

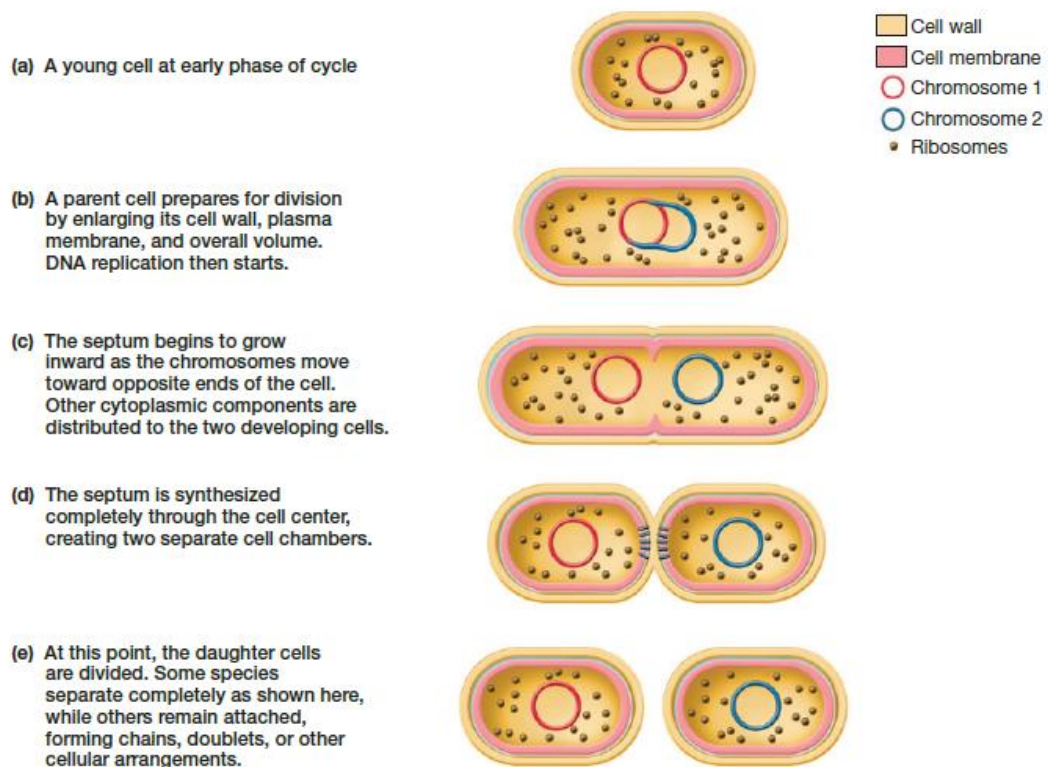
Growth of bacterial cultures:

Growth is defined as an increase in cellular constituents which leads to a rise in cell number. As we are aware, microorganisms reproduce by binary fission or by budding. In order to study growth, normally one follows the changes in the total population number. The cells copy their DNA almost continuously and divide again and again by the process called **binary fission**. **Binary fission** which has been described earlier as the form of asexual reproduction in single-celled organisms by which one cell divides into two cells of the same size. Fortunately, few prokaryotic populations can sustain exponential growth for long. Environments are usually limiting in resources such as food and space. Prokaryotes also produce metabolic waste products that may eventually pollute the colony's environment. Still, you can understand why certain bacteria can make you sick so soon after infection or why food can spoil so rapidly. Refrigeration retards food spoilage not because the cold kills the bacteria on food but because most microorganisms reproduce very slowly at such low temperatures.

Binary fission:

In the process of binary fission, the cell elongates and the DNA is replicated. Cell wall and plasma membrane begin to grow inward and cross-wall forms completely around the divided DNA. At the end the cell separates into two individual cells similar to the parent cell and contains all the contents a cell requires for its living including DNA

Few bacterial species reproduce by budding. In this method, the cell forms a small initial growth, it enlarges and then it separates from the parent cell. Some filamentous bacteria like actinomycetes reproduce by producing chains of conidiospores and a few bacteria simply fragment.



Growth Curve:

The increase in cell number or growth in population is studied by analyzing the growth curve of a microbial culture. Bacteria can be grown or cultivated in a liquid medium in a closed system or also called as batch culture. In this method, no fresh medium is added and hence with time, nutrient concentration decreases and an increase in wastes is seen. As bacteria reproduce by binary fission, the growth can be plotted as the logarithm of the number of viable cells versus the time of incubation. The curve plotted shows four basic phases of growth; the lag, log, stationary, and death phase

Lag Phase: As the cells are introduced into the new medium, no immediate increase in cell number occurs. During this phase, the cells are undergoing a period of intense metabolic activity involving synthesis of enzymes and various other molecules required to divide in the coming phase. This phase can vary considerably in length depending on the nature of the medium and the microorganism. The medium may be different from the one the microorganism was growing in previously. The cells may be old and depleted of ATP, essential cofactors and ribosomes; these must be synthesized before growth can begin. So, the microorganism requires time to recover and young, vigorously growing cultures and fresh medium are to be used for the lag phase to be short.

