

An Inquiry-Based Enzyme Lab

Norris Armstrong

Biology Division

University of Georgia

Room 403 Biological Sciences Bldg

Athens, GA, 30622

narmstro@uga.edu

Abstract: A laboratory commonly used in introductory biology classes is the breakdown of starch by the enzyme amylase. These labs tend use a “cookbook” format in which students are given explicit, step-by-step instructions that make it possible to easily complete the exercise without developing a good understanding of many of the concepts being taught. This workshop presents a three-week laboratory series that uses a guided-inquiry approach and collaborative teams to help students understand experimental design, the collection and analysis of data, and communication of results in the context of \biochemistry and enzyme function. The workshop will include a discussion of strategies designed to support the inquiry format and to help insure that students are well prepared for the lab. This laboratory series was designed for an introductory biology class but could be adapted for use in more advanced classes as well.

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Introduction

A laboratory commonly used in introductory biology classes is the breakdown of starch by the enzyme amylase. These labs often use a “cookbook” format in which students are given explicit, step-by-step instructions that make it possible to successfully complete the exercise without developing a good understanding of many of the concepts being taught. This workshop presents a series of labs that use a guided-inquiry approach to help students understand experimental design, the collection and analysis of data, and communication of results in the context of basic biochemistry and enzyme function. Compared to a traditional cookbook lab, no additional equipment and little additional preparation is needed for the inquiry lab.

This laboratory series was designed for an introductory biology class but could be adapted for use in more advanced classes as well. The complete lab consists of four modules taught in three 2-hour lab periods. Each lab includes a pre-lab homework assignment, the lab itself, and a post-lab writing assignment. This lab has been used successfully in 18 sections of a non-science major’s introductory biology laboratory enrolling approximately 300 students each semester.

Learning Objectives

At the completion of this laboratory series students will

- Have practiced designing and carrying out an experiment.
- Understand the difference between qualitative and quantitative results.
- Understand how to use a spectrophotometer.
- Understand the appropriate use of a control.
- Understand the preparation and use of a standard curve.
- Practice collecting and graphically representing data.
- Understand basic enzyme function and behavior.

Student Outline

Lab 1 (Week 1)

Objective 1: Use a *qualitative* method to determine whether starch is present in five mystery solutions and, if so, the relative concentration of starch in these solutions.

Background

On the lab bench, there are five solutions containing unknown concentrations of starch, labeled in bottles: A, B, C, D, and E. It is possible that one of the solutions does not contain starch. Using notes from your homework (Appendix B) as a guide, discuss and agree on an approach with your group that will allow you to:

1. Determine if each of unknown tubes contains starch. Do any of the tubes contain NO starch? How can you tell?
2. Place the 5 test tubes in order of starch concentration, from highest to lowest.

You may not use the spectrophotometer for this experiment. Before you begin to carry out your experiment, show or describe your experimental design to your GLA.

Available tools and equipment

Five bottles, labeled A-E, each containing a starch solution of unknown concentration
I₂KI (Note: I₂KI can stain clothing and skin. Gloves are available if you wish to use them.)
Pipettes
Test tubes
Test tube rack

Ideas to consider

- ❖ Information from the pre-lab reading may be helpful (Appendix B).
- ❖ Before you begin your experiment, practice using the pipette to draw up liquid and release it. Be careful and exact in your measurements. Notice that the pipette has two different numbering systems. How does the numbering work?
- ❖ Why is it important to use a different pipette for each solution you work with?
- ❖ How much liquid fits in a test tube? How do you find out how much solution to use?
- ❖ What do qualitative results tell you?

Objective 1 Experimental Design

Carry out your experiment and take notes describing what you did, how the experiments would help you answer your question, and what you found out. Realize that you will be compiling your notes together into a single report later. Your description of should be clear and complete enough so that someone else (as well as yourself, at a later date!) can follow your logic and repeat your tests if necessary. When you are done, please dump the test tube contents in the sink and place your test tubes into the broken glassware box.

Use the following guidelines to write up the experiment your group proposes to carry out.

Research Question:

- Context*: Provide the observations/knowledge that helped you choose your question.
- Question*: State what you hope to learn or conclude from your experiments.
- Justification*: Explain why your question is interesting or important.
- Hypothesis*: State what you think the answer to your question would be, and what was your guess about the results of your tests; i.e. your predictions.

Methods:

- Tests*: Explain how your tests will provide answers to your question; include what these tests are designed to uncover.
- Detail*: Provide sufficient detail so that another classmate could replicate your methods.

Predicted Results:

- Observations*: Thoroughly describe what you expect to see as the results of your tests.

- Data*: Describe how you will collect the data on your observations.
- Analysis*: Describe how you will use quantitative data to conclude if your hypothesis is correct?

Objective 2: Use a *quantitative* method to determine whether starch is present in five mystery solutions and, if so, the concentrations of starch in these solutions.

Background:

The results you obtained from the previous experiment are *qualitative*. They tell you which solutions have more/less starch than others but not much more than that. Often, it's desirable to know the absolute concentration of a solution – *quantitative* results. Absolute concentrations of a solution are generally determined using a spectrophotometer.

Using notes from your homework as a guide, your group should agree on an approach to:

1. Determine whether there is starch in each solution
2. Determine the concentration of starch in each solution.

Before you begin your experiment, show or describe your experimental design to your GLA.

Additional tools and equipment available

Distilled water
0.02 g/liter starch solution
Spec tubes (**These tubes are very expensive, so please be careful with them.)
Spectrophotometer

Ideas to consider

- ❖ What information do you lack about the mystery solutions?
- ❖ What supplies and methods are available that you could use to determine this information? (Hint: think about pre-lab questions 6-8.)
- ❖ What information will the spectrophotometer tell you about your solutions? What does absorbance refer to?
- ❖ Based on your qualitative results and knowing what information the spec will tell you about your solutions, what do you expect the relationship between absorbance and concentration will be?
- ❖ How can you use the information provided by the spec to determine the exact starch concentrations in your samples?

Objective 2 Experimental Design

Carry out your experiment and take notes below describing what you did, how these experiments would help you answer your question, and what you found out. Realize that you will be compiling your notes together into a single report later. Your description should be clear and complete enough so that someone else (as well as yourself, at a later date!) can follow your logic and repeat your tests

if needed. When you are done, please dump the test tube contents in the sink and place your test tubes into the broken glassware box. **Do not throw away the spectrophotometer tubes!**

Use the same guidelines as for objective 1 above to write up the experiment your group proposes to carry out. Graph paper will be provided.

Spectrophotometry

A spectrophotometer works by shining a light on one side of a sample solution and measuring how much light passes completely through the sample to a detector on the other side. If a substance is present that absorbs light, then not all of the light will make it through.

A good simulation of how a spectrophotometer works can be found at:

<http://www.chm.davidson.edu/java/spec/spec.html>

Why is the spectrophotometer set to a particular wavelength (580 nm)?

Most substances absorb light at one wavelength or another. For example, if you are wearing a shirt that is UGA red, that shirt is absorbing all visible light except for the wavelength that corresponds to red. Red light either passes through or bounces off the fabric and is able to reach our eyes. As a result, you see the color red. Likewise, most plants are green because they absorb most visible light except for the wavelength corresponding to green.

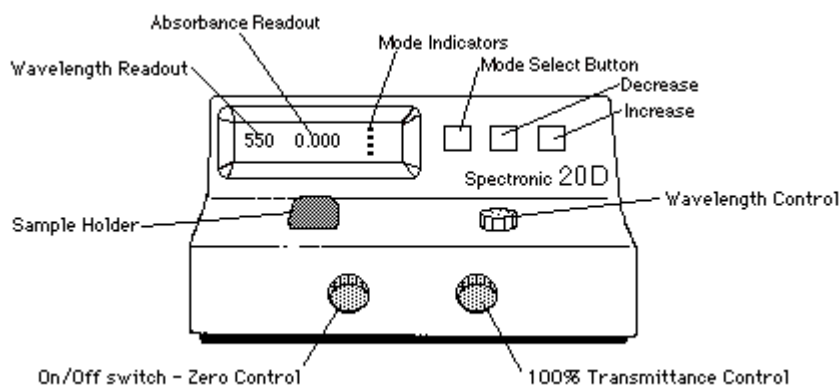
Different substances absorb different wavelengths of light so it is necessary to set the spectrophotometer to detect the wavelengths that correspond to the substance you want to measure. Also, some substances don't absorb light well at all. These substances will appear clear or white depending on whether the light passes through or bounces off them. To measure the concentration of molecules that do not absorb light, dyes must be added that change color when they bind to the substance. Potassium iodide, when it is bound to starch, absorbs light that has a wavelength of 580nm.

How do I work this machine?

Turn on the instrument with the **zero adjust/on-off control** and allow it to warm up for about fifteen minutes. Turn the **wavelength control knob** to select the wavelength you want to use; in our lab this will be **580 nm**.

Looking at your spec, notice that there is a button labeled “**Mode.**” There are four different modes: (1) **transmittance**, which measures how much light passes through the sample; (2) **absorbance**, which measures how much light is absorbed by the substance; (3) **concentration**; and (4) **factor**. We are not concerned with concentration and factor modes. The readings that you might get from them are not related to the concentrations of the solutions you will be measuring. The concentration and factor modes should both set to 1. Notify your GLA if they are not.

Calibrating your spec before beginning your experiment and between samples is essential for obtaining good consistent results.



Note: You will need at least 4 ml of solution in the spec tube to get an accurate reading.

- 1st. Set the mode to transmittance. With no sample tube in place and the cover closed, turn the zero adjust knob to bring the meter to 0% transmittance. Where is the zero adjust knob? Locate it on the diagram of the spectrophotometer and on your machine. *At this point, no light is shining on the detector. This step ensures that the measuring scale reads 0 under these conditions.*
- 2nd. You will need what is called a “blank” tube to calibrate your spec so that the meter reads 100% transmittance. Essentially, your blank tube is acting as a control.
- 3rd. Make sure the outside of the tube is clean and dry by wiping it with a chemwipe. *Try not to handle the tube on the sides—hold it at the very top. The grease and dirt from your fingerprints can absorb light!*
- 4th. Insert the tube into the cuvette holder in the spectrophotometer carefully pushing it all the way down. Align the tube so that the reference line on the cuvette holder matches the line on the tube.
- 5th. Close the cover of the cuvette holder. Adjust the light control knob on the spectrophotometer so that the meter reads 100% transmittance. *Putting the tube into the cuvette holder causes a window inside the spectrophotometer to open allowing light from a lamp to shine on the tube. Water and the glass in the tube can absorb a little light even though the tube doesn't contain any sample. By setting the scale on the spectrophotometer to 100% transmittance using this “blank,” you can distinguish between light absorbed by your sample and light absorbed by your “blank” tube.*
- 6th. Now you're ready to measure your samples! Prepare your spec tube with your samples that you want to measure. Wipe the outside of the tube to remove water and fingerprints and insert into the cuvette holder. Set the mode to absorbance. Align the tube, close the cover, and read the absorbance from the meter. Do not let your samples sit in the spec for very long since light can cause chemical changes to your samples and change the reading that you obtain.
- 7th. Because the scale used by the spectrophotometer can drift over time, you should repeat calibration steps (1) - (5) before measuring the absorbance for each sample.

Lab 2 (Week 2)

Objective: Determine how much starch a given amount of amylase enzyme is able to convert into sugar per minute.

Background

When you add amylase enzyme to starch, what happens? How could you measure the rate at which the enzyme converts starch into sugar? Another way to think of this is how much starch does the enzyme break down into sugar in a certain period of time.

Using the notes from your homework, discuss and agree on an approach with your group that will allow you to determine the amount of starch a given amount of amylase converts into sugar per minute.

Before you begin to carry out your experiment, show or describe your experimental design to your GLA. This week, **it is crucial that your calculations are correct!** Make use of previous lab work and prelab homework to inform your calculations. Be sure to check them with your GLA.

Available tools and equipment

I₂KI (Note: I₂KI can stain clothing and skin. Gloves are available if you wish to use them.)

Tris buffer, pH 7

0.02g/liter starch solution

Amylase at a concentration of 0.1g/liter

Pipettes

Spec tubes (**These tubes are very expensive, so please be careful with them.)

