

Bi 1X, Spring 2013

Week 2

- Session 1: Growth Curves
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Background:

Difference between members of the same bacterial species.

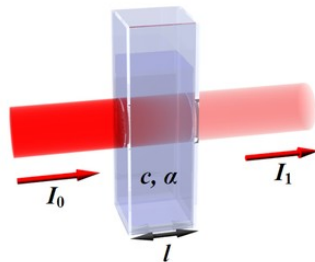
The physiology of a bacteria depends not only on its set of genes and specific regulatory mechanisms, but also on its growth rate. The rate of bacterial division influences important global parameters such as the number of RNA polymerases and ribosomes (components of protein expression) in a cell. To better understand the concepts involved in these differences, we will measure the bulk growth rate of different *E. coli* strains isolated from different animals around the world. We measure cell growth using spectroscopy and optical density as outlined in the following section. The included formulas will prove useful in analyzing your spectroscopic data.

Beer's Law and Optical Density

It is often extremely useful to know the concentration of cells in a liquid culture, and can be used in various kinetic measurements of fitness and protein production. Light scattering is the primary method by which we measure cell density. In the following section, we will discuss the basic physical concepts that allow determination of concentration as well as the formulas that relate optical properties to concentration.

The basic physics are as follows:

1. Light, with a wavelength comparable to the size of the object being measured (600 nm), enters the sample at an initial intensity (I_0)
2. Cells, having a different index of refraction as the surrounding medium, randomly reflect and scatter light out of the incident light path (scattering with no change in momentum). The amount of scattering is proportional to the amount of cells at any given point in the sample. (Caveat: to get an accurate reading, cell density must be low enough that light is only scattered *once* in its journey through the sample. Often, this means that cells must be serially diluted to get an accurate reading.)
3. Upon exiting the sample, the light intensity is reduced by $I_1 = I_0 e^{-\alpha c l}$, where α is the wavelength-dependent molar absorption coefficient of the species in question, l is path length – usually 1 cm in modern spectrophotometers, and c is the concentration, this is known as *Beer's Law*. See the figure below:



4. The transmission efficiency of the sample is the ratio of the final and initial light intensity, $T = I_f/I_0$, where the measured quantity, called “absorbance” is $A = \ln(1/T) = alc$. The general rule of thumb is that accurate absorbance readings lie in the range $0.01 < A < 1$. (Serial dilutions of the sample will be necessary to bring A within this range.)

Your Mission:

- Measure reference growth rate in minimal media for the lab strain *MG1655*.
- Measure the growth rate of the different wild-type isolates under the same conditions.
- Determine the correspondence between OD₆₀₀ and cell density.

Protocol:

Prior to your involvement:

1. Groups of two will be assigned a particular strain in which to assess growth rates.
2. Overnight cultures of the *E. coli* strains were inoculated into 50 mL of media, and incubated on a shaker at 37°C for 1 hour prior to the start of the experiment. This ensures that the so-called “lag phase” has passed, and that (slow) exponential growth has begun.

Procedure:

1. Every 10-15 minutes remove a 1mL sample of your cultures using a sterile pipette. Deposit this into a 1.5 mL cuvette and immediately bring to the spectrophotometer (the “spec”).
2. Carefully insert the cuvette into the spec in the correct orientation. Make sure the spec is set to read **OD₆₀₀**, and measure the absorbance. Record the reading and the **EXACT** clock time.

NOTE: You should have been given a “blank”, i.e. a sealed cuvette with sterile media. Absorbance is always measured relative to the blank. **Be sure to measure the absorbance of the blank before every culture measurement!**

3. Properly dispose of the sample cuvette, and repeat these steps for the next 1- 2 hours.
4. Take the last cuvette that you OD’d and follow the next few steps.
 - a. For the moment, let us assume $OD_{600}1 = 10^9$ cells / mL. With that in mind, properly dilute your sample with LB to ~ 100 cells / mL. This will require serial dilution – try to be accurate in your pipetting! Bracket your dilution by a factor of 10 on either side. For example, if you decide to dilute your cells 10^3 times, do another 10^2 and 10^4 dilutions. **Make sure to write down your dilution factor!**
 - b. For each of the diluted samples, evenly spread 200 μ L with beads on a LB plate. **Make sure to clearly label your names, date, OD reading, and amount of dilutions on the plate.**

