

BACTERIAL GROWTH CURVE

I. OBJECTIVES

- To determine the growth rate of bacteria under different temperature and aeration conditions
- To establish growth curves for an unknown bacterial species and observe the different phases of growth
- To demonstrate skill and knowledge in the use of the spectrophotometer.

II. INTRODUCTION

Bacteria normally reproduce by binary fission, forming two equal-sized progeny cells and thus doubling their number with each division. This type of cell division is called exponential; i.e., one cell divides to form two, each of these cells divides so that four cells are obtained, and so forth in a geometric progression. The unit of microbial growth is "generation time", which is the time required to achieve a doubling of the population size and is designated as t_{gen} . Generation time can be estimated by determining cell numbers during the period of active cell division and is expressed mathematically as:

$$t_{gen} = \frac{t \ln 2}{\ln X_t - \ln X_0} = \frac{t \log 2}{\log X_t - \log X_0}$$

In the above equations, t is the elapsed time during which growth is measured, and X_0 and X_t are the number of bacteria at times zero and t , respectively. The presence of 2 in the numerator of the equations is due to the fact that when doubling of the population number occurs, the quantity X_t/X_0 (called the doubling constant) will be equal to 2. Note that $\ln 2 = 0.693$ and $\log 2 = 0.301$. Also note that $\log 10^n = n$. So it is easier to use the log form rather than the ln form of the equation.

The generation time of many bacteria is usually several hours. However, under optimum conditions, *E. coli* has a generation time of about 20 minutes. Thus it can be calculated that a single *E. coli* cell will produce about a thousand progeny in 3.3 hours and over a million in 6.6 hours.

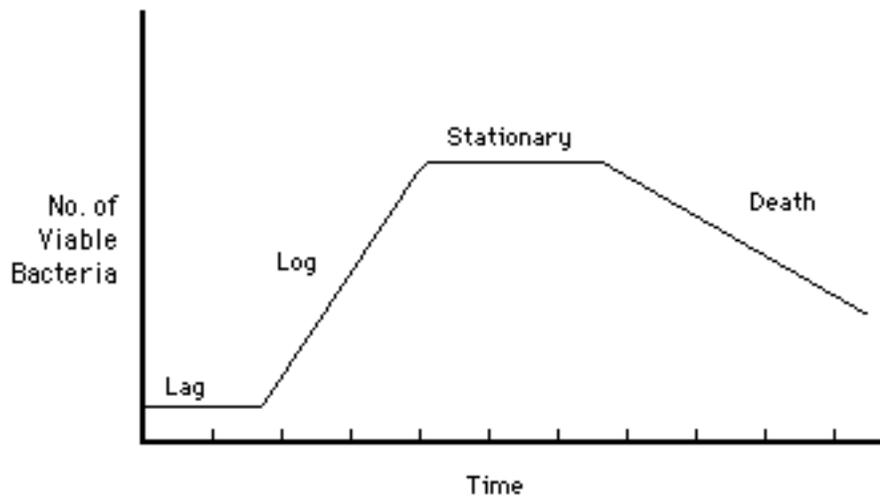
If the generation time and the elapsed time are known, then the number of generations can be estimated as follows:

$$\text{Number of generations} = t / t_{gen}$$

Since the optical density of the culture is proportional to the cell density, measuring the turbidity of the culture solution can be used in estimating numbers of bacterial cells, if a growth curve for the conditions used has already been established. This is the most common method used to rapidly estimate bacterial numbers. Other methods, such as viable plate counts, can also be used for determining bacterial growth curves but are often more tedious than turbidity measurements.

A bacterial population follows a characteristic growth curve which has four phases: the lag phase, the log or exponential growth phase, the stationary phase, and the death phase.

1. **Lag phase:** During this phase, there is no increase in cell number; rather, bacteria are preparing for reproduction and synthesizing DNA and various inducible enzymes needed for cell division.
2. **Log phase:** This phase, also called the exponential phase, follows the lag phase and starts with a rapid increase in bacterial number. At this stage, the logarithm of bacterial biomass increases linearly with time so that numbers of bacterial cells in a given interval of time is proportional to the biomass of bacteria present.
3. **Stationary phase:** The number of bacteria reaches a maximum in this phase and does not increase further (the growth rate is exactly equal to the death rate). This phase is sometimes called the plateau stage. A bacterial population may reach stationary growth when a required nutrient is exhausted, when inhibitory end products accumulate, or when physical conditions are inappropriate for growth. The duration of the stationary phase varies, with some bacteria exhibiting very long stationary phases.
4. **Death phase:** Eventually, the number of viable bacterial cells begins to decline, signaling the onset of the death phase. No further divisions occur in this phase. Death rate, in many cases, follows the same kinetics as the exponential growth.



