RAMA UNIVERSITY

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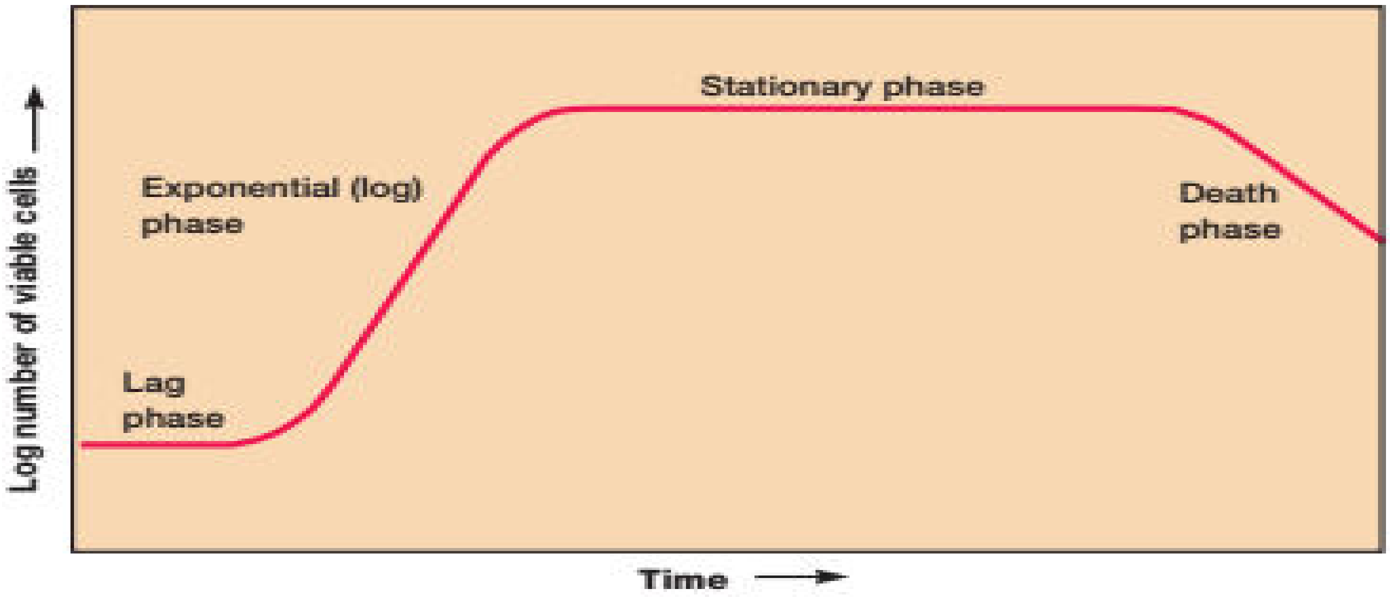
FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Microbial Growth kinetics

The Bacterial growth curve
In closed system or batch culture, the microbial growth curve is typical S-shaped or has sigmoidal nature.



Four characteristic phases of the growth cycle are recognized.

1. **Lag Phase**.

Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2. **Exponential (log) Phase**.

The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression.

The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation.

The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time is derived.

Exponential growth is **balanced growth.**
That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, **unbalanced growth** results

3. **Stationary Phase**.

Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors:

1. exhaustion of available nutrients;
2. accumulation of inhibitory metabolites or end products;
3. exhaustion of space, in this case called a lack of "biological space".

This **stationary phase** usually is attained by bacteria at a population level of around 109 cells per ml.

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence.

Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4. **Death Phase**.

If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.).

During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

The death of a microbial population, like its growth during the exponential phase, is usually logarithmic (that is, a constant proportion of cells dies every hour).

Generation time: The **time** taken by the bacteria to double in number during a specified **time** period is known as the **generation time**. The **generation time** tends to vary with different organisms.

E.coli divides in every 20 minutes, hence its **generation time** is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes.

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on.

The **generation time** is the time interval required for the cells (or population) to divide.

G (generation time) = t (time, in minutes or hours) / n (number of generations)

Example: Suppose that a culture tube is inoculated with one cell that divides every 20 minutes. The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2n where n is the number of generations. The resulting population increase is exponential or logarithmic.

