MICROBIAL GROWTH

Class – M.Sc. Biotechnology Code- MDSE(BT)-404B (Medical Biotechnology)

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Microbial growth can result in cell enlargement eventually leading to cell division.

Under certain conditions growth can occur without cell division, for example, when cells are synthesizing storage compounds, e.g. glycogen or poly β -hydroxybutyrate.

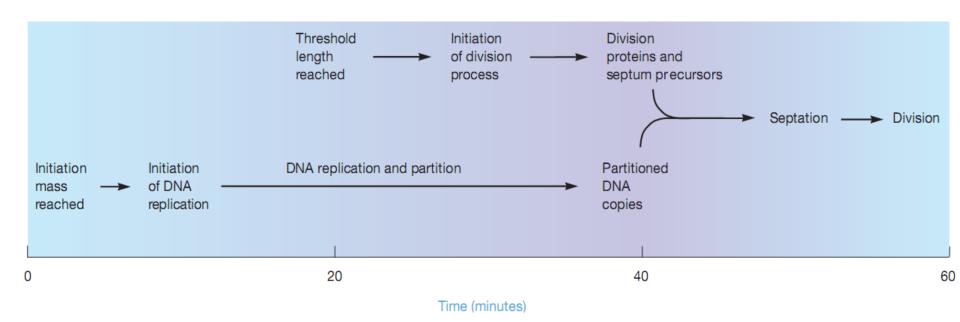
In this situation the cell numbers remain constant, but the concentration of biomass continues to increase.

This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells. Their growth results only in increased size.

Growth may be defined as an increase in cellular constituents. It may refer to an increase in cell volume or cell number.

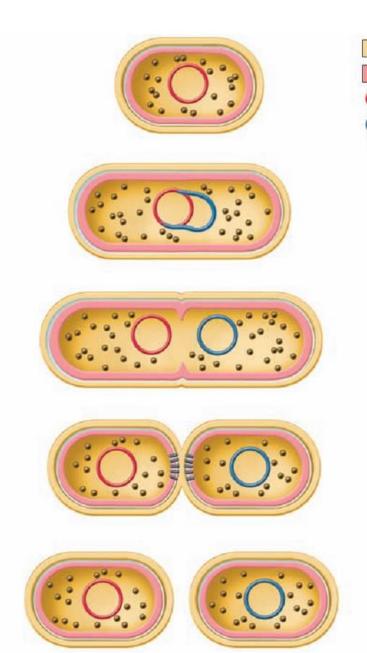
Most prokryotes (bacteria) reproduce by binary fission.

Cell Division: is the process by which a parent cell divides into two or more daughter cells.



(a) A young cell at early phase of cycle

- (b) A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume.
- (c) The septum begins to grow inward as the chromosomes move toward opposite ends of the cell. Other cytoplasmic components are distributed to the two developing cells.
- (d) The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.
- (e) At this point, the daughter cells are divided. Some species separate completely as shown here, while others remain attached, forming chains, doublets, or other cellular arrangements.



Cell wall

Cell membrane

Chromosome 1
Chromosome 2
Ribosomes

In prokaryotes, cell division or cytokinesis involves several steps:

- (1) selection of the site where the septum will be formed;
- (2) assembly of a specialized structure, the Z ring, which divides the cell in two by constriction;
- (3) linkage of the Z ring to the plasmamembrane and perhaps components of the cell wall;
- (4) assembly of the cell wallsynthesizing machinery; and
- (5) constriction of the Z ring and septum formation.

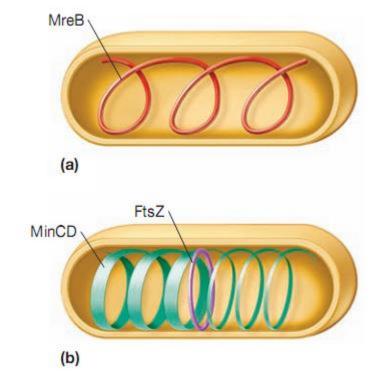


Figure 6.4 Cytoskeletal Proteins Involved in Cytokinesis in Rod-Shaped Bacteria. (a) The actin homolog MreB forms spiral filaments around the inside of the cell that help determine cell shape and may serve to move chromosomes to opposite cell poles. (b) The tubulin-like protein FtsZ assembles in the center of the cell to form a Z ring, which is essential for septation. MinCD, together with other Min proteins, oscillates from pole to pole, thereby preventing the formation of an off-center Z ring.

