An In-class Growth Curve Critical-Thinking Active-Learning Activity

Resource Type: Curriculum: Classroom

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Abstract

This is a two-part paper-based in-class activity for a class of any size that deals with the concept of growth curves. Given a set of data, students should be able to plot a growth curve and calculate the doubling time and growth rate. Students then predict how the growth rate will be affected by changing nutrients, oxygen, and physical conditions (such as pH, temperature, and salt). Students will demonstrate that they have successfully learned the exercise objectives when they can plot the growth curve, calculate the growth rate, and predict the changes in the growth curve given new information. The understanding of the growth curve concept may be assessed by exam questions.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Objectives.

Following this exercise the students will be able to:

1. use an appropriate data set to plot a growth curve.
2. calculate growth rate and generation time.
3. predict the effects of changing nutrients, oxygen, and physical conditions (such as pH, temperature, and salt).

Background.
Student prerequisite knowledge and skills:

- Binary fission
- Logarithms and logarithmic graph paper
- Standard plate count and colony-forming units
- Factors affecting growth
- Metabolism

These concepts will also be discussed in class.

PROCEDURE

Materials.
Handouts—Student and Instructor versions.

Student version.
Student Handout Part 1: Calculating Growth Rate and Doubling (Generation) Time
Student Handout Part 2: Predicting the Effects of Changing Conditions on Growth Rate

Instructor version.

1. Briefly discuss how *Escherichia coli* has been used as a model organism. *E. coli* is undoubtedly the most widely studied of the bacteria. Many of the classical experiments in microbiology were conducted using this organism because it grows quickly, has few nutritional requirements, and is metabolically versatile. Studying how microorganisms grow under varying conditions can provide information not only on how to control their growth, but about the physiological or molecular processes that occur as the environmental conditions change.

Bacterial growth is affected by many different factors, including nutrients, temperature, pH, and salt. In this experiment, you will be looking at the effects of a given environmental parameter on the growth of *E. coli.*

One method used to demonstrate bacterial growth is the growth curve. This involves measuring the increase in the number of cells over time. The number of cells can be measured using either serial dilution and plate counts or by measuring the absorbance (or optical density) of a broth culture.* In the first method, the number of cells would be reported as colony-forming units per milliliter of culture (CFU/ml). Optical density is unit-less. In either case, when measured over time, the data points can then be plotted on semilog paper. When plotted on semilog paper, you can easily calculate the doubling time and growth rate. In this exercise the number of CFU/ml are provided.

*It should be acknowledged that plate counts reflect the number of viable cells in the culture. When using optical density the total number of cells, whether viable or not, are measured.

2. Use as an in-class activity or as homework.

Part 1. Student Handout Part 1
This activity allows students to practice how doubling time and specific growth rate is calculated using an exponentially growing culture of *E. coli.* After a brief discussion about the use of semilog paper to plot exponentially increasing numbers as a linear function, have students work individually or in small groups to complete Student Handout Part 1.
On this handout, students use data representing the change in the number of viable cells in a logarithmic phase *E. coli* culture over time, they plot the data on the semilog paper provided, draw a straight line connecting the data (it is linear), and use the graph and instructions provided to calculate the doubling time and specific growth rate. Instructions are also provided for calculating the doubling time using mathematics. Either or both methods can be used.

Part 2. Student Handout Part 2
Student handout 2 asks students to use the growth curve they drew and predict how it will change if the growth conditions change. Instructors can use all or some of these options.

On semilog paper, an exponentially-growing population of bacteria is shown by a straight line. Optimal growth rate is represented by the line with the steepest slope. When conditions change (increasing salinity, increase or decrease of temperature) to less than optimal, the slope of the line decreases.

Safety Issues. Not applicable.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
Students will demonstrate that they have successfully learned the exercise objectives when they can plot a growth curve, calculate the growth rate, and predict the changes in the growth rate given new data sets.
Assessment would include giving similar questions on a later quiz or exam. If used as a homework assignment, then points for the exercise may be given.

Field Testing.
This activity has been tested in medium (30 to 50 students) and large (>100 students) classes. Primarily, nonmajors or allied health majors were in these classes. The feedback has been positive. On tests where this material was the principle topic being assessed, students scored an average of 77% (n > 100 students). During the lecture when this exercise was used, student comments included:
"I thought that it was fun to do the sample problems, the logarithms, and to talk about the Great Salt Lake. It was good that you asked open-ended questions and had us apply the different bacterial conditions (such as temperature, pressure, pH, etc.) when asked if we were to design a model that would enable the bacteria to have exponential growth."
"To make today's class/lecture better I would suggest to eliminate the repeat discussion on growth rate/doubling rate. The handout was sufficient for practice."
"I really liked the group work we did today. It helped us talk it out and understand the math procedures behind finding doubling time and the number of cells present in the bacteria."

