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How to Measure Growth of a Bacterial Culture: Bacterial Growth Kinetics and Growth Curves

--Manuscript Draft--

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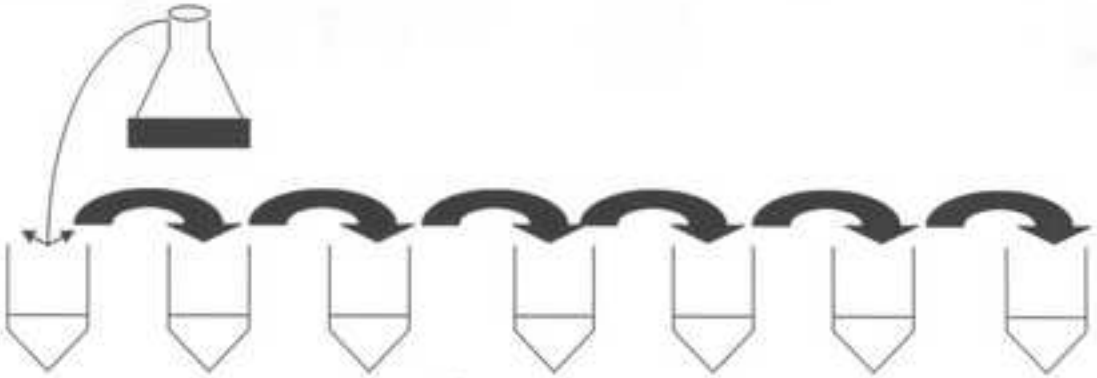
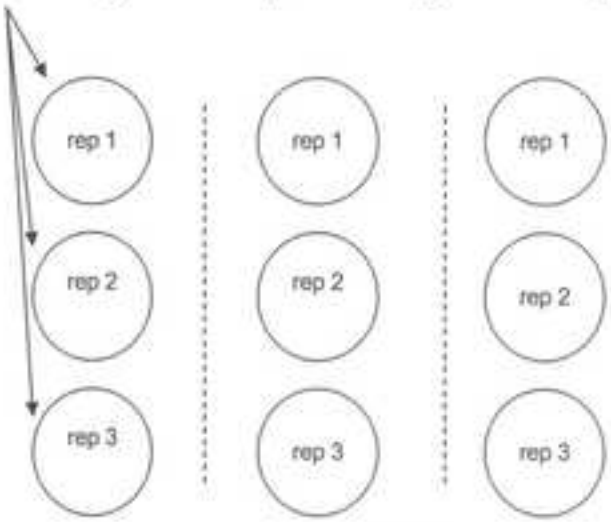
Phase	Characteristics
1. Lag Phase	Slow growth or lack of growth due to physiological adaptation of cells to culture conditions or dilution of exoenzymes due to initial low cell densities.
2. Exponential or Log Phase	Optimal growth rates, during which cell numbers double at discrete time intervals known as the mean generation time.
3. Stationary Phase	Growth (cell division) and death of cells counterbalance each other resulting in no net increase in cell numbers. The reduced growth rate is usually due to a lack of nutrients and/or a buildup of toxic waste constituents.
4. Death Phase	Death rate exceeds growth rate resulting in a net loss of viable cells.

1. Make a 10-fold dilution series:



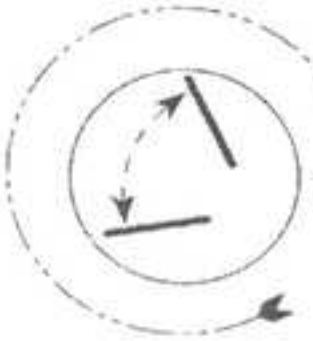
2. For **one** dilution, transfer 0.1 mL of suspension to each plate. After inoculating all replicate plates in **one** dilution, go to 3.

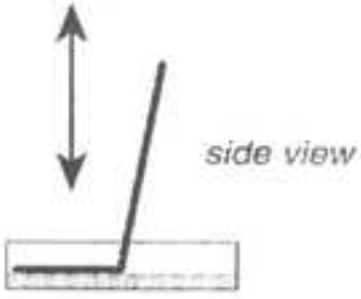


3 For each plate, sterilize a glass hockey stick spreader in a flame after dipping it in ethanol. Let the spreader cool briefly. Go to 4.

4 Briefly touch the spreader to the agar of an inoculated plate to cool, away from the inoculum. Then, spread the inoculum by moving the spreader in an arc on the surface of the agar while rotating the plate.

