C C 9;Unit 3: MICROBIAL GROWTH IN FOODS

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Microbial growth refers to increase in cell number, but not in size of the cells. In bacteria, growth is always followed by division of the cells by fission leading to increase in cell number. This increases the population size. Accordingly, there are more number of bacterial cells than cells larger in size. Growth rate in microorganisms is very high and microorganisms multiply very fast in a suitable environment. Under favourable conditions microorganisms multiply rapidly and maintain their small size. However, there is no change in their morphology.

This unit is covered in 6 sections. They are

1) Growth in bacteria
2) Bacterial growth curve
3) Diauxic growth
4) Synchronous cultures
5) Microbial growth in food- Bacteria, yeasts and molds
6) Factors affecting microbial growth in food

1) Growth in bacteria

Bacteria multiply by binary fission. A single bacterial cell divides into two after the development of a transverse cell wall between them. Many bacteria can divide in less than 15-20 min under favourable conditions. During the cell division complex biochemical changes takes place. These are synthesis of polymers, sub-cellular components and other required materials.

Several events take place before cell division (Fig 3.1). Nutrients from surrounding environment are transported into the cells. These nutrients are converted into cellular components and enzymes, etc. The nuclear content increases which is sufficient for both the cells. Elongation of the cells takes place. The cell contents are distributed evenly. The cytoplasmic membrane invaginates at the center of the elongated cell. A transverse cell wall is formed, presumably where mesosomes are present, leading to separation of two cells. Thus one cell becomes two in a very short period.

If cell division occurs in bacteria, which are in chain form, for ex. streptococci, these cells remain together. If the next cell division takes place at right angles to the plane of the first division, it appears as a tetrad (ex: *Pediococcus* spp.). If division occurs in their plane, it results in a group of 8 cells (ex: *Sarcina* spp.). If cell division takes places in different planes it may result in a bunch of cells like in the case of *Staphylococcus* (Fig.3.2.).

Growth rate and generation time of bacterial growth

Most bacteria divide by binary fission and the number increase in geometric proportion.

$$1 \longrightarrow 2 \longrightarrow 4 \longrightarrow 8 \longrightarrow 16 \longrightarrow 32 \longrightarrow 64 \longrightarrow 128$$

or 2^n, where, n is the number of generations

Generation time

The time required for the bacterial cells to double its population is known as generation time. It differs based on the organism and growth conditions. For some bacteria it may be few minutes and for some other, it may be several hours (Table-3.1). It depends on the type and amount of nutritients and environmental conditions like temperature, pH, amount of oxygen present, the presence of inhibitory compounds, etc.

The growth rate of any bacterial population can be calculated, if the number of generations in a given time is known. The bacterial growth rate can be expressed in terms of a mathematical expression. For this, the initial bacterial number, the number of bacterial cells at the end of a given time and the time duration are required.

$$n = 3.301 \times \log b/B$$

Where, n: number of generations after a known time period
b: Number of bacterial cells after ‘n’ generations
B: number of bacterial cells at time zero,

The generation time is determined by dividing the duration of time (t) by the number of generations (n).

Generation time =t/n

2) Bacterial growth curve

It is a graphical representation of various growth phases of a bacterial population. To understand this, an experiment is conducted by inoculating a small number of bacterial cells into a growth medium. Cell number is estimated in terms of viable count after regular intervals. The viable count is plotted in log numbers against time period. Alternatively, cell count is estimated by colorimetry or by direct microscopic methods. However, in these methods, dead cells also contribute to the turbidity. Microscopic observation or turbidity cannot differentiate live and dead cells. If the bacterial growth curve is plotted by using the data collected by colorimetry, the decline phase of the growth curve cannot be seen, as decreased absorbance is not depicted.

A typical bacterial growth curve has four major phases and there are three transitional phases separating these phases. (Fig.3.3). Let’s discuss them.

a**) Lag phase**: It is an initial growth phase and no visible increase in the cell number in this phase is noted. Cell enlargement takes place along with synthesis of new cellular components, DNA, cell organelles, enzymes, proteins, etc. Cells may need time to adjust to the new physical or chemical environment. During this phase of adjustment synthesis of additional cellular components for the new cell takes place. Cells are active with increased quantity of proteins, RNA, DNA and other components. The duration of this phase varies with type of bacteria and other factors. Dormant cells may take long time. If water activity is reduced in a food, duration of this phase can be extended. There is a lag in cell division. All the cells do not complete this phase at same time. Gradual increase in cell number is attained by the end of lag phase, hence, a slow upward increase in the graph is seen. The rate of multiplication increases with the time and reaches the maximum at the end of this phase. Cells are sensitive to the unfavorable conditions.

