# Bacterial Growth Curves: Analysis through $OD_{600}$ measurements

## Introduction

Bacteria are single cell organisms often characterized by their size, shape, vulnerability to specific antibiotics, and pathogenicity, among other properties.[1] Under the appropriate conditions (e.g., temperature, nutrients), bacteria can reproduce through binary fission, producing more bacteria over time.[1,2] Microbiologists will often study bacterial growth to better understand the different growth phases for a specific strain, such as the time it takes to double the population; this "doubling time" is a characteristic specific to the species and strain analyzed.[1] Additionally, analysis of bacterial growth can aid in determining the best experimental conditions for growing bacterial colonies (aggregates of clustered bacteria) according to the needs of the scientist.

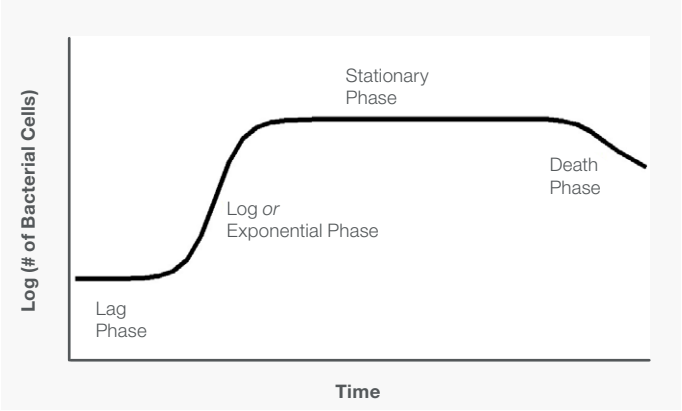


**Figure 1. Example diagram for a typical bacterial growth curve.**

Bacterial growth is characterized through four different phases: lag, log/exponential, stationary, and death phases (Figure 1). The lag phase is the first phase of bacterial growth and outlines the time it takes for bacteria to begin multiplying. In this phase, the bacteria begin to form the enzymes required to initiate growth. As described previously, the bacterial growth arises from binary fission, where one bacterium divides into two bacteria, which in turn produces two more bacteria each, and so on. This behavior will result in an exponential growth in the number of bacteria formed as a function of time and outlines the second growth phase: exponential or log phase.[2] During this phase, nutrients within the growth medium (*e.g.*, broth, agar) are being consumed. At some point, enough nutrients have been consumed such that the bacteria can no longer keep reproducing at an exponential rate, reaching a point of diminishing returns where the number of living bacteria no longer changes (stationary phase). Once the nutrients are diminished, and in the presence of waste byproducts produced during growth, the bacteria cannot survive indefinitely and will begin to die, leading to the death phase.

There are several different methods that can be employed to monitor the growth of bacterial colonies, including detecting nucleic acid sequences specific to the bacteria being grown and detecting of the structure of the bacteria through antibodies, among others.[1] One commonly employed method involves growing cultures on solid growth media from aliquots of the original growth culture collected at different time intervals during the experiment. The number of viable (living) colonies are then counted and reported for each time point. This method allows for the direct monitoring of the number of bacteria grown at a given time and is specific to living bacteria as dead colonies are unable to grow. Unfortunately, this technique is time-consuming as the bacteria must grow overnight before it can be counted.

Another frequently used technique to monitor bacterial growth involves UV-Visible spectroscopy. In principle, this technique uses light across the UV-Visible range of the electromagnetic spectrum to probe electronic transitions within a given analyte (molecule or biomolecule). Typically, data is reported as an absorption spectrum which describes these transitions. However, in the context of bacteria this technique is different. As the bacterial colonies grow, they become large enough to scatter light, which appears as an absorption feature across the entirety of the UV-Visible spectrum. With an increase in the number of colonies, the measured "absorbance" will increase as well. By measuring the "absorbance" at long wavelengths (600 nm is typically used by convention – $OD_{600}$), the growth of the bacteria can be monitored much more quickly than using the viable colony counting method, giving an almost instantaneous observation of growth.

