e. the log phase is known as the trophophase. Many products of primary metabolism are of great industrial significance which includes alcohols, organic acids, amino acids, polysaccharides and vitamins. The commercial significance of a few primary metabolites is given in Table 7.

Table 7: A few primary metabolites and their commercial significance

Primary metabolites	Commercial significance
Alcohols	
Ethanol	Alcoholic beverages; automobile fuel
Glycerol	Raw material for many fermentation products such as 1,3-propanediol; manufacture of explosives
1,3 propane-diol	Synthesis of polypropylene terephthalate for fiber and textile applications, also used for adhesives, laminates, resins, detergents and cosmetics
Organic Acids	
Citric acid	Preservative; anti-foam agent; treatment of textiles
Lactic acid	Acidulant; manufacture of polylactate (biodegradable polymer)
Amino Acids	
Aspartic acid	Flavor enhancer; aspartame (sweetener) production
Glutamic acid	Flavor enhancer
Lysine	Feed supplement
Phenylalanine	Aspartame production
Nucleotides	Flavor enhancer
Polysaccharides	
Dextran	Molecular sieve chromatography
Xanthan gum	Food application, oil recovery; paint industry
Vitamins	Food and feed supplements

Some microorganisms produce compounds during deceleration and stationary phases. These compounds which are not produced during the trophophase are, therefore, not linked to the energy metabolism of the cell and, therefore, appear not to have any obvious function in cell metabolism. Such compounds are commonly known as secondary metabolites and the phase in which they are produced (equivalent to the stationary phase) is known as idiophase. The secondary metabolites (also known as idiolites) are synthesised from the intermediates and from products of primary metabolism. For example, a vast group of secondary metabolites known as terpenoides and steroids are synthesized from isopentyl pyrophosphate via mevalonate pathway.

It may be noted that the secondary metabolites are not produced by all microorganisms. These have been found to be produced by many filamentous bacteria and fungi. Unlike primary metabolites, the secondary metabolites are produced as a group of closely related compounds. For example, several different types of penicillins and tetracyclines are produced by fungi and actinomycetes. Some of the important class of industrially important secondary metabolites includes penicillins, tetracyclines, streptomycins macrolide antibiotics, terpenoids and steroids.

Effect of Environmental Factors on Microbial Growth

Microbial growth is greatly affected by the chemical and physical conditions of their environments. Our understanding of the way the environment affects the growth of microorganisms helps in explaining the distribution of microorganisms in nature, in growing them optimally in laboratory and also in controlling their growth. Several environmental factors affect the growth of microorganisms. Amongst them, the major ones are temperature, pH, water activity and oxygen concentration. Pressure and radiation also affect the growth of microorganisms.

Effect of temperature

There is a wide variation in temperatures of different regions of the world but one microorganism or the other has been found to be present in these habitats. Thus, we have microorganisms growing at temperatures as low as about -10°C and as high as about 110°C . In general, temperature affects the growth of the microorganisms in two opposing ways. As the temperature rises, the enzymatic reactions inside the cell take place at increasing rates and, therefore, result in faster growth. At the same time, the protein denaturation also increases. However, at a certain temperature, known as the optimum temperature, the growth rate is the highest. Above this temperature, protein denaturation and enzyme inactivation overtakes the rate at which enzymatic reactions take place inside the cell. This results in decrease of growth rate above the optimum temperature till a temperature is reached where the growth rate becomes zero. This temperature is known as the maximum temperature. There is no growth at temperatures higher than the maximum temperature. The lower temperature range (below the optimum temperature) also has a temperature at which no growth takes place probably because of gelling of plasma membrane. The gelling results in slowing down of transport processes to such a level that no growth takes place. This temperature is known as the minimum temperature of growth. Thus, there is no growth of microorganisms at temperatures less than the minimum temperature. Every organism has a minimum temperature, an optimum temperature and a maximum temperature and these three temperatures are known as the cardinal temperatures, which are the characteristic features of each organism. It may be noted that these cardinal temperatures are not very rigid and can vary slightly depending on the culture and environmental conditions. It has been found that the optimum temperature of a microorganism is always nearer to its maximum temperature than to its minimum temperature.

Depending on the value of the optimum temperature, the microorganisms have been broadly classified into the following four groups:

- a) Psychrophiles, with low temperature optima (about $0-15^{\circ}\text{C}$).
- b) Mesophiles with mid range temperature optima (about $20^{\circ} - 45^{\circ}\text{C}$)

- c) Thermophiles with high temperature optima (about 55⁰ - 70⁰ C).
- d) Hyperthermophiles with very high temperature optima (about 80⁰ - 110⁰ C).