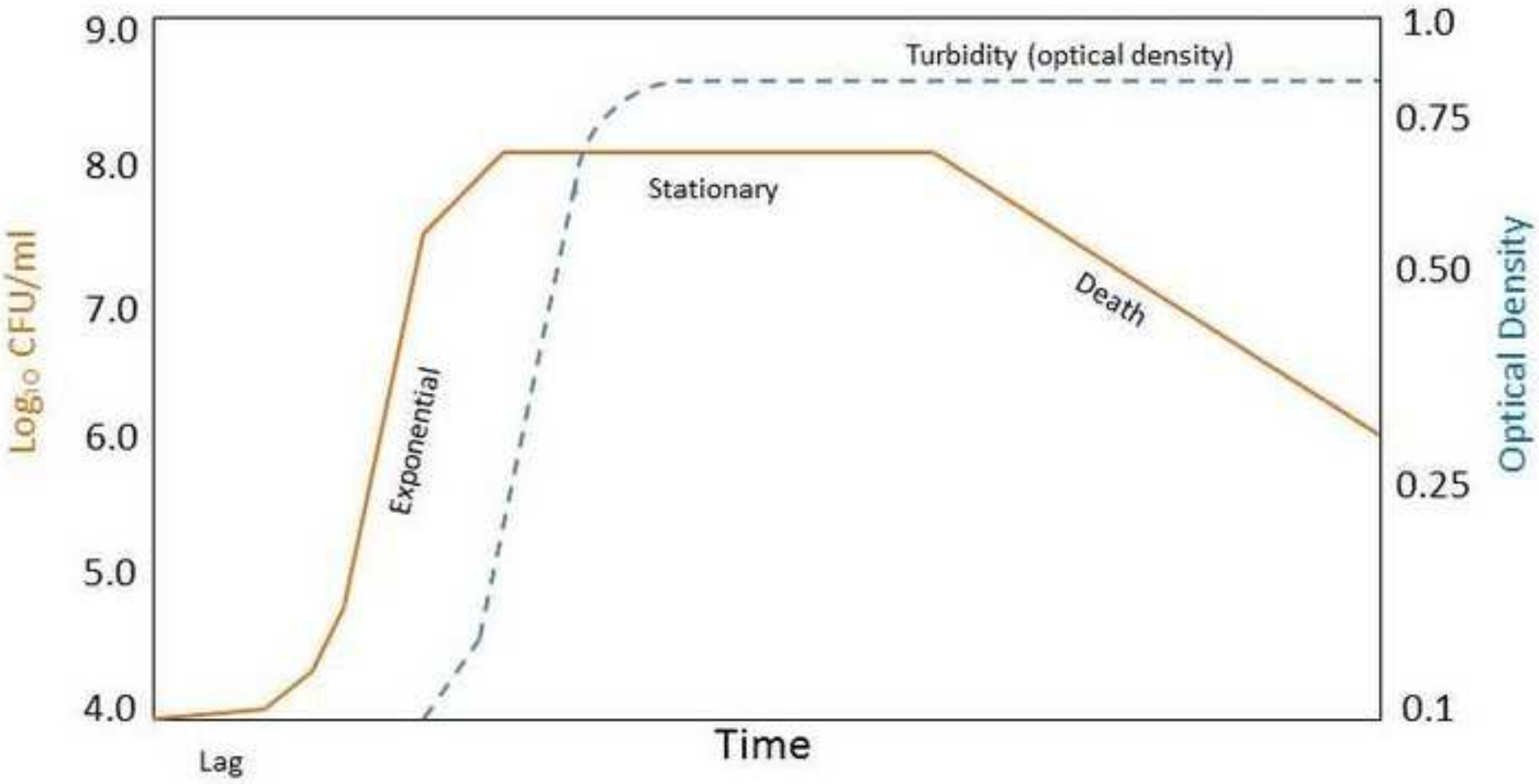
Continue until the inoculum has been absorbed into the agar. Repeat 3 and 4 for the other replicates. Then, go to 5.





5 Repeat steps 2, 3, and 4 for each dilution. When done, let the agar solidify, tape the plates together, and incubate them upside down for one week.