It is important to keep in mind that this is not a true measurement of the absorbance of a sample, but an indirect measurement of the scattering of light off the newly formed bacterial colonies. Additionally, this method will measure scatter off any material capable of scattering light indiscriminately, including both living and dead bacteria. While this method can provide a faster method of monitoring bacterial growth, it will inherently have error in the analysis compared with counting viable colonies.

In this experiment, you will be monitoring the growth of *Escherichia coli (E. coli)* K12 cultures as a function of time through two different methods: viable colony counting from cultures grown on plates and $OD_{600}$ measurements. Using the data from both methods, you will construct a growth curve for the formation of *E. coli* as a function of time. The experiment described herein is loosely based on the experiment outlined by McKernan.[3]

## References


1. McGoverin, C.; Steed, C.; Esan, A.; Robertson, J.; Swift, S.; Vanholsbeeck, F., Optical Methods for Bacterial Detection and Characterization, *APL Photonics*, **2021**, 6.
2. Harvey, R. A.; Champe, P. C.; Fisher, B. D., *Microbiology Second Edition*; Lippincott Williams & Wilkins, **2006**.
3. McKernan, L. N., Using a Simple Escherichia coli Growth Curve Model to Teach the Scientific Method, *Am. Biol. Teach.*, **2015**, 77, 357-362.


# Experimental

## Materials

- Thermo Scientific™ GENESYS™ 40 or 50 UV-Visible Spectrophotometer
- Disposable cuvettes (plastic cuvettes are appropriate)
- Lint-free lab wipes
- *Escherichia coli (E. coli)*, Strain: K-12
- Luria Bertani (LB) Broth (1X)
- 50 mg/mL streptomycin
- 10% Bleach solution
- Metal or disposable inoculating loops (10 µL)
- Metal spreader
- LB Agar plates (with 50 µg/mL streptomycin)
- 250 mL baffled flask
- 2 L baffled flask
- Incubator
- Shaker
- Bunsen burner or alcohol burner (optional)
- Igniter (e.g. matches, lighter, spark igniter)
- Pipettor with appropriate pipette tips
- Permanent marker (preferably with a fine tip)

## Safety

Note, in this lab students will be using a source of heat to sterilize inoculating loops. Care should be taken to ensure students and instructors do not burn themselves or allow the flame to become uncontrolled. Instruct students on proper fire safety, as well as applicable fire extinguishing methods. Additionally, students should be wearing proper personal protective equipment, including gloves, when performing this experiment or disposing of materials.

## Instructions

### Part A – Plating Bacterial Colonies

A1. Clean/disinfect the lab bench.

A2. Obtain (3) agar plates and the *E. coli* strain. Label the bottom of the plates appropriately (e.g. student initials, date, *E. coli* strain)

*NOTE: When removing the lid from the agar plate, ensure the lid is placed face up on the lab bench so as to avoid introducing any additional bacteria into the agar plate.*

A3. Set up a Bunsen burner or alcohol lamp.

a. Ensure safe lab procedures are followed when handling an open flame.

*NOTE: Skip this step if using disposable inoculating loops.*

A4. Using a sterile inoculating loop, pick and spread the *E. coli* on an agar plate. See Figure 2a for a visual description for spreading bacteria colonies on a plate for growth.

*Instructions for Spreading/Streaking Plates:*

a. Using the sterile inoculating loop, pick the *E. coli* colony and spread it in one quadrant of the agar plate. Running the inoculating loop over the flame will sterilize the loop (see Figure 2b).

b. Turn the plate 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

c. Using the cooled, sterilized loop, spread from the first quadrant into the next quadrant.

d. Turn the plate another 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

e. Using the cooled, sterilized loop, spread from the second quadrant into the next quadrant.

f. Turn the plate another 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

g. Using the cooled, sterilized loop, spread from the third quadrant into the next quadrant.

*NOTE: If using disposable inoculating loops, sterilization is not necessary. Dispose of the loop after each use, then use a new one each time the plate is turned.*

A5. Repeat step A4 on a second agar plate.

A6. Using just a sterile inoculating loop (no *E. coli*), spread on the final agar plate without *E. coli*, repeating the procedure outlined in step A4. This will serve as your negative control.

A7. Extinguish the flame.

