

BIOLOGY 1240: BIOLOGY LABORATORY

SPRING SEMESTER — 2001

BEER-LAMBERT LAW I

October 30, 2001

Instructor Version

BEER-LAMBERT LAB: I. INSTRUCTOR GUIDE

Introduction

This guide is intended to give help to biology lab instructors in the Beer-Lambert light extinction lab exercises.

The philosophy of the labs is to use group learning and problem-based teaching techniques so that students discover simple mathematical models of light extinction in biological (and non-biological) objects.

The basic organization of the labs will be: Instructor guided discussion; then lab bench group work; then instructor guided discussion; lab bench work; and so on. Lab bench group work is described in the student handout.

The class discussion periods are critical to the learning process. The instructor must use this time to evaluate if all students are mastering the material and to ensure that all groups are proceeding in the correct direction.

Overall Objectives of the Beer-Lambert Lab

We want the students to learn (1) General: learn what a mathematical model of a biological process is by constructing and testing one in the lab; how to reason about models that fit data; use spreadsheets to analyze data (2) Specific: For light extinction, to eliminate implausible models; to perform calculations using the equation for the Beer-Lambert Law; to appreciate that the Law applies to aquatic and terrestrial systems.

Structure of These Notes

Each section is written to **follow** the lab activity. The notes include *Learning Objectives* and *Discussion Points*. Learning objectives are the concepts the students should have before moving to the next step. The Discussion points are suggestions on how to get the class to understand the learning objectives.

Hints From the “Experts”

1. *Be Enthusiastic.* Some of your students may be less than thrilled about thinking on their feet in lab. As in most endeavors, enthusiasm in teaching can take you a long ways and even get your students to like you and your subject.
2. *Make Student Switch Roles.* Most lab groups will be dominated by one individual. Minimize this by (1) forcing different students to use the computer between activities, (2) learn all their names and call on them for answers to questions that follow in this guide.
3. *Use Your Own Examples.* If the students can't relate to eutrophication as a reason for calculating photosynthesis rate, try global warming.
4. *Hold Class Discussions.* These exercises depend on you keeping all the students together and

on track. The class discussions are designed for this. Don't let students get ahead. This is one of the reasons why we have the passwords.

For Section 2: How Much Photosynthesis Occurs in a Lake

This section motivates the quantitative problem with a topic possibly relevant to the students. They should know about most of the subjects mentioned (e.g., aquaculture, fishing, etc.). Some of this will be covered in the Recitation session, but some students will miss.

The main point you should impress on the students is that we are studying only one abiotic factor among many other factors including biotic factors. This is a central concept in modeling: simplify, make predictions from the simple assumptions, test the predictions. It's not strange, except we're using some math to make the predictions.

Some questions to test student comprehension:

1. What is the chemical formula for photosynthesis?
2. What is a limiting factor?
3. What is Liebig's Law of the Minimum?
4. What does Liebig's Law have to do with modeling? (It helps us simplify the system.)
5. Do you think lakes really are limited by light? What other abiotic factors might be important? What biotic factors?
6. How can we solve the problem of photosynthesis in the lake? (Assume light is the only variable. Solution: (a) determine light at depth, (b) determine how photosynthesis depends on light, (c) sum total amount of photosynthesis in a column of water, and (d) sum all columns of water in the lake.

After Step 1: Concept Map

We're asking the students to again try to link together causal processes as they affect photosynthesis.

Learning Objectives

(1) identify the key factors or variables that influence photosynthesis (e.g., carbon dioxide, water, light, etc.) (2) identify some of the interactions among the key causal agents (e.g., weather) (3) simplify a conceptual model that has too many variables.

Discussion

1. Get the attention of the students away from computers.
2. Call on all groups for input, taking part of the solution from each.
3. If one group is way off base, don't ignore or berate: put their diagram up separately.
4. List as many of the factors as students mention and leave these on the board for later discussion.
5. After all have given input, get the class consensus on the best diagram to pursue. You will need to anticipate next week's lab where you will study photosynthesis in an aquatic plant.