Psychrophiles are present in environments which are constantly cold such as Polar Regions of the world. They are rapidly killed when brought to room temperature. A great care is, therefore, taken to ensure that they are maintained at low temperatures during their handling in the laboratory. Mesophiles are commonly found in warm-blooded animals and in terrestrial and aquatic environments. Thermophiles are found in soils and fermenting materials such as compost piles and silage where temperatures can be as high as 70⁰ C. Hyperthermophiles are found in environments near the hot springs in nature and other artificial hot environments such as geysers. The temperature range for the growth of a few selected groups of microorganisms is shown in Table 8. The table also gives the cardinal temperatures of a well-studied mesophile, *Escherichia coli*, which has 10⁰, 37⁰ and 45⁰ C as its minimum, optimum and maximum temperatures respectively, in a particular growth medium. It may be noted that the temperature range for *Escherichia coli* is 35⁰ C and its optimum is 8⁰ C below the maximum temperature but is 27⁰ C above its minimum temperature.

Table 8: Minimum, optimum and maximum temperatures of a few microorganisms

Microorganism	Minimum Temperature (°C)	Optimum Temperature (°C)	Maximum Temperature (°C)
Procaryotes			
<i>Bacillus stearothermophilus</i>	40	65	72
<i>Bacillus subtilis</i>	8	39	53
<i>Escherichia coli</i>	10	37	45
<i>Pseudomonas fluorescens</i>	4	30	40
<i>Pyrolobus fumarii</i>	90	106	113
<i>Staphylococcus aureus</i>	7	37	46
<i>Thermus aquaticus</i>	30	70	80
Eucaryotes			
<i>Aspergillus niger</i>	10	25	32
<i>Piriformospora indica</i>	8	25	35
<i>Polaromonas vacuolata</i>	-5	4	12
<i>Saccharomyces cerevisiae</i>	7	28	40
<i>Trichoderma viride</i>	10	28	37

Effect of pH

The pH affects the growth of microorganisms as does the temperature. It is not difficult to find microbes that grow in acidic environment of pH as low as 1 – 2 and in alkaline environment of pH as high as 10 – 11. However, each microbial species has a definite pH growth range (normally 2 – 3 units) and an optimum pH for growth. Microbes growing in acidic environment, known as acidophiles, have growth optimum between pH 1 and 5.5; microbes growing in the neutral pH range, known as neutrophiles, grow best between pH 5.5 and 8.5 and microbes growing in the alkaline pH range, known as alkaliphiles, have their growth optimum between pH 8.5 and 11. Further, there are organisms having optimum pH of 11 or higher and, are, therefore, known as extreme alkaliphiles. Most bacteria grow well between pH 6 – 8 and very few proliferate at pH less than 4. On the other hand, most fungi grow well at pH values less than 5. When microorganisms grow, they release waste products (such as organic acids) that may lower the pH of their environment. Some microorganisms produce ammonia by degrading amino acids and make their growth medium alkaline. If this pH change is very large, their environment becomes inhospitable for their further growth. If good microbial growth is desired, the changes in pH of the medium must be avoided. To control the changes in the pH, buffers are normally added to the growth medium. Phosphate is a commonly used buffer which is made by mixing weak acid ($\text{H}_2\text{PO}_4^{2-}$) and its conjugate base (HPO_4^{2-}). Peptides and amino acids in complex medium also have a strong buffering effect. Table 9 gives the pH range of a few common substances found in nature and the microbes which grow optimally in a particular pH range.

Table 9: pH range of a few common substances and of a few microbes which grow optimally in that range

pH range	Examples of common substances	Examples of microbes
Acidic	Lemon juice, pineapple, tomato, orange, acid soil	<i>Thiobacillus thiooxidans</i> , <i>Sulfolobus acidocaldarius</i>
Neutral	Cheese, bread, rain water, milk, pure water, saliva, blood, sea water	<i>Lactobacillus acidophilus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>
Basic	Alkaline soil, soap, household ammonia	<i>Bacillus alcalophilus</i>

Effect of water activity

Osmotic pressure

Microorganisms can experience three kinds of environments in terms of solute concentration around them. When microorganisms grow in an environment where the solute concentration inside the cell is higher than the solute concentration outside, they are said to be growing in a **hypotonic** environment. In these environments, water tends to move into the cell. However, the rigid cell wall of bacteria, fungi and algae limits the amount of water that can go inside the cell and thereby prevent the cell from swelling and bursting. In an environment where the solute

concentration outside the cell is the same as that inside the cell, they are said to be growing in an isotonic environment. If the solute concentration outside the cell is greater than that inside the cell, they are said to be growing in a hypertonic environment. In such an environment, water tends to come out of the cell thereby dehydrating it. This leads to cessation of cell activity. Thus the solute concentration in the environment, which is linked to the osmotic pressure, influences the growth and reproduction of microorganisms. The osmotic pressure is basically the amount of pressure that is required to be applied to a solution to prevent the flow of water across a membrane within the solution. For example, if a dialysis bag containing 5% sucrose solution is put in beaker containing water, then water will tend to flow into the bag and dilute sucrose solution. The osmotic pressure of the solution will be higher if the sucrose concentration in the solution is higher. However, this flow of water can be prevented by exerting pressure across the membrane; the amount of minimum pressure required to prevent this flow of water will be the osmotic pressure of the solution. The osmotic pressure of the culture environment is also important for growing bacteria that lack or have a weak cell wall. The culturing of such bacteria requires proper concentration of the media constituents so that the osmotic pressure does not cause lysis of the cell.