A8. Place covers on each agar plate and place the plates in the incubator. The plates must be placed in the incubator upside down.

A9. Set the temperature to 37 ˚C and allow to incubate overnight.

### Part B – Overnight Culture

B1. Following incubation, check for colony growth on your agar plate. Check with your instructor to make sure the colonies are acceptable to be used for the overnight culture.

B2. Clean/disinfect the lab bench.

B3. Fill a 250 mL baffled flask with 50 mL of LB broth.

B4. Calculate the volume of 50 mg/mL streptomycin needed to result in a final concentration of 50 µg/mL in 50 mL of solution:

B5. Add the calculated amount of 50 mg/mL streptomycin to the LB broth.

B6. From one of the plates, pick a single bacterial colony using a sterile inoculating loop.

B7. Dip the inoculating loop with the picked colony in the baffled flask and stir it a little.

B8. Remove the loop and properly dispose of it or, if it is not disposable, flame the inoculating loop as shown in Figure 2b to sterilize. Allow to cool before putting away.

B9. Place the flask in the incubator and allow to incubate at 37 ˚C overnight (maximum of 16 hours). Ensure the solution is able to shake during the incubation. The shaker should be set to 300 rpm.

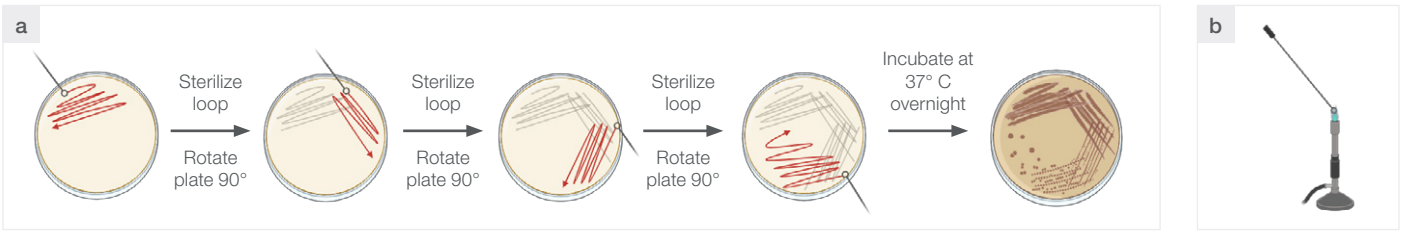


**Figure 2 - (a) Scheme demonstrating streaking a plate. (b) Depiction of inoculating loop sterilization. Images created with BioRender.com.**

## Part C – Bacterial Growth Curve

C1. Clean/disinfect the lab bench.

C2. Turn on the UV-Visible spectrophotometer.

C3. Open the "Fixed" Application and select the following instrument parameters:

a. Wavelength ($\lambda_1$): 600 nm

b. Factor ($F_1$): 1.000

c. ABS mode

C4. Calculate the volume of 50 mg/mL streptomycin needed to result in a final concentration of 50 µg/mL in 612 mL of solution:

C5. Add the calculated amount of 50 mg/mL streptomycin to enough LB broth to result in a final solution volume of 612 mL.

C6. Bring the mixture of LB broth and streptomycin to 37 ˚C.

C7. Measure the blank/baseline for the experiment using the UV-Visible spectrophotometer:

a. Fill a 1.0 cm cuvette with 2 mL of the warmed LB broth.

b. Place the filled cuvette in the spectrophotometer cuvette holder.

c. Close the sample compartment and press the "Blank" button.

d. Remove the cuvette and dispose of the solution and cuvette in the appropriate waste disposal receptacles.

C8. Prepare the batch culture by transferring 12 mL of the overnight culture to the warmed broth. Keep the batch culture held at 37 ˚C and shaking at 300 rpm for the remainder of the experiment.

C9. Remove 5 mL from the batch culture using a sterile pipette.

C10. Using 2.0 mL of the reserved batch culture, fill a 1.0 cm cuvette and place in the UV-Visible spectrophotometer sample holder.

C11. Press "Measure" to measure the absorbance of the sample. Record the measured absorbance and growth time in Table 1 in the Lab report section of this experiment.