These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

Then inspection of the results in table 6.1 will show that

$$N_t = N_0 \times 2^n.$$

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant *(k)*.** This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (g), can now be calculated. If the population doubles ($t = g$), then

$$N_t = 2\,N_0.$$

Substitute $2N_0$ into the mean growth rate equation and solve for k.

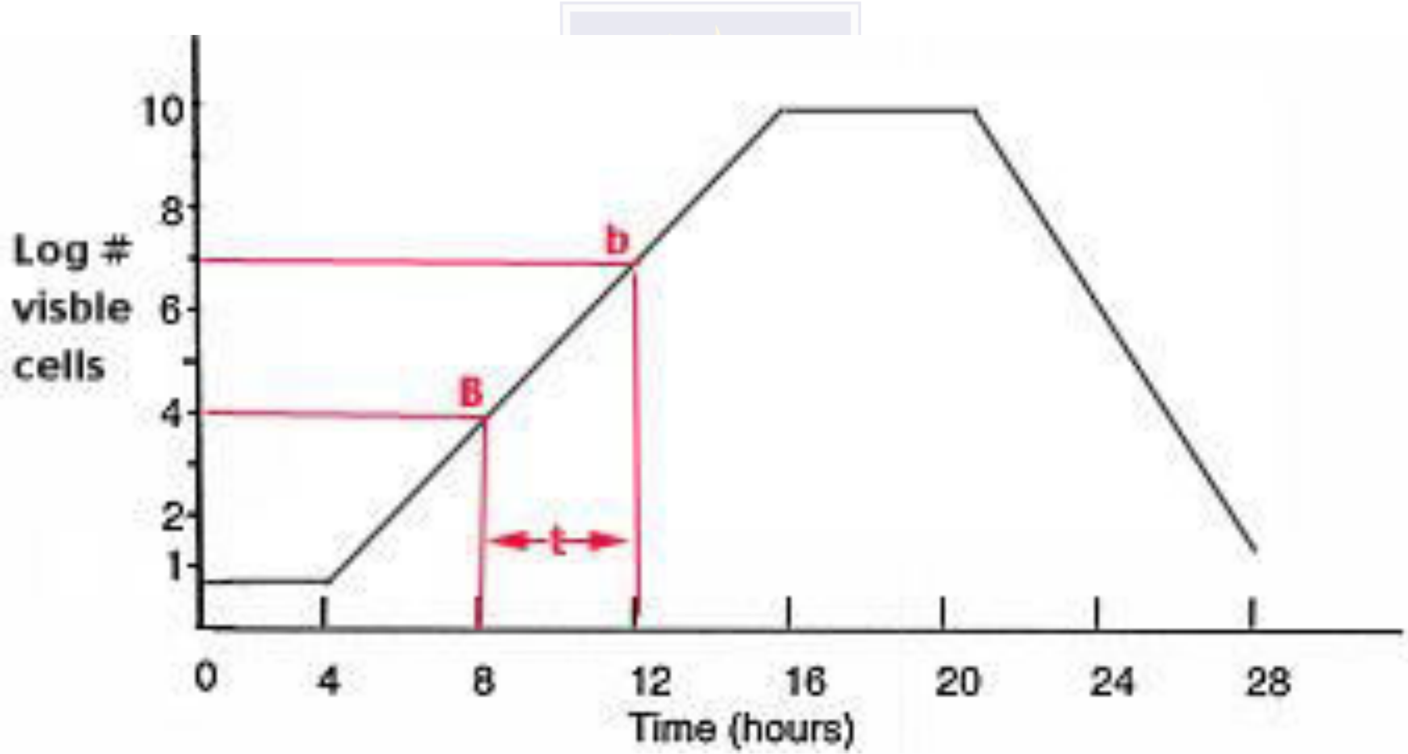
$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

Example: What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?



Arithmetic growth
Arithmetic growth refers to the situation where a microbial population increases by a constant number of persons (or other objects) in each period being analysed.

