Factors influences on growth

Temperature:

The optimum temperature for microorganisms varies widely; some have optima between 5 and 10 °C, whereas at the other extreme, some have optima at 80 °C or above. Microorganisms can be divided into four groups on the basis of their temperature optima:

- **Psychrophiles**, which have low optima, e.g. ***Flavobacterium***, optimum **13 °C**
- **Mesophiles,** which have mid-range optima, e.g. ***Escherichia coli***, optimum **39 °C**
- **Thermophiles**, with high optima, e.g. ***Bacillus stearothermophilus***, optimum **60 °C**
- **Hyperthermophiles**, with very high optima, e.g. ***Thermococcus celer***, optimum **88 °C.**

Oxygen:

Microorganisms also vary in their requirements for **oxygen**. Some grow only in the **presence of oxygen**, whereas others grow only in the **absence of oxygen**.

To the latter group of microorganisms, oxygen is actually toxic, probably because they are unable to remove toxic products of oxygen metabolism, such as **hydrogen peroxide**. On the basis of their requirements for oxygen, microorganisms can be separated into several groups, as outlined below:

- **Obligate aerobes**, which will grow only in the presence of oxygen,

e.g. ***Micrococcus luteus***

- **Facultative aerobes**, which can grow in the absence of oxygen, but grow better if oxygen is supplied, e.g. ***Escherichia coli***

- **Microaerophilic aerobes**, which require oxygen at lower concentrations than atmospheric, e.g. ***Spirillum volutans***
- **Obligate anaerobes**, which will grow only in the absence of oxygen, e.g. ***Desulphovibrio***.

In small-scale culture, such as on agar in Petri dishes or in universal containers of broth media, oxygen diffuses directly from the air to the microorganisms. However, if aerobic microorganisms are grown on a larger scale, such as in a laboratory fermenter, it is necessary to aerate the culture, usually by bubbling sterile air through the medium. This supplies the microorganisms with oxygen, where otherwise , The rate of diffusion and poor solubility of oxygen in water would mean that insufficient oxygen was available. If it is necessary to culture obligate anaerobes, for example in a hospital laboratory, they are grown on agar plates placed in a special container referred to as an anaerobic jar, a container which can be filled with a mixture of hydrogen and carbon dioxide to replace the air. The jar contains a catalyst which will remove any residual oxygen, to ensure anaerobic conditions.

We have seen that microorganisms vary widely in their tolerance to environmental **temperatures** and **availability of oxygen**; they also have a wide range of environmental **pH** values at which they can grow. Most microorganisms have a pH **optimum** between 5 and 9, but a few species can grow at pH values outside this range. In general, fungi tend to be more tolerant of acid conditions than bacteria, with optima at pH 5 or below. These pH values refer to the extracellular environment, and although this may vary widely, the intracellular pH remains nearly neutral. The pH of culture media is kept relatively constant by the use of buffer solutions, such as phosphate buffers. During the growth of a microorganism, the pH of the medium may change due to the production of acidic or alkaline products of metabolism and this may be regulated by the addition of appropriate sterile buffer solutions during the growth phase.

Figure: An anaerobic jar for the incubation of cultures under anaerobic conditions. Air in the jar is replaced with an oxygen-free gas mixture, such as hydrogen and carbon dioxide, or a chemical catalyst is placed in the jar which removes oxygen from the atmosphere

Growth of cultures

Under favorable conditions, the number of single-celled microorganisms will double at regular intervals. This is because each of the two daughter cells produced will have the same potential for growth as the original parental cell. **The time required for the number of cells to double is known as the mean doubling time**. Table : 3 shows how the number of cells will increase, starting with a single cell, assuming a doubling time of 20 minutes. The table shows both the arithmetic number of cells, and the number expressed as a logarithm to the base 10.

Table : Increases in the numbers of bacterial cells with a doubling time of 20 minutes

Time /minutes	Number of divisions	Number of cells	Log_{10} number of cells
0	0		
20	1	2	0.3
40	2	4	0.62
60	3	8	0.9
80	4	16	1.2
100	5	32	1.5
120	6	64	1.8
140	7	128	2.1
160	8	256	2.4
180	9	512	2.7
200	10	1024	3.0

- Calculation the number of microbial cells after n generations (Generation time : The time taken for a bacterial population to double in number).

If the original inoculums is 10000(10^4) cell / cm^3 or ml

After 1 generation : $10000 \times 2 = 10000 \times 2^1 = 20000/cm^3$

After 2 generation : $10000 \times 2 \times 2 = 10000 \times 2^2 = 40000/cm^3$

After 3 generation : $10000 \times 2 \times 2 \times 2 = 10000 \times 2^3 = 80000/cm^3$

After n generation : $10000 \times 2 \times 2 \times 2 \times \ldots = 10000 \times 2^n$ cell/cm^3

Therefore = $\mathbf{N = No \times 2^n}$

Where **No** = original number of cells

N = number of cells after **n** generations .