C12. Repeat steps C8 – C11 every 20 min. until the end of the lab period.

C13. Add bleach to the batch and overnight cultures and properly dispose of the solution.

C14. Using the aliquots collected at 0, 60, 120, 160 min, serially dilute 1:10 with LB broth for a total of 6 serial dilutions per time point as described below:

a. Dilution 1: Add 1.0 mL of batch culture to 9.0 mL of LB broth. Cap and mix by shaking.

b. Dilution 2: Add 1.0 mL of Dilution 1 to 9.0 mL of LB broth. Cap and mix by inverting.

c. Dilution 3: Add 1.0 mL of Dilution 2 to 9.0 mL of LB broth. Cap and mix by inverting.

d. Dilution 4: Add 1.0 mL of Dilution 3 to 9.0 mL of LB broth. Cap and mix by inverting.

e. Dilution 5: Add 1.0 mL of Dilution 4 to 9.0 mL of LB broth. Cap and mix by inverting.

f. Dilution 6: Add 1.0 mL of Dilution 5 to 9.0 mL of LB broth. Cap and mix by inverting.

C15. Set up a Bunsen burner or alcohol lamp.

C16. For each time point, pipet 100 µL of Dilution 4, 5 and 6 onto separate plates.

C17. Sterilize a metal spreader by running it over a flame. Allow to cool.

C18. Use the sterilized or single-use spreader to spread the solution across the entire surface of the agar plate in a circular motion.

C19. Re-sterilize the spreader, if applicable, and repeat for each plate.

C20. Sterilize the spreader, if applicable, once again and allow to cool before putting away.

C21. Wait a few minutes for the sample to diffuse in the agar, then incubate each plate overnight at 37 ˚C. Like in Part A, the plates should be placed upside down in the incubator.

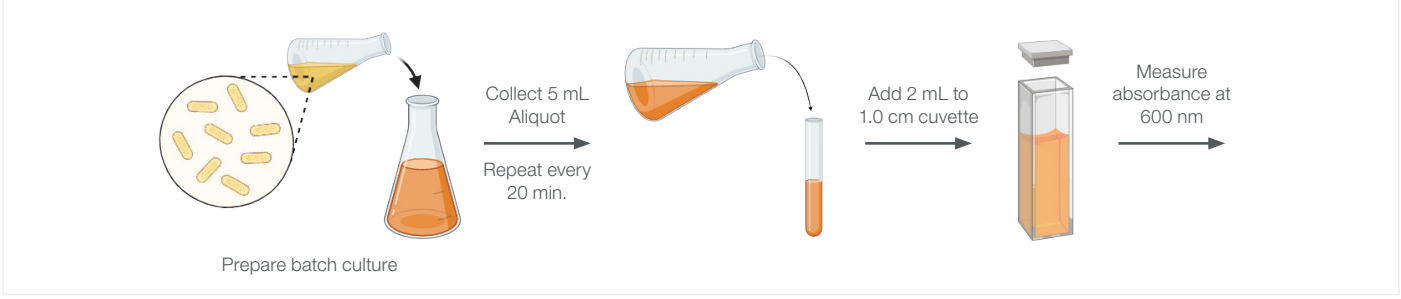


**Figure 3. General OD 600 procedure from Part C. Created with BioRender.com.**

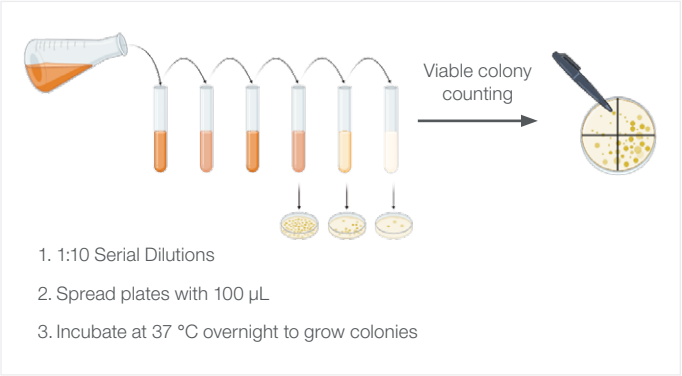


**Figure 4. Viable cell counting procedure. Created with BioRender.com.**

### Part D – Viable Colony Counting

D1. Using the plates spread from Part C, count the number of viable colonies in each plate which can be counted for each time point. It can be helpful to use a marker to count each colony on the bottom of the plate to avoid re-counting errors. Consult your instructor for other helpful methods for keeping track of the colony number (*e.g.*, count in batches, use a handheld counter, etc.)

   a. If the plate has too many colonies, use a marker to divide the plates into quadrants. Count one quadrant and multiply by 4 to estimate the total number of colonies on the plate (See Figure 4).