SUPPLEMENTARY MATERIALS

References.

Ingraham, J. 1987. Effect of temperature, pH, water activity, and pressure on growth. *In* F.C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. ASM Press, Washington, D.C.

Appendices and Answer Keys.
Answer Key

PART 1: Calculating Growth Rate and Doubling (Generation) Time Using Semilog Graph Paper

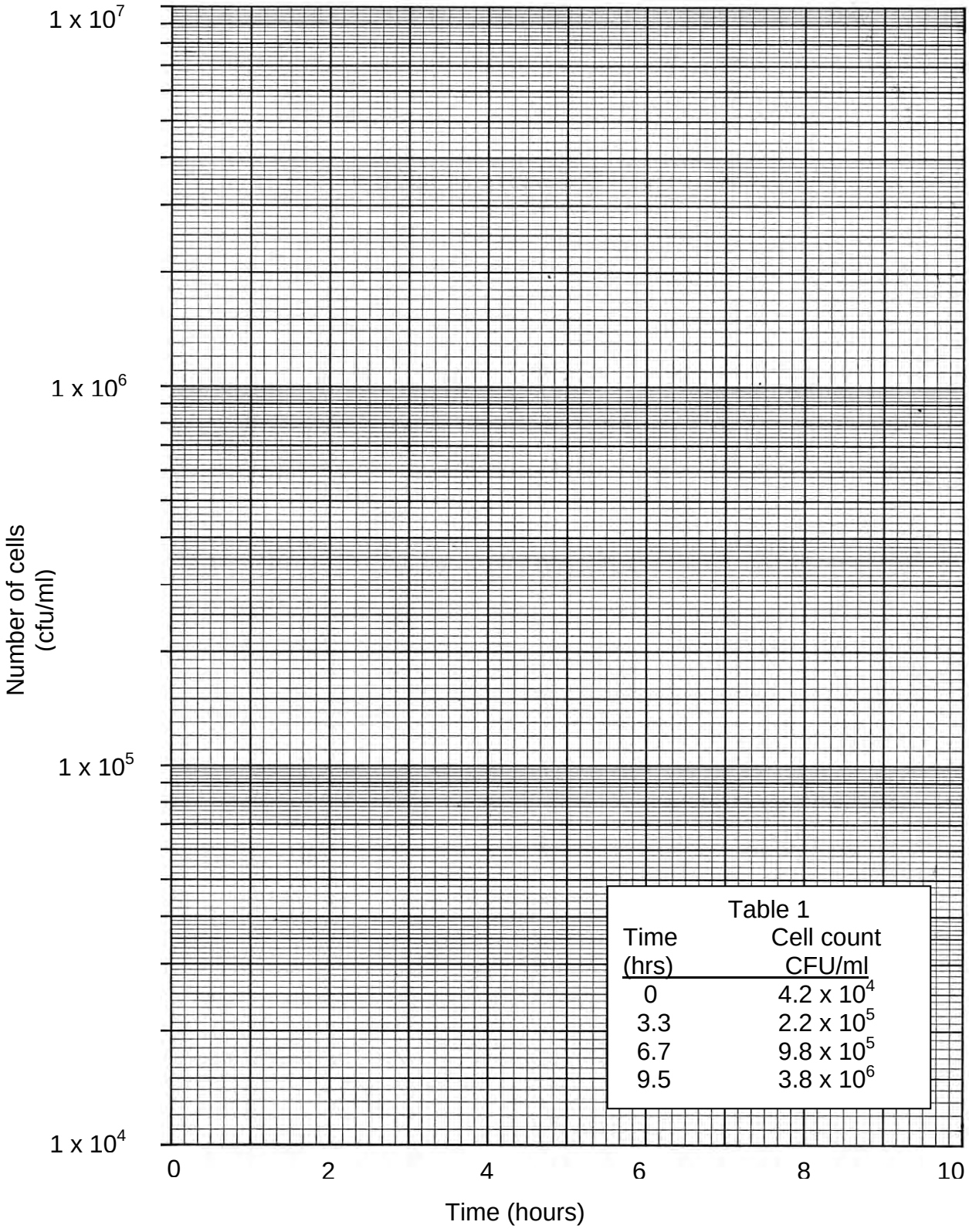

Table 1

Time (hrs)	Cell count CFU/ml
0	4.2×10^4
3.3	2.2×10^5
6.7	9.8×10^5
9.5	3.8×10^6

PART 1: Calculating Growth Rate and Doubling (Generation) Time Using Semilog Graph Paper

Bacterial growth is affected by many different factors, including nutrients, temperature, pH, and salt. In this experiment, you will be looking at the effects of a given environmental parameter on the growth of *Escherichia coli*.