Test tube rack

Spectrophotometer

Glass bottle

Guiding Questions:

- ❖ When using the spectrophotometer, what does absorbance refer to? What can absorbance tell you about your samples?
- ❖ Even after you determine absorbance, you're still missing critical information: the amount of starch present. How would you determine this? Hint: think about the first lab, Objective 2.
- ❖ How can you tell if the concentration of starch changes over time? How can this information tell you if and how quickly and enzyme is working (enzyme activity rate)?
- ❖ What solution belongs in your "blank tube"? Do you need to add I₂KI or not?
- ❖ What would be an appropriate measure you could take to ensure your data were accurate?
- ❖ When do enzyme reactions stop? Why do you want to consider this when planning your experiment?
- ❖ How much of each solution should you use?
- ❖ Consider the equipment that you have available to use. Besides test tubes, you also have a glass bottle. In general, the more pipetting that you do, the more error you introduce into your experiment. This is particularly significant when you are working with very small quantities. Is there a way that you could use the glass bottle to make a "reaction solution" that you could then pipette into individual test tubes as needed? Hint: Refer back to question on making sweet tea (7) from the pre-lab for enzymes I.
- ❖ Previously, you diluted stock solutions of starch using water. Why would you want to use Tris buffer instead of water when making dilutions of your starch solutions for this experiment?
- ❖ How much iodine will you need? (Hint: your test tubes should contain iodine and reaction solution in a 1:3 ratio.) Also, **I₂KI will stop amylase enzyme activity.** Considering this, when would you want to add I₂KI to your test tubes?

You should make up a 30 ml bottled reaction solution containing the following:

1. **Starch at a concentration of 0.066g/liter.** Use the following equation and what you've learned in the past few labs to determine how much starch you will need to add in order to achieve this particular concentration.

Concentration of initial stock solution	X	Volume of initial stock solution	=	Concentration of final solution	X	Volume of final solution
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2. **Amylase at a concentration of 0.33g/liter.** Remember that once you add the amylase, the experiment has begun! **Do not add the amylase until you are ready to begin!**
3. Calculate the amount of Tris buffer that is needed to bring the solution to 30 ml.

Experimental Design

After thinking through the previous questions, use this space to consolidate what you plan to do and how these experiments will help you answer your question. Carry out the experiment and take notes below describing what you did and what you found out. Realize that you will be compiling your notes together into a single report later. Your notes should be clear and complete enough so that someone else (as well as yourself, at a later date!) can follow your logic and repeat your tests if needed. When you are done, please dump the test tube contents down the sink and place your test tubes into the broken glassware box. **Do not throw away the spectrophotometer tubes!**

Use the same guidelines as for objective-1 above to write up the experiment your group proposes to carry out.

- You will need to create a graph of your data. Graph paper will be provided.
- After your have finished your experiment, you will want to determine the following information for your friends at the microbrewery:
 1. The enzyme you used was at a concentration of _____ mg/ml.
 2. _____ mg of starch was broken down in _____ minutes.

Lab 3 (Week 3)

Objective: Determine the optimal conditions for amylase enzyme activity. Choose a single variable, either temperature or pH, to investigate.

Background

When you add amylase enzyme to starch, what is happening? How do external conditions, such as temperature or pH, affect enzyme activity? Will an enzyme convert starch into sugar more rapidly under some conditions than others? Will some conditions completely inhibit or prevent enzyme activity? Under what conditions will the enzyme work best?

Using the notes from your homework, discuss and agree on an approach with your group that will allow you to determine the optimal conditions for amylase enzyme activity, choosing either temperature or pH to investigate.

Before you begin to carry out your experiment, show or describe your experimental design to your GLA. This week, it is crucial that your calculations are correct! Make use of previous lab work and pre-lab homework to inform your calculations. Be sure to check them with your GLA.

Available tools and equipment

I₂KI (Note: I₂KI can stain clothing and skin. Gloves are available if you wish to use them.)

Tris buffered pH solutions: pH 5, 6, 7, 8, 9

0.02g/liter starch solution

Amylase at a concentration of 0.1g/liter

Pipettes

Spec tubes (**These tubes are very expensive, so please be careful with them.)

Test tube rack

Spectrophotometer

Water baths, for heating and cooling: (ice, room temperature, 30° C, 50° C, 70° C)

Ideas to consider

- ❖ If you vary pH or temperature, how do you think this will affect an enzyme? Why? What can you measure to determine if enzyme activity is affected?
- ❖ Based on what you know from the previous lab's experiment to determine the rate of enzyme activity, how long should you run your experiment?
- ❖ What solution belongs in your "blank tube"? Do you need to add I₂KI or not?
- ❖ What can you use as your control? Is your control the same as your "blank"?
- ❖ How can you stop enzyme activity (Hint: think back to last weeks experiment)?
- ❖ What are appropriate amounts of each solution to use? Look at your notes from the previous experiment. Do these amounts seem reasonable for this experiment? Which solutions will be the same and which will be different this time?
- ❖ Will you want to make dilutions of your starch solutions using water or Tris buffer this time? Why?

Experimental Design

After thinking through the previous questions, consolidate what you plan to do and how these experiments will help you answer the question. Carry out the experiment and take notes describing what you did and what you found out. Realize that you will be compiling your notes together into a single report later. Your notes should be clear and complete enough so that someone else (as well as yourself, at a later date!) can follow your logic and repeat your tests if necessary. When you are done, please dump the test tube contents down the sink and place your test tubes into the broken glassware box. **Do not throw away the spectrophotometer tubes!**

Use the same guidelines as for objective-1 in the first enzyme lab to write up the experiment your group proposes to carry out.

- After you have finished your experiment, record the following information for your friends at the microbrewery:
 1. The enzyme you used was at a concentration of _____ mg/ml.
 2. The enzyme works best at under the following conditions:

Assignment: Lab Report

Audience: You know better than anyone what you did, why, what you observed, and what you can conclude. Therefore, you are the expert and your goal is to explain your experiments and logic to others who are interested in your work. Keep in mind that, as the expert, you need to explain your methods, results and conclusions clearly enough so that someone reading your report can replicate your experiment.

Criteria for Evaluation: A rough draft of your report will be due next week. Your final lab report (due in 3 weeks) will be graded using the rubric on the following page. A shortened version of this rubric will be used for the rough draft.

Keep the following in mind when composing your report

1. Format

- Your lab report should be approximately 5 to 7 pages, double-spaced.
- Use the rubric to organize the report under section headings

2. Introduction

- Don't start with your aim; instead explain the different aspects of your experiment such as the justification before stating your hypothesis or question.
- Instead of defining terms, state how they related to your tests.

3. Figures and Tables

- Use tables to simplify the description of your tests and observations, but remember more is not always better; try to consolidate as much information on as few tables as possible (within reason).
- Tables and figures should still be well explained in the text, and they should be placed near that text.
- Provide labels for tables and figures (including axes).

4. Writing Help

- There are great resources from the UGA writing center for everything from the writing process, including grammar, organization, and help citing and paraphrasing at: <http://www.english.uga.edu/writingcenter/writing/index.html>

5. Plagiarism –is a violation of the University's Academic Honesty Policy. Those unclear on the meaning of plagiarism should refer to *A Culture of Honesty*: "Plagiarism includes, but is not limited to, the following acts when performed without appropriate attribution:

- Paraphrasing all or part of another person's written or spoken words without notes or documentation within the body of the work;
- Presenting an idea, theory or formula originated by another person as the original work of the person submitting that work;
- Repeating information, such as statistics or demographics, which is not common

knowledge and which was originally compiled by another person;

- Purchasing (or receiving in any other manner) a term paper or other assignment that is the work of another person and submitting that term paper or other assignment as the student's own work.”

Lab Report: Guidelines

Assignment: You have just designed and completed an experiment that determined the conditions at which an enzyme operates best. Now, thinking like a biologist, write a lab report that describes your experiment.

Introduction (25%)

_____ *Context:* Provide the observations/knowledge that helped you choose your question. Include relevant background information from your readings that led you to ask the question.

_____ *Question:* Describe the question you tried to answer by conducting experiments.

_____ *Justification:* Explain why your question was interesting or important.

_____ *Prediction:* State what you thought the answer to your question would be, and your initial predictions about the results of your tests.

Methods (12.5%)

_____ *Tests:* Describe your experiments and *exactly* how you tried to answer the question.

_____ *Interpretation:* Explain how your tests will provide answers to your question including what these tests were designed to find.

_____ *Detail:* Provide sufficient detail so that someone else could replicate your methods.

Results (25%)

_____ *Observations:* Thoroughly describe the results of your tests.

_____ *Graphs/tables:* Produce graphs and tables to display your data effectively.

_____ *Table of Data:* Table of experimental data was present and clearly labeled.

_____ *Graph 1:* Graph showing the absorbance of samples at different pH over time or relating the change in absorbance to pH was clearly labeled.

_____ *Graph 2:* Graph relating absorbance to concentration of starch (Standard Curve) was present and clearly labeled.

Conclusions (25%)

_____ *Claims:* Provide the answer to your question based on your findings.

_____ *Explanation:* Explain why you got the results you did.

_____ *Reflection:* State if your initial hypothesis was correct

_____ *Critical Reasoning:* Evidence from outside scientific sources.

_____ *Future Tests:* Explain what you could have done better or differently if you extended your project.

Grammar and Style (12.5%)

_____ *Grammar/spelling:* Follow correct conventions for grammar and spelling.

_____ *Format:* The ideas are organized in a logical format.

_____ *Clarity:* The paper is clear and concise.

Notes for the Instructor

Safety:

The reagents used in this lab are non-toxic. I₂KI can stain clothing and skin. Gloves should be provided for those who wish to use them.

Classroom Management

The goal of these labs is for the students to discover fundamental concepts regarding experimental design and procedures as they apply to biochemistry and enzyme analysis through an inquiry-based approach. Towards this end, the lab handouts provide minimal instructions and require students to develop their own experimental procedures. This increases the number of errors students are likely to make and lengthens the time required to complete the lab. In addition, many students will have had little experience with inquiry based-labs and may be uneasy when they are not given step-by-step instruction of what to do. To help ensure that the class proceeds as smoothly and successfully as possible, each lab is divided into three sections.

1. Pre-lab homework assignment.

In our experience, many students either do not read the lab or do so only minutes before the class is scheduled to start. The success of the labs described here demands that students be prepared for the work they will carry out. To encourage students to be prepared for each lab, we require them to complete and turn in a pre-lab homework assignment. These assignments ask the students to do two things:

- The problems in the assignment ask students to apply many of the same concepts and skills they will need to use in lab. At the beginning of the lab, the instructor asks different students to share some of their answers with the rest of the class. This process enables the instructor to identify and address misconceptions and other problems the students may be experiencing and to address these issues before the students start their experiments.
- The students are asked to design experiments to meet the objectives described in the lab and to turn in their work to the instructor at the beginning of the lab. Information on how to do this is provided in the lab and in the homework problems themselves. This helps to ensure that the students have at least looked through the lab and should be relatively familiar with the task they need to accomplish before they come to class.

2. Conducting their experiments

Before beginning their experiments, the students gather in their assigned lab groups to compare ideas for the experiment they need to conduct. Working collaboratively in this way helps the students detect errors and formulate more effective experimental plans. After developing a consensus approach, the students present this plan to the instructor and, if needed, address potential problems before proceeding.

The instructor monitors the work performed by each group and points out potential problems or errors when they arise. The instructor tries to avoid telling the students what to do. Rather

s/he tries to help the students develop their own solutions by asking guiding questions or pointing out analogous situations that students may be more familiar with.

3. Recording their results.

The students are asked to write down their experiments, results, and conclusions to turn in at the end of the day. The goal of this task is to encourage students to discuss their experiment and results with each other and the instructor as well as to write down what they know while the information is still fresh in their minds. In this respect, obtaining an “accurate” result is not of primary concern. Rather, the students should demonstrate that they have thought through their experiment and interpreted their results in an appropriate manner. The instructor may provide oral or written feedback to these assignments if desired.

Starch measurement

Iodine-potassium iodide solution (I_2KI) contains a mixture of iodine (I_2) and potassium iodide (KI). By itself, the solution appears brown at higher concentrations but is a yellowish orange color when diluted in water. The iodine inserts into the middle of the helical structure formed by intact amylose molecules (starch and glycogen) and the solution changes to a bluish-red. The greater the color change, the more amylose that is present. This color change only occurs in the presence amylose and not with smaller carbohydrate fragments because these smaller fragments do not have the necessary helical structure. **Note:** Iodine stains skin and clothing, so be sure to wear gloves while handling it.

Amylase activity

Amylase was the first enzyme to be isolated. This enzyme cleaves the α -1,4-glycosidic bonds found in starch and glycogen molecules breaking large polymers down into mono-, di- and trisaccharides. Amylase can be obtained commercially from most biological supply companies, many health stores, or from the students themselves (saliva).

The optimal conditions for amylase function depend on the source of the enzyme. For example, human pancreatic amylase works over a broad range of pH (5-10.5) with an optimum at a pH of 7.0 (Sky-Peck and Thuvasethakul, 1977) while amylase from the fungus *Streptomyces* works over the range of pH 5.5-8.5 with an optimum at a pH of 6 (Dey and Agarwal, 1999). Similar variation in optimum temperatures should also be expected.

