

Lab Exercise 13: Growth Curve

OBJECTIVES

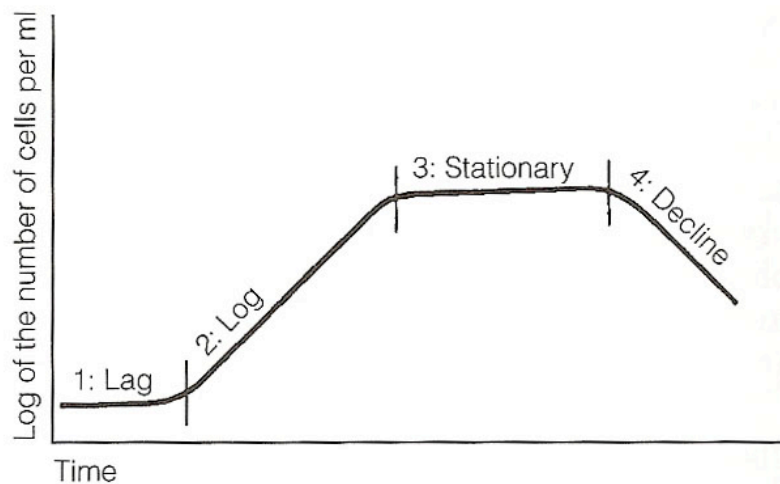
1. Know the different phases of a standard growth curve.
2. Understand and perform direct measurement of bacterial growth through serial dilutions and standard plate counts.
3. Understand and perform indirect measurement of bacterial growth through spectrophotometer readings and optical density measurements.

INTRODUCTION

Bacterial population growth studies require inoculation of viable cells into a sterile broth medium and incubation of the culture under optimum temperature, pH, and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a **population growth curve**, which is constructed by plotting the increase in cell numbers versus time of incubation and can be used to delineate stages of the growth cycle. It also facilitates measurement of cell numbers and the rate of growth of a particular organism under standardized conditions as expressed by its **generation time**, the time required for a microbial population to double.

The stages of a typical growth curve (figure below) are:

1. **Lag phase:** When the cells are adjusting to their new environment. During this phase, cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of the cycle. Although the cells are increasing in size, there is no cell division and therefore no increase in numbers.
2. **Logarithmic (log)/Exponential phase:** Under optimum nutritional and physical conditions, the physiologically robust cells reproduce at a uniform and rapid rate by binary fission. Thus there is a rapid exponential increase in population, which doubles regularly until a maximum number of cells is reached. The length of the log phase varies, depending on the organisms and the composition of the medium, although the average may be estimated to last 6 to 12 hours.
3. **Stationary phase:** During this stage, the number of cells undergoing division is equal to the number of cells that are dying. There is no further increase in cell number and the population is maintained at its maximum level for a period of time. The primary factors responsible for this phase are the depletion of some essential metabolites and the accumulation of toxic acidic or alkaline end-products in the medium.
4. **Decline or death phase:** Because of the continuing depletion of nutrients and buildup of metabolic wastes, the microorganisms die at a rapid and uniform rate. This decrease in population closely parallels its increase during the log phase. Theoretically, the entire population should die during a time interval equal to that of the log phase. This does not occur, however, since a small number of highly resistant organisms persist for an indeterminate length of time.

