

The Growth of Bacterial Populations (page 3)

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The Bacterial Growth Curve

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.

When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve** (Figure 3 below).

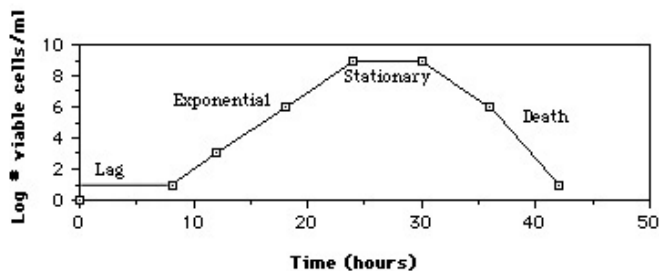


Figure 3. The typical bacterial growth curve. When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase). Note the parameters of the x and y axes. Growth is expressed as change in the number viable cells vs time. Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

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 (below) derive.

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".

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.

Growth Rate and Generation Time

As mentioned above, bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour. Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, have especially long generation times, and this is thought to be an advantage in their virulence. Generation times for a few bacteria are shown in Table 2.

Table 2. Generation times for some common bacteria under optimal conditions of growth.

Bacterium	Medium	Generation Time (minutes)
<i>Escherichia coli</i>	Glucose-salts	17
<i>Bacillus megaterium</i>	Sucrose-salts	25
<i>Streptococcus lactis</i>	Milk	26
<i>Streptococcus lactis</i>	Lactose broth	48
<i>Staphylococcus aureus</i>	Heart infusion broth	27-30
<i>Lactobacillus acidophilus</i>	Milk	66-87
<i>Rhizobium japonicum</i>	Mannitol-salts-yeast extract	344-461
<i>Mycobacterium tuberculosis</i>	Synthetic	792-932
<i>Treponema pallidum</i>	Rabbit testes	1980

Calculation of Generation Time

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

$G = t/n$

t = time interval in hours or minutes

B = number of bacteria at the beginning of a time interval

b = number of bacteria at the end of the time interval

n = number of generations (number of times the cell population doubles during the time interval)

$b = B \times 2^n$ (This equation is an expression of growth by binary fission)

Solve for n:

$$\log b = \log B + n \log 2$$

$$n = \frac{\log b - \log B}{\log 2}$$

$$n = \frac{\log b - \log B}{.301}$$

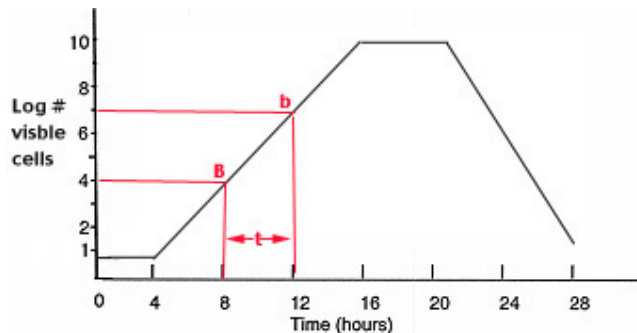
$$n = 3.3 \log b/B$$

$$G = t/n$$

Solve for G

$$G = \frac{t}{3.3 \log b/B}$$

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?



$$G = \frac{t}{3.3 \log b/B}$$

$$G = \frac{240 \text{ minutes}}{3.3 \log 10^7/10^4}$$

$$G = \frac{240 \text{ minutes}}{3.3 \times 3}$$

$$G = 24 \text{ minutes}$$

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