Week-1: Qualitative and Quantitative measures

Assignments due:

1. Enzymes I pre-lab homework
2. The in-class experimental design for objectives 1 and 2 needs to be shown to the instructor before students leave lab. (Rubric: 2 points – good effort, 1 point – cursory effort, 0 points – no effort.)

Suggested Teaching Strategies:

- **Homework:** In this lab, the students will make qualitative and quantitative measurements of a starch solution and will prepare a standard curve. Go over the pre-lab questions 1-7 as a

class to make sure that the students understand the concepts being covered. This is a great time to review some of the skills they may have learned in previous labs including concentrations, graphing, etc. Do not go over questions 8-13 until the students have discussed them in their groups and have prepared an experimental plan for you to look over.

Give the students an overview of what will be happening over the next three weeks. Remind them that they will be writing up the results of their individual experiments from the third week as a report.

- **Experimental Plans:** Divide your students into groups. These groups will last for the three-week duration of the lab. The students need to work with their groups to figure out what specific steps they will need to take to complete the assignment. They should get started with their work as soon as possible in order to ensure that they finish on time.

Potential Problems:

Objective 1. Qualitative Measures. The students are asked to do two things:

1. Rank five tubes according to their respective starch concentrations (e.g. A has more than B).
 - Most groups are able to quickly complete this task by adding Iodine-Potassium Iodide (I_2KI) to each of the tubes and comparing the colors in each tube. The more intense/darker the blue coloration, the more starch that is present. The most common mistake students make when completing this task is by adding too much I_2KI or by not adding the same amount to each tube. Though I_2KI changes color only when bound to starch, large amounts of the chemical can make it difficult to observe this color change.
2. To determine if any of the tubes contain no starch.
 - The stock I_2KI solution is fairly dark and, when diluted into a tube containing a dilute starch solution, it is essentially impossible to tell if a color change has occurred. The only way to be certain to add the I_2KI to a tube that is known not to contain starch (i.e. contains only water). This tube can then be compared to the unknown tubes to determine if there is a difference in color. The primary difficulties students experience with this task are similar to those described above, namely that they add too much I_2KI or do not add the same amount to each tube.

Objective 2. Quantitative Measures. This module is taught the same day as module-1. The students must determine the exact concentrations of starch in each tube. Many of the groups will struggle and may not complete this part of the lab. However, most should progress to the point that they realize that they will need to prepare a standard curve. Despite the example in the homework assignment (Q#8) many (most?) will not have a clear idea of what a standard curve is. You will probably need to give the students hints and ask guiding questions when they develop their experimental plan at the beginning of the lab. It is important that the students try to work out the answers for themselves. Many will want to simply wait until you tell them what to do. Try to avoid this.

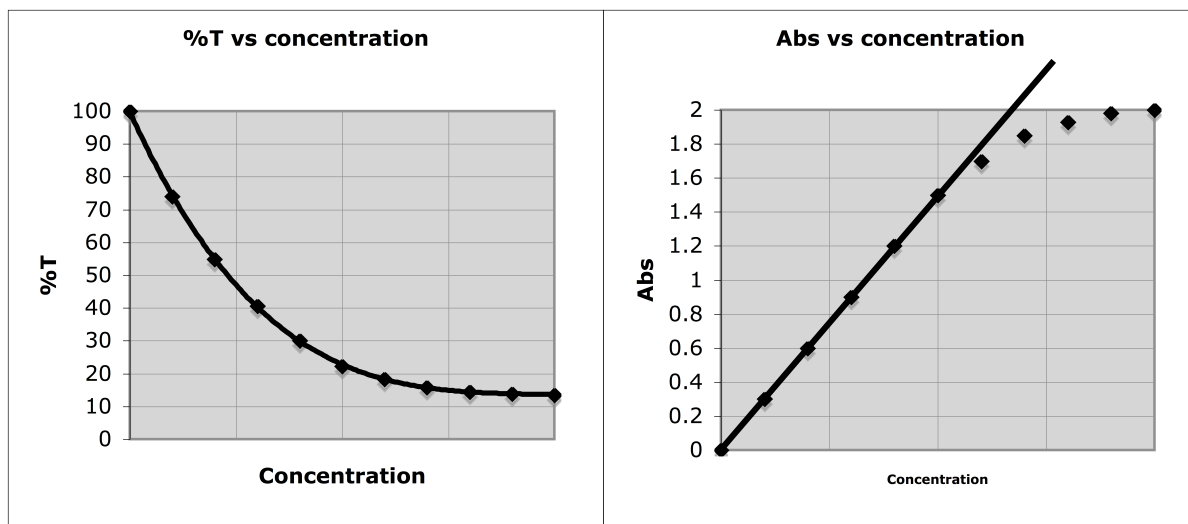
To complete this part of the lab, the students will need to carry out three tasks:

1. Dilute a stock solution of starch into a range of concentrations that can be used to create a standard curve.
 - Students find this to be one of the more difficult tasks associated with this module. They usually know that they need to dilute the stock starch solution to different concentrations but do not know how to make specific dilutions or how to determine the new starch concentrations of dilutions they have made. This can potentially be addressed by the instructor using guided questions and by making sure the students understand the concepts and skills associated with making dilutions when going over the pre-lab homework problems at the beginning of the class.
2. Measure and record the absorbance/transmittance of their known and unknown starch solutions using the spectrophotometer.

Information how to use the spectrophotometer is provided in the lab handout and the students are introduced to the idea of standard curves and how to use them as part of the pre-lab homework assignment. Despite this, students frequently have questions with this part of the lab. Some of the more common questions:

- How does the spectrophotometer work and why are we using them?
 - It shines a light through a sample in a test tube and measures how much of the light passes through (transmittance/absorbance). If a substance is present that absorbs light, not all of the light will pass through. By measuring how much light is able to pass through, it is possible to determine the concentration of the substance.
- Why are we setting the spectrophotometer to 580 nm?
 - Different substances absorb different wavelengths of light. The colors we see around us correspond to the wavelengths of light that are not absorbed by the substance you are looking at. E.g. plants appear green because they absorb most visible wavelength of light except green.
 - We need to set the spectrophotometer to look for the specific wavelength of light absorbed by the substance(s) we want to measure. Iodine bound to starch absorbs light with a wavelength of 580 nm.
- What are we measuring on the spec? Should we use absorbance or transmittance?
 - Transmittance is easier for students to understand (measured from 0-100%) but will not give them a straight line when they plot out their standard curve. To make a standard curve that is easier to use, have the students use Absorbance $(A) = \log_{10}(1/T)$ and gives values from 0-2. The spec should automatically give them Abs values. An example of how the same samples plotted out as %T and Abs is shown below.
 - A common mistake is assuming the Abs value is same as the concentration.
 - One common problem occurs when students change the settings on the spectrophotometer before reading the instructions (for example changing the

wavelength being measured and/or maxing out the calibration dials). This requires the instructor to reset the spectrophotometer so students can get accurate readings.



- The spectrophotometer does not give very accurate readings for values greater than an absorbance of 1.5. If students get readings much higher than 1.5, they should dilute their sample and take a new reading.

3. Graph out their results from known starch solutions in task-2 to create a standard curve and use this curve to determine the starch concentrations in their unknown tubes.
 - Once students realized that they would need to make a standard curve, the primary problems our students experience with this part of the lab is the concept of continuous versus categorical data and how to graphically represent this information. For example, some students insist on plotting the results from their known starch solutions in a bar graph even though more appropriate examples of standard graphs (line graphs) are provided in the pre-lab homework assignment and discussed in class. Other students use inappropriate or inaccurate axes. As a result, these students often have difficulty determining the concentration of their unknown samples. This can be addressed by the instructor using guided questions and by making sure the students understand the concepts and skills associated with graphing data when going over the pre-lab homework.
 - A second problem students experience is derived from the fact that some of them have become accustomed to solving problems similar to the one posed here by calculating the slope of a line mathematically using programmable or graphing calculators. This can lead to two different types of errors.
 - The assay method being used only works within a limited range of starch concentrations. Solutions with concentrations outside of this range will give inaccurate readings and, if included in the calculations being made, will lead to an inaccurate estimate of the slope of the standard curve.

- Once the student obtains a formula for the relationship between concentration and absorbance/transmittance, s/he rarely questions the accuracy of this formula. As a result, unknown concentrations are determined simply by plugging the values into the formula and assuming the answers obtained are correct even if these answers are nonsensical.
- These issues can be addressed by requiring the students to graph their data out on paper, which will help them visualize the data better. Alternatively, the instructor can ban the use of graphing calculators.

Note: Depending on your students' skills, some or many of the groups may not complete the second objective by the end of the lab. This is expected and built into the design of the laboratory. The students will have additional chances to prepare and work with standard curves in the second and third weeks of the lab. If your students experience a great deal of difficulty with the standard curves, you can spend additional time addressing this topic while covering the homework problems for the subsequent weeks' labs.

Week-2: Enzyme Rates:

This lab is composed of two parts and asks students to apply what they learned the previous week in order to determine the rate at which an enzyme promotes a chemical reaction.

1. Prepare a standard curve for determining starch concentrations. This assignment reinforces work carried out the previous week and is supported by questions involving standard curves on the pre-lab homework assignment.
2. Measure the change in starch concentration over a period of time with and without enzyme. The students are usually able to figure out what they need to do with a little guidance from their instructor.

Assignments due:

3. Enzymes II pre-lab homework
4. The in-class experimental design needs to be shown to the instructor before students leave lab. (Rubric: 2 points – good effort, 1 point – cursory effort, 0 points – no effort.)

Suggested Teaching Strategies:

- **Homework:** It is important that the students understand what a standard curve is and how to use it for this lab. The pre-lab homework has the students work with several examples of standard curves that deal with concentrations of a substance dissolved in water and this should be enough to get them headed in the right direction. Feel free to go over the homework as a class (except for Q#9) to help those students who are still having trouble. Students may give just a yes/no answer for question number 5. Whether the students understand this answer may be revealed by their answers on questions 6 and 7.
- **Experimental Plans:** Have the groups show their experimental plans after they have come to a consensus and before they begin their experiments. Students might take an excessive amount of time determining the appropriate dilutions of the sucrose stock and may need help figuring out how to solve this problem.

Potential Problems:

- Preparing solutions. The students will generally make several separate solutions of starch and enzyme in separate tubes and use a different tube for each time point. As a result, each solution is likely to have slightly different beginning starch and enzyme concentrations because of measurement errors. This will mess up the experiment's results before it even starts. A better approach is to make a single solution of starch and enzyme ("master mix" and aliquot (divide) it up into separate tubes that can be used for different time points. In this way, each time point tube is guaranteed to start out with the same starch and enzyme concentrations.
- When measuring starch concentrations the students may attempt to speed up the process by using multiple spectrophotometer tubes or may use the same tube but do not rinse the tube out between samples. Because each spec tube is similar to but not exactly the same as another, measuring the exact same solution in different spec tubes will give slightly different readings. Not rinsing between samples will alter their measurements because some of the old sample may still be in the tube.
- Using enzymes. Students are unfamiliar with how quickly enzymes work and often add either too much or too little of the enzyme or add the enzyme too early (i.e. before they are ready for the experiment to begin). As a result, the reactions go to completion before the experiment finished or show little change in starch concentration. Students can be allowed to discover this problem and develop a solution on their own. However, if time is a concern, the easiest remedy is to recommend that students use a specific amount of enzyme in their experiments.
- Though it is pointed out to them in the lab handout, students forget that I_2KI inhibits amylase activity. If they add it to their solutions before running the experiment, they will not see any change in starch concentrations. Conversely, if they simply set aside tubes while doing something else, the reaction will continue longer than they intended and before they have had a chance to measure starch concentrations.
- Students often try to determine the rate of an enzymatic reaction using a single time point and 0. More accurate rates can be determined if two or three time points are used.
- Some students may think that it will be okay to use the standard curve from the day before. This is not a good idea because they will be using new solutions and reagents that may be slightly different than the previous week. They will also be using different test tubes and conditions such as humidity, temperature, etc may also be different. All of this can change the results they obtain.

Week-3: Enzyme properties. This lab asks the students to apply what they have learned the previous two weeks to determine how environmental conditions such as pH or temperature affect the activity of an enzyme. The students need to select an environmental variable and design an experiment to test how changes in this variable affect the rate of a reaction catalyzed by an enzyme. Students will need to carry out the three activities.

1. Prepare a standard curve for determining starch concentrations.
2. Prepare experimental samples with which to examine enzyme activities under different environmental conditions.
 - Students usually prepare the reagents for each sample tube individually. As a result, measurement errors can cause the students' results to be highly variable and erratic. A better solution is for them to prepare a "master" solution containing all of the common components and dividing this among the different sample tubes they plan to examine. This will help to ensure the each tube contains the same concentrations of all of the reagents.
3. Measure absorbance at different time points to determine enzyme reaction rates.
 - Students often try to determine the rate of an enzymatic reaction using a single time point. More accurate rates can be determined if two or three time points are used.