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*It should be acknowledged that plate counts reflect the number of viable cells in the culture. When using optical density the total number of cells, whether viable or not, are measured.

DETERMINING THE GENERATION TIME

1. Plot the data from Table 1 using the graph paper provided.
2. Draw a straight line to fit the data.
3. Choose a value representing the number of cells in the culture (e.g., 8×10^4 CFU/ml).
4. Determine the time at which this number of cells was attained.

   How much time is represented by each division?

5. Double the value in step 3 and determine the time at which that number of cells was reached. (i.e., 1.6×10^5 CFU/ml for our example).
6. The difference between these times is the doubling time or the generation time. In other words, it is the amount of time required for the population of cells to double in number. Record the doubling time in the space below.

DETERMINING THE GROWTH RATE

The growth rate is defined as the number of doublings per unit time (most often this is generations per hour). Hence, if the generation time is the time taken to double the number of cells, the growth rate is the reciprocal of the doubling time. For example, if the doubling time were 90 minutes or 1.5 hours/generation, the growth rate would be ~0.67 gen/h.

USING MATH TO CALCULATE GROWTH RATE AND DOUBLING TIME

First calculate the number of doublings that have occurred during the time period of the experiment:

$$\text{\# doublings} = \frac{\log_{10}[3.8 \times 10^6] - \log_{10}[4.2 \times 10^4]}{\log_{10}2}$$

(Notice that $\log_{10}2$ is 0.301.)

Then divide the number of doublings by the time taken to grow that number of cells (i.e., 9.5 hours). This is the growth rate (generations/hour). The doubling time is then the reciprocal of the growth rate. That is, doubling time = 1/growth rate.

The following example of another experiment might help you check your math. Suppose 3×10^4 bacteria were inoculated into a culture and incubated for 24 hours. After incubation, the number of cells was determined to be 6×10^7 cells. Calculate the growth rate and the doubling time.

$$\text{\# doublings} = \frac{\log_{10}[6 \times 10^7] - \log_{10}[3 \times 10^4]}{\log_{10} 2} = \frac{7.778 - 4.477}{0.301} = \frac{3.301}{0.301} = 10.967 \text{ doublings}$$

growth rate = # doublings/time = 10.967 doublings/24 hours = 0.457 doublings/hour
= 0.457 generations/ hour

doubling time = 1/growth rate = 1/0.457 = 2.19 hours/generation

Part 2: Predicting the Effect of Changing Conditions on Growth Rate

1) Assume that the original growth curve was conducted in broth that had an optimal pH for this organism, pH7. **Predict what the growth curve would look like if you grew cells at pH 6.3. Explain your thinking.**

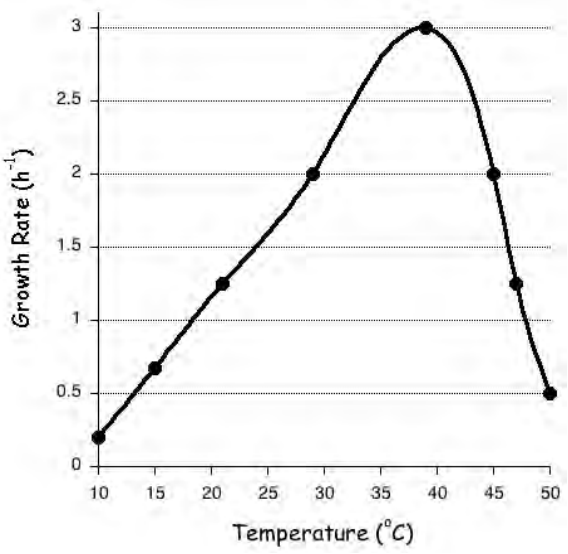
2) Assume that the original growth curve was done at a less than optimal salt concentration, 2%. **Predict what the growth curve would look like at a more optimal salt concentration of 0.5%. Explain your thinking.**

