

# The Growth of Microorganisms

## Course

Medical  
Microbiology

## Unit VI

Concepts of  
Medical  
Microbiology

## Essential Question

How do bacteria  
grow in culture?

## TEKS

130.207(c)2FGH  
4BC

## Prior Student Learning

Intro to  
Microorganisms,  
Understanding  
Binary Fission

## Estimated time

Lab: 50 minutes,

Term review and  
explanations:  
30 minutes

## Rationale

Recognizing the conditions necessary for microbial growth is vital to disease prevention and treatment.

## Objectives

Upon completion of this lesson, the student will be able to:

- Graph the four stages of the bacterial growth curve and identify the four stages
- Identify different requirements for bacterial growth
- Correlate turbidity readings or cell counts to colony growth phases

## Engage

Picture one little dandelion in your yard that grows up, makes the cute little puff ball and you blow it to make a wish (let's say 50). Now each one of those little fuzzy seeds grows up in your yard to make another little puff ball the next week ( $50 \times 50 = 2,500$  seeds). By week three your 50 little plants have turned into 2,500 little plants with now 125,000 seeds. Bacteria work much the same way except each one just makes one new bacterium at a time in a process called binary fission. The upside for them is some can replicate in as little as 20 minutes (not the week our dandelion needed). So how can we track bacterial replication and why do we need to?

## Key Points

### I. Definition of key terms

- A. Binary Fission: the method used for bacterial cell division or replication.
- B. Generation time: the time required for one bacterium to divide into two bacteria, aka doubling time. Can be as fast as 17-20 min for quick growing bacteria or as long as hours or days for slow growing bacteria. (note: these are under 'ideal' conditions in the lab)  
Other common bacteria include:

<i>Escherichia coli</i>	17-20min	common intestinal flora or pathogen
<i>Bacillus megaterium</i>	25 min	common research bacteria used for making artificial penicillin
<i>Streptococcus lactis</i>	26-48 min	used to make buttermilk and cheese
<i>Staphylococcus aureus</i>	27-30 min	common human pathogen
<i>Lactobacillus acidophilus</i>	66-87 min	common intestinal flora ('good' bact.)
<i>Mycobacterium tuberculosis</i>	13-15 hrs	pathogen that causes Tuberculosis
<i>Treponema pallidum</i>	33 hrs	pathogen that causes Syphilis

- C. Logarithmic growth: The period of bacterial growth during which cells divide at a constant rate

- D. Thermophiles: Bacterium that grow best at high temperatures like 50° to 60°C (often found in the environment near hot springs)
  - E. Mesophiles: a Bacterium that grows best at moderate temperatures like 20° to 40°C (Human body temperature is 37°C) It can be diagnostically significant if a bacteria or fungal culture can grow at reduced temperatures (25°C) or increased temperatures (42°C)
  - F. Psychrophiles: Bacterium that grow best at cold temperatures like 10° to 20°C (often found in the environment near arctic seas)
  - G. Obligate Aerobe: a bacterium that requires Oxygen for growth
  - H. Obligate Anaerobe: bacterium that cannot grow in the presence of Oxygen
  - I. Facultative anaerobes: a bacterium that grows either with or without Oxygen like *E. Coli*
  - J. Microaerophilic: a bacterium that requires a reduced level of Oxygen to grow like *Campylobacter sp.*
  - K. Canophilic: a bacterium that grows better in the presence of increased Carbon Dioxide (CO<sub>2</sub>) like *H. influenzae*
- II. Bacterial growth
- A. The lag phase – when the bacteria are getting ready to divide
  - B. The logarithmic or exponential phase – growth of bacteria
  - C. The stationary phase – when bacterial growth begins to slow
  - D. The death phase – cell death due to the depletion of nutrients, crowding from growth, and drop in pH
- III. Factors influencing bacterial growth
- A. Temperature -- microorganisms grow over a broad range of temperatures but the optimal growth temperature for most medically important bacteria is 20 – 45C
  - B. Gaseous composition of the atmosphere – see terms above for aerobic, anaerobic, facultative anaerobic, microaerophilic, and capnophilic
  - C. Neutral pH is optimal – most media will be a 7 or 7.5 pH
  - D. Nutritional requirements – all human pathogenic bacteria are heterotrophs and require outside nutrients. Some can use a wide variety of organic compounds as carbon sources; others are fastidious and require additional metabolites, vitamins, purines and pyrimidines such as *Haemophilus influenzae*. (some must be cell cultured, like *Chlamydia*, or will not grow in culture at all due to nutritional needs)