Assignments due:

1. Enzymes III pre-lab homework
2. The in-class experimental design needs to be shown to the instructor before students leave lab. (Rubric: 2 points – good effort, 1 point – cursory effort, 0 points – no effort.)

Suggested Teaching Strategies:

- **Homework:** In this lab, the students will put into practice what they have (hopefully) learned the previous two lab sessions in order to identify conditions (pH or temperature) that are close to optimal for an enzyme (amylase) to work. The homework is meant to remind them of these skills as well as provide hints as to how to use them to complete this lab's assignment.
- **Experimental Plans:** The students need to get together in their groups and figure out what specific steps they will need to take to complete the assignment. They should get started with their work as soon as possible in order to ensure that they finish on time. It might not be a bad idea for them to subdivide the work, e.g. 1-2 people set up the standard curve while the rest get the enzyme reactions ready.

Potential Problems:

- Students may still be using too little or too much enzyme. To ensure that the students are able to finish this last enzyme lab, it may be a good idea to recommend that students use a specific amount of enzyme in their experiments.
- Remind students that they should make a "master mix" of the enzyme reaction they want to carry out that can be aliquoted to separate containers for the different conditions they want to test. Alternatively, instead of using separate test tubes in the water bath/incubator for each time point, they can use a single large flask and take out only as much as they need each at each time point.

- Some students may still want to use the standard curve from the day before. Remind them that this is not a good idea because they will be using new solutions and reagents that may be slightly different than the previous week. They will also be using different test tubes and conditions such as humidity, temperature, etc may also be different. All of this can change the results they obtain.
- Ask the students when they want to add I_2KI to remind them why this is important. As soon as they collect a sample, they should immediately add KI to stop the reaction.
- Remind students that they will want more than one time point to accurately determine the rate of an enzymatic reaction.

TA Training

In order for the labs described here to work effectively, it is important that the students not be given detailed instructions on how to carry out their experiments or to evaluate their data. Students can become frustrated with this approach, especially if they have not had previous experience with inquiry, and may frequently ask what they should do. Alternatively, the students may sit and do very little waiting to be given more explicit instructions. Ideally, the instructor will help these students by asking guiding questions, pointing out possible flaws in their logic, and using analogies. This can be difficult for graduate teaching assistants who may have had little experience with classes taught using inquiry themselves. Ironically, it may be the most motivated TAs who experience the most difficulty as they can become very concerned when the students experiments don't work particularly well. The instructor can remind the TAs that "getting the right answer" is not the primary goal of the lab. Rather, one of the more important skills that the lab is trying to teach is how to design, carry out, and interpret experiments as well as to critically evaluate work of others whether it is presented at a scientific conference or in a news article on TV or in the paper.

Assessment

The lab includes both formative and summative assessments that includes the following components:

1. Pre-lab homework questions – questions are designed to encourage students to think about and apply concepts they will need to use during the upcoming lab. A small number of points are awarded for correct answers to encourage the students to take the assignment seriously.
2. Pre-lab experimental plan questions – questions are designed to encourage students to plan out the experiments they will do during lab. This is a formative assessment. Points are awarded not for correct answers but rather for showing evidence of having thought through the work they will be doing.
3. In lab writing: Experimental Design – The students are to work with their peers and critically evaluate the experimental designs they prepared before coming to lab. They are also expected to write out their revised plan, their predictions for their experiments, the results they obtain, and what they would conclude based on their findings as they carry out the experiment. This assignment encourages them to begin the writing process as they work rather than to delay until shortly before a summative lab report is due to be turned in.

4. Summative Laboratory Report – We are using a modified lab report format that encourages students to make connections between the claims they are making and the evidence they have collected using prompts such as
- Introduction: What are the questions I am trying to answer?
 - Methods: What did I do?
 - Results: What did I observe?
 - Discussion: What can I claim? How do I know? .

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About the Author

Norris Armstrong received a B.S. from Southampton College at Long Island University and a Ph.D. in Zoology from Duke University. He is currently an Assistant Professor in the Genetics Department at the University of Georgia where he teaches large introductory Biology classes and laboratories for science and non-science majors. His research examines approaches such as writing-to-learn, cooperative learning, and inquiry to promote learning in very large classes.

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Appendix A: Equipment and Materials

The supplies and materials for this largely identical to those required by a traditional cookbook lab. The primary difference is how these materials are provided to the students. The reagents used in this lab are non-toxic though I₂KI can stain clothing and skin. Gloves should be provided for those who wish to use them.

Week 1:

For each group of 3-5 students

100 ml	I₂KI solution • 2g KI
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	<ul style="list-style-type: none"> • 1g Iodine • H₂O to 100 ml • Store in a dark bottle or wrapped in tin foil at RT
100 ml	0.02% (0.2g/liter) starch solution <ul style="list-style-type: none"> • Easiest is to make 100 mls of a 10x stock solution (0.2g in 100 ml) and then dilute an aliquot 1:10 to make a working stock solution. • <u>NOTE: Starch must be heated to go into solution effectively.</u> This can take several hours.
100 ml	Unknown starch sample "A" (0.064 g/liter) <ul style="list-style-type: none"> • 32 ml working stock + 68 ml dH₂O
100 ml	Unknown starch sample "B" (0.14 g/liter) <ul style="list-style-type: none"> • 70 ml working stock + 30 ml dH₂O
100 ml	Unknown starch sample "C" (0.12 g/liter) <ul style="list-style-type: none"> • 60 ml working stock + 40 ml dH₂O
100 ml	Unknown starch sample "D" (0.032 g/liter) <ul style="list-style-type: none"> • 16 ml working stock + 84 ml dH₂O
100 ml	Unknown starch sample "E" (0.096 g/liter) <ul style="list-style-type: none"> • 48 ml working stock + 52 ml dH₂O
2x	Test tube racks
1x + spares	Spectrophotometer tubes
5 each	1 ml and 5 ml Pipettes
1x	Wax pencils for labeling spectrophotometer tubes
1x	Spectrophotometer capable of visible light at 580 nm

For each laboratory section of 15-20 students

1 box each small, med, lrg	Gloves
1 box of 500-1000	Disposable Test tubes (13 x 100mm)
1 liter	Beaker for waste fluids

Week 2:

Same as for Week-1

Also include:

For each group of 3-5 students

100 ml	50 mM Tris, pH 7 <ul style="list-style-type: none"> • Make from working stock for lab (see below)
1x	50 ml Erlenmeyer flasks

For each laboratory section of 15-20 students

1 liter	50 mM Tris, pH 7 • 6.055 g/liter • Adjust pH with HCl or NaOH as needed
1 box	1 ml disposable syringes
1 box	5 ml disposable syringes
50 ml	Amylase enzyme • 1gm / 100 ml cold water • Keep on ice while not in use

Week 3:

Same as for Week-2

Also include:

For each group of 3-5 students

100 ml	50 mM Tris, pH 5 • Make from working stock for lab (see below)
100 ml	50 mM Tris, pH 6 • Make from working stock for lab (see below)
100 ml	50 mM Tris, pH 7 • Make from working stock for lab (see below)
100 ml	50 mM Tris, pH 8 • Make from working stock for lab (see below)

For each laboratory section of 15-20 students

1 liter	50 mM Tris, pH 5	See instructions for preparing above
1 liter	50 mM Tris, pH 6	
1 liter	50 mM Tris, pH 7	
1 liter	50 mM Tris, pH 8	
1x	100 ml beaker	
3x	Water baths set for 30, 50, and 70 °C	
1x	Ice bath	
100	50 ml disposable conical tubes	For incubation of sample solutions
5x	Wire racks for 50 ml conical tubes	

Example laboratory set up.



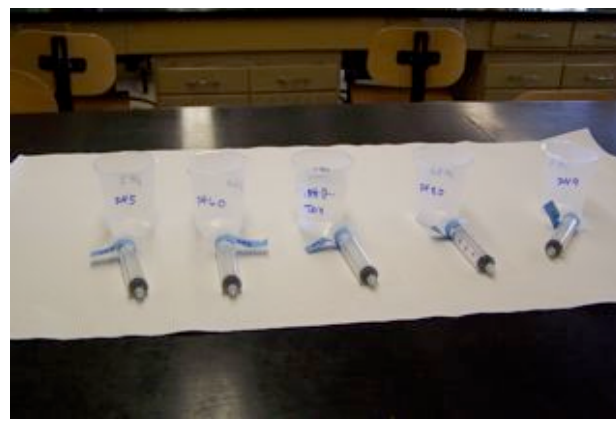
Week-3 group station



Week-3 group station



Tris Buffer stock



Tris Buffer station



Week-3 Water Baths



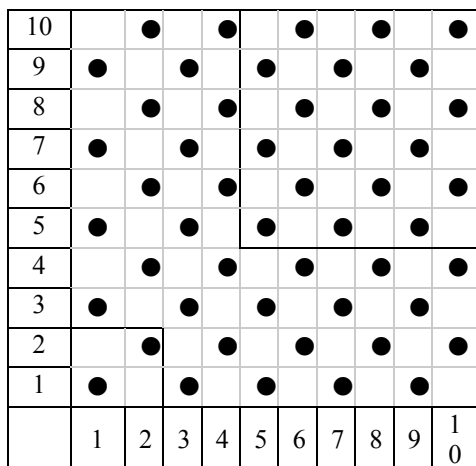
Laboratory Bench preparation

Appendix B: Pre-lab Homework Week-1 and Answer Key

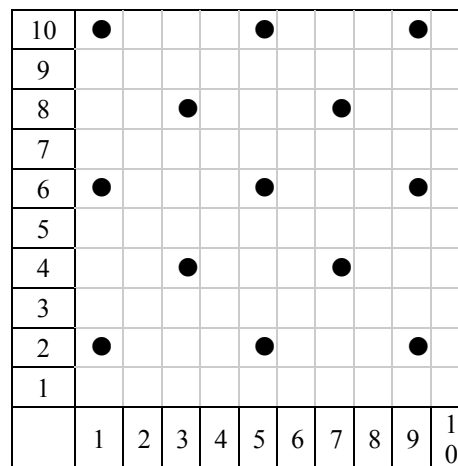
There are many ways to measure, calculate, weigh, quantify, compute, or simply count the characteristics of things that you might want to investigate. For some of the more common ways of doing this, standard units of measurement are used to ensure that scientists are able to understand each other while discussing what they have learned. It is also important to be able to convert between different units of measurement when needed. Below are problems that will give you practice with the types of measurements you will be making in the coming week's lab.

Scientific Units Conversions Chart

1 inch = 2.5 centimeter	Weight measured in grams (g)	Volume measured in liters (L)
1 mile = 1.6 kilometers	(mg) milligram = 1/1000g	(ml) milliliter = 1/1000L
1 ounce = 28.57 grams	(µg) microgram = 1/1000mg	(µl) microliter = 1/1000ml
1 quart = 1.10 liters	(ng) nanogram = 1/1000 µg	(nl) nanoliter = 1/1000 µl
°F = (°C)(9/5) + 32		



Grid A



Grid B

1. The graph above shows the distribution of dots in two 10 x 10 cm grids.
 - a. In which grid is the concentration of dots the highest?
 - b. What is the concentration of dots in both grids in dots/cm?

Grid A:

Grid B:
 - c. Which has the higher concentration of dots, the 2 x 2 section in the lower left corner of grid A or the 6 x 6 section in the upper right corner? How do their concentrations compare to the concentration of the whole grid?
 - d. What conclusion regarding concentrations can you draw from your answer to the previous question?
2. Indicate which of the following is the most and least concentrated. Which two show the same concentration?

	50 g/liter	0.5g/ml	5 mg/ml	500mg/liter	5g/100ml
Most concentrated					
Least concentrated					
Same concentration					
Concentration in mg/ml					
Answers:	50	500	5	0.5	50

Hint: From the conversions chart you know that there are 1000ml in one liter, so you can perform the conversion

$$\frac{500\text{mg}}{\text{liter}} = \frac{500\text{ mg}}{\text{liter}} \times \frac{1\text{ liter}}{1000\text{ml}} = \frac{500\text{ mg}}{1000\text{ml}} = \frac{0.5\text{mg}}{\text{ml}}$$

Try converting all the concentrations above to the same units, say mg/ml and then you can easily compare them in the bottom row.