In this laboratory exercise, you will work in groups and follow the normal growth curve of an unknown bacterial species to determine its generation time at different temperature and aeration conditions.

Turbidity, which is a measure of the growth of microorganisms, is determined via an instrument called a spectrophotometer. The basis for the use of this instrument is that less light passes through a turbid solution than through a clear solution; i.e., the more turbid a solution (more growth), the less the amount of light transmitted through the solution.

Inside a spectrophotometer, light is focused through a lens system to an entrance slit. The light rays are refocused by a second lens to an exit slit. Between the second lens and the exit slit is a monochromatic grating which separates the white light into its component wavelengths in much the same way as a prism does. By proper rotation of the monochromatic grating, specific light wavelengths may be passed on through the exit slit to a photocell. This cell is connected directly to a galvanometer that translates the electrical output of the activated photocell into a specific transmittance value.

In between the exit slit and the photocell is a chamber where samples may be placed. As previously mentioned, a clear specimen will yield 100% transmittance, while a turbid sample will deflect a considerable portion of the light rays, resulting in a lower percent transmittance. The greater the density, the lower the percent transmittance.

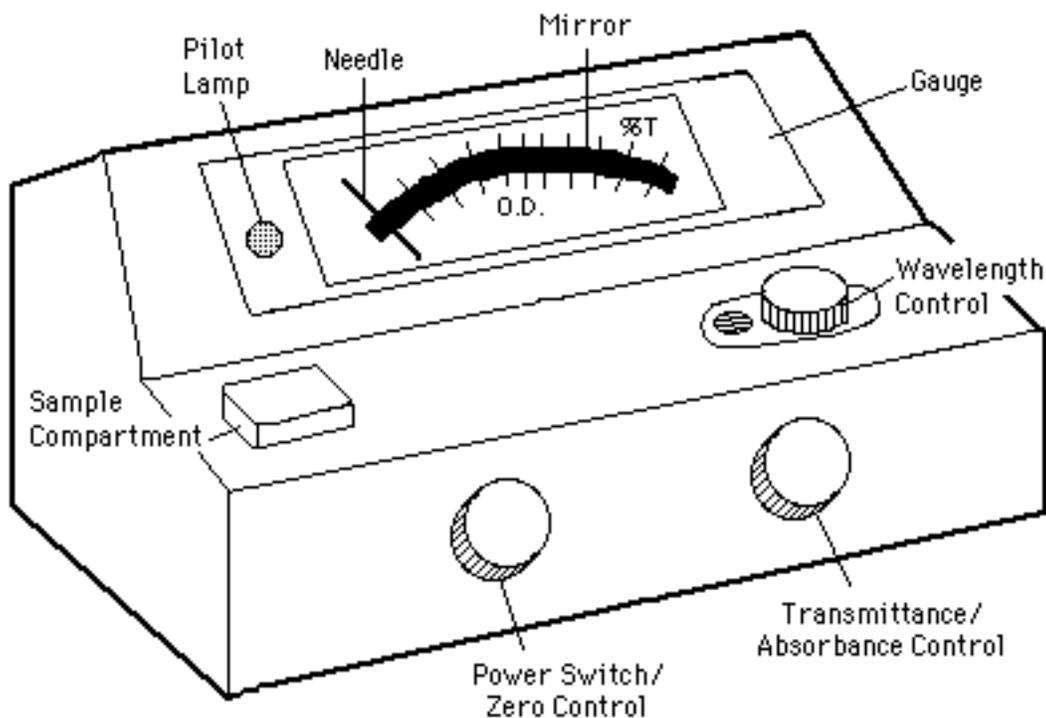
The sample is placed in a special test tube called a "cuvette", which fits the spectrophotometer snugly. Cuvettes are very expensive and care should be exercised in handling them. To cut down on the costs, we use regular small test tubes in place of cuvettes. If the cuvette is rotated within its holder in the spectrophotometer, the reading would vary. For this reason, cuvettes contain a white line at the lip that should be adjusted to the line on the machine's cuvette-holder. This is to keep the cuvette from contributing to the reading of the sample. You can mark a vertical line on the test tube yourself.

The wavelength selection is important and should vary with the color of the suspension medium. However, it should not be changed during the experiment. A 420 nm selection is usually used if the medium is nearly colorless; 550 nm if the solution is yellowish.