3) Assume that the original growth curve was done using a well-aerated medium. **Predict what the growth curve would look like if the culture was grown under anoxic conditions. Explain your thinking.**

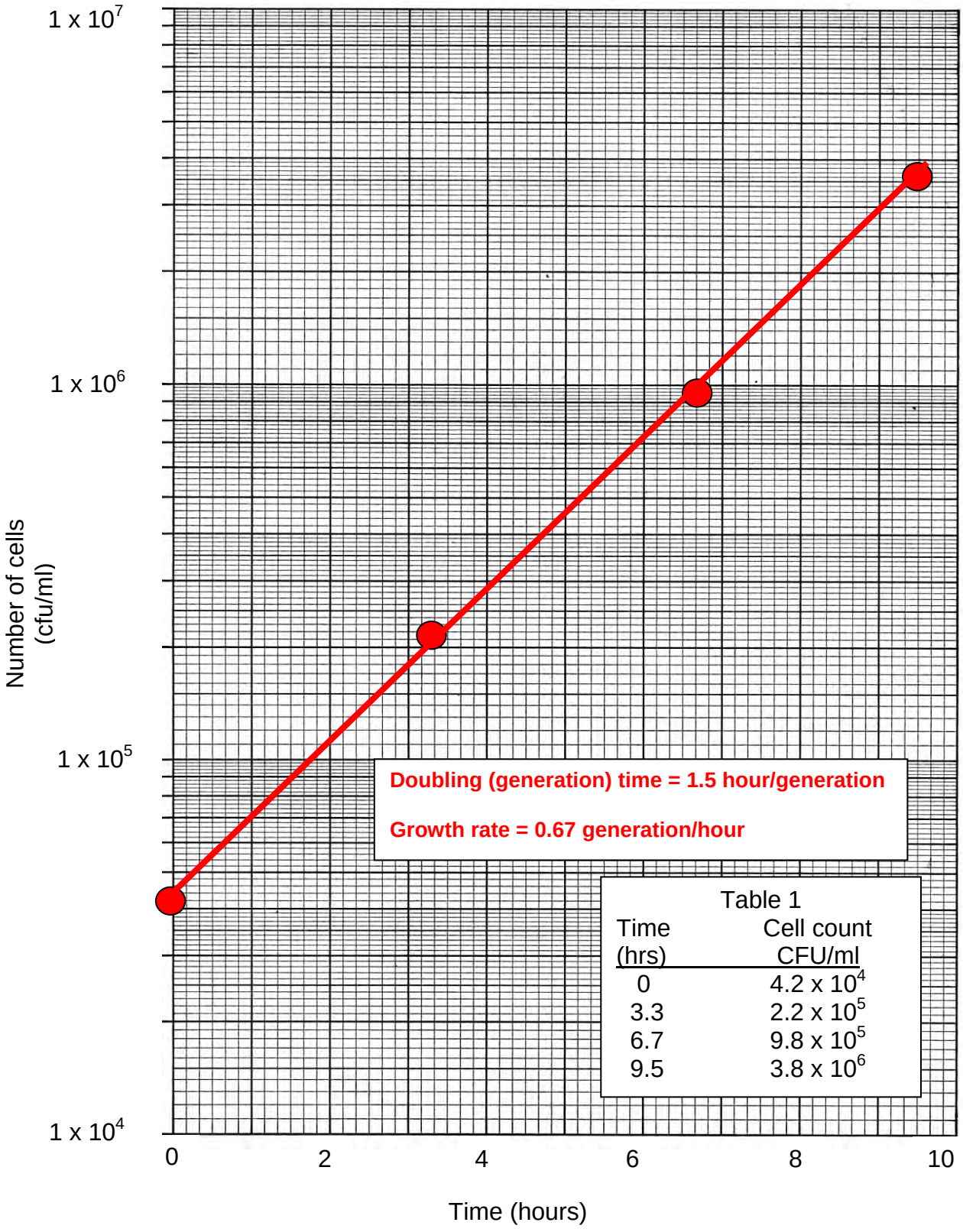
4) Assume that the original growth curve was done using a rich, high nutrient medium like Luria-Bertani broth. **Predict what the growth curve would look if the culture was grown in a defined, minimal salts medium (with glucose). Explain your thinking.**

5) Growth rates provide useful information because they can tell us something about optimum growth conditions. For example, if you were to plot a series of growth curves at various temperatures and calculate the growth rates, you could generate a graph similar to the one at the right.