You should have something like the attached figure, but the exact picture is not critical.

For Section 4: The Relation of Light and Depth

Learning Objectives

- (1) Make a qualitative prediction that gives the students a framework for “reasonable” behavior.
- (2) Understand the scaling of radiation to be a fraction of a maximum value (that at the surface),
- (3) eliminate impossible quantitative ranges for the scaled variable.

Discussion

The students will have seen 2 curves in Recitation, so they certainly can produce those two curves. The students should suggest three or four curves like those in Fig. 1 page.

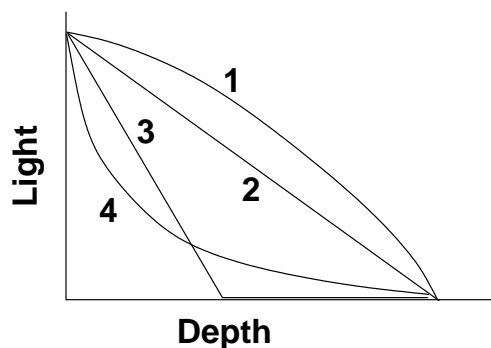


FIGURE 1: Four possible light curves.

Questions to Ask

1. Which curve is most likely? Why?
2. What does each curve predict about light at great depth? (Curves 1 and 2, say it will go negative.)
3. What is wrong with curve 3? (In a column of water greater than the point where light is 0, would you expect complete darkness? Imagine you are photon, bumping into water particles, curve 3 suggests that there is an absolute depth below which not a single photon will, even by chance, go beyond?)

Before going on, make the point: *The students have created alternative predictive models. They have made a preliminary guess as to which is most likely. The next step is to test the conjectures against nature.*

For Section 5: Data Collection in Aquaria

Learning Objective

To see the effect of distance (depth) and turbidity on light concentration.

Warnings While Doing the Experiment

1. Before the experiment, demonstrate the use of the light meter.
2. The lights are hot and bright. Don't touch or look directly into them. You might want to wear protective glasses.
3. If you put water on the hot bulbs, they will explode. [TAs: maybe you don't want to make a big deal of this!]
4. Make the room dark: turn off the overhead lights and draw the blinds tightly.
5. Check the paper inside the aquaria; keep it as flat as possible. The paper may not last the entire week.
6. The dye must be well mixed.
7. Always place the light meter sensor in the same position at the end of the aquaria: centered on the bulb.
8. Use the smallest range that does not

For Sections 6 and 7: Analyze the Data and Guessing an Equation

Learning Objectives

- (1) How to propose a possible equation for a set of data; (2) how to eliminate implausible equations;
- (3) appreciate how any equation will be an approximation to the data; (4) reinforce graphing skills.

Plotting the Data

For this segment, students will plot all three treatments by hand on the attached graph paper.

1. Plot luxes versus distance. A lux is a unit of illumination (lumens per m^2) and has no direct physical translation to energy fluxes. The proper measure of light for plants is micro-Einsteins measured as $\mu\text{moles-photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. We have some photon flux meters for photosynthetically active wavelengths, if you or a student wishes to see the connection. It would be a good exercise in calibration.
2. Make sure all the students do the plots. This is a skill we want to reinforce. You will have to walk around the room and check.
3. The graphs should be complete: units on all axes. The students should not "connect the dots."

Finding an Equation

This activity is one of the major objectives of the lab exercise. Guessing an equation that fits some data is an art just like looking at a squishy, semi-transparent thing in a jar and guessing that is a cnidarian. It's the same principle: at this level of sophistication, we do not ask the students to derive the equation from first principles. We'll be satisfied if they remember a few equations from earlier math classes and can mentally picture whether or not the equations could look like the data.

The students probably will draw a blank. Don't let them give up too soon. They will remember more than they think if they spend some time at it.