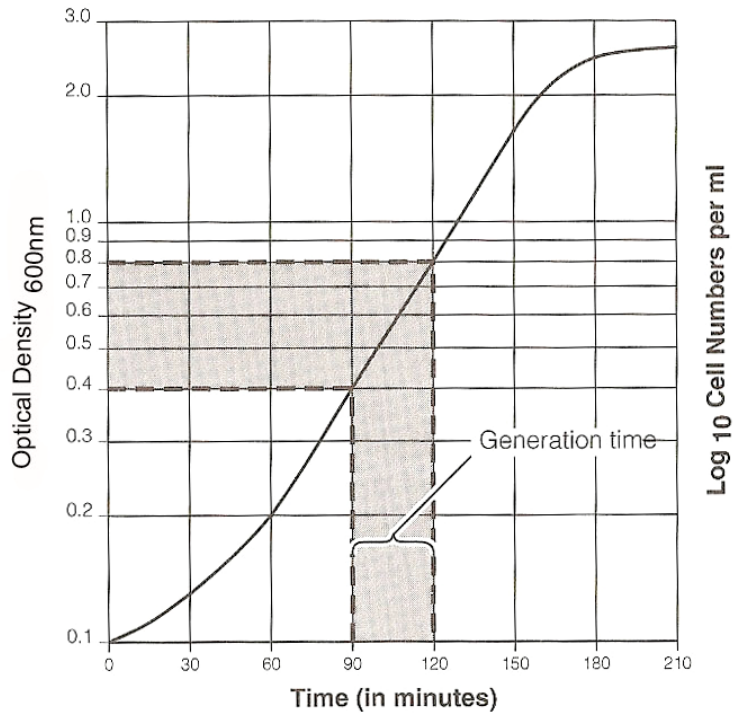


Construction of a complete **bacterial growth curve** requires that aliquots of a 24-hour shake-flask culture be measured for population size at intervals during the incubation period, however, such a procedure does not lend itself to a regular laboratory session. This experiment is designed to include only the lag, log and possibly stationary phases of population growth. Upon completion of this experiment, you will plot the data collected during this experiment by using two values for the measurement of growth. The **direct method** requires that you use serial dilution to plate out cells at 30 minute intervals in order to calculate the number of **colony forming units (CFUs)** at a given time. The **indirect method** uses spectrophotometric measurements of the developing turbidity at the same 30-minute intervals, as an index of increasing cellular mass (assumed to correlate with an increase in the number of cells).

You will determine generation time with indirect and direct methods by using data you collect, once it has been plotted onto a graph like the one shown below. Indirect determination is made by simple extrapolation from the log phase as illustrated in the figure below. Select two points on the optical density (OD) scale, such as 0.2 and 0.4, that represent a doubling of turbidity. Using a ruler, extrapolate by drawing a line between each of the selected optical densities on the ordinate and the plotted line of the growth curve. Then draw perpendicular lines from these end points on the plotted line of the growth curve to their respective time intervals on the abscissa. With this information, determine the generation time as follows:

$$GT = t_{(O.D. \ 0.4)} - t_{(O.D. \ 0.2)}$$

$$GT = 90 \text{ minutes} - 60 \text{ minutes} = 30 \text{ minutes}$$



The direct method uses the log of cell number scale on the growth curve and the following formula:

$$GT = t \log 2 / [\log b - \log B]$$

Where GT = generation time; B = number of bacterial CFUs at some point during the log phase; b = number of bacterial cells at a second point of the log phase; and t = time in hours or minutes between B and b .

LAB EXERCISES

I. Indirect growth measurements- the spectrophotometer and optical density

Table supplies	Team supplies
Spectrophotometer	Side arm flask with <i>E. coli</i> in TSB (each table will have a different start time for inoculation)
Media blanks	

Protocol:

1. Starting at time 0, you will do the following every 30 minutes for a total of 4 time points:

1. **Set** and **calibrate** the spectrophotometer. To do this, set the wavelength knob (top of instrument) to 600 nm. Then, adjust the meter needle to zero by rotating the zero control knob (left side, front of instrument, see figure below).
2. **Blank** the spectrophotometer. To do this, insert a test tube containing the medium you are using (called a blank) into the sample holder. Adjust the meter needle to read 100 % transmittance by rotating the light control knob (right side, front of instrument, see figure below).
3. Remove the blank from the instrument.
4. Swirl culture flask to resuspend organisms and carefully pour medium into side arm. Wipe down the side arm if it is at all dirty on the outside. Insert side arm into sample holder. Try to maintain the same orientation of arm to flask for each time point reading.
5. Read and record the % transmittance **and** the optical density (OD) of the culture.



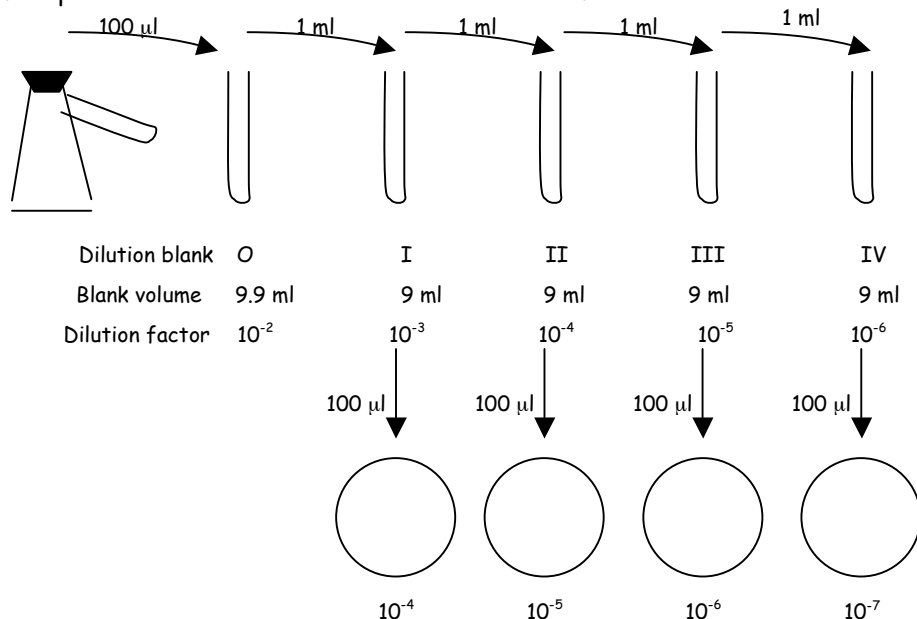
II. Direct growth measurements: serial dilutions and standard plate counts