- E. Moisture -- water is necessary for the metabolic activities of microorganisms
- F. Salt requirements -- some microorganisms are high-salt tolerant

#### IV. Determining Bacterial Growth

- A. Measuring cell mass
  - 1. Cell weight
  - 2. Turbidity – as bacteria grow in a culture broth, the broth becomes cloudy. Turbidity can be measured with a spectrophotometer and a growth curve can be identified by charting data on graph paper.
- B. Measurement of cell number.
  - 1. Direct cell counts under a microscope – good estimate but does not differentiate between live and dead cells
  - 2. Direct plate count – by growing dilutions of broth cultures on agar plates one can determine the number of colony-forming units per mL and provides a count of viable cells only (unit used is CFU/mL) Bacterial growth measurements are important in urine cultures and are useful in food and environmental micro.)
  - 3. Density measurement – using a sample of a culture in Log Phase can be correlated to CFU/mL of the culture and is often used to prepare dilutions for antimicrobial susceptibility testing.

#### Activity

- I. Complete the Growth of Microorganism Key Terms.
- II. Complete the Bacteria Growth Curve Laboratory Investigation.
- III. Complete alternate paper lab – group activity to show growth.
- IV. Use a graphic organizer to sort bacterial growth times and temperatures to get students more familiar with the terms.

#### Assessment

Laboratory Investigation Rubric

#### Materials

The Growth of Microorganisms Key Term Definitions

Spectrophotometer (Ask AP Biology or Chemistry instructor)

Nonpathogenic Escherichia coli cultures (other cultures may be used)

Broth tubes

Incubator at 37°C

Graph Paper

Inoculating loops

Bunsen Burner

Gloves

Goggles

Laboratory coats or aprons

Biohazard container  
Surface disinfectant  
Paper towels

*Microbiology for Health Careers*, e. Fong, et al., Delmar publishers, 5th edition; chapter 12

Great Resource for the Instructor:

<http://www.umsi.edu/~microbes/introductiontobacteria.pdf>

### **Accommodations for Learning Differences**

For reinforcement, the student will draw a diagram showing the typical growth curve of a bacterial population. Explain the processes that occur in each phase and factors that play a role.

For enrichment, the student will research and report on why antibiotics must be taken for ten days and the consequences of noncompliance.

### **National and State Education Standards**

National Healthcare Foundation Standards and Accountability Criteria  
Foundation Standard1

1.32 Analyze diagrams, charts, graphs and tables or interpret healthcare results.

Foundation Standard 7

7.12 Describe the methods of controlling the spread and growth of microorganisms.

7.31 Apply safety techniques in the work environment.

### **TEKS**

130.207 (c)2(F) Collect and organize qualitative and quantitative data and make measurements with accuracy and precision using tools such as calculators, spreadsheet software, data-collecting probes, computers, standard laboratory glassware, microscopes, various prepared slides, stereoscopes, metric rulers, electronic balances, hand lenses, Celsius thermometers, hot plates, lab notebooks or journals, timing devices, Petri dishes, lab incubators, dissection equipment, meter sticks, and models, diagrams, or samples of biological specimens or structures;  
(G) Analyze, evaluate, make inferences, and predict trends from data;  
(H) Communicate valid conclusions supported by the data through methods such as lab reports, labeled drawings, graphic organizers, journals, summaries, oral reports, and technology-based reports.

