EXERCISE 20

Determination of a Bacterial Growth Curve: The Role of Temperature

Objectives

After completing this exercise, you should be able to:

1. Identify the four phases of a typical bacterial growth curve.
2. Measure bacterial growth turbidimetrically.
3. Interpret growth data plotted on a graph.
4. Determine the effect of temperature on bacterial growth.

Background

Most bacteria grow within a particular temperature range (Figure 20.1). The *minimum growth temperature* is the lowest temperature at which a species will grow. A species grows fastest at its *optimum growth temperature*. And the highest temperature at which a species can grow is its *maximum growth temperature*. Some heat is necessary for growth. Heat probably increases the rate of collisions between molecules, thereby increasing enzyme activity. At temperatures near the maximum growth temperature, growth ceases, presumably due to inactivation of enzymes.

The effect of temperature on bacteria can be determined by measuring the population growth rate. The phases of growth of a bacterial population are shown in the figure on page 140. During log phase, the cells are growing at the fastest rate possible under the conditions provided. **Generation or doubling time** is the time it takes for one cell to divide into two cells—or the time required for the population of cells to double. The shorter the generation time, the faster the growth. The growth of a bacterial population can be determined by inoculating a growth medium with a few cells and counting the cells over time. However, bacteria in suspension in a broth scatter light and cause the transparent broth to appear turbid. This turbidity can be measured with a spectrophotometer (Appendix C) to determine bacterial growth. Although turbidity is not a direct measure of bacterial numbers, increasing turbidity does indicate growth.

Changes in the logarithmic absorbance scale on the spectrophotometer correspond to changes in the number of cells. Consequently, a growth curve can be obtained by graphing absorbance (Y-axis) vs. time (X-axis). The rate of growth is indicated by the slope of the lines; faster growth produces a steeper (higher-number) slope (Figure 20.2).

In this exercise, we will plot growth curves for a bacterium at different temperatures to determine the preferred temperature range of this species.

Materials

Flask containing nutrient broth

Spectrophotometer tubes (2)

Sterile 5-ml pipettes (11)

Sterile 10-ml pipette

Spectrophotometer

Culture

Escherichia coli

Techniques Required

Aseptic technique, Exercise 10

Pipetting, Appendix A

Spectrophotometry, Appendix C

Graphing, Appendix D

Procedure

Each student group is assigned a temperature: 15°C, room temperature, 35°C, 45°C, or 55°C.

1. Aseptically transfer 2–4 ml of nutrient broth to one of the spectrophotometer tubes. This is your control to *standardize* the spectrophotometer. Read Appendix C.
2. Aseptically inoculate a flask of nutrient broth with 10 ml of *E. coli*.

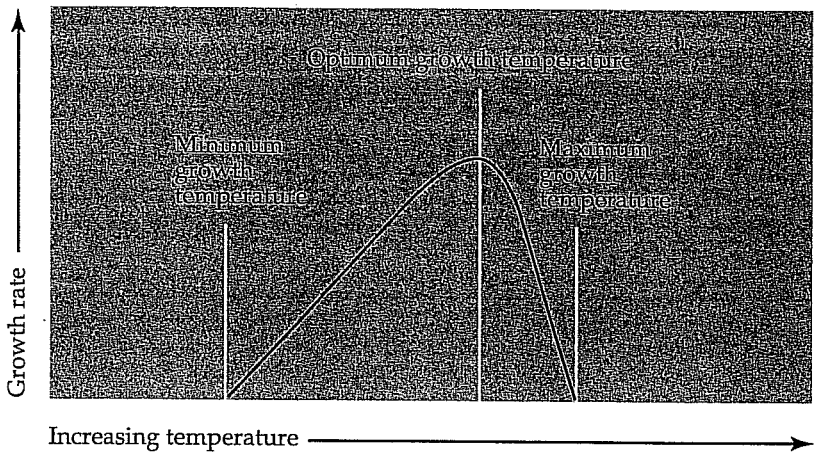


Figure 20.1
Growth response of bacteria within their growing temperature range.