Click here to download Photo or Graphic File: Ian Pepper_Bacterial Culture_Figure 2.jpg



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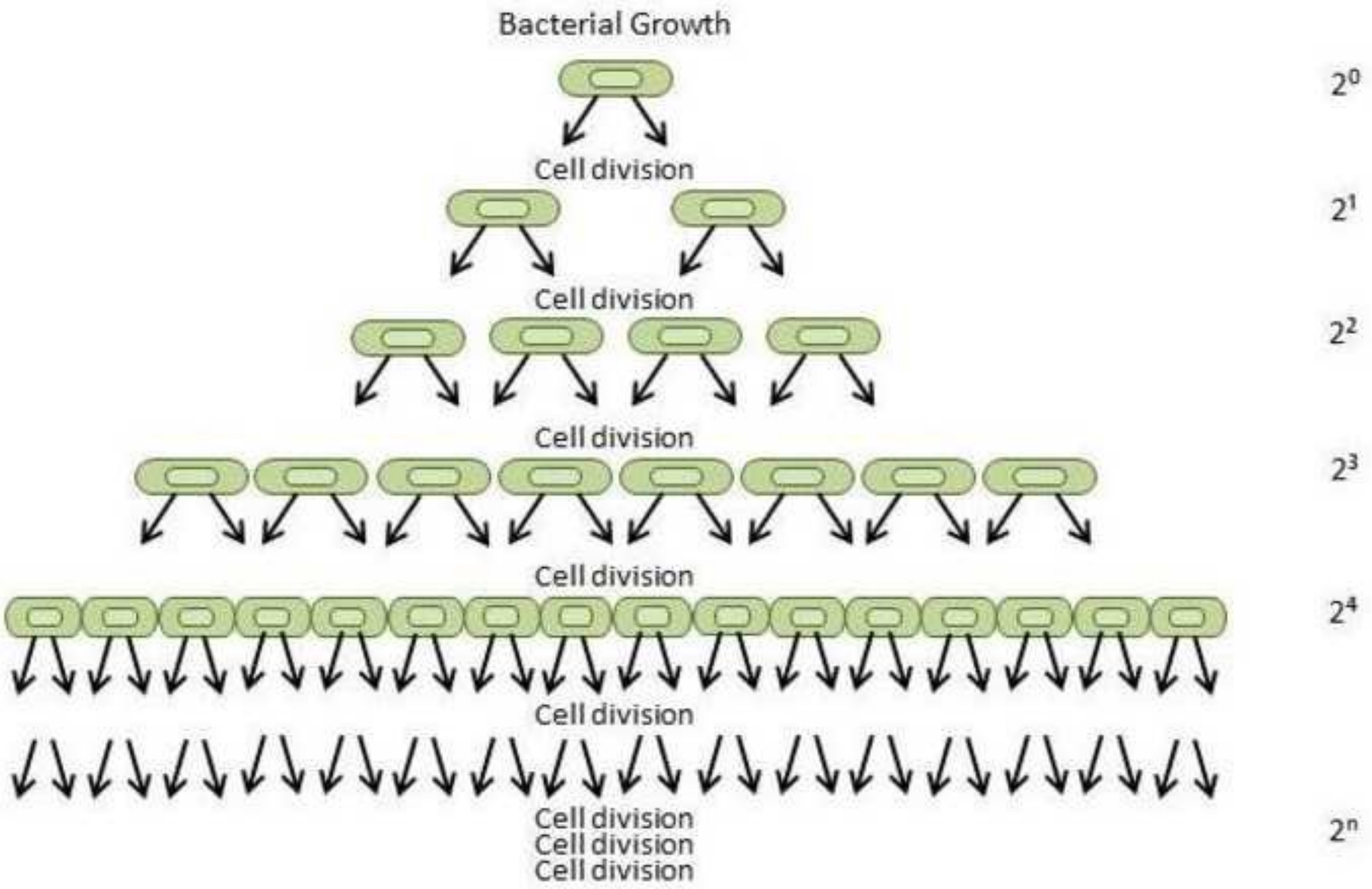

Photo or Graphic File
Click here to download Photo or Graphic File: Ian Pepper_Bacterial Culture_Table 3.docx

***E.COLI* CULTURE**	**DILUTIONS TO BE PLATED**		
T_0	10^{-1}	10^{-2}	10^{-3}
T_1	10^{-1}	10^{-2}	10^{-3}
T_2	10^{-2}	10^{-3}	10^{-4}
T_3	10^{-3}	10^{-4}	10^{-5}
T_4	10^{-4}	10^{-5}	10^{-6}
T_5	10^{-5}	10^{-6}	10^{-7}
T_6	10^{-6}	10^{-7}	10^{-8}
T_{7*}	10^{-5}	10^{-6}	10^{-7}
T_{8*}	10^{-4}	10^{-5}	10^{-6}

*Lower dilutions take into account lower populations due to death phase.

