

Bacterial Growth:

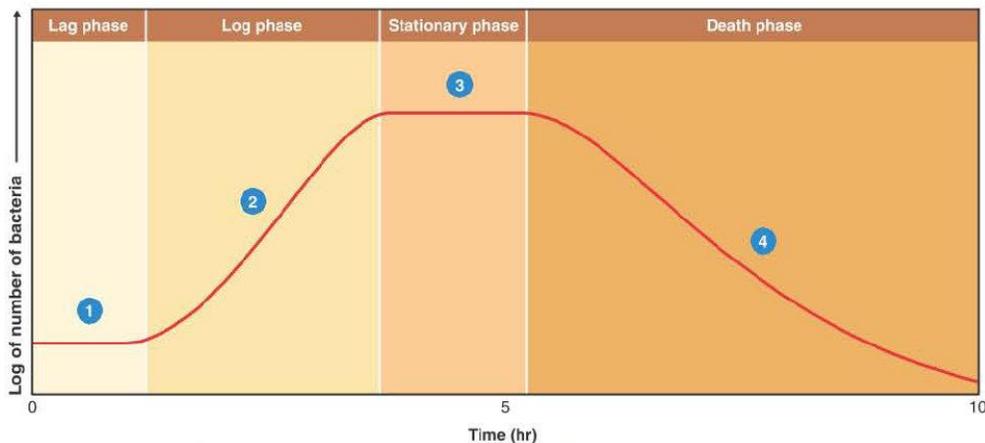
Bacterial Growth:

When we talk about microbial growth we are really referring to the *number* of cells, not the *size* of the cells. Microbes that are "growing" are increasing in number, accumulating into *colonies* (groups of cells large enough to be seen without a microscope) of hundreds of thousands of cells or *populations* of billions of cells. Although individual cells approximately double in size during their lifetime, this change is not very significant compared with the size increases observed during the lifetime of plants and animals.

Therefore Bacterial Growth refers to increase of the size of a bacterial population (*i.e.* Increase of the number of bacterial cells) over time.

Phases of Growth

When a few bacteria are inoculated into a liquid growth medium and cultivated in broth, they usually are grown in a batch culture; that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. It is possible to plot a bacterial growth curve that shows the growth of cells over time. Population growth of microbes reproducing by binary fission in a batch culture can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases: the lag, log, stationary, and death phases.



- 1 Intense activity preparing for population growth, but no increase in population.
- 2 Logarithmic, or exponential, increase in population.
- 3 Period of equilibrium; microbial deaths balance production of new cells.
- 4 Population is decreasing at a logarithmic rate.

Key Concept

Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.

The Lag Phase:

- When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs. This period is called the lag phase.
- It is not a time of inactivity; rather cells are synthesizing new components like ATP, essential co-factors, Ribosomes, enzymes requires to grow on a new media.
- Eventually however, the cells begin to replicate their DNA, increase in mass, and divide. As a result, the number of cells in the population begins to increase.

Exponential Phase

- During the exponential (log) phase, microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the environmental conditions. Their rate of growth is constant during the exponential phase; that is, they are completing the cell cycle and doubling in number at regular intervals.
- Therefore the cellular reproduction is most active during this period, and generation time reaches a constant minimum.
- Because the generation time is constant, a logarithmic plot of growth during the log phase is a straight line.
- The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies.
- The growth rate during log phase depends on several factors, including nutrient availability. When microbial growth is limited by the low concentration of a required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present.
- The rate of growth also increases with nutrient concentration but in a hyperbolic manner much like that seen with many enzymes. The shape of the curve is thought to reflect the rate of nutrient uptake by microbial transport proteins. At sufficiently high nutrient levels, the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration.

Stationary Phase

- In a closed system such as a batch culture, population growth eventually ceases and the growth curve becomes horizontal. This stationary phase is attained by most bacteria at a population level of around 10^9 cells per milliliter. Protist cultures often have maximum concentrations of about 10^6 cells per milliliter.
- Final population size depends on nutrient availability and other factors, as well as on the type of microorganism.
- In stationary phase, the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease to divide but remain metabolically active.
- The reasons microorganisms enter the stationary phase are as follows:
 - (i) Nutrient limitation; if an essential nutrient is severely depleted, population growth will slow and eventually stop. Microbes enter the stationary phase for other reasons besides nutrient limitation.

- (ii) Limitation of availability of O₂; Aerobic organisms often are limited by O₂ availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O₂ concentration adequate for growth.
- (iii) Accumulation of toxic waste products; Population growth also may cease due to the accumulation of toxic waste products. This seems to limit the growth of many cultures growing in the absence of O₂.
- (iv) Harmful changes in pH, all may play role.

For example, *Streptococci* can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited. Finally, some evidence exists that growth may cease when a critical population level is reached. Thus entrance into the stationary phase may result from several factors operating in concert.

Senescence and Death

- Cells growing in batch culture cannot remain in stationary phase indefinitely. Eventually they enter a phase that for many years was described simply as the "death phase".
- During this phase, the number of viable cells often declines exponentially, with cells dying at a constant rate.
- It was assumed that detrimental environmental changes such as nutrient deprivation and the buildup of toxic wastes caused irreparable harm to the cells. That is, even when bacterial cells were transferred to fresh medium, no cellular growth was observed. Because loss of viability was often not accompanied by a loss in total cell number, it was assumed that cells died but did not lyse.
- Some microbiologists think the cells are only temporarily unable to grow, at least under the laboratory conditions used. This phenomenon, in which the cells are called viable but non-culturable (VBNC), is thought to be the result of a genetic response triggered in starving, stationary phase cells. Once the appropriate conditions are available (e.g., a change in temperature or passage through an animal), VBNC microbes resume growth. (*VBNC microorganisms could pose a public health threat, as many assays that test for food and drinking water safety are culture based.*)
- The second alternative theory to a simple death phase is programmed cell death. In contrast to the VBNC hypothesis whereby cells are genetically programmed to survive, programmed cell death predicts that a fraction of the microbial population is genetically programmed to die after growth ceases. In this case, some cells die and the nutrients they leak enable the eventual growth of those cells in the population that did not initiate cell death.
- Experiments reveal that some microbes have a very gradual decline in the number of culturable cells. This decline can last months to years. During this time, the bacterial population continually evolves so that actively reproducing cells are those best able to use the nutrients released by their dying brethren and best able to tolerate the accumulated toxins. This dynamic process is marked by successive waves of genetically distinct variants. Thus natural selection can be witnessed within a single culture vessel.