b**) Log phase**: This phase is also known as exponential growth phase. In this phase, cells divide steadily and at a constant rate. This results in a straight line in the graph, when drawn against time. Cells are smaller in size and active during this phase. The entire population is active in this phase. Hence, cells from this phase are preferred to be used as inoculum for metabolite production. During this phase, generation time is very short and constant. The growth rate is maximum and constant. However, at the end of this phase decline in the multiplication rate results in a decline in the graph curve. This is due to the accumulation of toxic metabolites like acids and change in the environment of the medium. Duration of exponential phase may vary depending on the type of bacteria and amount of nutrients and environmental conditions.

c) **Stationary phase**: In this phase, the number of new cells formed is equal to the number of cells dead. As a result, the growth curve appears as flat during this phase. However, it turns downwards and reaches maximum at the end of this phase due to increased death rate.

d) **Death Phase**: In this phase, the number of viable cells decreases exponentially resulting in the graph to move downwards. If the initial population at the beginning of this phase is 1 million, it becomes ½ million, ¼ million and so on with the increase in the incubation period. In this phase, death rate is constant and varies from bacteria to bacteria. Depletion of nutrients and accumulation of toxic components are the main reasons for death phase. Gram negative bacteria die faster than Gram positives. Spore formers can survive long period.

3) Diauxic growth

It is the growth of a bacterial population wherein growth can be seen in two separate phases. This is due to the preferential use of one carbon over the other. If the results of diauxic growth are plotted, a graph as shown in Fig. 3.4 can be obtained. This has two exponential growth phases separated by one transient lag phase. This is due to the catabolic repression of induced enzyme synthesis. This feature indicates that bacteria try to survive in the environment by making use of the all possible resources available to it. For example, if the cells growing in xylose containing medium are added to a medium containing both glucose and xylose as carbon sources to grow, bacterial cells first use xylose for this metabolic activities and later it tries to survive by using glucose, which is already present in the medium. However, cells require some time to change its metabolic activities to use a pentose sugar by synthesizing the enzymes required to metabolize xylose. Hence, a lag phase can be seen in between two log phases.

4) Synchronous cultures

In a growing culture, bacterial cells have different stages of the growth cycle. Some may be very young, some may be well developed and some about to die. It is very difficult to predict the behavior of the population in this condition. Hence, there is a requirement of having all cells at the same stage of the growth. This can provide behavioural information related to the whole population. A synchronus culture is thus defined as the population of bacteria that contains cells that are all in the same growth stage.

A population of bacteria can be synchronized by manipulating the physical or environmental conditions. When bacterial cells are inoculated into a fresh medium and incubated at less than optimum temperature for around 10-12 h, bacteria start assimilating nutrients from the medium. However, cell division will not take place at this temperature. Once, the medium is transferred to optimum temperature, all bacterial cells start diving at the same time leading to synchronous growth. Filtration is another method to get synchronous cultures (Fig 3.5). The bacterial cell population grown is filtered through a filter which can retain bacteria over it. Later this is inverted and fresh medium is allowed to pass through it. All bacterial cells adhered to the filter start diving simultaneously leading to the synchronous cultures. However, this can be maintained for a few generations only.

5) Microbial growth in food- Bacteria, yeasts and molds

Food is a rich medium for the growth of microorganisms**.** It contains carbohydrates, proteins, fats, minerals, etc. which can support the growth of microorganisms. Some microbes can grow in the foods and cause health hazards to the consumers (ex: *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, *E.coli*, *Listeria monocytogenes*, *Clostridium* spp. etc.). The growth of some other microbes may lead to spoilage of foods (several molds, yeasts and bacteria). These microbes may bring enzymatic changes, produce off flavours and change the colour. All this happens due to metabolizing of various components present in the foods. This makes the food unfit for consumption. Some other microbes can convert the food from one form to another leading to development of several fermented foods (Ex: lactic acid bacteria, *Saccharomyces*, *Aspergillus oryzae*, etc.). As discussed earlier, the generation time may be as short as few minutes for several microbes. Hence, foods may get spoiled rapidly, if it is not protected. However, several factors can influence the growth of these microorganisms.

6) Factors affecting microbial growth in food

Several factors affect the growth of microorganisms and their metabolic activity in foods changing the characteristics of the food itself. These changes may be considered as beneficial, if it happens during fermentation leading to production of fermented foods like curd/cheese. When the changes it leads to spoilage of the foods or causes pathogenicity to the consumers, they are considered harmful. Factors affecting the growth of microbes are intrinsic factors, extrinsic factors, implicit factors, processing factors and interaction among these factors.

Intrinsic factors are factors pertaining to the food itself (ex: pH, water activity, redox potential, nutrient content, presence of inhibitory compounds and mechanical barriers, etc.). Extrinsic factors are factors associated to the environment in which the food is stored (ex: relative humidity, temperature and gas composition, etc.). Implicit factors are factors associated to the microorganisms themselves (ex: interactions among different microorganisms growing in the food and between the food and microorganisms, ability to tolerate various stress conditions in the food, production of antimicrobial compounds like bacteriocin, and growth promoting compounds). Processing factors are factors related to the processing of the foods like freezing, cooling, drying, heating, etc. Interaction among these factors in combination may either promote or decrease the growth of microorganisms.