Photo or Graphic File
Click here to download Photo or Graphic File: Ian Pepper_Bacterial Culture_Table 2.docx

E.coli *culture*	**Dilutions needed and Tube #**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
T_0	10^{-1}	10^{-2}					
T_1	10^{-1}	10^{-2}					
T_2	10^{-1}	10^{-2}	10^{-3}				
T_3	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
T_4	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		
T_5	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
T_6	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
T_7	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
T_8	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	

Photo or Graphic File

Click here to download Photo or Graphic File: Ian Pepper_Bacterial Culture_Figure 4.jpg

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Environmental Science Education Title: How to Measure Growth of a Bacterial Culture: Bacterial Growth Kinetics and Growth Curves

Overview:

Bacteria are the most abundant life forms on earth other than viruses, which are not considered to be "alive." Bacteria are found in every ecosystem on Earth and are vital for everyday life. For example, bacteria affect what people eat, drink, and breathe, and there are actually more bacterial cells within a person's body than mammalian cells. Because of the importance of bacteria, it's preferable to study particular species of bacteria in the laboratory. To do this, bacteria are grown under controlled conditions in pure culture, meaning that only one type of bacterium is under consideration. Bacteria grow quickly in pure culture, and cell numbers increase dramatically in a short period of time. The rate of cell increase over time allows for a "growth curve" to be developed. This is important to utilize or inoculate known numbers of the bacterial isolate, which is done for many reasons, such as to enhance plant growth, increase biodegradation of toxic organics, or the industrial production of antibiotics or other natural products. Bacteria's ability to grow and reproduce quickly is due to the fact that they are the simplest of life forms known as prokaryotes. Reproduction occurs via binary fission in which one bacterial cell divides and becomes two cells (**Figure 1**). The time needed for cell division is known as the mean generation time, or doubling time, which is the time needed for the number of cells to double.

To understand and define the growth of a particular microorganism, cells are placed in a flask, where the nutrient supply and environmental conditions are controlled. If the liquid medium supplies all the nutrients required for growth and environmental parameters conducive to growth, the increase in numbers can be measured as a function of time to obtain a growth curve. Several distinct growth phases can be observed within a growth curve (**Figure 2**). These include the lag phase, the exponential or log phase, the stationary phase, and the death phase. These phases correspond to distinct periods of growth and associated physiological changes (**Table 1**).

Overall, it is often critical to determine bacterial growth kinetics for a given bacterial isolate, in order to know the number of bacterial cells present in the liquid medium. There are different ways to measure growth in a liquid medium including turbidity measurements using a colorimetric spectrophotometer or culturable assays. Turbidity measurements rely on the fact that the more cells present in the liquid medium, the more turbid the liquid becomes. Cultural methods involve measuring the number of viable culturable cells in the liquid medium as a function of time. Note, however, that cultural assays can only be used for bacteria that are, in fact, culturable.

Procedure:

1. Preparation for Experiment

1.1. 2 days before experiment.

1.1.1. Inoculate a 50-mL flask of trypticase soy broth (TSB) medium with *E. coli*.

1.1.2. Incubate overnight at 27 °C. This relatively long incubation period results in a stationary phase population of cells of approximately 10^9 CFU/mL.

1.2. 1 day before experiment.

1.2.1. Use 100 µL of the prepared culture to inoculate 250 mL of TSB (in a 500-mL flask).

1.2.2. Mix thoroughly, remove 5 mL, and refrigerate immediately at 4 °C. This is T = 0 or T_0, and yields approximately 4 x 10^5 CFU/mL.

1.2.3. Place the flask of the remaining *E. coli* (245 mL) in a 37 °C shaking incubator. Remove 5 mL aliquots of culture every hour, up to 8 hr.

1.2.4. Store each aliquot at 4 °C. Designate these cultures T_1 through T_8.

1.3. Remove aliquots of *E. coli* from the refrigerator and place on ice, if transporting. Keep all cultures on ice until use.

1.4. Set up a series of dilution tubes to obtain various dilutions of each *E. coli* culture (**Figure 3**). Microfuge tubes are convenient for this function. Each dilution tube should have 900 µl of dilution fluid (sterile saline). A dilution series is needed for each *E. coli* culture (T_0 through T_8) (**Table 2**).

1.5. Begin dilutions by adding 100 µl of *E. coli* from the tube labeled T_0 (the initial *E. coli* culture) to tube A. Tube A is the 10^{-1} dilution of T_0.