Water activity

The availability of water is generally expressed as water activity, a_w , which is the ratio of the vapor pressure of air in equilibrium with the solution to the vapor pressure of pure water. Thus water activity values vary from zero to 1.0. The water activity of pure water is thus 1.0. To have an idea of the water activity, the values of a few of the common natural substances are: human blood (0.995), sea water (0.98) jams (about 0.8) and cereals and dry fruits (about 0.7). It may be noted that water activity is inversely related to osmotic pressure of a solution. If a solution has high osmotic pressure, its water activity is low and vice-versa.

Although most microorganisms grow quite well at high water activities (about 0.95 or higher), a few microorganisms have developed the ability to grow in environments of low water activity. This is because such microorganisms have evolved a mechanism by which they increase their internal solute concentration and thereby decrease the internal water activity in relation to their environment's water activity. The maintenance of higher internal solute concentration is a must for the growth of such microorganisms, as water along with nutrients present in the environment has to flow into the cell. Such microorganisms are known as osmotolerant. For example, the yeast *Saccharomyces rouxii* can grow in sugar solution of as low as 0.6 water activity.

Halophiles

In nature, the osmotic effects are of interest in environments of high salt concentration. The halophilic microorganisms found in sea water (or halophiles) grow optimally in an environment with water activity of sea water besides having a specific requirement for the sodium ion. The halophiles can belong to the category of mild halophiles which grow at low (about 1-6 %) NaCl concentration, moderate halophiles which grow at moderate (7-15 %) NaCl concentration and extreme halophiles which grow at very high (15-30 %) salt concentration. It is to be noted that all halophiles have an absolute requirement of sodium ion for their growth.

Compatible solutes

As mentioned above, those microorganisms which grow in environments of low water activity increase their internal solute concentration. This is achieved by either

- (a) pumping inorganic ions, such as K^+ , inside the cell from the medium, or
- (b) synthesizing an organic solute.

The solutes synthesized by the cell for increasing its total internal solute concentration must not inhibit the biochemical processes within the cell. Such compounds are known as compatible solutes because they do not inhibit the metabolic activities of the cell. These solutes are highly water soluble sugars, sugar alcohols, other alcohols or amino acid and their derivatives. Some of the common compatible solutes are as follows:

- Amino acid type and their derivatives – glycine betain, ectoine, proline, glutamic acid.
- Carbohydrate type – sucrose, trehalose
- Alcohol type – glycerol, mannitol
- Salt type – potassium chloride.

Effect of oxygen

Oxygen requirement of microorganisms

On the basis of the requirement of oxygen, microorganisms have been divided into two main categories: aerobes whose growth is dependent on the presence of oxygen and anaerobes which can grow in the absence of oxygen. Almost all multicellular microorganisms such as fungi, algae and protozoa depend on oxygen for growth. However, amongst prokaryotes, there are several bacteria which can grow in the absence of oxygen. There are also variations amongst microorganisms in terms of their need for oxygen and their tolerance of oxygen. Accordingly, they have been divided into several groups depending on how their growth is affected by oxygen (Table 10). Amongst aerobes, there are species which can grow at full oxygen tensions and carry out aerobic respiration process of metabolism. Such organisms are known as aerobes or obligate aerobes. There are several aerobes which can tolerate very high concentrations of oxygen (hyperbaric oxygen). On the other hand, there are aerobes which can grow at levels much less than that present in air (which contains about 21 % oxygen). Accordingly, such organisms are known as microaerophiles. In addition, there are many microorganisms which can be termed as facultative aerobes (or facultative anaerobes) because they can grow under both aerobic as well as under anaerobic conditions.

The second category consists of anaerobic organisms which cannot use oxygen in their metabolism. Amongst anaerobes, there are organisms which can tolerate oxygen and grow in its presence but they do not use it for their metabolic activity. Such organisms are known as aerotolerant anaerobes. On the other hand, there are several groups of microorganisms which are inhibited or even killed in the presence of oxygen. Such organisms are known as obligate (or strict) anaerobes (Table 10).

