MICROBIAL GROWTH IN FOODS - FAQs

1. Explain about various steps in bacterial cell division.

   Before cell division takes place in bacteria, nutrients from surrounding medium or environment are transported into the cells. These nutrients are converted into cellular components and enzymes, etc. The nuclear content is increased which is sufficient to both the cells. Elongation of the cells takes place. The cell contents are distributed evenly into two sets. The cytoplasmic membrane invaginates at the center of the elongated cell. A transverse cell wall is formed, presumably from where mesosomes are present, leading to separation of two cells. Thus one cell becomes two in a very short period. However, in some cases the cells may not get separated and appear as a chain like in the case of streptococci or remain in bunches as in the case of *Staphylococcus.*

2. Explain about the generation time in bacterial population.

   Bacterial cells divide by binary fission. As a result the population doubles and the number increases in geometric proportion. The time required for the cells to divide for the population to become double is known as generation time. The generation time is not the same to all the organisms and not the same for same organism under different conditions. For some bacteria it may be a few minutes and for some others, it may be several hours. It depends on the type and amount of nutritional components and environmental conditions like temperature, pH, amount of oxygen present, the presence of inhibitory compounds, etc.

3. What is a bacterial growth? Indicate its various stages.

   Bacterial growth curve is a graphical representation of various growth phases of a bacterial population. To draw this, an experiment is conducted by inoculating a small number of bacterial cells into a growth medium and the cell number is estimated in terms of viable count, by taking samples from the incubated medium after regular intervals. The viable count in plotted in log numbers against time period. Alternatively, cell count can be estimated by colorimetry or by direct microscopic methods. However, in these methods, dead cells also contribute to the turbidity or dead cells cannot be distinguished from the living cells under normal microscopic observation. A typical bacterial growth curve has four major phases and there are three transitional phases separating these phases. These are lag phase, log phase, stationary phase and death phase. However, if the growth is plotted using the data from turbidometry, death phase cannot be seen. The stationery phase extends for long duration.

4. Explain about the lag phase of a bacterial growth curve.

   Lag phase is an initial growth phase and during this phase there is a visible increase in the cell number. 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, and it is a phase of adjustment to synthesis additional cellular components required for the new cell. However, cells are active and increase in the quantity of proteins, RNA, DNA and other components. The duration of lag phase is not the same for all bacteria and depends on various other factors. Dormant cells may take longer time. If water activity is reduced in a food, duration of this phase is extended. There is a lag in cell division. As all the cells have not completed this phase at same time, there is a gradual increase in cell number by the end of this 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 unfavourable conditions.

5. What is the log phase? Indicate its significance.
   Log phase is also known as exponential growth phase. During this phase, cells divide steadily and at a constant rate, resulting in a straight line in the growth curve, when the data in logarithmic data is 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 use 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 there will be a decline in the multiplication rate and results in a decline in the curve of the graph. This is due to the accumulation of toxic metabolites like acids and change in the environment of the medium. During exponential phase, growth rate is reciprocal of generation time (g), [R= n/t]. Duration of exponential phase may vary depending on the type of bacteria and amount of nutrients and environmental conditions.

6. What do you know about death phase of a bacterial population?

   Death phase is the last phase of the bacterial growth curve. The number of viable cells decreases exponentially in this phase resulting in the graph to move downwards in a straight line. Ex: 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, rate of death is constant and varies from bacteria to bacteria. Depletion of nutrients and accumulation of toxic components like acids, are the main reasons for death phase. Gram negative bacteria die faster than Gram positives, especially those, which can produce endospores.

7. What is known as diauxic growth?

   It is the growth of a bacterial population wherein growth can be seen in two separate phases due to the preferential use of one carbon over the other. This has two exponential growth phases separated by one transient lag phase. This is due to the catabolic repression of induced enzyme synthesis. For example, if the cells growing well in xylose containing medium are added to a medium containing both glucose and xylose as carbon sources and incubated, bacterial cells first use xylose for its 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.