Hints to give:

1. The curve is not linear
2. Adding another linear term (e.g., a constant) to a linear equation will not change its shape. So, $y = mx + b + A$ won't help.
3. Simple arithmetic operations can make non-straight lines:
 - (a) Multiplication: $y = x(mx + b) = mx^2 + xb$ (parabola, or quadratic)
 - (b) Division: $y = 1/x$ (inverse function)
 - (c) General Powers: $y = ax^b$ (power function)
4. Trig functions: A segment of a cosine curve might fit the data.
5. Exponential: $y = ae^{-bx}$ (exponential function)

For Section 7: Class Models

You can combine the discussion of this section with that of Section 6; i.e., it may not be effective to have the students discuss the two class models in groups. Your objective here is to end up with two models that might be plausible. One of the class models must be the negative exponential (the Beer-Lambert Law), the other will probably be a parabola or the inverse function. The students should write these two equations in the boxes.

Now you and the students must do some reasoning about the equations. Below are points to make about the some plausible models.

This is the hardest part of the lab. You should stress the following three points: (1) any equation is an approximation,

Polynomial (quadratic equation)

$$I(z) = a_2z^2 + a_1z + a_0$$

The polynomial equation has 3 undesirable properties: (a) some parameter values give physically impossible numbers: negative light; (b) some parameter values show quantitatively implausible patterns (light increasing then decreasing: $a_2 < 0$); (c) a polynomial that fits over a range of data will fail if depth is allowed to increase: the graph of the function will increase if z is large enough; and (d) there is no single constant (coefficient, parameter) that corresponds with the surface light intensity. Item (d) is not so serious, but it makes the equation a little less “natural” in its description of the phenomenon.

Inverse

$$I(z) = a/z^b$$

The inverse function might not be such a bad model. As it is written, it goes to infinity as depth goes to zero (infinite light at the surface). As such, there is again no coefficient that corresponds with surface intensity. It is related to the “inverse-square-with-distance” concept that they will have heard about in Recitation. Remind them that we are concerned with **attenuation**, not the distance effect.

Exponential

$$I(z) = I(0)e^{-az}$$

The exponential has nice properties: (a) it is always positive; (b) it continually decreases (if the exponent is negative); (c) it goes to zero as depth increases; (d) it does not go to infinity as distance goes to 0.0; (e) it can be easily scaled to any value by multiplying by a constant: $y = e^{-3x}$ is 1.0 when $x = 0$, and $y = 0.1e^{-3x}$ is 0.1 when $x = 0$. This scaling factor is a coefficient that corresponds to the surface intensity ($I(0)$).

This equation is the Beer-Lambert Law. The Appendix to this document “derives” the appearance of e in this situation. The exponential is not just an empirical constant here; it emerges from the notion that over a finite distance of medium, a constant proportion of the incoming light is attenuated. e appears automatically when the finite intervals are made infinitely small.

Power

The power function

$$y = I(0)C^{bz}$$

is interesting since it is very similar to the exponential model, where $C = 2.718\dots$. If students play with this on their calculators over a range of C values, they may find that $C \approx 3$ is not a bad fit. If this equation is suggested, point out that it is a generalization of the exponential model. As such, it has the disadvantage that there are 3 parameters (not 2 like the exponential). This makes it more general, but more complicated. It does have a coefficient ($I(0)$) that corresponds to surface intensity, as does the exponential model.

Others

If a student comes up with an equation that you do not know, have them graph some values. Short of that, you need to do a little bit of reasoning about the equation yourself: what will happen at 0? what will happen at $z = \infty$? This is definitely an area where it is no dishonor to say “I don’t know; why don’t you graph a few values?”

Section 8: Beer-Lambert

In the end, you will tell them the generally accepted form is the Beer-Lambert Law:

$$I(z) = I(0)e^{-az}$$

where z is depth, $I(z)$ is light intensity at depth z , $I(0)$ is light intensity at the “surface” (depth 0), and a is a constant that characterizes a particular body of water (or any fluid).

Some features to emphasize:

1. The surface of the water should always be $z = 0$. The students will have to subtract 15 cm from the distances shown in the aquarium data sheet.
2. It works for any depth z . This means it will work for the entire distance of our water column of 108 cm, as well as through 1 aquarium: from 0 to 31 cm.