(i) Intrinsic factors:

Several intrinsic factors can influence the growth of microorganisms. The pH, known as negative logarithm of hydrogen ion concentration, can affect the growth of microorganisms. Based on the pH requirements bacteria are classified as acidophiles (pH < 6.0), neutrophiles (pH 6-7) and alkaliphiles (pH > 7.0). Many bacteria can grow better in the pH range of 6-8, yeasts between pH 4-5 and 6.0 and fungi between 4 and 10.0. Many lactic acid bacteria have the ability to grow in acidic range of foods. Change in the pH affects the enzyme activity, permeability of the membranes, solubility of the cellular constituents and shifts the metabolic activity.

Water activity (a_w) is referred as the ratio of the vapour pressure of water above the material (p) to the vapour pressure of pure water (P0). [$a_w = P/P0$]. It is an index of available water in the foods. Water is an essential requirement, as a solvent, for transport of nutrients and waste, hydrolysis of proteins, fats, polysaccharides, etc. If the water activity of the food is less than the optimum growth for the microorganisms, the lag phase of microbes extends by several hours. Hence, foods can be preserved for longer period if the water activity is reduced/controlled. Water activity in foods can be reduced by adding sugars, salt or by drying.

The natural antimicrobial compounds like short chain fatty acids or essential oils or natural antimicrobial compounds like lysozyme in eggs, lactoperoxidase in milk can inhibit the growth of microorganisms. Some of the antimicrobial compounds may be bacteriostatic (inhibit the growth by stopping the division) and some bactericidal (kill the bacterial cells). Depending upon the compound, membrane damage, inactivation of enzymes or nuclear material may vary. Some may cause mutations by alkylation or deamination.

(ii) Extrinsic factors

Several external factors can influence the growth of microorganisms.

Relative humidity (RH) is the amount of free moisture present in the air. It is an essential measure of the water activity of the gas phase. Water activity and relative humidity are interrelated. If any food with low water activity is kept in a place having a high RH, water will enter the food through the gas phase. This will finally affect the quality and acceptability of foods.

Temperature: Temperature plays a major role in the growth of microorganisms. Based on the temperature requirement growth of microorganisms are divided into three groups : psychrophilic bacteria (12-15 °C), mesophilic bacteria (30-40 °C) and thermophilic bacteria (50-70 °C). Besides this, some of the mesophylls can also grow well at lower temperatures. Ex: *Lister* ssp. Higher temperatures can inhibit the growth or kill the microorganisms, due to cytoplasmic membrane disruption, enzyme inactivation and denaturation of proteins and DNA, etc.

Oxygen: Based on oxygen requirement bacteria can be grouped into obligate aerobes, obligate anaerobes, facultative anaerobes, microaerophilic and aero tolerant anaerobes. Obligate aerobes cannot grow without oxygen (ex: *Bacillus*). Obligate anaerobes cannot grow in the presence of oxygen (ex: *Clostridium*). Facultative anaerobes can grow with or without oxygen (ex: *Staphylococcus, E. coli*). Microaerophilic requires less concentration of oxygen (ex: lactic acid bacteria). Aero tolerant anaerobes do not require oxygen, but can tolerate oxygen at certain concentration (ex: *Enterococcus*). Superoxide dismutase, catalase and peroxidase are required to inactivate the free radicals, like superoxide, singlet oxygen, hydroxyl and peroxide anion, generated by oxygen. As anaerobic microorganisms do not produce enzymes, they cannot survive in the presence of oxygen.

Processing factors: The food processing methods like freezing, cooling, drying, heating, etc., hamper the growth of microorganisms. These methods either remove free water from the cells or cause damage to cell membranes.

Microorganisms can inhibit or stimulate the growth of each other. Microorganisms are always present in mixed groups. The goal is self-preservation, hence, they try to compete for food. The resulting interaction may be either beneficial or harmful. For example *Saccharomyces* produces alcohol, whereas, film yeasts (*Deabaromyces*) breakdown this alcohol into carbondioxide and water. In yoghurt symbiotic association between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* can be seen. The presence of micrococci in milk stimulates the growth and acid production by lactic acid cultures due to the production of catalase. The bacteriocins (ex: nisin, pediocin) produced by some lactic acid bacteria can inhibit the growth of pathogenic bacteria.

In conclusion, we can say that bacteria multiply by binary fission. The generation time varies in the bacteria. While growing in a medium bacterial cells appear in different phases of growth. These are lag phase, log phase, stationary phase and death phase. This indicates that all bacterial cells are not in the same phase of the growth. As bacterial cells are too small in size they do not have place to store many enzymes. To survive in the nature, bacteria show preference over the use of carbon sources. As a result they take additional time (lag phase) to synthesize enzymes required to assimilate a new carbon source. To know the behavioural information related to the whole bacterial population, synchronous cultures, wherein all cells are in the same growth stage, are required. Bacteria growth in foods is influenced by several internal, external, processing parameters or combination of all these.