Class supplies	Team supplies
Shaking 37° C incubator	Side arm flask containing <i>E. coli</i> in TSB (each table will have a different start time of inoculation)
	4 dilution blanks (9.9 ml water)
	16 dilution blanks (9 ml water)
	16 TSA plates
	p200 pipette
	p1000 pipette
	Pipette tips for both pipettes
	Turn table
	Metal spreader
	Alcohol

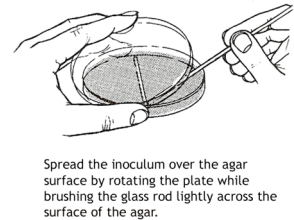
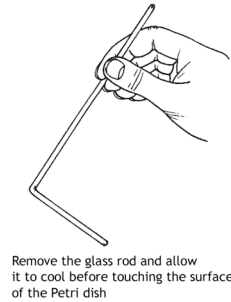
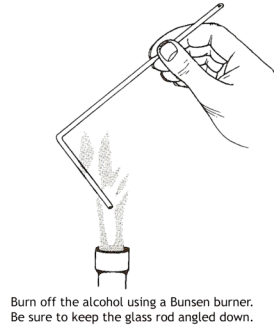
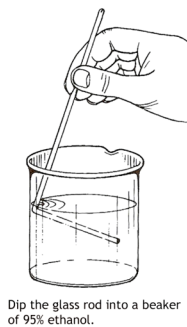
Protocol:

1. Your flask will be inoculated by the instructor prior to class and allowed to incubate at 37° C in a shaking incubator during lecture.
2. Starting at time 0, you will do the following every 30 minutes for a total of 4 time points (see figure below):

1. Label one (1) 9.9 ml dilution blank O. Label four (4) 9 ml dilution blanks I- IV. Label 4 plates with time point (i.e. 0, 30m, 60m, 90m) and dilution factor (i.e. 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7}).
2. Remove 100 μ l of culture from the side-arm flask and add to dilution blank O. Mix thoroughly by rolling in hands. DO NOT shake the dilution blanks as material will spill out.
3. Remove 1 ml from dilution blank O and add to dilution blank I. Mix thoroughly by rolling in hands.
4. Remove 1 ml from dilution blank I and add to dilution blank II. Mix thoroughly by rolling in hands.
5. Repeat serial dilution with blanks III and IV.



6. Top spread 100 μ l from dilution blanks I, II, III, and IV onto the TSA plates. Sterilize spreader by dipping in alcohol and then *briefly* flaming to burn off excess alcohol (see figure below).



7. Allow to dry and then incubate inverted at the appropriate temperature.

DATA AND OBSERVATIONS

Day one:

1. Record your indirect measurements of OD and % transmittance in the table below.

Day two:

1. Collect your plates from the 37°C incubator. Count the number of distinct colonies on each plate.

2. For each time set, keep only those plates which contain between 30-300 colonies, discard all the rest. If more than one plate contains 30-300 CFUs, average the numbers and record only one for each time set.

3. Determine the number of CFUs/ml of the **original culture** for each of the time sets and record the data in the table below.

Incubation Time (min)	Optical Density (600nm)	% transmittance	Bacterial concentration (CFU/ml)	Log of CFU/ml

4. Based on the instructions in the introduction to the lab, calculate the generation time using your CFU/ml calculations. Remember that B and b can be any two time points, but the most accurate would be those that give the longest interval:

Direct Generation Time= _____

5. Plot the OD_{600nm} on the Y axis and incubation times on the X axis of the provided semi-log graph paper.

6. Identify the log phase (if present) for the graph and determine the generation time of your bacterial culture using the indirect method described in the introduction to this lab exercise. Remember that the log phase is represented by the straight-line portion of the curve.



Indirect Generation Time= _____

DISCUSSION

1. Does the term growth convey the same meaning when applied to bacteria and to multicellular organisms? Explain.
2. Were the generation times from your direct and indirect measurements similar? Give a possible explanation for why or why not?
3. Was your bacterial culture in log growth during this experiment? If not, provide a possible growth phase for your culture and explain why.
4. Did any of your classmates have a culture in log growth?
5. Why do variations in generation time exist:
 - a. among different species of microorganisms?
 - b. within a single microbial species?
6. The generation time and growth rate of an organism grown in the laboratory can be easily determined by constructing a typical growth curve. Would you expect the growth rate of the infectious organisms found in an abscess that developed from a wound to mimic the growth curve obtained in the laboratory? Explain.