3. You want to make homemade Ginger Ale and download some recipes from the Web. One recipe calls for a 20g/liter solution of sucrose (table sugar). You only need 400 milliliters of the solution and making a whole liter would be wasteful. How many grams of sucrose would you need to make just 400 milliliters? Show your work.

$$\frac{20\text{g}}{\text{liter}} \times \frac{1\text{ liter}}{1000\text{ml}} = \frac{\text{g}}{1000\text{ml}} = \frac{\text{g}}{400\text{ ml}}$$

4. A friend of yours offers to help but doesn't read the recipe carefully. Instead, s/he mixes 265 grams of sugar with water and has a final volume of 725 ml. What is the concentration of this solution in grams/liter? Show your work.

$$\frac{265\text{g}}{725\text{ ml}} = \frac{\text{g}}{\text{liter}}$$

5. A different recipe asks for a solution with a final concentration of 100 mg of sucrose per milliliter of (100 mg/ml). If you had 270 ml of this solution instead of one ml, what would the concentration be?
6. Despite a few mistakes, your Ginger Ale came out pretty good. To make things easier the next time you want to make a batch, you prepare a stock solution of sugar that is 500 grams/Liter. Using this, you will need to make solutions that are 1/2, 1/10 and 1/100 times more dilute. You will need 100 mls of each of these solutions. Describe how you would make them.

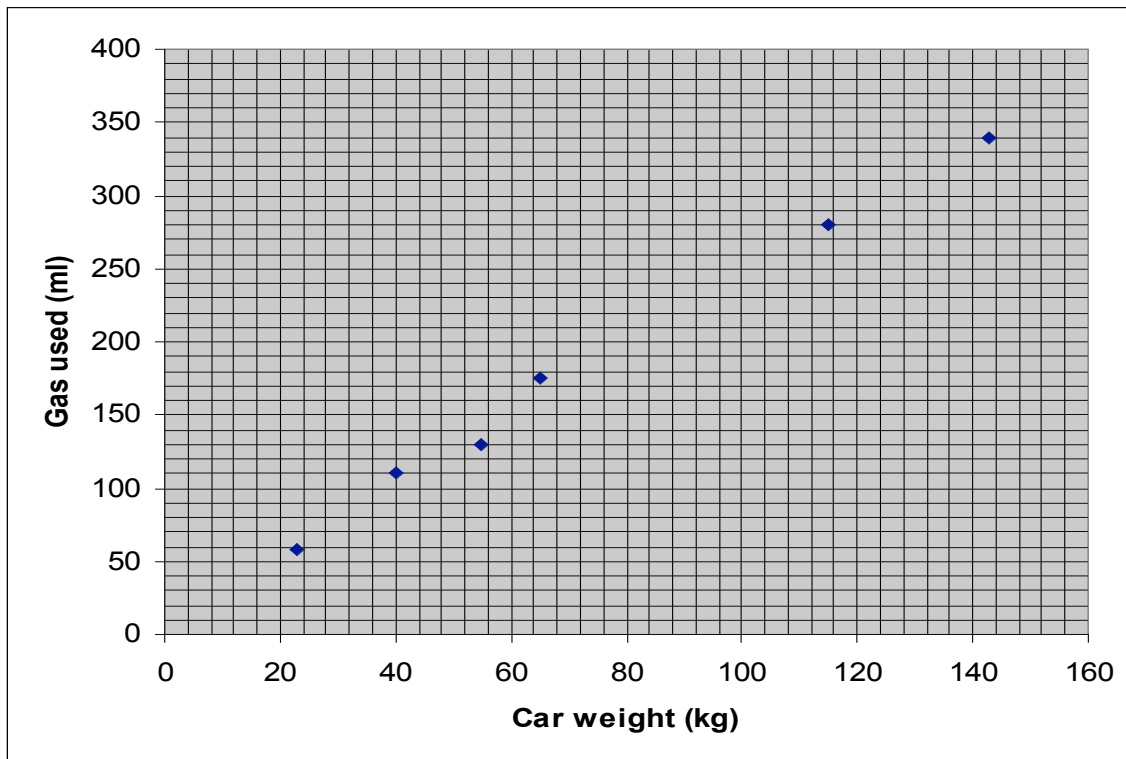
1/2 times more dilute = ____ volume of concentrate [500g/l] plus ____ volume water

1/10 time more dilute = ____ volume of concentrate [500g/l] plus ____ volume water

1/100 times more dilute = ____ volume of concentrate [500g/l] plus ____ volume water

7. You are hosting a taste test of your Ginger Ale and you want to compare it to some sweet tea. You need to make up 6 identical glasses of sweet tea for the test, each with 100ml of sweet tea. Instead of making up each of the 6 glasses separately, you decide to save time and increase accuracy for comparison by making enough for all and then pouring 100ml into each class. How

much fresh brewed tea and sugar would you need to combine so that the final concentration of sugar was 500g/liter and you had 600ml total?



8. Your cousin is building a go-cart for a race and you are helping. Heavier go carts go faster but weight affects gas usage. The graph above shows the relationship between the size of last year's go-carts and the amount of gas they used during the race. Using this chart, answer the following questions.
- What overall conclusion can you draw from the data depicted on this graph?
 - How much gas would you expect a car that weighs 40 kilograms to use?
 - Predict how much gas a car that weighs 100 kilograms would use? Show how you determined this.
 - You cousin bought a gas tank that will hold 200 ml of gas. What is the heaviest car you could make?

Would Dr. Atkins approve?

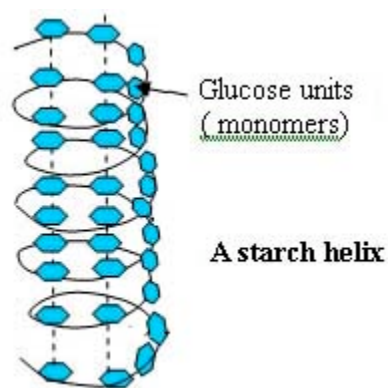
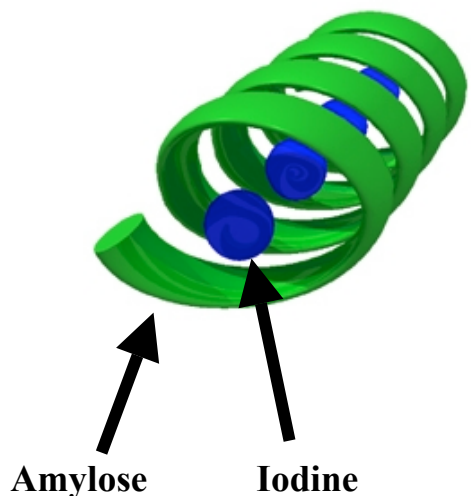
How do you measure something you cannot see? Researchers must often do just that. For example, starch is one of the major forms of digestible carbohydrate present in many foods and, as a result, starch content is of interest to many different groups. Farmers and food manufacturers evaluate the quality of grain (wheat, barley, corn), in part, by measuring how much starch is present. Fruit growers look at how much starch has been converted into sugar to determine the optimal time for harvesting. Nutritionists and many consumers keep track of the starch content of different foods to maintain a healthy diet.

Unfortunately, it is not possible to “see” starch molecules or distinguish them from other molecules just by looking at them. But, there are dyes that can help detect the presence of starch, and machines that can be used to quantify the amount of dye that binds the starch.

IKI: Iodine-potassium iodide solution.

Iodine-potassium iodide solution contains a mixture of iodine (I_2) and potassium iodide (KI). By itself, the solution is a yellowish orange color. However, the

Iodine solution changes to a bluish-red in the presence of amylose (starch and glycogen). The iodine inserts into the middle of the helical structure formed by intact amylose molecules. This color change only occurs in the presence amylose and not with smaller carbohydrate fragments because these smaller fragments do not have the necessary helical structure. **Note:** Iodine stains skin and clothing, so be sure to where gloves while handling it.



Starch Solution

Amylose is a large molecule but the glucose monomers it is made of contain many polar-covalent chemical bonds. As a result, starch and glycogen can dissolve in water relatively easily. A stock solution of starch will be available on the side bench in the lab for you to use. The concentration of the solution will be indicated on the side of the bottle.

9. Using the information provided above, propose a test that would allow you to determine the relative amounts of starch in different tubes (i.e. which tubes have more starch and which tubes have less)
10. What sort of results would you expect to see if there were:
 - a. Different concentrations of starch in two tubes?

- b. How could you tell which tube contained the most starch?
 - c. How could you tell if there was the same concentration of starch in two tubes?
 - d. How could you tell if there was no starch in a tube?
11. Use the information describing spectrophotometers (below) to answer the following questions.
 - a. When you measure a solution in a sample tube, the spectrophotometer indicates 100% transmittance. What does this mean? (Hint: think about absorbance.)
 - b. What is a blank and what is it used for?
 - c. You want to measure the concentration of starch in your samples and need to create a blank. Would you want to include iodine? Why or why not?
12. Describe how you could have used the spectrophotometer to examine the relative concentrations of starch in each tube.
13. When measuring your starch samples, what sort of results would you expect to see if there were: (Hint: remember absorbance and transmittance).
 - a. Different concentrations of starch in two tubes?
 - b. How could you tell which tube contained the most starch?
 - c. How could you tell if there was the same concentration of starch in two tubes?
 - d. How could you tell if there was no starch in a tube?
14. You need to determine how much starch in each test but in terms of grams/liter. Describe how could you use the spectrophotometer to do this? (Hint: remember the stock starch solution available on the side bench.)

Pre-lab Homework Answer Key

1a/b. Grid A = 50 dots in 100 squares = 0.5 dots/square

Grid B = 13 dots in 100 squares = 0.13 dots/square

1c. The smaller, large, and whole grids have the same concentration.

1d. Concentration is independent of volume.

2.

	50 g/liter	0.5g/ml	5 mg/ml	500mg/liter	5g/100ml
Most concentrated		X			
Least concentrated				X	
Same concentration	X				X
Concentration in mg/ml	50	500	5	0.5	50

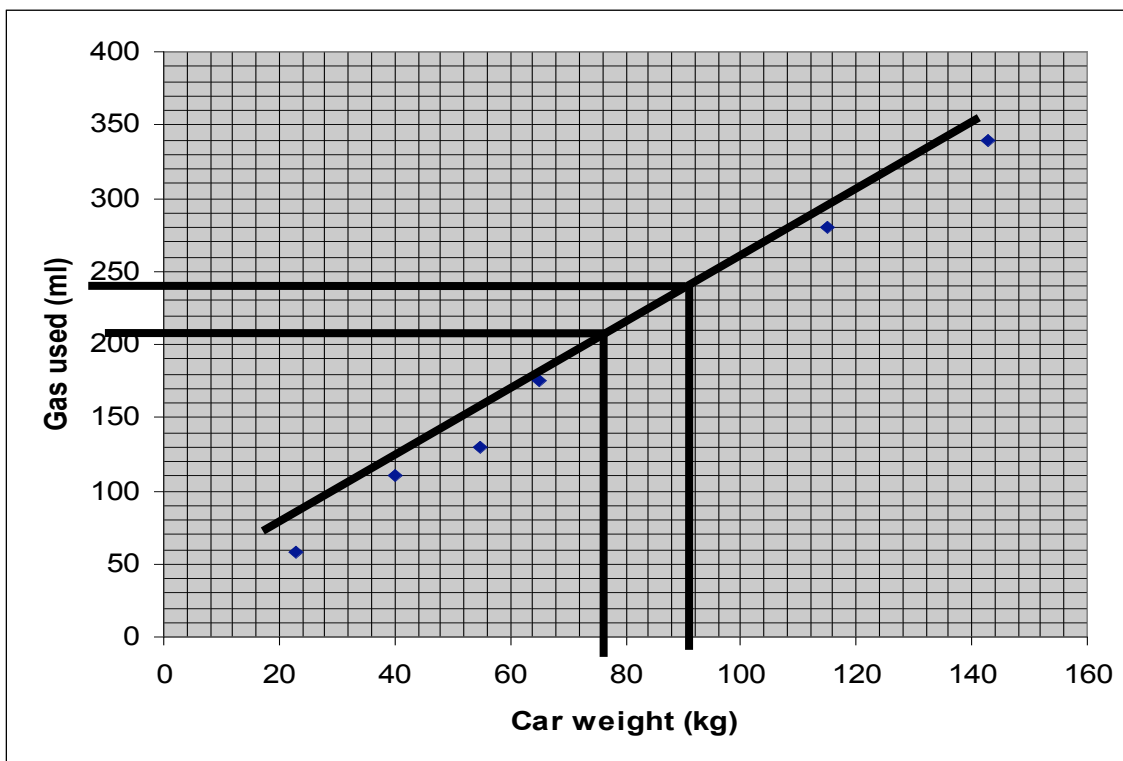
3. 8g/400ml

4. 365.5g/L

5. It would be the same, concentration is independent of volume.

6. $1/2 = [50/50]$, $1/10 = [10/90]$, $1/100 = [1/99]$

7. $\frac{500}{1000ml} = \frac{Xg}{600ml}$ $X = (500)(600)/1000 = 300 \text{ g}$



8.

a. As weight goes up, gas used goes up.