1.6. Vortex the 10^{-1} tube for 5 sec.

1.7. Subsequently, add 100 µl of Tube A to the next tube of saline, Tube B. Tube B is a 10^{-2} dilution of T_0. Repeat the needed dilution series for each *E.coli* culture, referring to **Table 2** to see the necessary dilutions for each *E. coli* culture. Remember to vortex each tube prior to transfer. It is also important to use a new pipette tip for each transfer.

1.8. Now plate 3 dilutions for each *E.coli* culture, according to the regiment specified in **Table 3**.

1.9. Label plates with the dilution and volume to be added to the plate. Make sure the label contains the time point plated (T_1 through T_8) identification. Use triplicate plates for each dilution.

1.10. Pipette 100 µl from each of the three dilutions to be plated. Add 100 µL of each dilution tube to be plated by pipetting the amount to the center of the agar plate (**Figure 3**).

1.11. Immediately spread the aliquot by utilizing a flame sterilized "L" shaped glass rod. If the aliquot is not spread immediately, it sorbs *in situ* on the plate, resulting in bacterial overgrowth at the spot of initial inoculation.

1.12. Repeat the plating for each dilution series for T_1 through T_8 cultures. Remember to sterilize the rod between plates and especially between different dilutions.

1.13. Once plates have dried for a few minutes, invert and place in 37 °C incubator overnight. Inverting the plates preclude condensation from falling onto the agar plate. Following this, store plates in refrigerator until next period.

1.14. Examine plates for uniformity of colonies and lack of contamination.

1.15. For each culture (T_0 through T_8), count triplicate plates at one dilution that contains between 30 and 300 colonies.

1.16. Calculate the number of cells per ml of original culture for T_0 through T_8 cultures. For example, the number of colonies resulting from a 10^{-4} dilution is 30, 28, and 32.

Mean number of colonies = 30 colonies

These arose from 0.1 ml of a 10^{-4} dilution

$$\text{Number of colonies per ml} = \frac{30 \times 10^{-4}}{0.1}$$

1.17. Plot $\log_{10}$ CFU/ml versus time (hours).

1.18. From the graph, identify the exponential phase of growth. Using 2 time points within the exponential phase of growth and corresponding cell numbers, calculate the mean generation time.

Results:

Following a dilution and plating experiment, the following data was obtained. At the beginning

of exponential growth designated here as time t = 0, the initial concentration of bacterial cells is 1,000/mL. At time t = 6 hr, the concentration of cells is 16,000/mL.

Now, $X = 2^n X_0$

Where: X_0 = initial concentration of cells = 1,000/mL
X = concentration of cells after time t = 16,000/mL
N = number of generations

$16{,}000 = 2^n \times 1000$

$2^n = 16$

$\log_{10} 2 = \log_{10} 16$

$n(0.301) = 1.204$

$n = \frac{1.204}{0.301} = 4$

Four generations in 6 hr.

Mean Generation Time = 6/4 = 1.5 hr.

Applications:

Knowledge of bacterial growth kinetics and bacterial numbers in a culture medium is important from both a research and commercial point of view. In research, it is often critical to know the numbers of bacteria in a sample, so the experiment can be replicated, if need be, with the exact same numbers. For example, during experiments in which bacterial inoculants are added to a soil, a minimum of 10^4 per gram of soil needs to be added to get the desired effect, such as enhanced biodegradation of toxic organic soil contaminants. Commercially, this can be important as in the case of commercially produced rhizobial inoculants, where known numbers of rhizobia are impregnated into a peat-based carbon medium (**Figure 4**). The medium is then used to inoculate legume seeds to enhance biological nitrogen fixation. Obviously, for commercial microbial products, quality control in terms of bacterial numbers is mandatory.

Commented [AW1]: The Applications section can't be scripted and shotlisted until it's clear what will be shown, both on a conceptual and visual level. Provide more concrete information here.

Commented [JR2R1]: Hi Andrew, Ian provided us with the new image of a root nodule that contains nitrogen fixing bacteria.

If further visual representation is necessary, Ian said we could also add a picture of a researcher pipetting from a broth culture into two flasks or a researcher pipetting from a broth culture into a peat-based carbon medium for enhanced nitrogen fixation (this would be a similar image to Figure 4). These would be obtained at the shoot.

Legend:

Figure 1: Exponential cell division. Each cell division results in a doubling of the cell number. At low cell numbers, the increase is not very large; however after a few generations, cell numbers increase explosively. After n divisions, there are 2" cells.

Figure 2: A typical growth curve for a bacterial population. Compare the difference in the shape of the curves in the death phase (colony-forming units (CFUs) versus optical density). The difference is due to the fact that dead cells still result in turbidity.