Group Work

The problem to find a method to estimate a is a group activity. The handout does not tell them how to do it. They need to ponder it in their group to see if they can remember anything about logarithms and exponents. Take at least 5 minutes to let them think. A hint you can give individual groups would be: “Get a out of the exponent and make a new equation that corresponds to a straight line.”

After they have had a chance to think about it, re-convene the class and ask for suggestions.

Section 9: Estimating a

Learning Objectives

(1) Discovering how to transform the data and equation to get a method of estimating a ; (2) using logs.

Discussion

There’s not that much to discuss. Make sure everyone knows how to do basic log manipulations. I recommend laboriously going through the algebra step by step:

$$\begin{aligned} I(z) &= I(0)e^{-az} \\ \ln(I(z)) &= \ln(I(0)e^{-az}) \\ &= \ln(I(0)) + \ln(e^{-az}) \\ &= \ln(I(0)) - az(1) \end{aligned}$$

Here are the math rules used in the above:

1. Line 2: the log of the right-hand-side of an identity (Line 1) equals the log of the left-hand-side of the identity.
2. Line 3: the log of a product is the sum of the logs
3. Line 4: the log of a base raised to a power is the power times the log of the base
4. Line 4: the natural log of e is 1 (the log of the base of the log is always 1: $\log_1 0(10) = 1$)

The result is a straight line with $-a$ being the slope, z is the independent variable, and $\ln(I(0))$ is the intercept. To re-inforce that it is a straight line, have the students write the slope and intercept in the box provided.

a is called the attenuation coefficient (or, “parameter”, “constant”).

Section 9.1: Estimating a for Student Data

Learning Objectives

(1) to apply the logs to their data to estimate a constant in an equation; (2) to see the effect of turbidity on extinction coefficients; (3) to use the concept of statistical variation around an underlying pattern (or model); (4) reinforce spreadsheet skills.

Operations

Before they work in groups, give the entire class this introduction:

You now know the following:

1. *Hypothesis: If the Beer-Lambert Law applies to your data, then light will decrease exponentially with distance.*
2. *If light decreases exponentially, then a plot of the log of light against distance will be a straight line.*
3. *Your problem is to estimate the constant that describes the degree to which light is attenuated. How to estimate this constant if the data fall on a straight line?*

Do not tell the class what to do with their data. Have the students work in their groups to discover for themselves. But here, in a nutshell, is the procedure they should discover:

1. Compute the \ln transform of the light readings, but do not transform the z (distance) values.
2. Plot transformed light on y-axis, z on x-axis.
3. Draw, by “eye” the best **single** straight line through the plotted points
4. Calculate the slope of the line by picking 2 points **from the line** (not 2 data points).

The students should do only the “no dye” treatment by hand. All three treatments will be done with MS Excel.

Discussion

Our students commonly make 2 mistakes when they complete the estimation: (a) they want to draw $n - 1$ short segments through each successive pair of data points, and (b) they want to calculate the slope by picking 2 data points. Don't let them make these errors. We want them to use the idea that there exist underlying patterns (negative exponential, in this case) but that this is masked by statistical variation. Having the students draw the line and using the line to get the slope is necessary.

Make sure everyone knows how to do the operations and gets reasonable values of a . Be sure they know what the units of a are: $1/\text{length}$, where length is determined by the ruler used to measure depth (e.g., cm or meters).

Using Spreadsheets to Estimate a

If time permits, the students will engage in two extra activities. First, have them do the no-dye aquaria experiment by hand: plot the logs on graph paper, eye-ball the best line, and estimate $-a$ from the slope of the line. After a group has done this and shown it to you, they should use MS Excel for all 3 treatments. They should:

1. Enter the data in the template: `My_Documents\BIOL1240_Aquaria_template.xls`
2. Compute the natural logarithms of the light data.
3. Plot **all three** data sets together on 1 graph.