Table 10: Classifications of microorganisms with respect to their requirement of oxygen

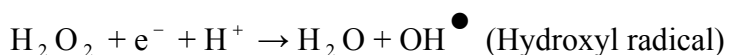
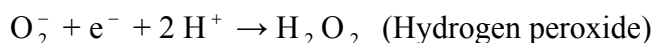
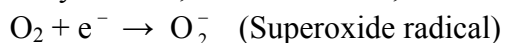
Category	Relationship to oxygen	Type of metabolism	Enzymes used in detoxification of toxic forms of oxygen
Aerobes			
Obligate	Oxygen required	Aerobic respiration	SOD and catalase
Facultative	Oxygen not essential for growth, but growth is better with oxygen	Aerobic respiration (when oxygen is present); anaerobic respiration or fermentation (when oxygen is not present)	SOD and catalase
Micro-aerophilic	Oxygen required at low levels	Aerobic respiration (limited capacity)	SOD (catalase may be present at low levels)
Anaerobes			
Obligate	Oxygen inhibitory or lethal	Anaerobic respiration or fermentation	None
Aerotolerant	Oxygen not required (growth is the same in the presence or in the absence of oxygen)	Anaerobic respiration or fermentation	SOD

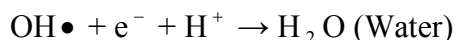
SOD = Superoxide dismutase

Generation of toxic forms of oxygen

The above mentioned relationships of microorganisms with oxygen are due to the effect of toxic oxygen derivatives on cellular constituents. It is known that oxygen is a powerful oxidant and is the best electron acceptor in respiration process in aerobic organisms but at the same time, it is inhibitory or lethal to anaerobic microorganisms. It has been found that oxygen *per se* is not inhibitory or lethal, but its derivatives are. The generation of toxic forms of oxygen in microbes is briefly mentioned below.

Oxygen accepts electrons and is readily reduced because its two orbital electrons are unpaired. Flavoproteins, quinones and iron-sulphur proteins present in a cell can carry out reduction of oxygen. The reduction of oxygen results in formation of superoxide radical, hydrogen peroxide and hydroxyl radical, which are toxic, as shown below:



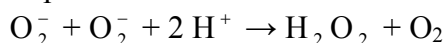


These reduced oxygen derivatives (except water) are extremely toxic because of their powerful oxidizing capacity. They can rapidly destroy cellular constituents and thereby kill microorganisms. Out of the three toxic forms of oxygen, the hydroxyl radical is the strongest oxidizing agent. However, its generation from hydrogen peroxide is limited because the enzyme catalase acts on hydrogen peroxide and converts it into harmless water and oxygen molecules (see below).

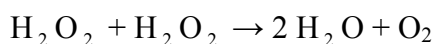
Removal of toxic forms of oxygen

Microorganisms possess enzymes that provide protection against the toxic forms of oxygen generated in most organisms, i.e. superoxide radical and hydrogen peroxide. Obligate and facultative aerobes usually contain superoxide dismutase (SOD) and catalase (or peroxidase) enzymes which catalyse the following reactions and thereby convert them to harmless H_2O .

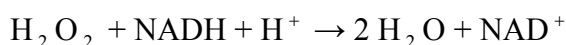
(i) Superoxide dismutase:



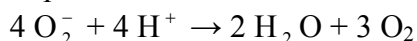
(ii) Catalase:



Peroxidase:



(iii) Superoxide dismutase and catalase in combination:



The aerotolerant anaerobes almost always have superoxide dismutase but may lack catalase. Some microorganisms like aerotolerant lactic acid bacteria use manganese ion instead of SOD to destroy the superoxide radical. All obligate anaerobes lack both these enzymes and, therefore, cannot tolerate oxygen.

Effect of pressure

Although most organisms survive and grow at a pressure of about 1 atm. (found on land and on surface of water), there are some organisms present in deep sea which can survive at hydrostatic pressure of about 500-1000 atm. Such organisms are known as barotolerant. These organisms are affected by pressure but not as much as non-tolerant organisms. On the other hand, some organisms which are barophilic grow rapidly at high pressures. Such organisms actually are unable to grow at pressures less than about 500 atm. The barophiles have been found amongst several bacterial species such as *Photobacterium* and some species of archaea such as *Pyrococcus*.