Some spectrophotometers have lids over the sample holder slot. The lid should be shut for reading the instrument. Since you will be using a spectrophotometer in this and in future lab exercises, it is necessary to make sure that you know how to use it. The following steps should be followed when a measurement is to be made:

1. Turn on the instrument by rotating the zero control knob clockwise. Wait 10 minutes for the spectrophotometer to warm up.
2. Select the proper wavelength by turning the knob that is located on top of the instrument.

3. Adjust the meter needle to zero (left margin) by turning the zero control knob on the left.
4. Fill a cuvette with 5 ml of the broth without any bacterial growth. This is your control blank tube. Place the blank tube in the sample holder and adjust the meter needle to 100% transmittance by turning the light control knob (on the right).
5. Remove the blank tube.
6. Insert the sample to be read and note the percent transmittance (%T) or optical density (O.D.). Continue to read any other samples you may have. If the number of samples is great, recheck and adjust the transmittance using your blank after every ten readings. Turn off the instrument, remove your blank or sample and clean the instrument. Note that you will be able to read both the % T (upper scale) and O.D. (lower scale) simultaneously.



Remember that:

1. If you are going to use a test tube in place of a cuvette, draw a vertical line near its lip, so you can line it up with the mark on the cuvette-holder. This makes your readings more comparable.
2. There is a mirror strip on the gauge. When reading a sample, close one eye and move your head until the needle and its image in the mirror are superimposed. Now take your reading.

III. LABORATORY SUPPLIES

10 ml culture of an unknown microorganism, kept at 30°C	1/tablesideside
50 ml BHI broth in 125-ml flasks, kept at 30°C	1/tablesideside
Spectrophotometer	4/lab
Water baths, 37°C & 30°C w/ & w/o shaker	1 of each/lab
Un-inoculated BHI broth in small test tube for blank	1/tablesideside
Cuvettes= small test tube	2/tablesideside
Flask weight	1/table
Liquid waste beaker	2/table

IV. PROCEDURE (The students at each tablesideside will work together as a group).

1. Your group will be assigned one of the following growth conditions:
 - a. 37°C incubator with shaking
 - b. 37°C incubator without shaking
 - c. 30°C water bath with shaking
 - d. 30°C water bath without shaking
2. Obtain a flask of BHI and transfer 5 ml of broth to a small test tube. This is your blank. Adjust the wavelength to 550 nm on the spectrophotometer and calibrate the machine to 0.0 O.D. or 100% transmittance with your blank. Take the blank out but do not discard it.
3. Inoculate your BHI flask with 10 ml of the culture provided and swirl the flask well.
4. Work quickly to withdraw 5 ml of the sample into a clean small test tube, using a pipette
5. Place the culture flask immediately at the assigned incubating temperature and aeration condition and record the time.
6. Measure the O.D. of your sample next. Record the 0 time absorbance. Discard the contents of your sample test tube. This test tube can be re-used for your next sample without washing.
7. At 15-minute intervals for up to 90 minutes, shake your flask vigorously and transfer 5 ml of the culture to your sample test tube.
8. Record the optical density at each time period. Use your blank each time to make sure your readings are correct and other students have not altered the machine's calibration. Your instructor will ask all groups to write their results in a table on the blackboard. Copy these results to your Results Sheet. If more than one group is doing a growth condition, get their average data.
9. Plot the optical density (y-axis) versus time (x-axis) for each growth condition on the same regular graph paper. If a log phase occurred at the test condition you

- used, go to the next step. Otherwise, use the data of the group(s) with a log phase and then proceed to the next step.
10. To determine the generation time, plot the optical density (y-axis) versus time (x-axis) on semi-log paper. Draw a straight line of best fit through the points making up the log phase. Next draw two horizontal lines cutting the y-axis where the optical density has doubled and then draw two vertical lines where the horizontal lines intersect the curve and note the time period between the two points on the x-axis. This is the generation time.

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Results of the Bacterial Growth Curve Exercises

NAME _____ DATE _____ GROUP NAME _____

1. Your assigned growth condition: _____

Incubation Time (min)	0	15	30	45	60	75	90
O.D.							

2. Collect the data from other groups in the lab and complete the following table. If more than one group is assigned the same growth condition, write their average results in the table below:

Growth Condition	O.D. at							
	0	15	30	45	60	75	90	
37°, shaking								
37°, no shaking								
30°, shaking								
30°, no shaking								

Draw the growth curve at different conditions on the same regular graph paper and label each curve carefully. Discuss the results.

3. Using semi-log graph paper, determine the generation time (t_{gen}) under the best assigned condition.