4. Show the trend lines for all data sets.
5. Write a 1-2 sentences on the spreadsheet comparing the attenuation coefficients for the 3 experiments and whether the differences make sense and why. (To insert a text box: On the Drawing toolbox, click on the Text Box tool and then at the point of the spreadsheet where you want the box to appear.
6. All groups must hand-in a printed copy of their spreadsheet.

The second activity is to have each lab bench work through the solved problems and the practice unsolved problems. The groups should do this on their own, using at most a few key hints from the instructors. The solved problems are worked out, so they should not need any help, but they will. We will also give you some additional practice problems to work on. These are on a separate sheet. Another separate sheet contains the answers to these practice problems. If you simply give them the answers, you will be doing them a very big disservice.

Section 10: Solved Problems

Learning Objectives

(1) To reinforce: (a) estimating a from data, (b) apply the Beer-Lambert equation in specific situations (depths and coefficients), (c) understand how a spectrophotometer works, (d) understand compensation point.

Discussion

The answers are supposed to be self-explanatory, so the students can do these outside of class. You can suggest that they try them on their own and email you questions. Or, you can do them in class **if** time is available.

Appendix: Why is e here?

This appendix gives a partial answer to the question: Why does light decrease as a negative exponential? A complete answer from first principles of photons scattering from spherical particles is very difficult. But the following provides an answer from the observation that a constant proportion of light is attenuated in a series of finite layers to the idea that there are an infinite number of layers (continuous medium).

Figure 2 shows the necessary geometry.

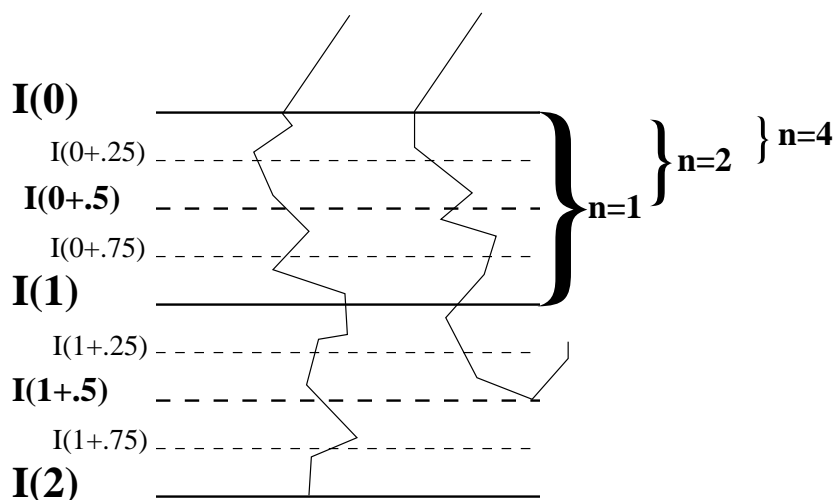


FIGURE 2: Imaginary layers in a continuous medium with light attenuation. Horizontal lines are imaginary layers; the two irregular lines are paths of 2 photons.

Assume light enters a medium at the top and is attenuated as photons move through the medium and are absorbed or scattered by the particles of the medium. Assume that each major layer ($I(0), I(1), I(2)$) attenuates the light entering by the same constant fraction R :

$$I_{i+1} = I_i R = I_i(1 - q) \tag{1}$$

where q is the fraction attenuated.

This works fine if the medium really is discrete and light passes through the space in jumps. But water (and real tree canopies) is continuous and light is continuously removed from the medium. What equation describes this situation?

To answer this, we subdivide the medium into progressively more layers, and we reason from finitely many to an infinite number of layers. The number e is the result of doing this as follows.