130.207 (c)4(B) Identify chemical processes of microorganisms;  
(C) Recognize the factors required for microbial reproduction and growth;

## **Texas College and Career Readiness Standards**

### **Science Standards**

1. Nature of Science: B3 Demonstrate skill in the safe use of a wide variety of apparatuses, equipment, techniques and procedures.
2. Foundation Skills: C2 Understand that a curve drawn on a defined set of axes is fully equivalent to a set of algebraic equations.
6. Biology: F1 Know that organisms possess various structures and processes (feedback loops) that maintain steady internal conditions.

### **Cross-Disciplinary Standards**

1. Foundational Skills: D1 Identify patterns of departures from patterns among data. D3 Present analyzed data and communicate findings in a variety of formats.

## The Growth of Microorganisms Key Terms

Binary Fission	The method used for bacterial cell division or replication
Canophilic	A bacterium that grows better in the presence of increased Carbon Dioxide (CO <sub>2</sub> ) like <i>H. influenzae</i>
Death phase	Cell death due to the depletion of nutrients, crowding from growth, and drop in pH
Facultative anaerobes	A bacterium that grows either with or without Oxygen like <i>E. Coli</i> .
Generation time	The time required for one bacterium to divide into two bacteria, aka doubling time. Can be as fast as 17-20 min for quick growing bacteria or as long as hours or days for slow growing bacteria. (Note these are under 'ideal' conditions in the lab)
Lag phase	When the bacteria are getting ready to divide
Logarithmic growth	The period of bacterial growth during which cells divide at a constant rate
Logarithmic or exponential phase	Growth of bacteria
Mesophiles	A bacterium that grows best at moderate temperatures like 20° to 40° C. (Human body temperature is 37° C) It can be diagnostically significant if a bacteria or fungal culture can grow at reduced temperatures (25° C) or increased temperatures (42° C)
Microaerophilic	A bacterium that requires a reduced level of Oxygen to grow like <i>Campylobacter sp.</i>
Moisture	Water is necessary for the metabolic activities of microorganisms
Obligate Aerobe	A bacterium that requires Oxygen for growth
Obligate Anaerobe	Bacterium that cannot grow in the presence of Oxygen
Psychrophiles	Bacterium that grow best at cold temperatures like 10°to 20°C (often found in the environment near arctic seas)
Stationary phase	When bacterial growth begins to slow
Temperature	Microorganisms grow over a broad range of temperatures but the optimal growth temperature for most medically important bacteria is 20 – 45C
Thermophiles	Bacterium that grow best at high temperatures like 50°to 60°C (often found in the environment near hot springs)
Turbidity	As bacteria grow in a culture broth, the broth becomes cloudy. Turbidity can be measured with a spectrophotometer and a growth curve can be identified by charting data on graph paper.

## The Growth of Microorganisms Key Terms

Binary Fission	
Canophilic	
Death phase	
Facultative anaerobes	
Generation time	
Lag phase	
Logarithmic growth	
Logarithmic or exponential phase	
Mesophiles	
Microaerophilic	
Moisture	

Obligate Aerobe	
Obligate Anaerobe	
Psychrophiles	
Stationary phase	
Temperature	
Thermophiles	
Turbidity	

# BACTERIA GROWTH STUDIES

NAME: \_\_\_\_\_

DATE: \_\_\_\_\_

## PURPOSE:

In this laboratory investigation, the student will graph the growth curve and identify the four stages.

## BACKGROUND INFORMATION:

## MATERIALS:

TSB of Nonpathogenic *Escherichia coli*  
Inoculating loop (metal reusable or plastic disposable)  
Spectrophotometer  
Black markers  
Test tube rack  
2 tubes of TSB  
Gloves  
Goggles  
Lab coat or apron  
37°C Incubator  
Bunsen burner (not needed with plastic loops)  
Surface disinfectant  
Paper towels

## PROCEDURE:

1. Students should work in groups of two.
2. Wash hands and put on gloves.
3. Assemble equipment and materials and prepare work area.
4. Set the spectrophotometer at 450nm and let it warm up 30 minutes before performing the readings. The meter on the spectrophotometer should read "0% Transmittance."
5. Obtain 2 tubes of TSB. Label one C (un-inoculated) for control and one S (inoculated) for sample.
6. Place tube C into the sample holder of the spectrophotometer. Turn the light control knob until the meter needle is on "100% Transmittance," Remove the control tube. The spectrophotometer is now standardized.
7. Flame and cool the loop.
8. Mix the culture of *E. coli* and inoculate tube S with 5 loop-fulls of the broth.
9. Flame and cool the loop.