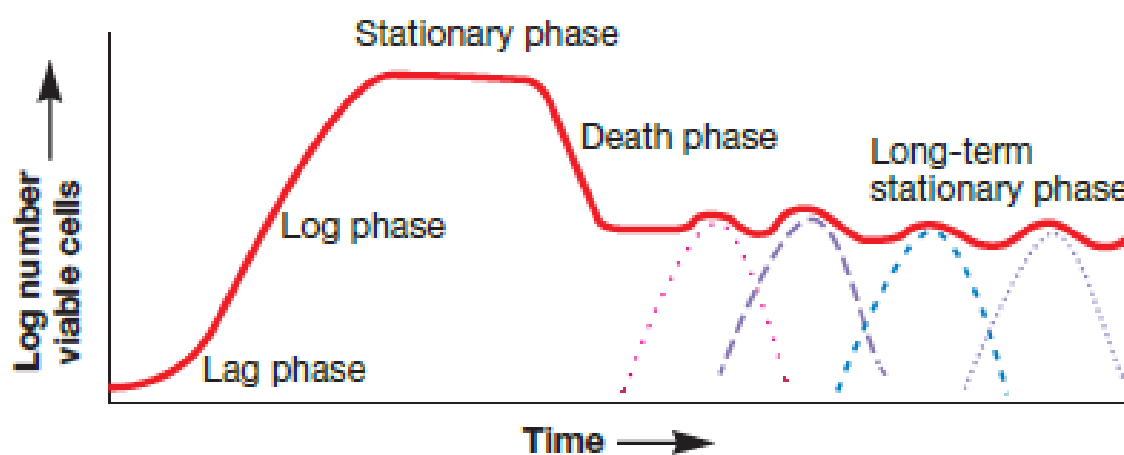


Figure 7.29 Microbial Growth Curve in a Closed System. The five phases of the growth curve are identified. The dotted lines shown during the long-term stationary phase represent successive waves of genetic variants that evolve during this phase of the growth curve.

Log Phase: In this phase the cell starts dividing in a logarithmic way and this is also called as exponential phase and the growth is balanced. Cellular reproduction is high during this period and the plot during this phase is a straight line. The cells are most active metabolically during this phase and the population is most uniform during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies. But during this phase, the microorganisms may be particularly sensitive to adverse conditions. On the whole, in this phase the cells are growing and

dividing and increasing in cell number. 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 (n = number of generations). Hence, $G=t/n$ is the equation from which calculations of generation time can be derived.

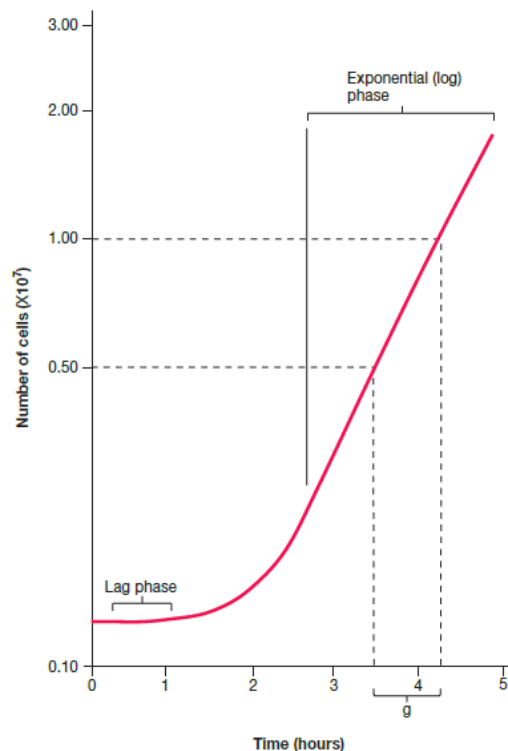


Figure 7.33 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

Table 7.9 Examples of Generation Times ¹		
Microorganism	Incubation Temperature (°C)	Generation Time (Hours)
Bacteria		
<i>Escherichia coli</i>	40	0.35
<i>Bacillus subtilis</i>	40	0.43
<i>Staphylococcus aureus</i>	37	0.47
<i>Pseudomonas aeruginosa</i>	37	0.58
<i>Clostridium botulinum</i>	37	0.58
<i>Mycobacterium tuberculosis</i>	37	~12
<i>Treponema pallidum</i>	37	33
Protists		
<i>Tetrahymena geleii</i>	24	2.2–4.2
<i>Chlorella pyrenoidosa</i>	25	7.75
<i>Paramecium caudatum</i>	26	10.4
<i>Euglena gracilis</i>	25	10.9
<i>Giardia lamblia</i>	37	18
<i>Ceratium tripos</i>	20	82.8
Fungi		
<i>Saccharomyces cerevisiae</i>	30	2
<i>Monilia fructicola</i>	25	30