Eqn 1 describes light attenuation over one large finite layer. Divide the layer in half so that now the interval is composed of $n = 2$ sub-layers (Fig. 2). In this geometry, it takes 2 steps to compute the light at layer 1:

$$\begin{aligned} I(0 + .5) &= I(0)(1 - q/2) \\ I(1) &= I(0.5)(1 - q/2) \end{aligned}$$

Since $I(0.5)$ in the second equation is defined in the one above, we substitute the first equation for

$I(0.5)$:

$$I(1) = [I(0)(1 - q/2)](1 - q/2) = I(0)(1 - q/2)^2$$

If we continue to subdivide each of the 2 sub-layers into 2 subsublayers ($n = 4$, Fig. 2), we have for 1 subsublayer

$$I(0.25) = I(0)(1 - q/4)$$

and for all 4 subsublayers

$$I(1) = I(0)(1 - q/4)^4$$

Extending this process to n layers

$$I(i + 1) = I(i)(1 - q/n)^n \quad (2)$$

What happens when $n \rightarrow \infty$? First, we need one of those little tricks that only a mathematician could dream up. We will multiply the power in Eqn 2 by 1, in this special form: $(-a/-a)$:

$$I(i + 1) = I(i)(1 - q/n)^{n(-a/-a)}$$

After re-arranging:

$$I(i + 1) = I(i) \left[(1 - q/n)^{-n/a} \right]^{-a}$$

Now as $n \rightarrow \infty$, $q/n \rightarrow 1/n$ and $-n/c \rightarrow -n$, so in the limit $n \rightarrow \infty$

$$I(i + 1) = I(i) \left[(1 - 1/n)^{-n} \right]^{-a} \quad (3)$$

The question now is: What is the limit of $(1 - 1/n)^{-n}$? Let

$$V_n = \left(1 - \frac{1}{n}\right)^{-n} = \left(\frac{n-1}{n}\right)^{-n} = \left(\frac{n}{n-1}\right)^n$$

let $n - 1 = m$, so that

$$V_m = \left(\frac{m+1}{m}\right)^{m+1} = \left(1 + \frac{1}{m}\right)^{m+1} = \left[\left(1 + \frac{1}{m}\right)^m\right] \left(1 + \frac{1}{m}\right)$$

As $n \rightarrow \infty$, so does m . In the last equality on the right, the limit of the rightmost expression is 1 ($1/m \rightarrow 0$). The other expression in square brackets is more interesting. If you substitute progressively larger integers for m , you get this series, letting y be the expression in brackets:

m	1	10	500	1000	10,000
y	2	2.59374	2.71557	2.71692	2.71815

The expression y evidently has a definite limit, and we call this number e . So, the series V_m and V_n both converge on e . As a result, in the limit as the number of sub-layers goes to ∞ and substituting the limit (e) in Eqn 3 we have:

$$I(i + 1) = I(i)e^{-a}$$

And since this equation can be used recursively:

$$I(i + 2) = I(i + 1)e^{-a} = I(i)e^{-a^2}$$

or, for any interval from 0 to i :

$$I(i) = I(0)e^{-ai}$$

where i is the total distance over which attenuation occurs. This is the Beer-Lambert Law.

In-Class Practice Problems: Biol 1240: Beer-Lambert Law

Remember that you will not have a spreadsheet during an exam. You should be certain that you can do these problems with a calculator. Show all of your intermediate calculations. So you can check your work, the final answer is in bold text after the problem.

1. Below are some data for light ($\mu\text{moles} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) as a function of lake depth (measured in meters). Calculate a and $I(0)$.

z	Light
10	90.0
20	12.5
30	0.75
40	0.15

Answer: $a = 0.2211$, $I(0) = 93.36$

2. If $a = 0.8$, $I(0) = 3,333$ micro-Einsteins, what is $I(8)$?

Answer: $I(8) = 5.538$

3. Two lakes, A and B, are side-by-side. Lake B is more turbid than lake A. In lake A, $a = 0.5$. In lake B, the light at 10 meters is exactly 0.5 that of lake A. What is the extinction coefficient for lake B?

Answer: For lake B $a = 0.569315$

Hint: you do not need any additional information. Use the Beer-Lambert Law formula for the two lakes and the data given in the problem.

4. In the spring, a lake has $a = 0.1/\text{meter}$, later in the summer $a = 0.4/\text{meter}$. If $I(0) = 3000$ all year, at what depth in the summer would you expect to see no suspended plant growth?

Answer: $z = 11.5$ meters