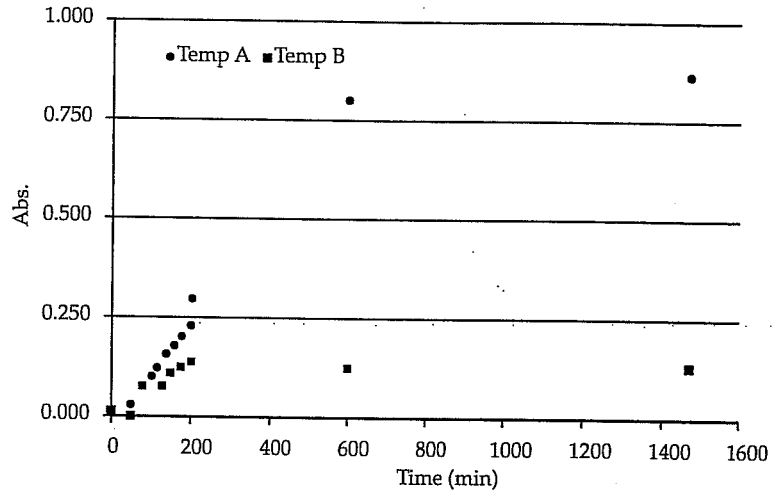
3. Swirl to mix the contents. Transfer 2–4 ml from the flask to the second spectrophotometer tube. Wipe the surface of the spectrophotometer tube with a low-lint, nonabrasive paper such as a Kimwipe. Place the tube in the spectrophotometer, wait about 45 sec, and measure the absorbance (Abs.) on the spectrophotometer.
4. Record all measurements.

After taking a reading, empty the spectrophotometer tube into disinfectant. Rinse the spectrophotometer tube with distilled water and discard the water into disinfectant.

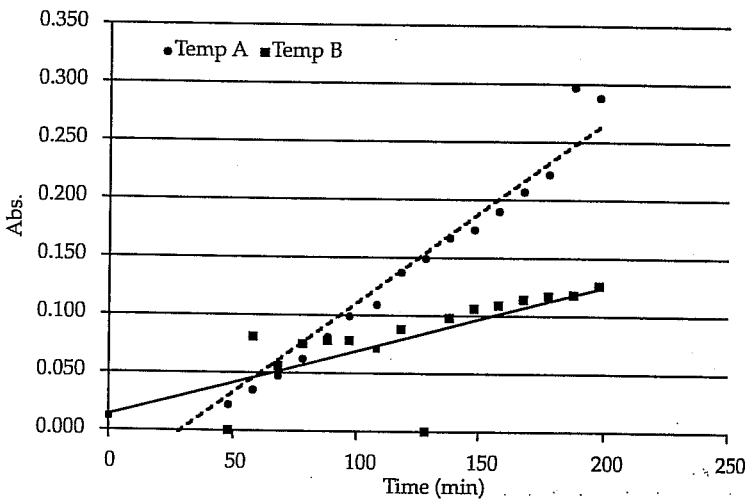
5. Place the flask at your *assigned* temperature.
6. Record the absorbance every 10 minutes for 60 to 90 minutes. Remove the flask from the water bath for the minimum time possible to take each sample. To take a sample, aseptically pipette 2–4 ml into the second spectrophotometer tube.
7. Graph the data you obtained. *Read* Appendix D before drawing your graph.
8. Determine the generation time. Select two points, (*a*) and (*b*), in the log phase during which the absorbance doubled and determine the number of minutes required for the culture to go from (*a*) to (*b*). See Figure 20.2.

Time (min)	Temp A	Temp B
0	0.011	0.012
48	0.022	*a* 0.045
58	0.035	0.081
68	*a* 0.047	0.056
78	0.062	0.075
88	0.081	0.078
97	*b* 0.099	0.078
108	0.109	0.071
118	0.137	0.088
128	0.149	*b* 0.089
138	0.167	0.098
148	0.174	0.106
158	0.190	0.109
168	0.207	0.114
178	0.222	0.117
188	0.297	0.118
198	0.288	0.126
600	0.800	0.125
1470	0.864	0.130
Slope between *a* and *b*	0.0018	0.0004
Generation time (min)	29	80

(a) Generation times. To determine the generation time. Select two points, (*a*) and (*b*), in log phase growth during which the absorbance doubled. Determine the time required for the culture to double. Use your graphing application to calculate the slope of the line. A higher (steeper) slope indicates faster growth.



(b) A graph of all the data shows logarithmic and stationary phases.



(c) A graph of the first three hours expands the log phase. You can compare the growth by comparing the slopes of the best-fit lines.

Figure 20.2

Showing bacterial growth using absorbance values. (a) The generation time is the number of minutes required for the absorbance to double. (b) Plotting all the data shows when the cultures reach stationary phase. (c) This graph is used to examine log phase. The best-fit lines show the rate at which the population is growing.

Exercise 20

LABORATORY REPORT

Determination of a Bacterial Growth Curve: The Role of Temperature

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Record your data below or in a computer application spreadsheet.