One model holds that the FtsZ filaments are shortened by losing FtsZ subunits (*i.e.*, depolymerization) at sites where the Z ring is anchored to the plasma membrane.

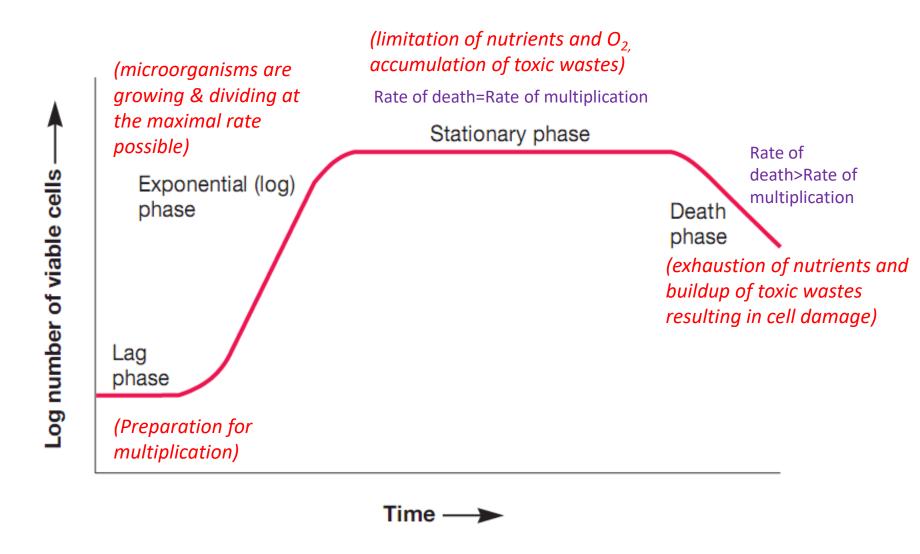


Figure 6.6 Microbial Growth Curve in a <u>Closed</u> System.

(Or batch culture)

Lag Phase:

No increase in cell number Synthesis of ATP, essential cofactors, ribosomes, new enzymes, etc.

It varies considerably in length with the *condition of the microorganisms* and the *nature of the medium*.

For example, it may be quite long if the inoculum is from an old culture, if medium is chemically different.

On the other hand, the lag phase will be short or absent if a young, exponential phase culture is transferred to fresh medium of the same composition.

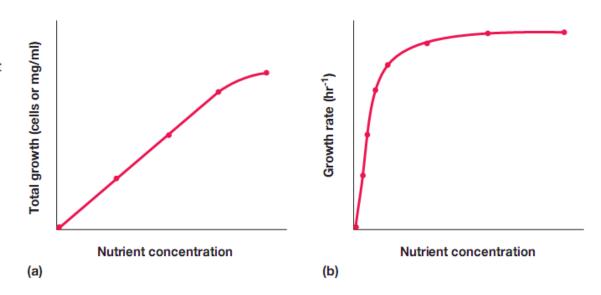
Unbalanced growth or lag is seen during shift-up and shift-down experiments. **Shift-up**: where a culture is transferred from a nutritionally poor medium to a richer one; and **shift-down**, where a culture is transferred from a rich medium to a poor one— to reorganize themselves metabolically.

The rate of microbial growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals.

Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps.

When microbial growth is limited by the low concentration of a required nutrient

Figure 6.7 Nutrient Concentration and Growth. (a) The effect of changes in limiting nutrient concentration on total microbial yield. At sufficiently high concentrations, total growth will plateau. (b) The effect on growth rate.



At sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration.

The Mathematics of Growth

During the exponential phase each microorganism is dividing at constant intervals. Thus the population will double in number during a specific length of time called the **generation time** or **doubling time**.