10. Start the turbidity readings by placing the S tube into the sample well and read the OD (absorbance). Record the results in the data section.
11. Take OD reading over the time period specified in the data section and return the TSB tube to the 37 C incubator between each reading.
12. Turn off the spectrophotometer upon completion of the readings.
13. Plot the readings on the graph paper as absorbance vs. time.
14. Clean work area with disinfectant.
15. Remove gloves and wash hands with disinfectant.

**DATA:**

Record the absorbance value for each sample reading starting at 0 time.

TIME (MIN)	ABSORBANCE
0:00	
5:00	
10:00	
15:00	
20:00	
25:00	
30:00	
35:00	
40:00	

**CONCLUSIONS:**

1. Plot the absorbance values vs. time on graph paper. Label the x and y axis. Each of the readings will represent the growth of the *E. coli* in the culture.
2. Label each part of the growth curve: Lag phase, Exponential growth phase, and the Stationery phase.
3. Why is the Death phase not represented on graph?
4. What is the correlation between the turbidity of the broth and the growth of the bacteria?
5. Why do you use an un-inoculated tube to standardize the spectrophotometer?

## Laboratory Investigation Rubric

Student: \_\_\_\_\_ Date: \_\_\_\_\_

Scoring Criteria	4 Excellent	3 Good	2 Needs Some Improvement	1 Needs Much Improvement	N/A
Problem is appropriately identified.					
Problem is precise, clear, and relevant.					
Association between the problem and the predicted results is direct and relevant.					
All variables are clearly operationalized.					
Demonstrates comprehension of the use of scientific concepts and vocabulary.					
All significant data is measured.					
Data is recorded effectively and efficiently.					
Data table is well designed to the task requirements.					
All graphs are appropriate.					
All data accurately plotted.					
Graph visually compelling; highlights conclusions of the study.					
Conclusion relates directly to the hypothesis.					
Conclusion has relevancy in resolution of the original problem.					
Conclusion relates the study to general interest.					

## **“Paper” Bacterial Growth Activity**

### **Procedure:**

1. Have one student be the starting '*E. coli*' and 'divide' by handing a sheet of paper to the next student. Clay or candy could be substituted for the paper.
2. Student 2 'divides' by tearing the paper into smaller and smaller pieces.
3. Repeat steps 1 and 2 taking 'measurements' at timed intervals.
4. Consider counting pieces, photographing torn pieces in one location or putting them into a cylinder to visualize.
5. The data can be used to continue the graphing portion of the lab as a class or in pairs.

### **Student Data and Conclusions:**

#### **DATA:**

Record the absorbance value for each sample reading starting at 0 time.

TIME (MIN)	Number of 'cells'
0:00	
5:00	
10:00	
15:00	
20:00	
25:00	
30:00	
35:00	
40:00	

#### **CONCLUSIONS:**

1. Plot the number of cells vs. time on graph paper. Label the x and y axis. Each of the readings will represent the growth of the bacteria in the culture.
2. Label each part of the growth curve: Lag phase, Exponential growth phase, and the Stationery phase.
3. Why is the Death phase not represented on graph?
4. When testing live bacteria we use a spectrophotometer and bacteria growing in a broth solution. Just like our paper version the bacteria replicate in the broth and the more bacteria the cloudier the solution. What is the correlation between the turbidity of the broth and the growth of the bacteria?
5. Why do you use an un-inoculated tube to standardize the spectrophotometer?