Effect of radiation

The major source of radiation on earth is sunlight which includes visible light, ultraviolet radiation and ionizing radiation. Visible light is beneficial because it is the source of energy for

photosynthetic organisms. However, even this visible light can kill microbial cells when it is present in sufficient intensity. The microbial species contain pigments like chlorophyll, cytochromes and flavins which are activated on absorption of light energy. These activated molecules on transfer of electrons to oxygen generate the highly oxidative singlet oxygen. Because of its very powerful oxidizing capacity, singlet oxygen can destroy a cell. Many organisms living on exposed surfaces, which include both photosynthetic and non-photosynthetic microorganisms, contain carotenoid pigments. These pigments can absorb energy of the singlet oxygen and convert it back into unexcited ground state. Ultraviolet radiation kill all kinds of microorganisms because of its high energy. Wave length of 260 nanometres is the most lethal UV radiation because it is absorbed by DNA, which results in formation of thymine dimers. The presence of thymine dimers in DNA inhibits DNA replication and thereby kills the microorganisms. Similarly, the ionizing radiations (such as X-rays and gamma rays) which are radiations of very short wave length or high energy, can cause mutations and be lethal at high doses. The ionizing radiations break hydrogen bonds, oxidize double bonds and destroy ring structures of molecules in a cell. Their destructive effects are enhanced by oxygen through generation of highly reactive hydroxyl radicals ($\text{OH}^\bullet$). However, some bacterial endospores can tolerate large doses of ionizing radiations.

Nutrient Uptake

Before the nutrients present in the environment could be used by microorganisms, they have to be taken up by the cell. Each cell possesses specific uptake mechanisms which allow the uptake of nutrients required by the cell and exclusion of those nutrients which are not required by it. Since in nature the microorganisms often live in nutrient- poor environments, they possess necessary mechanisms which allow them to transport the nutrients into the cell against their concentration gradient. We know that microorganisms have cytoplasmic membrane which is made up of lipid bilayer. This layer is essentially impermeable to ions and polar molecules because of its lipophylic interior. However, molecules which are lipophylic in nature (cholesterol, for example) can easily pass through it. Such molecules can pass through the membrane if their concentration outside is higher than inside the cell. Such transport process is known as passive diffusion or simply diffusion. The rate of passive diffusion obviously depends on the concentration gradient between the cell exterior and its interior (Fig. 7). It decreases as the nutrient accumulates within the cell. Besides lipophylic molecules, some small molecules such as water and gases (oxygen, carbon dioxide etc.) are also taken up by the cell through passive diffusion. However, the permeability for transport of large molecules, ions and polar molecules is conferred to the cytoplasmic membrane by two types of membrane proteins known as pumps and channels. While pumps are able to transport molecules against concentration gradient by using a source of energy such as ATP or light, channels allow the uptake through the membrane in a downhill direction only. Thus, the pump action illustrates the active and group translocation modes of transport, and the channel action illustrates the passive and facilitated diffusion mode of transport.

Facilitated diffusion

Like passive diffusion, facilitated diffusion also takes place only if there is concentration gradient of a nutrient across the membrane. However, the rate of diffusion across the membrane

is enhanced by the presence of carrier proteins (or transport proteins) which are embedded in the cell membrane. These transport proteins are also known as permeases. It may be noted that the rate of transport reaches a plateau above a specific gradient due to saturation of the carrier protein (Fig. 7). The carrier proteins are specific: while some may transport only a particular substance, others transport a group of closely related substances. Although a carrier protein is involved in facilitated diffusion, it is truly a diffusion process. A concentration gradient across the membrane is required for transport of nutrients and no metabolic energy is required in this mode of transport. This means that if the concentration gradient disappears, the transport of the nutrient will also stop. The mechanism of the transport in facilitated diffusion is as follows:

The transport protein spans the cytoplasmic membrane with some of its portions protruding out of the membrane on both sides. When a solute molecule binds to the outside of the protein, it changes in conformation and releases the molecule in the cell cytoplasm. The protein then regains its original shape and becomes ready to bind to another molecule. The net effect of this process is that the lipid-insoluble solutes can enter the cell in the presence of a concentration gradient. The facilitated diffusion seems to be more prevalent in eukaryotes than in prokaryotes. The solute glycerol is transported by this mechanism in *Escherichia coli* and many other bacteria whereas eukaryotes use it for transport of a number of sugars and amino acids.

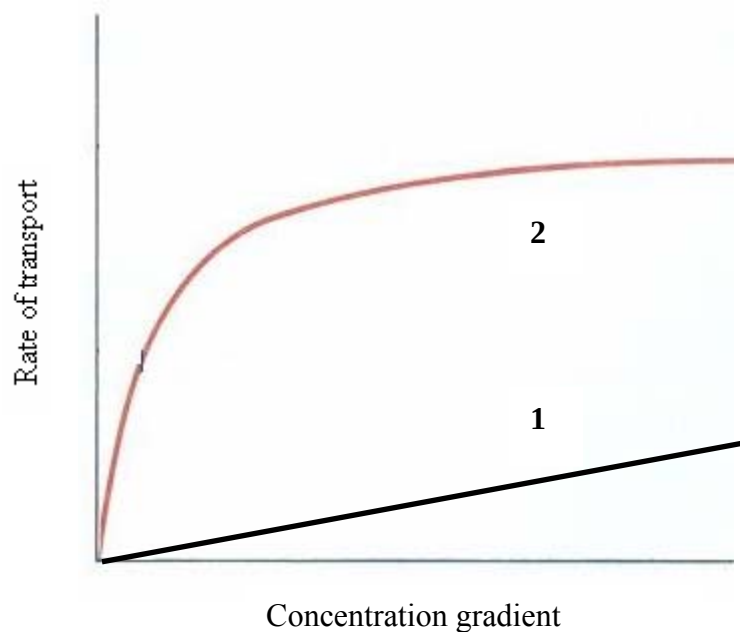


Fig 7: The effect of concentration gradient of a solute on rate of its transport through passive diffusion (1) and through facilitating diffusion (2). Note that at high concentration gradient, the rate of transport through facilitated diffusion shows saturation effect

