Bi 1X, Spring 2012

Week 2

- **Session 1: Growth Curves**

Background:

Cell challenge with anti-bacterial substances and Spectroscopy

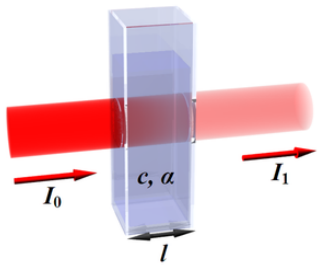
While most bacteria pose no threat to humans, there are a number of species that can cause very serious infections. One major challenge facing biomedical scientists is how to kill off these bacteria after an infection has taken hold, a challenge that has become more and more challenging as the abuse of antibiotics has lead to the widespread occurrence of antibiotic resistant strains. To better understand the concepts involved in combating bacterial infection, we will measure the bulk growth rate of *E. coli* in media after challenge with various anti-bacterial substances. 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 $I_1 = I_0 e^{-\alpha l c}$, 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, $T = I_1/I_0$, 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 baseline growth rate in rich media.
- Measure how various anti-bacterial substances influence actively growing E Coli.
- Determine the correspondence between OD_{600} and cell density.

Protocol:

Prior to your involvement:

1) Groups of two will be assigned a particular mixture in which to assess growth rates.

   i) Growth 1-6: NZC media

2) Overnight cultures of wild type (C600) *E. coli* were inoculated into 50mL of each of these media, and incubated on a shaker at 37°C for 15 minutes 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 culture using a sterile pipette. Deposit this into a 1.5mL 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 **OD_{600}**, 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 <u>before</u> every culture measurement!***

3. Properly dispose of the sample cuvette, and repeat these steps for the next 45 minutes.

4. After the first hour is up, take one more measurement, and then add your assigned amendment.

   i. Growth 2: Add nothing
   ii. Growth 2: Add $3x10^{-6}$ moles Ampicillin (263uL of stock)
   iii. Growth 3: Add $3x10^{-6}$ moles Kanamycin (148uL of stock)
   iv. Growth 4: Add $3x10^{-6}$ moles Rifampicin (250uL of stock)
   v. Growth 5: Add 10uL T7 stock (10^6pfu/uL)
   vi. Growth 6: Add 10uL T7 stock (10^6pfu/uL) and $3x10^{-6}$ moles Kanamycin

5. 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 200µL with beads on a LB plate. **Make sure to clearly label your names, date, OD reading, and amount of dilutions on the plate!**
c. Incubate the plates overnight at 37°C. Later, we can count colonies and determine the exact correspondence between OD_{600} and cell density.

6. Continue to take OD measurements for the rest of the class period. Make sure to switch to the new blank after you have made your amendments.

7. Afterwards you should have a list of times and absorbances in your notebook. We will combine data of the whole class for data analysis. With the blank's absorbance subtracted from each sample value, make a plot of absorbance vs. time for each group of data on the same figure.

Sample Data Table:

Time	**OD_{600}**	**Dilution Factors at *OD_{600} 0.1*, *OD_{600} 0.5*, and *maximum OD_{600}***	**Number of Colonies at *OD_{600} 0.1*, *OD_{600} 0.5*, and *maximum OD_{600}***