- b. 120 ml
 - c. About 240 ml
 - d. About 84 kg
9. Add IKI to each tube to see which turns more/less blue
- 10.
- a. One more blue than the other
 - b. Bluest
 - c. Same intensity of blue
 - d. Compare tubes with a tube containing just water
- 11.
- a. All of the light shined on a sample passes through
 - b. To set the spectrophotometer to read 100% transmittance when a sample containing none of the molecule you want to measure is present
 - c. The IKI solution creates the color you want to measure when it mixes with starch. It may also absorb some of this light even when no starch is present and you want to set the spectrophotometer to ignore this.
12. Tubes that have more starch will absorb/transmit different amounts of light.
- 13.
- a. Different absorbance levels
 - b. Higher absorbance levels
 - c. Same absorbance levels
 - d. No absorbance, same as the blank
14. Prepare a set of sample tubes with known amounts of starch by diluting the stock solution and measure absorbance in spec. Collect absorbance data and plot in graph similar to question #8 with absorbance on one axis and starch concentration on the other. Measure the absorbance of the unknown samples and use graph to determine what concentrations these correspond to.

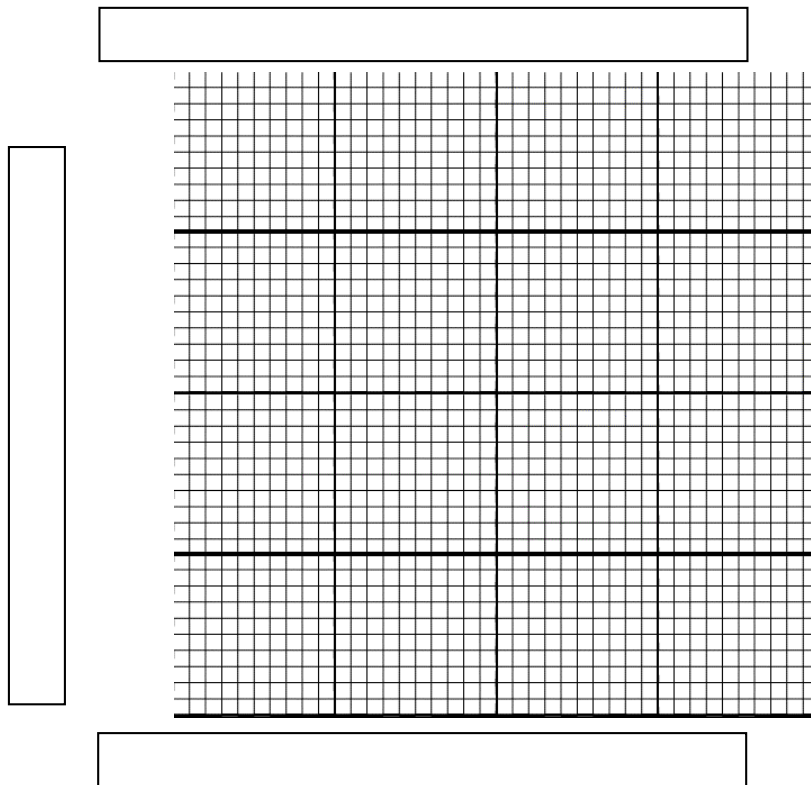
Appendix C: Pre-lab Homework Week-2 and Answer Key

In the previous lab, you were asked to determine the absolute concentration of starch in a set of test tubes. To do this successfully you needed to create and use graphs of your data. Below are some problems that will give you practice creating and using graphs.

You get a job at the post office and are responsible for setting up the new package sorting system. To do this you will need to find out how a package's size is related to its weight. You measure the several packages that arrive one day and collect the following data.

Package Volume (cm ³)	Package Weight (grams)
54	150.5
27	70
136	344.5
78	225
44	115
56	155
33	93

1. Is there a relationship between package size and weight? Graph out the data below to find out. Be sure to include labels for the axes and a Title for the graph.



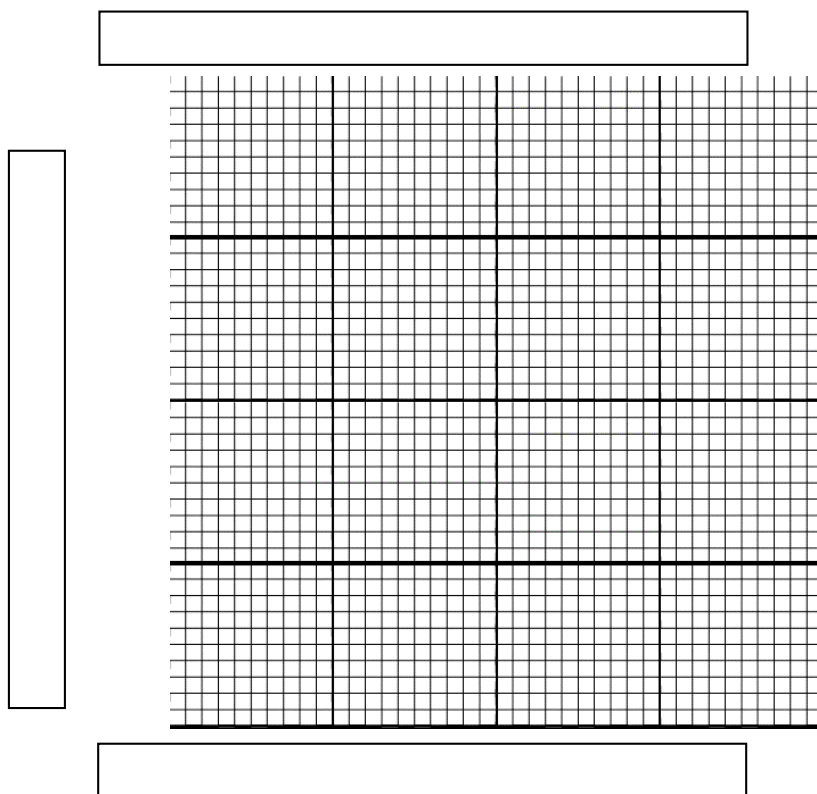
2. The package sorter can determine the weight of each package but not its volume. Packages over 100 cm³ must be inspected manually. According to your data, how heavy would a package probably be if it needed to be inspected?

You work in the lab at a local hospital. It's Saturday evening and a large part of your night will be spent determining blood alcohol levels for patients admitted through the emergency room. To do this, you will use a spectrophotometer and chemical that changes color when it reacts with alcohol.

Alcohol Concentration (g/ml)	Absorbance
0.01%	0.03
0.02%	0.05
0.05%	0.15
0.1%	0.22
0.2 %	0.55

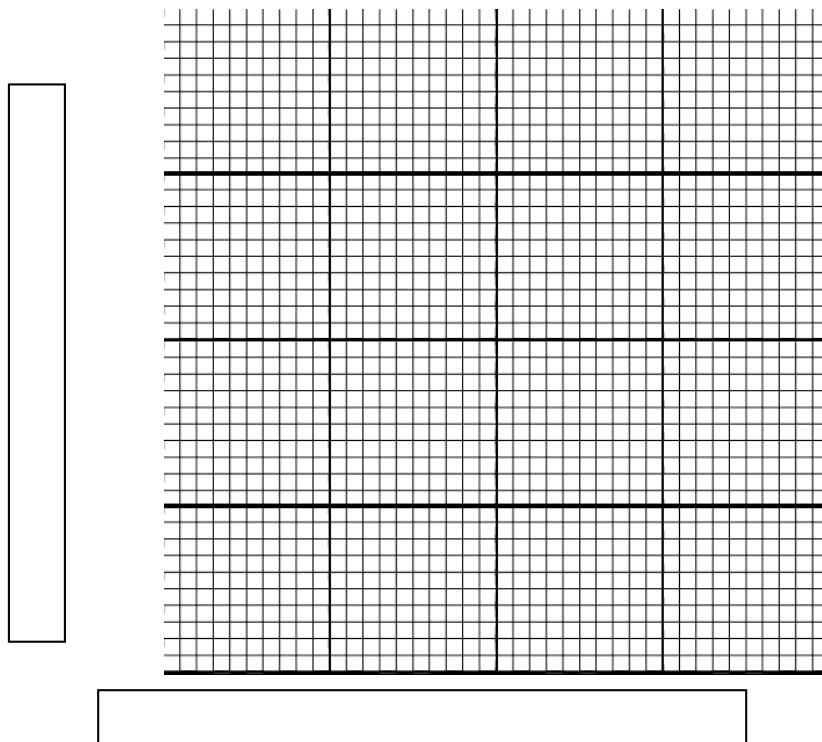
0.5 %	1.25
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3. You make a set of alcohol solutions to use as a reference. Data from these reference samples are shown above. Use this information to prepare a standard curve below. Be sure to label the axes correctly.



4. You test the blood of 5 patients that evening and get the data shown below.

Patient	Symptoms	Absorbance	% Blood Alcohol
1	Poor balance, impaired memory and speech	0.20	
2	Lightheaded, talkative	0.05	
3	Comatose, respiratory distress	1.1	
4	Nauseous, uncoordinated	0.45	
5	Semiconscious	0.7	



The police call and ask how to be certain that person is sober before being released. They have a Breathalyzer but don't have the manpower to check each detainee repeatedly.

You know that the liver breaks down alcohol over time. The question is, how quickly? You examine samples from two of your patients at the hospital for several hours. Plot out your data (below) in graph above. Be sure to include the proper axes.

Time	% Blood Alcohol	
	John	Jane
0	0.1	0.08
1 hr	0.084	0.067
2 hr	0.070	0.054
3 hr	0.056	0.041
4 hr	0.040	0.027

5. Do John and Jane break down alcohol at a constant rate?
6. How fast do they each break down alcohol in % blood alcohol/hour?
 - a. John:
 - b. Jane:
7. The police will not release a detainee until their blood alcohol drops below 0.02%. If John was picked the following weekend with a blood alcohol of 0.12%, when could the police release him? What if it were Jane with same blood alcohol?
 - a. John:
 - b. Jane:

8. Answer the following questions based on the article at the end of the pre-lab homework.
- What hypothesis/idea did Ishida examine in his study?
 - How did Ishida test to see whether his hypothesis might be correct?
 - What is the function of amylase in the brewing process?
 - Where does amylase used in beer making come from?
 - Why is mold needed for synchronous fermentation?

The article pointed out how an enzyme can promote a chemical reaction. In this case, amylase broke starch down into sugar. Enzymes promote thousands of different reactions in your body every second. Industry is finding many uses for many enzymes to make different products.

Some friends of yours have decided to set up an organic brewery and need your help. They have found a source of organic amylase enzyme that they can use for fermentation. Unfortunately, the enzyme is very expensive and they don't want to buy more than is necessary. How much they need will depend on the enzyme's activity (how quickly amylase is broken down in the presence of the enzyme). They ask you, how do you measure the speed of an enzyme?

9. Describe an approach that would allow you to determine how quickly a given amount of amylase enzyme would convert starch into sugar.

After you have completed your experiment, you should be able to give your friends the following information:

_____ amount of enzyme that can break down _____ amount of starch in one minute. (Hint: think about questions 5-7 of the homework and how you use the spectrophotometer to measure starch concentrations.)

Brewing Beer like the Ancient Egyptians

Modified from Ishida, H., 2002. Insight Into Ancient Egyptian Beer Brewing Using Current Folkloristic Methods. MBAA TQ 39(2): 81-88

Beer has been produced for thousands of years and modern techniques enable billions of gallons to be brewed annually. However, despite beer's long history and our current efficiency at producing it, how brewing methods were first developed and perfected in ancient times is poorly understood. Our knowledge of early beer production has been limited to interpretations of images expressed in wall paintings, reliefs, models, etc., such as those found in the tombs of Egyptian pharaohs, and the writings of ancient historians. Recently, this began to change with the publishing of a study by Hideto Ishida, a biochemist and Senior Manager at the Kirin Brewery.

Ishida's study was based on two observations. The first observation was that modern commercial breweries use a variety of techniques to make beer but that all of these different approaches require a critical set of events to take place in order to brew beer successfully. The second observation was that many indigenous cultures still make fermented alcoholic beer-like beverages using methods that have been passed down from generation to generation. Ishida believed that, just as with commercial breweries, even though different cultures may use different brewing methods, these methods would also be constrained by general rules that were likely to be similar from one culture to the next.

Furthermore, Ishida also believed the rules that apply to home-brewing today would probably have applied to the ancient Egyptians as well.

Do traditional methods for brewing beer follow similar rules?