Active transport

Microorganisms often live in environments where the concentration of nutrients is very low compared to that in the cytoplasm. In order to grow in these environments they must have the ability to transport these nutrients against their concentration gradient. The organisms achieve it

at the expense of energy. Thus through active transport process, a solute is transported to higher concentration against its concentration gradient with an input of metabolic energy. Since carrier proteins are involved in this mode of transport also, it resembles facilitated diffusion in that respect. The mechanism of transport is more or less the same as in the case of facilitated diffusion except that an energy source is required to transport the solutes against their concentration gradient.

For active transport, several bacteria, archaea and eukaryotes employ ABC transporters (ATP Binding Cassette Transporters). The membrane-spanning domains of the transporter protein form a pore in the membrane and the nucleotide binding domains of the protein bind and hydrolyze ATP. The hydrolysis drives the uptake process. The ABC transporter system also contains special substrate binding proteins (also known as periplasmic binding proteins) which are either present in the periplasmic space of gram-negative bacteria or are attached to membrane lipids on the external face of cytoplasmic membrane in gram-positive bacteria. The periplasmic binding protein binds to the solute molecule and then interacts with the membrane transport protein to transport the solute inside the cell with an input of energy from hydrolysis of ATP to ADP by ATP- hydrolyzing protein. The process by which an ABC transporter transports a solute inside the cell is schematically shown in Fig.8.

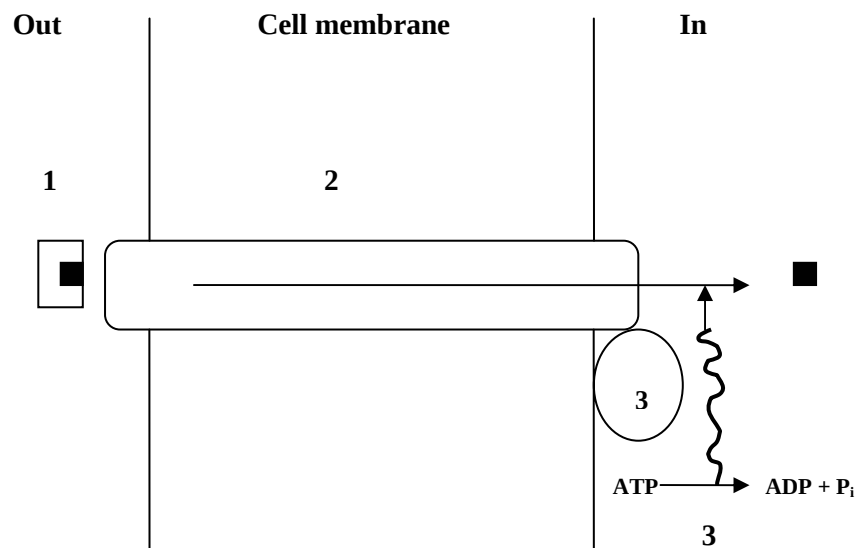


Fig. 8: Schematic diagram for transport of a solute across the cytoplasmic membrane using ABC transporter system of active transport. Solid square represents the solute, (1) the periplasmic binding protein, (2) the carrier protein and (3) the ATP hydrolyzing protein

Group translocation: The phospho transferase system (PTS)

In group translocation, the transported molecule is chemically altered during its passage across the cytoplasmic membrane. Since the process involves an input of metabolic energy for transporting a molecule against its concentration gradient, it is similar to active transport process. Many prokaryotes use this mechanism for transport of a number of sugars into the cell. This system has been best studied in *E.coli* where it is involved in transport of sugars such as glucose, fructose and mannose. The phosphotransferase system (PTS) comprises a family of proteins, five of which are required to transport a given sugar molecule. A small protein called HPr and Enzyme I are non-specific proteins as they are involved in transport of a number of sugar molecules. The other three proteins termed as Enzyme IIa, Enzyme IIb and Enzyme IIc are specific proteins as they are involved in transport of a specific sugar molecule. HPr, Enzyme I and Enzyme IIa are cytoplasmic proteins. Enzyme IIb is present on the inner membrane surface. Enzyme IIc is an integral membrane protein embedded in the membrane. The energy for the PTS is derived from the hydrolysis of energy rich compound, phosphoenolpyruvate (PEP). In PTS mode of transport, a high energy phosphate group is transferred from PEP to enzyme I through the intermediacy of Enzyme I, HPr and Enzyme IIa. During the uptake of sugar molecule through the membrane protein (Enzyme IIc), the phosphate group is transferred from phosphorylated Enzyme IIb to the incoming sugar molecule. In case of glucose, it is phosphorylated at 6th OH-position and appears in the cytoplasm as glucose-6-phosphate. Other sugars may appear as sugar-1-phosphate (for example, mannitol after transport appears as mannitol-1-phosphate). The mechanism of PTS in *E.coli* is shown in Fig. 9.