8. What are synchronous cultures? Indicate its significance.

It is known that during the growth of a bacterial population, different bacterial cells have different stages of the growth cycle. Some may be very young, some are well developed and some are 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. The output from this type of study can be considered to that of a single cell. To achieve this, cultures need to be grown synchronously. However, synchronous may last only for a few generations. The synchronous growth of bacteria results in step like growth indicating that all the cells in that population are dividing at the same time.

9. Indicate various methods used to get synchronous cultures.

A population of bacteria can be synchronized by manipulating the physical or chemical conditions. When bacterial cells are inoculated into a fresh medium and incubated at less than optimum temperature for around 10-12 h, bacterial cells 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. The bacterial cell population grown at normal conditions is filtered through a filter which can retain bacteria over it. Later this can be 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. In another method bacterial cells are grown in the medium devoid of thymine. In the absence of it bacterial cells grow but cannot divide as DNA multiplication could not happen in the absence of thymine. As soon as thymine is added all the cells start dividing synchronously. Thus we can achieve synchronous cultures.

10. What is the significance of microbial growth in foods?

Food is rich medium for the growth of microorganisms**.** It contains carbohydrates, proteins, fats, minerals, etc. which can support the growth of all kinds of microorganisms. Some microbes can grow in the foods and produce toxins that can cause health hazards in 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. These microbes may bring enzymatic changes, produce off flavours and change the colour of the food by metabolizing various components present in the foods and make 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 the generation time of microorganisms, especially of bacteria, is as short as few minutes, food may get spoiled rapidly, if it is not protected. However, several factors can influence the growth these microorganisms.

11. Classify the factors that affect the growth of microbes in foods.

Several factors affect the growth of microorganisms and their metabolic activity in foods. These factors can be classified in the following way:

(i) intrinsic factors: Factors pertaining to the food itself (ex: pH, water activity, redox potential, nutrient content, presence of inhibitory compounds and mechanical barriers, etc.)

(ii) extrinsic factors: Factors associated to the environment in which the food is stored (ex: relative humidity, temperature and gas composition, etc.)

(iii) implicit factors: 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)

(iv) processing factors: Factors related to the processing of the foods (freezing, cooling, drying, heating, etc.)

(v) interaction among the factors as indicated above. It is a complex system the combined effects may either promote or decrease the growth of microorganisms.

12. What is known as water activity? How it can influence the growth of bacteria in foods?

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 for the microorganisms, it extends the lag phase by several hours. Hence, foods can be preserved for longer period if the water activity is reduced by adding sugars, salt or drying.

13. Classify bacteria based on temperature and oxygen requirement for its growth.

Temperature plays a major role in the growth of microorganisms. Based on the temperature requirement for their growth, bacteria are divided into three groups: psychrophilic bacteria (12-15 °C), mesophilic bacteria (30-40 °C) and thermophilic bacteria (50-70 °C).

Based on oxygen requirement bacteria can be grouped into 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, however, growth is better in presence of oxygen, ex: *Staphylococcus, E. coli*), microaerophilic (requires less concentration of oxygen, ex: lactic acid bacteria) and aerotolerant anaerobes (do not require oxygen, but can tolerate oxygen at certain concentration, ex: *Enterococcus*). Anaerobic microorganisms do not produce enzymes

like superoxide dismutase, catalase, peroxidase, etc., to inactivate the free radicals generated by oxygen, like superoxide, singlet oxygen, hydroxyl and peroxide anion, hence cannot survive in the presence of oxygen.

14. Provide some examples to beneficial association among different bacterial cultures.

In yoghurt symbiotic association between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* can be seen. Amino acids (Glutamic acid & Proline) released by *Lb. bulgaricus* enhances growth of *Str. thermophilus* and the acid production by *Lb. bulgaricus* is enhanced by formate and CO_2 released by *Str. thermophilus*. The presence of micrococci in milk stimulates the growth and acid production by lactic acid cultures due to the production of catalase.