As a first step in his research, Ishida needed to collect traditional recipes for brewing beer. This was actually not too difficult as beverages similar to beer are made by indigenous cultures around the world. In all of these cultures, beer is produced from starch rich foods by the process of fermentation.

Fermentation plays a very important role in many societies, in particular those that live in hot climates. Fermentation provides a way for cultures that do not have modern refrigeration to increase the average shelf life of food. This is because fermentation can produce conditions in the food that kill off most harmful microorganisms and prevent food from spoiling. The fermentation process also produces vitamins that can supplement the diet. If foods are stored for too long, however, they can easily be converted to alcohol. During storage, the fermentation process can continue and even solid materials such as half-baked bread and cooked rice balls can produce alcohol if they are allowed to stand within the household for an extended time.

Ishida collected information on fermented foods and beverages from indigenous cultures in Asia, Africa, and Latin America. Even though these cultures fermented many different types of food using a variety of techniques, when Ishida compared recipes from all of these cultures, he found a number of similarities. In particular, almost every culture he looked at used two specific steps in order to ferment food to produce alcohol. One was the preparation of a “starter” that would contain desirable microorganisms for fermentation. The second was to convert starch present in the food into fermentable sugars using heat and amylase enzymes.

Starter Preparation

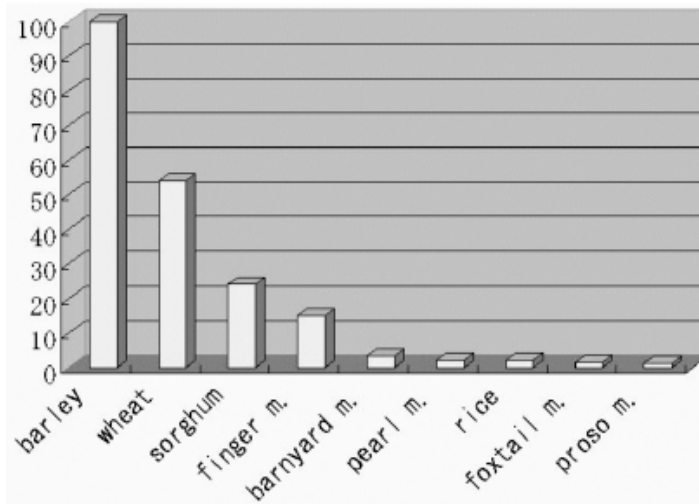
A starter is a culture of microorganisms that is used to initiate fermentation. Starters are prepared by inoculating food with bacteria and mold. It is these microorganisms that will actually carry out the fermentation process. Commercial breweries generally use starter cultures that contain a single strain of yeast that has been carefully prepared and maintained. In contrast, because they are prepared under non-sterile conditions, traditional starters will generally contain a whole host of microorganisms. This can be a problem. Most of the microorganisms that might inoculate a traditional starter would not produce a very good product if used for fermentation. At best, the results would be undrinkable; at worst it could lead to food poisoning. As a result, steps must be taken to ensure that the right organisms populate the starter when it is prepared.

A variety of techniques can be used in order to create a useful starter that gives rise to a product with consistent and desirable characteristics. One common technique is to add plants such as hops, red pepper, or various herbs to control the growth of undesirable microorganisms. Plants such as these can influence the types of organisms that grow in the starter by altering the pH, providing nutrients for beneficial microorganisms, or by being a source of desirable microorganisms. A second approach is the addition of lactic acid to the starter. Lactic acid can easily reduce the pH of the food to below 5, which inhibits the growth of many spoilage microorganisms and imparts a desirable sour taste with a hint of bitterness. Lactic acid can be provided through the fermentation of sugar in the starter by lactic acid bacteria. Alternatively, dilute solutions of lactic acid can be added directly to the starter.

Preparation for Fermentation

Fermentation involves the breakdown of simple sugars by and bacteria for energy. Lactic acid and/or alcohol are produced as products. Alcoholic fermentation requires a larger amount of fermentable sugar than is generally available in most foods. To provide enough sugar for alcoholic fermentation, starch in the food must be broken down into glucose by amylase enzymes through a process called saccharification.

Some plants contain within their cells enough amylase for saccharification. Barley, for example, contains large amounts of amylase that is normally used by the growing seedling to breakdown starch for energy. This amylase enzyme can be activated and saccharification initiated simply by allowing the grain to germinate (sprout) in a process called malting. Other foods cannot be processed in this manner. For example, rice, the principal food of eastern Asia, contains relatively low levels of amylase and alcohol production from this food requires amylase from other sources to be added. The most common sources of additional amylase enzyme are the koji molds (*Aspergillus oryzae* and *Rhizopus* sp).



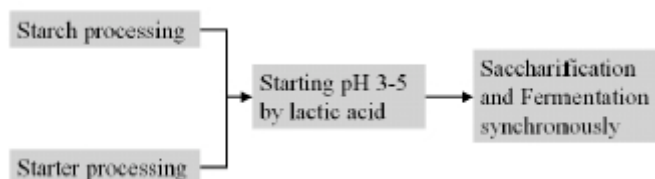
Amylase activity in foods commonly used in traditional beer production.

molds
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foods.
present
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Synchronous fermentation.

In modern breweries, starch hydrolysis is completed prior to fermentation to ensure that an

ample supply of sugars will be available. concentrations can also be raised adding fruit juice or honey to the mash. In many traditional cultures, starch breakdown and fermentation take place at the same time in a process called synchronous fermentation. Synchronous fermentation works well as in traditional societies, because mold enzymes can function under conditions as are also optimal for amylase fermentation.



A common pathway for making traditional fermented beverages. Most harmful microorganisms can't propagate at a pH lower than 4.3. Amylase from barley can't saccharify starches at low pH in fermenting liquor but amylases from molds (such as yeast) can. By taking advantage of the antimicrobial effects of native plants and lactic acid fermentation by bacteria, in combination with other technologies (such as mold propagation) traditional societies are able to prepare pure starter cultures suitable for alcoholic fermentation. .

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Synchronous fermentation, however, is not used in all traditional brewing methods. For example, in some cultures saccharification is carried out at relatively high temperatures, which would kill off most of the microorganisms that might be present. As a result, starter must be added after saccharification in order for fermentation to begin. Cultures that use barley as the source of amylase must also carry out saccharification and fermentation in separate steps. This is because barley amylase does not work well at the low pH levels that occur during synchronous fermentation. Thus, production of alcohol can require unique processing conditions depending on the raw materials being used.

Was brewing in ancient Egypt similar to today's traditional methods?

The ancient Egyptians portrayed many different aspects of daily life through wall paintings and stone reliefs. Depictions of the production of bread and beer have enabled scholars to infer some of the techniques that may have been used for these tasks. Additional information on beer production has been provided by writings of the alchemist Zosimus who lived in Egypt in the third century A.D. In all, Zosimus wrote 28 books on alchemy including one on yeast that includes recipes for brewing beer.

Zosimus provided recipes for two types of starter made from the powdered grist of barley malt that is similar to methods for making home-brewed alcoholic drinks today. One method corresponded with the current commercial process of saccharification in which bread was dispersed in hot water (probably around 70°C) in order to convert long chain starches into fermentable sugars. Heating of the batter-like "bread" for saccharification was carried out in preheated clay pots. The various steps of milling, sieving, and mashing (making the dough) in heated clay pots, etc., can be seen in images from a relief in the tombs of Niankhkhnum and Khnumhotep. It is likely that this approach would have required the addition of a separate starter after saccharification was finished as the relatively high temperatures in the clay pots would have killed off any microorganisms that might have been present.

The second approach for making starter involved half-baking bread containing leaven (a mixture of yeasts, lactic acid bacteria, and other microorganisms) from the previous fermentation. Which microorganisms would have been present in this fermented dough would have been influenced by the quality/quantity of water used, the baking conditions, and the amount of ventilation during bread storage. The composition of the starter would also have been affected by partial baking of the bread, which would have discouraged the growth of spoilage microorganisms and improved the shelf life of the starter.

The way in which saccharification was carried using the bread method would have been influenced by the raw material that was used. In the Old Kingdom, barley malt was used and would have provided sufficient amylase enzyme for saccharification by itself. However, the shape of the bread displayed in depictions of the brewing process from the Middle Kingdom suggests that, by this time, normal wheat had replaced malted barley. Wheat contains lower quantities of amylase and it would not have been possible to make beer with a high alcohol content without the use of mold to supply additional amylase enzyme. Interestingly, the bones of ancient Egyptians who died at the time of the Middle Kingdom were recently reported to contain the antibiotic tetracycline, which is produced, by some types of mold. It is possible that these antibiotics may have originated from mold used for the production of beer

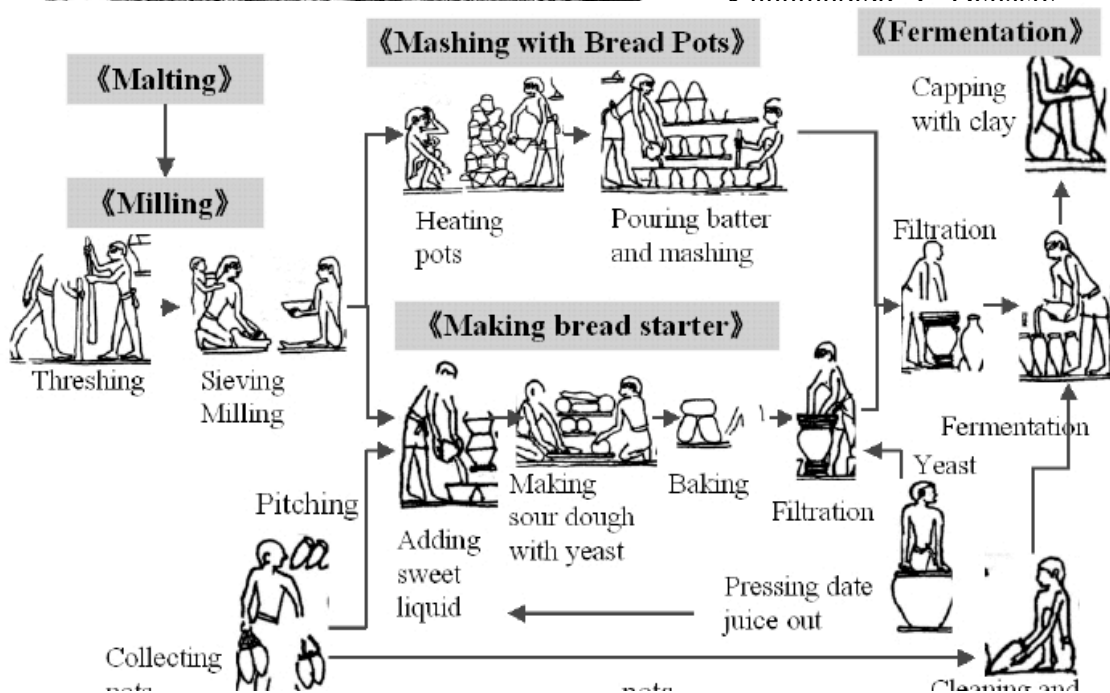
Summary

Comparing traditional methods for home brewing alcohol with historical depictions of the ancient Egyptian process, it is possible to speculate how beer was produced in ancient times. In the Old Kingdom, beer was made using barley which, by itself, would have provided enough amylase enzyme for saccharification to take place. After saccharification had been completed, starter containing lactic acid bacteria and mold would have been added to initiate fermentation.

In the Middle Kingdom, increased production and use of wheat for brewing would have required the addition of amylase enzymes from other sources for saccharification. The mold present in the starter would have been able to provide the enzyme needed. The technical advance of use of mold present in the starter both for fermentation and as a source of amylase enzyme would have enabled the development of synchronous fermentation improving both the quality and the yield of the fermentation process.