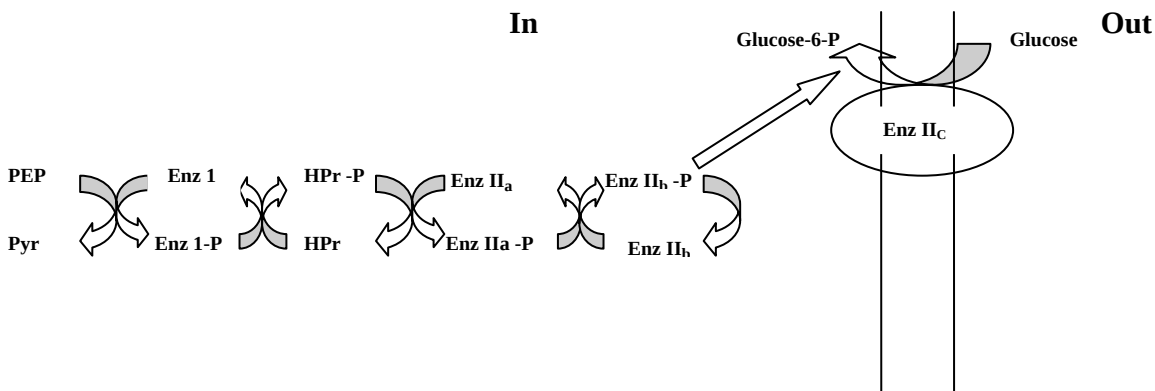


Fig. 9: Schematic diagram for transport of solute (sugar) by phosphotransferase system (PTS) of *Escherichia coli*. Note that glucose appears as glucose -6- phosphate in the cytoplasm and energy is supplied by PEP (phosphoenolpyruvate). EI, EIIa, EIIb and EIIc are the enzymes that along with HPr protein take part in the transport process

The overall reaction for transport of glucose by PTS can be written as:

$$\text{Glucose (outside)} + \text{PEP} \rightarrow \text{Glucose - 6 -phosphate (inside)} + \text{Pyruvate}$$

The salient features of the four transport processes mentioned above are summarized in Table 11.

Table 11: Modes of transport employed by bacteria for uptake of nutrients

Mode of Transport	Requirement of concentration gradient	Involvement of carrier protein	Requirement of metabolic energy	Form of transported molecule
Passive diffusion	Yes	No	No	Unaltered
Facilitated diffusion	Yes	Yes	No	Unaltered
Active transport	No	Yes	Yes (in the form of ATP or ion gradient)	Unaltered
Group translocation	No	Yes	Yes (through high energy compound such as phosphoenol-pyruvate)	Altered (mainly in the form of phosphorylated molecules)

Ion motive pumps

Several molecules are transported across the membrane by using ion motive pumps. Basically the membrane proteins which are embedded in the cytoplasmic membrane function as ion motive pumps and use the energy contained in ion gradients. The most common form of energy for ion motive pumps is perhaps the proton gradient, which is generated in the electron transport process. These membrane transport proteins do not possess any special periplasmic solute binding proteins (compare it with ABC transporter system of active transport process). The lactose permease of *E. coli* is one such membrane protein which transports lactose across the cell membrane by using proton gradient. In this case, the transport of lactose takes place along with the transport of protons through the permease. The energy for this transport is provided by the proton gradient because there is a higher concentration of protons outside the membrane than inside. Such a linked transport system where two molecules are transported in the same direction is known as symport or co-transport. In this type of transport, it is believed that the binding of protons to the transport protein results in conformational changes which affect its affinity for the solute.