Exponential phase or log phase 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. The individual cells may take slightly longer than others to go from lag phase to the log phase, and they do not all divided precisely together. If they divided together and the generation time is same, the number of cells in a culture would increase in a stair – step pattern, exactly doubling every 20 min or a particular time – a hypothetical situation called Synchronous growth. In an actual culture, each cell divides sometime during the 20 min generation time, with about 1/20 cells dividing each minute – a natural situation called nonsynchronous growth or asynchronous growth which appears as a smooth line, not as steps.

Organisms in a tube of culture medium can maintain logarithmic growth for only a limited time. As the number of organisms' increases, nutrients are used up, metabolic wastes accumulate, living space may become limiting factor and aerobes suffer from oxygen depletion

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". 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. Starving bacteria frequently produce a variety of starvation proteins, which make the cell much more resistant to damage. They increase peptidoglycan cross-linking and cell wall strength. The Dps (DNA-binding proteins from starved cells) protein protects the DNA. Bacterial pathogens like *Salmonella typhimurium* become more virulent when starved.

Death Phase: Due to the conditions during the stationary phase, the death phase is seen as there is a decline in the number of viable cells. This phase also is like the log phase where the cell number is declining in a logarithmic way. The cell is said to be dead if it does not revive itself and reproduce when incubated again in a fresh medium. In this phase, the number of live cells decreases at a logarithmic rate, as indicated by the straight downward sloping diagonal line. The duration of this phase is as highly variable as the duration of log phase. Both depend primarily on the genetic characteristics of the organism.

Mathematics of Growth:

Microbial growth during the exponential phase is very important and of interest to microbiologists and the analysis applies to microorganisms dividing by binary fission. The time required by a cell to divide is called the generation time or doubling time. In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or $2^0, 2^1, 2^2, 2^3, \dots, 2^n$ (where n = the number of generations). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature. This might vary from organism to organism depending upon the environmental conditions etc. For example in *E.coli* the generation time is 20 min and hence after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. The population is doubling every generation; hence the increase in population is always 2^n where n is the number of generations. The resulting population increase is exponential or logarithmic.

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.

Measurement of Microbial Growth:

A number of techniques are available in order to measure growth of microbial populations. Either population number or mass may be calculated and growth leads to increase in both.

Direct measurement of cell numbers:

Bacteria or microorganisms can be counted directly on the plate and also called as plate counting. Advantage of this method is that it measures the number of viable cells. Disadvantage is that, it is time consuming and expensive as one needs media and other conditions need to be maintained. Bacteria counted on plate counts are referred to as colony forming units as a single cell or a clump of

bacterial cells can lead to a colony which contains many cells. The colonies when they are counted in plate count method are to be present sparsely for accurate counting as overcrowding can lead to incorrect counting. To solve this, one has to adapt the serial dilution method in order to get an accurate count.

Serial dilution and pour and spread plate: Supposing one has to accurately count the number of cells given in a solution, then serial dilution needs to be performed. A 1ml of the sample is taken and transferred to a tube containing 9ml of sterile water and this process can be repeated until we reach a considerable dilution (say 10^6 to 10^7). Once the original inoculum is diluted one needs to perform a pour plate or a spread plate technique in order to count the number of bacteria present in the diluted sample and then the original sample. In pour plate method the diluted sample is poured into the petriplate and then the medium which is at nearly 50°C is poured over the inoculum and mixed by gentle agitation. With this method, colonies grow within the nutrient agar as well as on the surface of the agar plate. As certain disadvantages are encountered in this method like heat sensitive microorganisms might not grow and also bacteria when they grow within the nutrient medium might not be useful for diagnostic purposes. In order to avoid these problems, spread plate method is mostly used (Fig. 3). A 0.1ml of the diluted sample is added to the surface of the nutrient medium and spread uniformly with the help of a glass spreader and after incubation, the colonies can be counted and the concentration of the bacterial cells in the original sample is calculated as follows:

Number of bacteria/ml = Number of colonies on plate x reciprocal of dilution of sample

Membrane Filtration: This method can be used in order to study if the quantity of the bacteria is very small as in aquatic samples like lakes, streams etc. Membranes with different pore sizes are used to trap different microorganisms. The sample is drawn through these special membrane filters and placed on an agar medium or on a pad soaked with liquid media. After incubation, the number of colonies can be counted and the number determined in the original sample. Selective media or differential media can be used for specific microorganisms. This is mostly used for analyzing aquatic samples.