   Going back to your original growth curve, was it obtained at the optimal temperature for this organism? If yes, explain how you knew. If not, can you tell at what temperature the growth curve was done?



Part 1: Calculating Growth Rate and Doubling (Generation) Time Using Semilog Graph Paper



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Part 1: Calculating Growth Rate and Doubling (Generation) Time Using Semilog Graph Paper

Bacterial growth is affected by many different factors, including nutrients, temperature, pH, and salt. In this experiment, you will be looking at the effects of a given environmental parameter on the growth of *Escherichia coli*.

One method used to measure bacterial growth is the growth curve. This involves measuring the absorbance (or optical density) of a broth culture over time, then plotting it on semilog paper. From this, you can easily calculate the doubling time and growth rate.

DETERMINING THE GENERATION TIME

1. Plot the data from Table 1 using the graph paper provided.
2. Draw a straight line to fit the data.
3. Choose a value representing the number of cells in the culture (e.g. 8×10^4 CFU/ml)
4. Determine the time at which this number of cells was attained.
5. Double the value in step 3 and determine the time at which that number of cells was reached. (i.e., 1.6×10^5 CFU/ml for our example.)
6. The difference between these times is the doubling time or the generation time. In other words, it is the amount of time required for the population of cells to double in number. Record the doubling time in the space below.

DETERMINING THE GROWTH RATE

The growth rate is defined as the number of doublings per unit time (most often this is generations per hour). Hence, if the generation time is the time taken to double the number of cells, the growth rate is the reciprocal of the doubling time. For example, if the doubling time were 85 minutes or 1.42 hours/generation, the growth rate would be ~0.71 generations/hour.

USING MATH TO CALCULATE GROWTH RATE AND DOUBLING TIME

First calculate the number of doublings that have occurred during the time period of the experiment:

$$\text{\# doublings} = \frac{\log_{10}[3.5 \times 10^6] - \log_{10}[4.2 \times 10^4]}{\log_{10} 2}$$

Then divide the number of doublings by the time taken to grow that number of cells (i.e., 9.5 hours). This is the growth rate (generations/hour).

Part 2: Predicting the Effect of Changing Conditions on Growth Rate

NOTE: The magnitude of the decrease in growth rate for each of these conditions is not important given the level of student for which this exercise was designed.

1) Assume that the original growth curve was conducted in broth that had an optimal pH for this organism, pH 7. **Predict what the growth curve would look like if you grew cells at pH 6.3. Explain your thinking.**
The slope of the growth curve would decrease (get less steep). The cells would not grow as fast at a less than optimal pH because proteins don't function as well and cells use energy to maintain their internal pH at 7.

2) Assume that the original growth curve was done at a less than optimal salt concentration, 2%. **Predict what the growth curve would look like at a more optimal salt concentration of 0.5%. Explain your thinking.**
The slope of the curve would increase (get more steep) because cells would grow faster at a more optimal salt concentration. Proteins and transport processes function more efficiently.

3) Assume that the original growth curve was done using a well-aerated medium. **Predict what the growth curve would look like if the culture was grown under anoxic conditions. Explain your thinking.**
If the medium was poorly aerated, the growth rate would be decreased because cells would be making ATP via fermentation, which is much less efficient than aerobic respiration.

4) Assume that the original growth curve was done using a rich, high nutrient medium like Luria-Bertani broth. **Predict what the growth curve would look like if the culture was grown in a defined, minimal salts medium (with glucose). Explain your thinking.**
In this case, the growth rate would be less because cells would be putting more energy into biosynthesis of building blocks like amino acids and nucleic acids instead of just transporting them from the environment. This leaves less energy for cell division. (see Ingraham, J. 1987. Effect of temperature, pH, water activity, and pressure on growth. In F. C. Neidhardt, (ed.), Escherichia coli and Salmonella typhimurium: cellular and molecular biology, vol. 2. ASM Press, Washington, D.C.).

5) Growth rates provide useful information because they can tell us something about optimum growth conditions. For example, if you were to plot a series of growth curves at various temperatures and calculate the growth rates, you could generate a graph similar to the one at the right.

Going back to your original growth curve, was it obtained at the optimal temperature for this organism? If yes, explain how you knew. If not, can you tell at what temperature the growth curve was done?
The optimal growth rate for this culture was about 3 at 40°C. The growth rate obtained from the original experiment was about 0.67, which occurs at both 15°C and 47°C, so we can't tell for sure.