The growth rate is sometimes expressed as the time taken for the population to double, or the mean doubling time

So far, we have considered cells only in the exponential phase of growth. Cell growth, with a limited supply of nutrients, does not continue indefinitely. **The growth of the population is normally limited** by either the exhaustion of one or more essential nutrients, or by the accumulation of toxic by-products of metabolism. Figure : 3 shows a complete growth curve for a microorganism. Four distinct phases of growth can be seen:

- **Lag phase**
- **Exponential (or logarithmic) phase**
- **Stationary phase**
- **Death phase.**

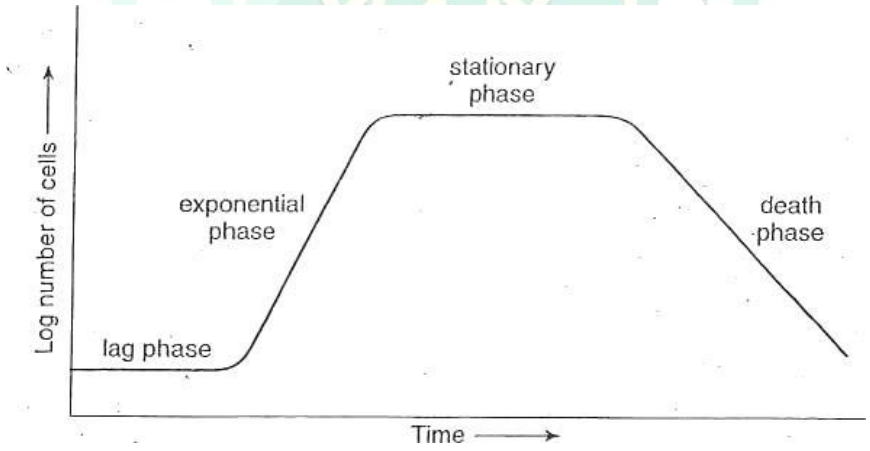

Figure:3 Typical growth curve for a bacterial culture

When fresh, sterile medium is inoculated with a culture of a microorganism, growth may begin immediately. There is a period of time in which the cells are synthesizing the enzymes required for the metabolism of nutrients present in the medium. This period of time is referred to as the **lag phase**, and can be seen as a period of adjustment to the culture conditions. A lag phase does not always occur. If, for example, cells which were already in the exponential phase were transferred to fresh, identical medium, exponential growth would continue at the same rate.

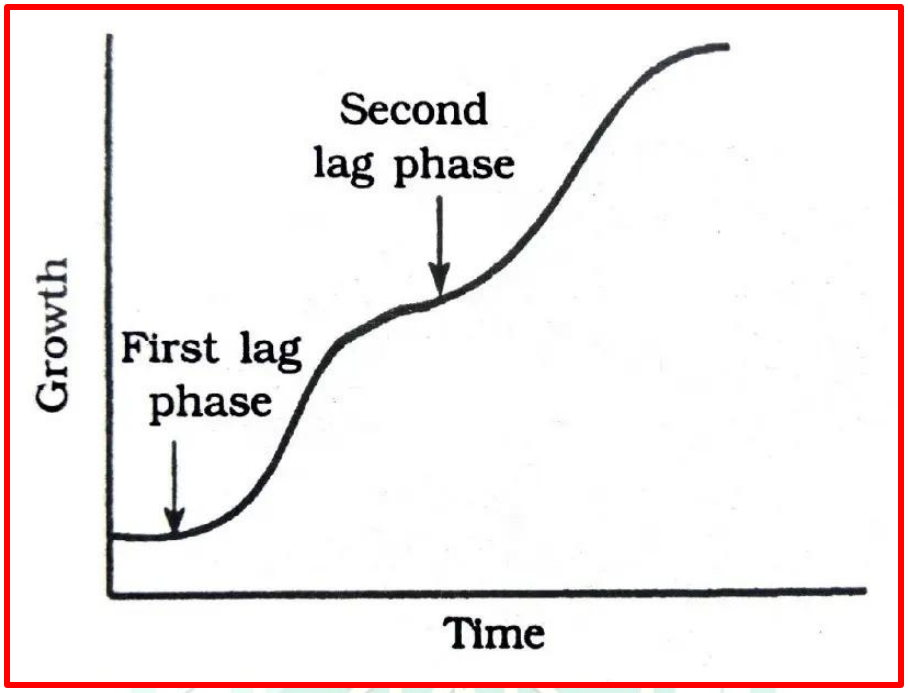

Figure : Diauxic growth of *E. coli* in a medium containing glucose and lactose

We have already described **the exponential phase** as a period of constant growth in the size of the microbial population, in which both cell numbers and cell mass increase in parallel. The growth rate constant is affected by both genetic and environmental factors; it varies from one species to another, and is influenced by such factors as the concentration of nutrients in the growth medium, temperature and pH. The exponential phase is followed by the **stationary phase**, in which the overall growth rate is zero. During this phase, slow growth of some cells may occur, which is balanced by the death of others, so that the total number of viable cells remains constant. This phase is followed by the death phase in which the number of viable cells progressively decreases. **Death of cells** may be accompanied by cell lysis so that both the total cell number and the viable cell count decreases.

Diauxic growth is sometimes observed if a microorganism is grown in a medium containing two different carbon sources. **Diauxic growth** is characterized by two distinct phases of exponential growth, separated by a brief lag phase. For

example, if *Escherichia coli* is grown in a medium containing both glucose and lactose, the glucose will be metabolized first. Glucose actually inhibits the synthesis of lactase (β-galactosidase) (referred to as catabolite repression) and only after the glucose has been used up will lactase be synthesized. Growth then resumes using lactose as an energy source.