The proton gradient is also used to transport the ions or solutes in the opposite direction. For example, a sodium transport system in *E. coli* transports sodium outward in exchange for the inward transport of protons. Here the energy for the transport of sodium is supplied by the proton gradient because of its higher concentration outside the cell. Such a linked transport system in which the movement of transported molecules takes place in opposite directions is known as antiport. The sodium gradient so generated can now be used for transporting other molecules by symport mechanism. For example in *E.coli*, the sodium gradient drives the uptake of sugars and amino acids. In such a transport system, it is believed that sodium ion

causes a conformational change in the transport protein, which allows it to bind to a sugar molecule. Subsequent orientation of its binding sites towards the cell cytoplasm causes the sodium ion and the solute to dissociate from it because of lower concentration of sodium in cell cytoplasm. In eukaryotes also, the sodium symport is used in uptake of sugars and amino acids. However, instead of proton motive force, ATP is used to derive sodium transport in eukaryotic cells. A schematic representation of symport and antiport systems is shown in Figure 10. Because energy is used in both symport and antiport modes of transport, they essentially represent active transport systems.

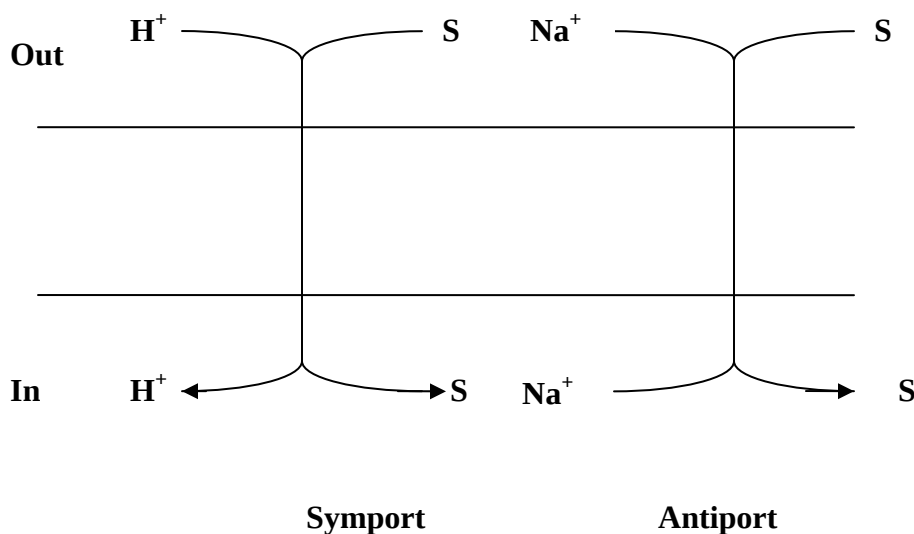


Fig.10: Active transport using proton gradient for transport of a solute (S) in symport process and sodium gradient for transport of a solute in antiport process. Note that the energy contained in protons derives the transport of solute in symport process, while the energy contained in sodium ions derives the transport of solute in antiport process

It may be remembered that the transport proteins are not present all the time in a cell and their concentration depends on environmental conditions. For example, while only a few molecules of lactose permease are present when lactose is absent in the external medium of *E.coli*, its concentration increases significantly when lactose is present in the medium. Furthermore, a microorganism may have more than one transport system for a particular molecule. When this happens, then one transport system may differ from the other in terms of the use of energy source, its affinity for the molecule and the nature of its regulation. Apparently this facility confers a competitive advantage to microorganisms in a variable environment.

Porins and the periplasmic binding proteins

While the cytoplasmic membrane of gram-negative bacteria is almost impermeable to even small hydrophilic molecules, the outer membrane is relatively permeable even though it is basically a lipid bilayer. This is due to the presence of proteins, known as porins, in this outer membrane.

These proteins work as channels for the entry and exit of hydrophilic low molecular weight molecules. Both specific and non-specific types of porins have been identified. While non-specific porins which form water-filled channels allow the passage of any small molecule, the specific porins possess a specific binding site for one or a group of structurally related molecules. Most of the porins contain three identical sub-units and associate in such a way so as to create small holes of about 1 nm diameter in the outer membrane. These porins do not allow the passage of large molecules and thereby these porins also do not allow the proteins and enzymes present in the periplasmic space to diffuse away from the cell. As mentioned in section 8.2, the periplasmic space is present between the outer surface of the cytoplasmic membrane and the inner surface of the outer membrane in gram-negative bacteria. Besides containing a number of proteins, enzymes and chemoreceptors, periplasm also contains binding proteins which are a part of ABC transporters involved in the nutrient uptake by active transport.

Suggested Readings

1. Microbiology (International Edition) by L. M. Prescott, J. P. Harley and D. A. Klein, published by McGraw Hill, 2003
2. Brock Biology of Microorganisms (International Edition) by M. T. Madigan and J. M. Martinko, published by Pearson Education International, 2006
3. Principles of Fermentation Technology (Second Edition) by P. F. Stanbury, A. Whitaker and S. J. Hall, published by Aditya Books Pvt. Ltd., New Delhi 1997.
4. Microbiology by M. J. Pelczar and R. Reid, published by Tata McGraw Hill, 2000.