Microscopic count: The Petroff-Hausser counting chamber or slide is easy, inexpensive and relatively quick method and also gives information about the size and morphology of the microorganisms. These specially designed slides have chamber of known depth with an etched grid on the chamber bottom.

$\text{Bacteria/mm}^3 = (\text{bacteria/square}) (25 \text{ squares}) / (50)$

Bacteria can be counted by taking into account the chamber's volume and any sample dilution. The disadvantage encountered in this method is that fairly large volume is required and also it is difficult to distinguish between living and dead cells. Microorganisms of larger sizes can be counted by using electronic counters such as coulter counter; where in the number of cells in a measured volume of liquid is counted. This method gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells.

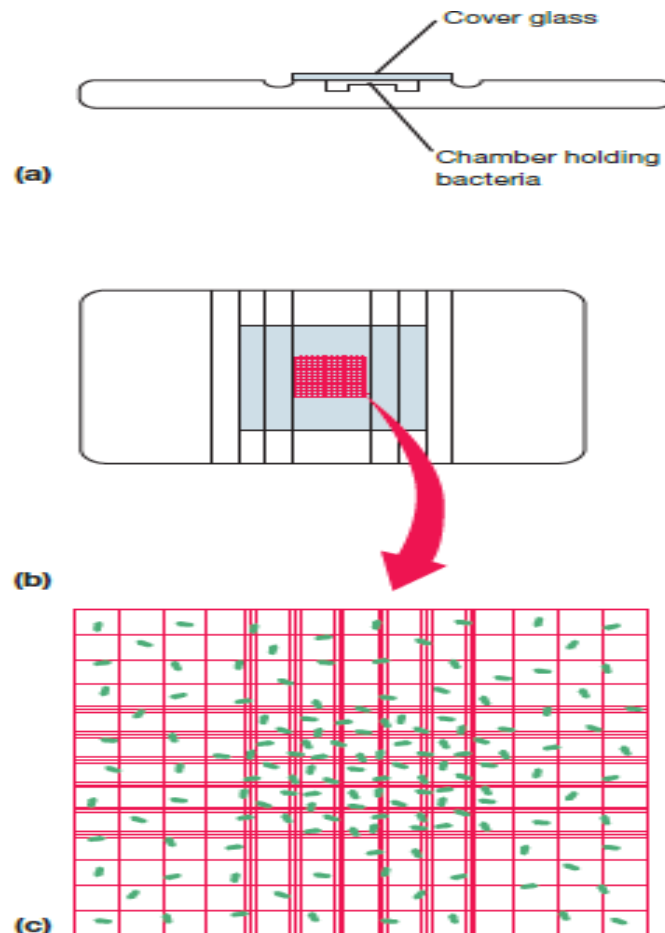


Figure 7.34 The Petroff-Hausser Counting Chamber. (a) Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension. (b) A top view of the chamber. The grid is located in the center of the slide. (c) An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at $\times 400$ to $\times 500$ magnification. The average number of bacteria in these squares is used to calculate the concentration of cells in the original sample.

Indirect methods of measurement of cell mass:

Population growth leads to increase in the total cell mass, as well as in cell numbers. The following methods can be used.

Turbidity: As bacteria grow/multiply in a liquid medium, the medium becomes turbid (Fig. 5). Spectrophotometer is used in order to measure the turbidity. A beam of light is transmitted through a bacterial suspension to a light-sensitive detector. The fact that microbial cells scatter light striking them, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. The extent of light scattering can be measured and is almost linearly related to bacterial concentration at low absorbance levels.

Dry weight: This method is mostly used for filamentous bacteria and moulds. The microorganism is grown in liquid medium, filtered or centrifuged to remove extraneous material, and dried in an oven and then weighed. It is time consuming and hence not very sensitive.

Continuous culture of Microorganisms:

Batch cultures : Nutrient supplies are not renewed nor wastes removed.

Continuous cultures : continuous provision of nutrients and removal of wastes takes place. The population can be maintained in the exponential phase and at a constant biomass concentration for extended periods.

These can again be categorized into two types:

Chemostat : Where sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed.

Turbidostat : It has a photocell that measures the absorbance or turbidity of the cell culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density.

Used in food and industrial microbiology.

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