D2. Back-calculate how many colonies were present in the diluted solution for each countable plate. Record your results in Table 2.

D3. Determine the bacterial cell count for each time point in CFU/mL.

# Lab Report

## Results Tables

| Growth Time (min) | Absorbance at 600 nm (A.U.) | Growth Time (min) | Absorbance at 600 nm (A.U.) |
|---|---|---|---|
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**Table 1. $OD_{600}$ Growth curve data.**

| Growth Time (min.) | Colony Count – Dilution | Colony Count – Stock |
|---|---|---|
| 0 | | |
| | | |
| | | |
| 60 | | |
| | | |
| | | |
| 120 | | |
| | | |
| | | |
| 180 | | |
| | | |
| | | |

**Table 2. Viable bacterial colony count data.**

Based on the data in Table 1 and 2, construct the growth curve for the samples analyzed herein. Include appropriate units, axes labels and significant figures in the graph. Attach the graph to the lab report questions. Include labels for the bacterial growth phases observed.

## Questions

1. From the data collected in this experiment, what phases of growth were you able to observe?

2. What was the doubling time for this strain of *E. coli* calculated using the viable colony counting data, and what was it when calculated using the $OD_{600}$ data? Use the equation below for your analysis:

$$t_D = \frac{ln(2)(t_2 - t_1)}{ln\left(\frac{B_2}{B_1}\right)}$$

*NOTE: Only the data collected in the exponential/log phase will be used for this analysis.*

3. From Part A, what did your negative control look like? Why did we need to make a negative control when streaking plates?

4. Why is it important the agar plates and LB broth have streptomycin?

5. What would you expect to happen to the growth curve if you added 24 mL of the overnight culture instead of 12 mL to the LB broth?

6. What would you expect your growth curve to look like if you incubated the batch culture at 25 ˚C instead of 37 ˚C?

# Notes for Instructors and Teaching Assistants:

Below includes an example of on $OD_{600}$ measurement coupled with a viable cell count for an *E. coli* (strain K12) growth experiment according to the procedure outlined in this lesson plan with the exception of the intervals at which the aliquots were measured. Note, the experimental procedure requires 5 hours to begin observing the end of the exponential/log phase. If your lab period only lasts 3 hours, consider having students compare their data to the figure below or complete the experiment in your lab for the full 5 hours to produce a data set for students to compare their data against.

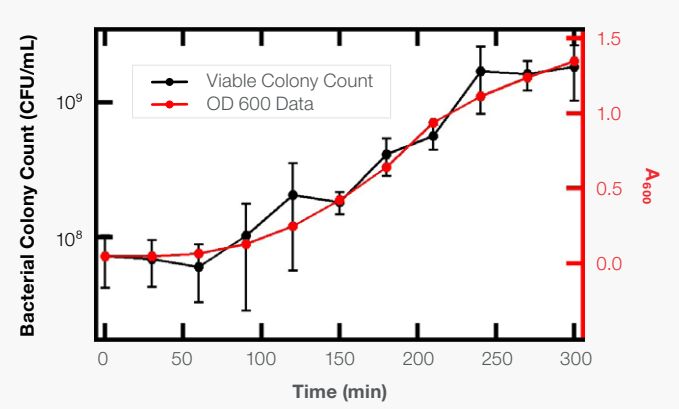


**Figure 5. Example OD 600 curve. The left axis correlates to the black data points (bacterial colony count) and is plotted on a logarithmic scale. The right axis represents the measured absorbance at 600 nm and correlates to the red data points. Both right and left axes were scaled to overlay well on one another.**

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