Suppose that a culture tube is inoculated with one cell that divides every 20 minutes.

Timea	Division Number	2 ⁿ	Population $(N_o \times 2^n)$	log ₁₀ N _t
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

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 (fast population growth)

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,

$$\log N_{t} = \log N_{0} + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_{t} - \log N_{0}}{\log 2} = \frac{\log N_{t} - \log N_{0}}{0.301}$$

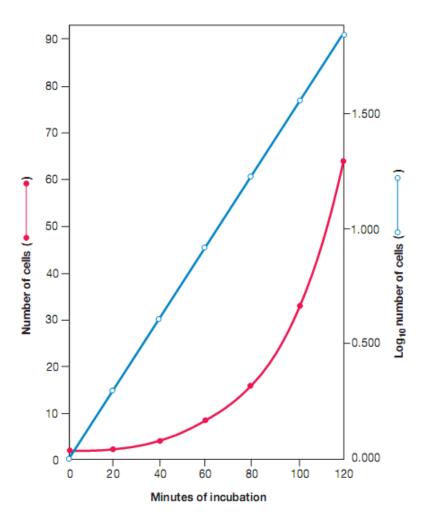


Figure 6.10 Exponential Microbial Growth. The data from table 6.1 for six generations of growth are plotted directly (→) and in the logarithmic form (→). The growth curve is exponential as shown by the linearity of the log plot.

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

$$N_t = 2N_0$$
.

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

$$k = \frac{\log(2N_0) - \log N_0}{0.301 g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301 g}$$
$$k = \frac{1}{g}$$

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

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

The mean generation time (g) can be determined directly from a semilogarithmic plot of the growth data (**figure 6.11**) and the growth rate constant calculated from the g value. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 10^3 cells to 10^9 cells in 10 hours.

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10 \text{ hr})} = \frac{9 - 3}{3.01 \text{ hr}} = 2.0 \text{ generations/hr}$$

 $g = \frac{1}{2.0 \text{ gen./hr}} = 0.5 \text{ hr/gen. or } 30 \text{ min/gen.}$

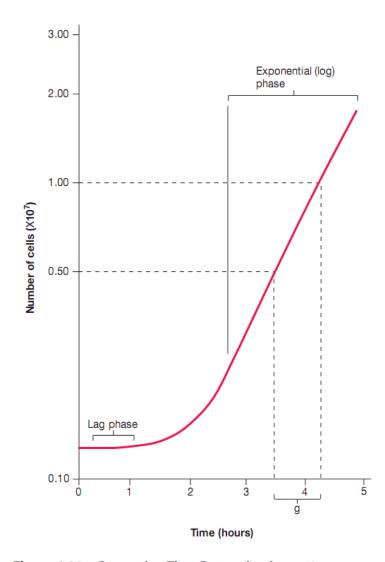


Figure 6.11 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

The generation time varies in different microorganisms

Table 6.2	Examples of Generation Times ^a				
Microorganism		Incubation Temperature (°C)	Generation Time (Hours)		
Bacteria					
Beneckea natriegens		37	0.16		
Escherichia coli		40	0.35		
Bacillus subtilis		40	0.43		
Staphylococcus aureus		37	0.47		
Pseudomonas aeruginosa		37	0.58		
Clostridium botulinum		37	0.58		
Rhodospirillum rubrum		25	4.6 - 5.3		
Anabaena cylindrica		25	10.6		
Mycobacterium tuberculosis		37	≈12		
Treponema pallidum		37	33		
Protists					
Tetrahymena geleii		24	2.2-4.2		
Scenedesmus quadricauda		25	5.9		
Chlorella pyrenoidosa		25	7.75		
Asterionella formosa		20	9.6		
Leishmania donovani		26	10-12		
Paramecium caudatum		26	10.4		
Euglena gracilis		25	10.9		
Acanthamoeba castellanii		30	11-12		
Giardia lamblia		37	18		
Ceratium tripos		20	82.8		
Fungi					
Saccharomyces cerevisiae		30	2		
Monilinia i	fructicola	25	30		

Thank You