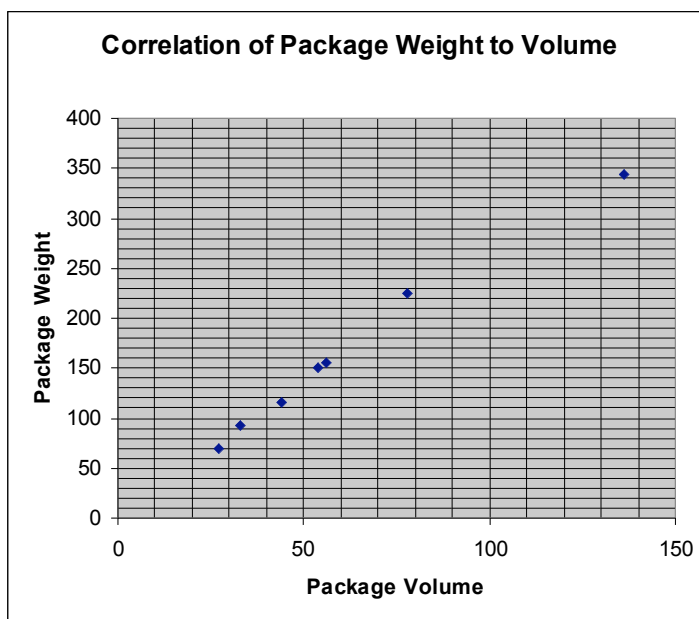


A depiction of beer making in reliefs from the tombs of Niankhkhnum and Khnumhotep, 5th Dynasty

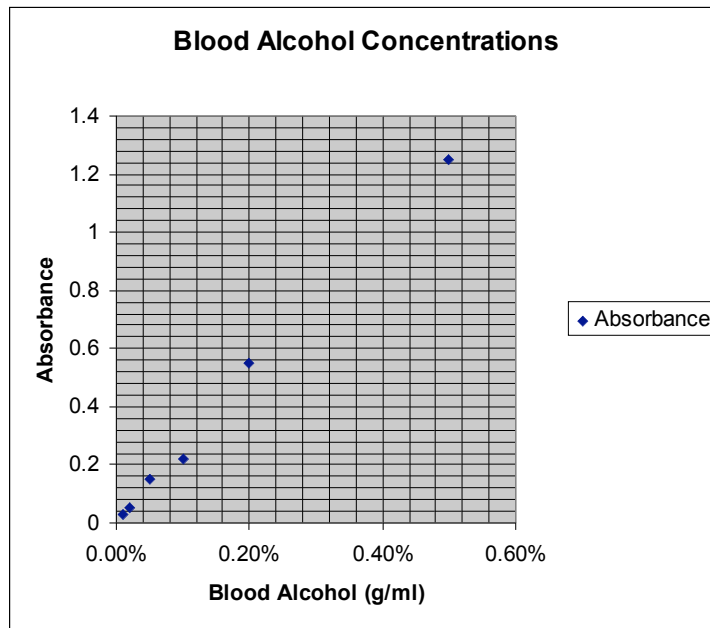


Answers to Enzymes II Pre-lab homework

1.



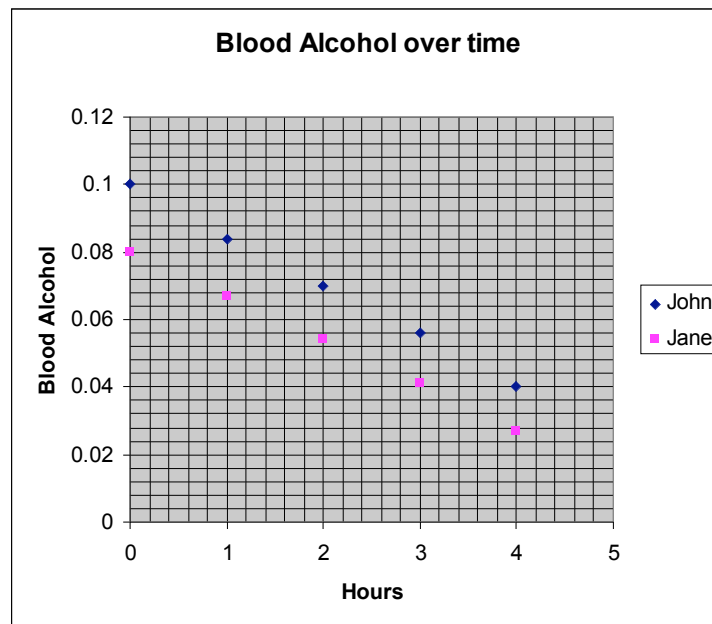
2. Package would be larger than approx 260 grams



3.

4. Patients

1. 0.08
2. 0.02
3. 0.46
4. 0.17
5. 0.27

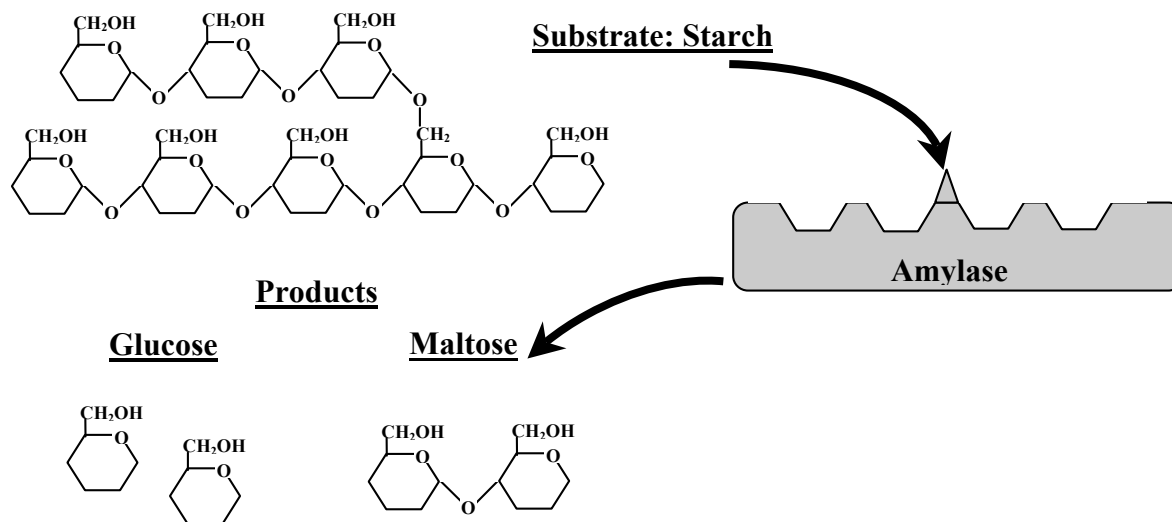


5. The students will need to determine the overall rate of change for two or more time periods. Both John and Jane are breaking down alcohol at a relatively constant rate.

6. John at 0.015%/hr Jane at 0.013% /hr
7. John after about 6.7 hrs Jane after about 7.7 hrs
8. Alcohol Article
 - a. Ishida thought that beer making in different cultures would be affected by the same factors and would likely be carried out in similar ways. These modern, traditional practices would also be likely to be similar to ancient methods of brewing.
 - b. He looked at traditional methods of brewing around the world and compared them.
 - c. Amylase breaks down starch into smaller subunits that can be fermented.
 - d. It can come from the grain itself or can be provided from an outside source such as mold.
 - e. The amylases from grains don't work well under the conditions required for fermentation. The amylases released by the mold do work under these conditions.
9. The experiment would require students to set up a standard curve for measuring starch content of samples. They would also need to expose starch to a known amount of amylase enzyme for a period of time. They could then compare how much starch was present at the start and how much after the starch had been exposed to the enzyme. The rate at which the amylase breaks down starch would be

Appendix D: Pre-lab Homework Week-3 and Answer Key

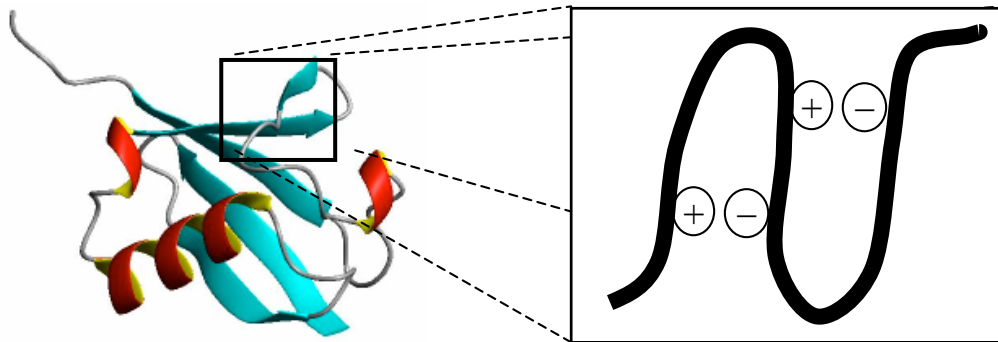
Enzymes are able to speed up chemical reactions because their amino acid chains fold to create a small pocket, called the "active site," in which a chemical reaction can take place more easily. Molecules with the correct shape can fit into the active site and take part in the reaction promoted by the enzyme. Most enzymes catalyze just one chemical reaction involving specific molecules. To speed up all of the different chemical reactions you need to live, your body produced over 5000 different enzymes.



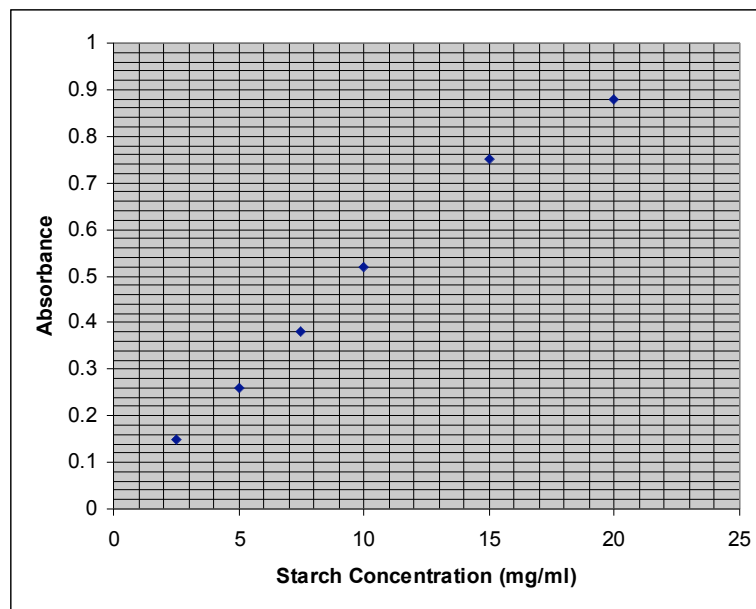
How an enzyme folds is critically important and determined by interactions between the amino acids in the enzyme. Interactions between hydrophobic groups, charges, and hydrogen bonds help

the enzyme keep its proper shape. If an enzyme unfolds or is not folded correctly, it is said to be denatured and the enzyme will not function properly.

Environmental conditions such as pH, temperature, and the presence of other molecules can influence the folding, and thus the activity of an enzyme. Most enzymes work best under specific conditions. For example, the enzyme pepsin works very well at pH 2 in your stomach but would not work well in your blood stream at pH 7. Bacteria that live in hot springs produce enzymes that can work in boiling water but would not work at room temperature.



Your friends have asked for your help with their microbrewery again. Use the graph shown here to help answer the following questions.



1. You have two solutions that contain starch. You examine these solutions using techniques you practiced the previous two weeks in lab. The first sample gives you an absorbance of 0.74. The second tube gives you an absorbance of 0.56. How much starch is in each tube?
2. The two samples started out with the same amount of starch. However, you had added one microgram (1 mg) of your enzyme to the second sample and then let both tubes sit for an hour

before measuring the amount of starch that was present. Why do you see a difference between the two samples?

3. How much starch do you think would have been in the second tube if you had let it sit for three hours? Explain your answer.
4. The recipe your friends are using requires 5 kg of starch for each batch of beer they make. How much enzyme would they need to completely breakdown this much starch into sugar in one hour?
5. The supplier told your friends that the enzyme works better at some temperatures than others but can't remember which temperature worked best. You treat the same amount of starch with enzyme at different temperatures and obtain the data below. In which of the temperatures did the enzyme work best? Would be the optimal temperature for the enzyme? Explain.

Temp (° C)	Absorbance
4	0.63
20	0.32
60	0.46
80	0.59
100	0.72

6. Your friends setting up the organic brewery are still experiencing some problems. They have found a new source of organically produced amylase enzyme but the company they bought it from did not know how active it was or what conditions under which it would work best. Describe an experiment that would let them determine the optimal conditions they should use to prepare their beer. (Pick just a single variable, either pH or temperature, to test.)

Answer key.

1. 15 mg/ml and 11 mg/ml
2. The enzyme digested part of the starch in the second tube into glucose. Glucose does not stain using KI. The absorbance of the solution would drop.
3. The enzyme broke down 4 mg of starch in 1 hour. In three hours, it should break down 12 mg. $15 - 12 =$ about 3 mg of starch left in the tube.
4. $5 \text{ kg} = 5,000 \text{ g} = 5,000,000 \text{ mg}$. One microgram of enzyme can breakdown 4 mg/hr. $5,000,000 / 4 = 1,250,000$ micrograms of enzyme = 1,250 milligrams = 1.25 g of enzyme.
5. The enzyme appeared to work best at 20° C. At this temperature, the most starch was converted into glucose as indicated by the lowest absorbance.
6. They would need to set up a standard curve to measure starch concentrations. They would then need to conduct an enzyme rate experiment like they did the previous week but with several tubes under different conditions (either pH or temp). Whichever condition showed the greatest reduction in starch concentrations would be closest to the optimal of the conditions tested.