The Bounds of Population Growth: Bacteria on the Move

Introduction and Objective:

The analysis of population dynamics and its relationship to available resources comprises an integral part of many biological studies. In the past, we looked at how cellular metabolic processes were predominantly controlled by chemical and material gradients; now we will see how these same principles hold true at larger scales. Humans and all organisms in search of survival typically seek out environments that are beneficial for growth, and today we will see how this applies to a bacterial population.

You will be presented with several bacterial populations subjected to various environmental conditions. Your objective is to analyze how these conditions affect population dynamics (i.e. time dependant growth) using data obtained through the use of spectrophotometry.

Materials and Methods:

Escherichia coli cultures (E.coli strain DH5α) were started using a concentrated cell stock of unknown concentration*. Approximately 2.5 microliters of this stock were used to inoculate each culture tube.

Experimental culture tubes, were comprised of 6 milliliters of Luria-Bertani (LB) broth treated in one of four ways. Treatments were **(1)** LB only (blue), **(2)** LB + 1% Glucose (green), (3) LB + 3% Glucose (purple), and **(4)** LB + diluted antibiotic (Ampicillin, 1 milligram) (red). Color codes for tubes are listed in parentheses.

After inoculation, the tubes were placed in a 37ºC shaker to promote bacterial growth. Tubes were removed at the following time points (hours post-inoculation): 0, 4, 6, 8, 12, 22. To arrest cell growth, removed cultures were stored at 4 C (refrigerator).

*To determine exact cell number of concentrated cell stocks, typically several dilutions are made and then plated on solid LB growth medium. This allows the researcher to count the number of cells on the plate, and recalculate the number in the original stock using the dilution factor.

Hypothesis:

Please enter your hypotheses in the table below

Treatment	**Relative Growth Rate (fastest…slowest)**	**Max Population (highest…lowest)**
LB		
LB + 1% glucose		
LB + 3% glucose		
LB + diluted antibiotic		

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Experimental Observations:

For each time point, record the relative turbidity of the broth.

Next, take absorbance readings at a wavelength of 600 nanometers using a spectrophotometer. To remove the interference that the LB medium has with the culture readings, take a reading of LB broth alone. To do this, add 4 milliliters of LB broth alone to the cuvette. Insert the cuvette into the spectrophotometer and record the reading at 600 nm. List this value next to the "**Blank OD =**" entries on your sheet. You will subtract this value from every culture OD reading you take from now on.

Measure the cultures for each time point of the appropriate treatment. Remember to subtract the blank value!

Calculate the number of cells from each OD reading. You can assume that for an OD value of 1, there are 3×10^7 cells per milliliter. For example, if you measure an OD of 0.340…

$$\frac{1\ (\text{OD})}{3 \times 10^7\ \text{cells}} = \frac{0.340\ (\text{OD})}{X\ \text{cells}}$$

$$1\ (\text{OD}) * X\ \text{cells} = 0.340\ (\text{OD}) * 3 \times 10^7\ \text{cells}$$

$$X\ \text{cells} = 1.02 \times 10^7\ \text{cells}$$

Treatment 1: LB **Blank OD =**

Time point	Visible observations	OD 600 (abs units)	Number of cells

Treatment 2: LB + 1% Glucose **Blank OD =**

Time point	Visible observations	OD 600 (abs units)	Number of cells

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Treatment 3: LB + 3% Glucose **Blank OD =**

Time point	Visible observations	OD 600 (abs units)	Number of cells

Treatment 4: LB + diluted antibiotic **Blank OD =**

Time point	Visible observations	OD 600 (abs units)	Number of cells

Analysis:

1. For each treatment, use the graph paper provided to chart the dependence of cells on time.

2. Record the maximum number of cells. What does this number represent?

3. How do the graphed results differ from the case for unlimited growth?

4. What factors in the experiment caused this to occur and what difference, if any, did you observe between the four treatments?

Conclusions:

How do your results relate to your hypothesis? Were your assumptions correct? Explain by relating the experimental data to your guesses about relative growth rate and maximum population.

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