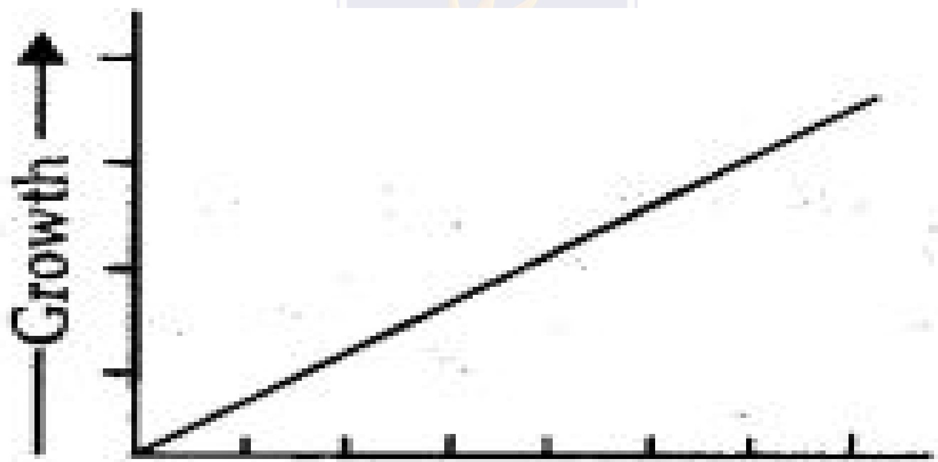


Fig.: Arithmetic growth curve

Geometric growth

Geometric growth refers to the situation where successive changes in a population differ by a constant ratio (as distinct from a constant amount for arithmetic change).

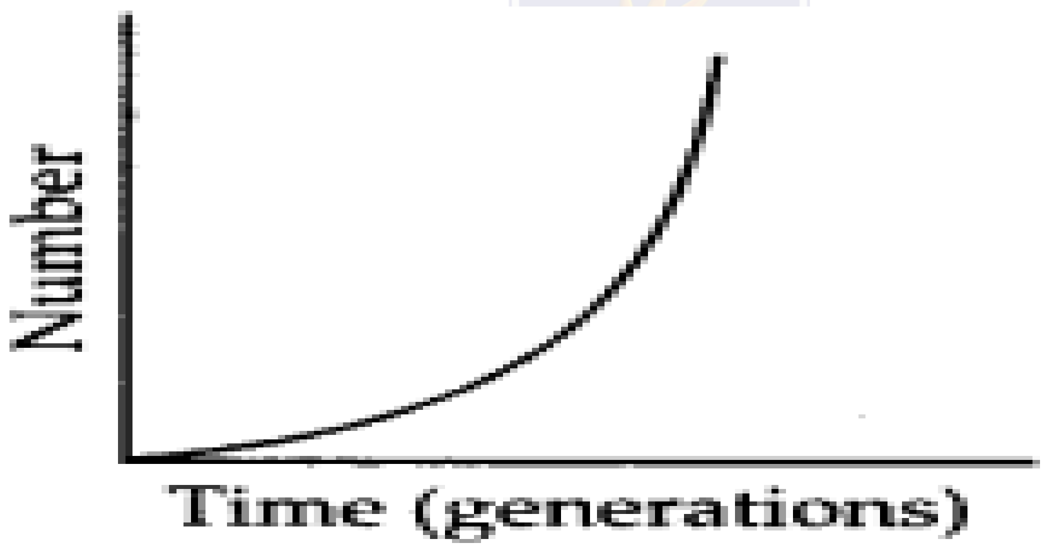


Fig.: Geometric growth curve

Exponential or Logarithmic Growth:

As growth of bacteria takes place through binary fission, a single bacterium (1) grows as 1,2,4,8,16 and so on, which can also be expressed as 1×2^0, 1×2^1, 1×2^2, 1×2^3, 1×2^4,……………….. 1×2^n respectively. This type of growth, in which the number of cells doubles during each unit time (generation time), is called 'exponential growth' or 'logarithmic growth'. Logarithmic growth is much faster than arithmetic growth (1, 2, 3, 4, 5, 6, 7….) or geometric growth (1, 2, 4, 8, 16, 32……).

Though, apparently it follows geometric growth, after few generations it grows as 1, 10, 100, 1000, 10000………. (10^0, 10^1, 10^2, 10^3, 10^4……..) Whose logarithmic values are 0, 1, 2, 3, 4……..respectively?