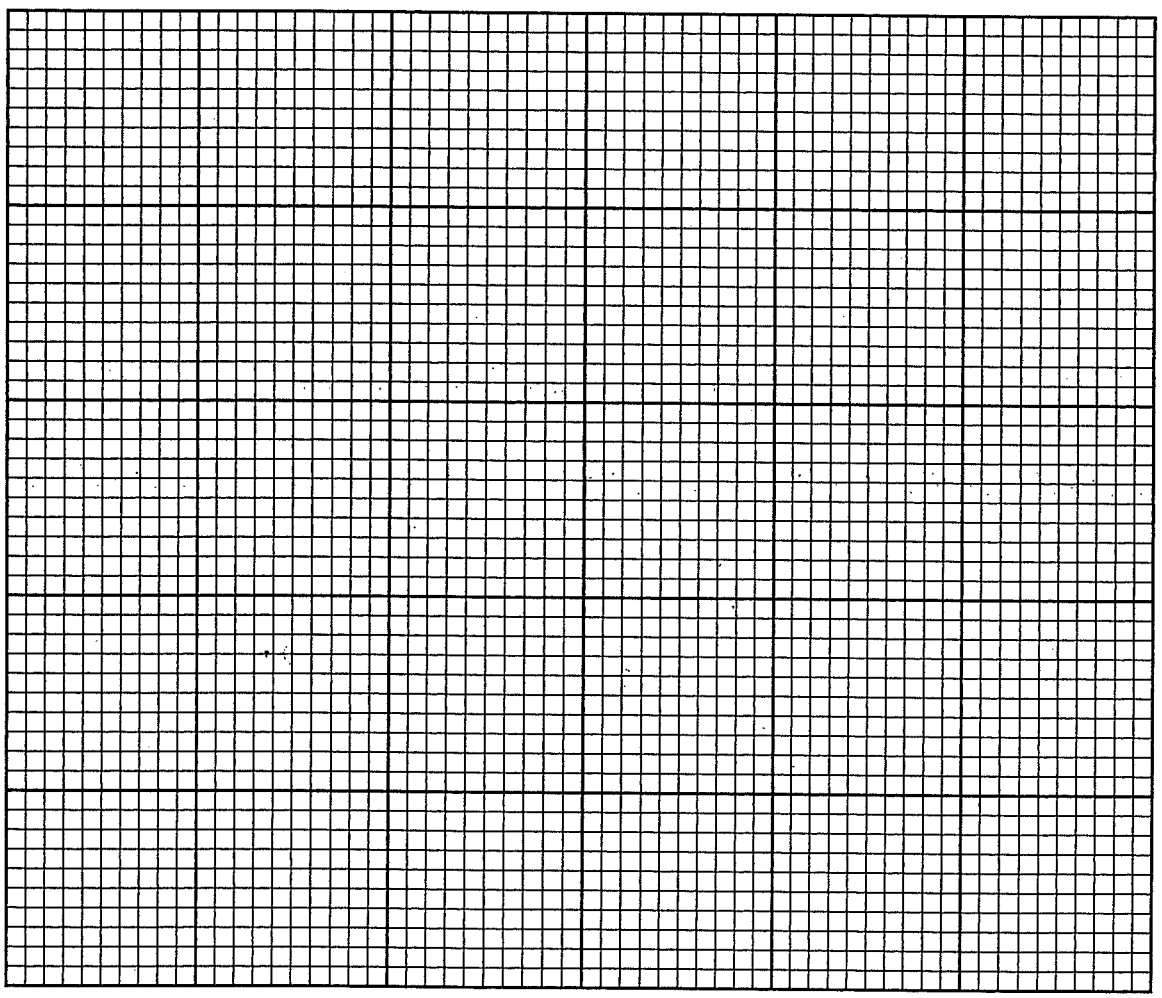
Temperature: _____

Time (min)	Absorbance	Time (min)	Absorbance
0			

Plot your data on graph paper or use a computer graphing application. Mark absorbance on the Y-axis and time on the X-axis. Obtain data for other temperatures from your classmates. Plot these data on the same graph using different lines or colors. Label the phases of growth.

Use your data and that of your classmates to fill in this table:

Temperature	Doubling Time (min)



Conclusions

1. Summarize the effect of temperature on growth of *E. coli*. _____

_____

_____

_____

2. From the class data, what is *E. coli*'s optimum temperature? _____ Does this agree with your textbook? _____

If not, provide a brief explanation. _____

Questions

1. Why aren't you likely to get lag and death phases in this experiment? _____

_____

_____

2. What is the optimum growth temperature for human pathogens? _____

3. Where in nature would you most likely find a thermophile? _____

A psychrophile? _____

4. What is the effect of temperature on enzymes? _____

_____

Can you use any examples from your experiments? _____

Critical Thinking

1. The following graph shows a likely relationship between bacterial growth and oxygen use in glucose broth. Explain the relationship.