

General Microbiology Laboratory

Lab Session 4 - The Bacterial Growth Curve

Introduction

Bacteria replicate exponentially by transverse binary fission in liquid (broth) culture. The complete construction of a bacterial growth curve (increase in cell numbers vs. time) requires measurement of population size at intervals over an extended period of time. One can directly measure the increase in viable cells (cell counts are the most accurate, but also the most tedious and time consuming method), or one can indirectly measure increase in growth as a function of increasing turbidity (i.e. increasing cell density) in a liquid medium. Either method allows for construction of a typical microbial growth curve (with a lag, log, stationary and death phase) as well as the determination of the generation time (the time required for a bacterial population to double during log phase growth). The purposes of this lab are to:

- 1) Use an indirect, spectrophotometric assay to determine the shape of a microbial growth curve;
- 2) Determine the generation time of a typical bacterium under ideal, defined conditions; and,
- 3) Evaluate the effects of environmental conditions on microbial growth and generation time.

A. Review the following for reference:

1. Lab Manual: Introduction - Pages 115 and 116.
Exercise #20 - Pages 123 and 124.
2. Text: Chapter 6 - Pages 123 to 130.
3. Pay particular attention to the following:
Phases of growth in culture. Measuring growth via Serial Dilution and Turbidity

B. Do the following during lab: Growth Curve

1. Preparation: Basically, this lab requires much preparation, as well as 8-24 hours of actual measurement time to determine a complete growth curve. Therefore, some of the following steps will have been completed before you come to lab:
 - A broth culture of *E. coli* was started yesterday afternoon,
 - Fresh, broth cultures of *E. coli* were started this morning,
 - Growth flasks with 100 ml of Trypticase Soy Broth were inoculated with *E. coli* this afternoon,
 - Growth flasks were placed on shaker platforms and rotated at ~100 rpm,
 - Recordings of Optical Density were made ~ every 15-20 minutes
 - Antibiotics will be added to flasks 3, 4, and 5 when the mo's are in the log phase of growth (optical density will be approximately 0.15).
2. Experimental Design: There will be nine Growth Flasks, including:
 - An uninoculated negative control, (i.e. a spectrophotometer blank): Flask 1
 - Two inoculated positive controls (i.e. ideal growth conditions): Flasks 2A and 2B
 - Six experimental flasks (i.e. six variables to compare to the untreated, positive control)

Flask	Inoculum	Treatment	Temperature	Purpose
1	None	None	25	- control
2A	<i>E coli</i>	None	37	+ control
3	<i>E coli</i>	Streptomycin (0.1 mg/ml) ^a	37	Antibiotic
4	<i>E coli</i>	Streptomycin (0.01 mg/ml)	37	Antibiotic
5	<i>E coli</i>	Vancomycin (0.1 mg/ml) ^a	37	Antibiotic
2B	<i>E coli</i>	None	37	+ control
6	<i>E coli</i>	None	24	Temperature
7	<i>E coli</i>	pH of medium: 4.75 ^b	37	pH
8	<i>E coli</i>	3.75% NaCl ^c	37	Salinity

^a Antibiotics are used at approximate therapeutic concentrations.

^b Fresh TSB is buffered with a phosphate buffer to approximately pH 7.0.

^c Fresh TSB contains 0.5% NaCl.

3. Measurements:

- a. Work in two groups:
 - Group #1 will do flasks: 2A, 3, 4, 5
 - Group #2 will do flasks: 2B, 6, 7, 8
 - You will share data so that each individual has data for all 8 flasks!
- b. You are responsible for the following:
 - Measurement and recording of Optical Density every 15-20 minutes in each of the flasks.

C. Results

1. You are responsible for collection and presentation of the following data:
 - Records of Optical Density at defined intervals in each of the 8 flasks.
 - Plotting data to determine the shape of the growth curve:
 - You will plot optical density (log scale) vs. time (in minutes).
 - You can plot all eight of the curves on a single piece of 2-cycle log paper, or you can plot Group #1 and Group#2 data separately.
 - Determination of Generation Time for each culture (#2 through #8) **during log growth**.
 - Discussing the significance of your observed measurements:
 - Effects of: Antibiotics, temperature, pH and salinity.
2. This is one of two or three labs for which you might be asked to prepare a formal report. The report can follow any format that you like, but the standard format below is certainly acceptable:
 - Introduction (which includes a statement of purpose),
 - Methods and Materials (which completely describes the supplies and procedures),
 - Results (which includes only a presentation of the collected data),
 - Discussion (which includes your interpretation of what the data mean),and,
 - Conclusions (a summary statement that relates back to your stated purpose in the introduction).

D. Techniques

Use of Spectrophotometer (Spec 20):

- Turn instrument on - Left hand knob
- Adjust wavelength of light to 550 nm- Top knob
- Set Transmittance to 0 % - Left hand knob
- Place Control Flask, #1 in instrument and set OD to 0 - Right hand knob
- Replace Control Flask with a Test Flask and read OD - On screen

Cautions:

- Be careful beyond reason when handling the sidearm flasks: they are expensive.
- Make sure the side arm of the flask is clean and inserted into the Spec 20 correctly.
- Try to be consistent in how you measure optical density.
- Try to not let any excess stray light enter the cuvette.
- The culture must be in the log phase of growth in order to determine the Generation Time.
 - The log phase of growth is indicated by a straight line when growth is plotted on log paper.
 - Use a ruler to make the straight line for log phase growth. Do not just "connect the dots".
 - It is important to plot the data carefully because you will use the plotted data (not the recorded data) to determine the generation time.
 - OD #1 (Optical Density #1, see Determination of Generation Time below) is any point on the straight line (i.e. you simply choose a starting point). It is not the first data measurement of Optical Density.
 - OD #2 is therefore two times Optical Density #1.
 - The difference (in minutes) between OD#1 and OD#2 is therefore the generation time.

1. Recordings of optical density: Group #1

Time Point	Time		Optical Density			
	Actual	Elapsed	Flask 2A	Flask 3	Flask 4	Flask 5
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						

2. Determination of Generation Time **During Log Phase of Growth**: Group #1

a. OD #1 (Start)	_____	_____	_____	_____
Time #1	_____	_____	_____	_____
b. OD #2 (2x OD#1)	_____	_____	_____	_____
Time #2	_____	_____	_____	_____
Time difference #1 (#2-#1)	_____	_____	_____	_____
c. OD #3 (2x OD#2)	_____	_____	_____	_____
Time #3	_____	_____	_____	_____
Time difference #2 (#3-#2)	_____	_____	_____	_____
Average Generation Time*:	_____	_____	_____	_____

*AGT = (Time difference #1 + Time difference #2) / 2

3. Recordings of optical density: Group #2

Time Point	Time		Optical Density			
	Actual	Elapsed	Flask 2B	Flask 6	Flask 7	Flask 8
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						

4. Determination of Generation Time **During Log Phase of Growth**: Group #2

a. OD #1 (Start)	_____	_____	_____	_____
Time #1	_____	_____	_____	_____
b. OD #2 (2X OD#1)	_____	_____	_____	_____
Time #2	_____	_____	_____	_____
Time difference #1 (#2-#1)	_____	_____	_____	_____
c. OD #3 (2x OD#2)	_____	_____	_____	_____
Time #3	_____	_____	_____	_____
Time difference #2 (#3-#2)	_____	_____	_____	_____
Average Generation Time*:	_____	_____	_____	_____

*AGT= (Time difference #1 + Time difference #2) / 2

E. Discussion and Conclusions