A Study of Fermentation by *Saccharomyces cerevisiae*

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Introduction

Enzyme activity is an important concept in many introductory biology courses. However, many protocols for enzyme experiments involve a degree of technical proficiency that is beyond first-year students. As a result, data are problematic and cannot lead to meaningful analysis and discussion.

This workshop, derived from an article in *American Biology Teacher* (Reinking, Reinking, and Miller, 1994) presents a simple method for investigating fermentation. In the basic reaction, a 7% yeast suspension is combined with a 1.0% glucose solution in fermentation tubes—conical centrifuge tubes in which the caps have small perforations. As CO_2 is produced, the yeast-sugar solution leaves through the hole. At regular intervals, students mark off the fluid level in the fermentation tubes and measure the concentration of glucose with a diagnostic test strip. A small amount of data manipulation is necessary to obtain the volume of CO_2 produced by fermentation. The basic reaction can be carried out within 15 minutes.

Once the procedure of the basic reaction is established, the laboratory investigation can be taken in multiple directions. Students can be challenged to develop their own extension of the basic reaction. They might, for example, study the effect of temperature or pH, or compare fermentation rates when various carbohydrates are substituted for glucose. If students study various carbohydrate substrates, they can address enzyme specificity, using substances such as Lactaid®, a β-galactosidase, or Beano®, an α-galactosidase, in conjunction with the carbohydrates. Alternatively, students can be asked to predict the effect of increasing substrate concentration on the rate of CO_2 production and then to repeat the basic experiment with multiple glucose concentrations. In either extension of the basic reaction, consistent data are relatively easy to obtain. Students, therefore, have ample opportunity for more extensive data analysis and deliberation. If students use various concentrations of glucose as substrate in a series of reactions, they can ultimately construct a graph that shows the effect of substrate saturation on enzyme activity. This is a concept that is addressed in most introductory biology courses, but is not one that students easily comprehend.

Preparation for this laboratory exercise is relatively easy. It does not take much time to transform conical centrifuge tubes into fermentation tubes. For the basic reaction, only 1.0% glucose solution and 7% yeast suspension must be prepared. For the instructor-directed version of Part II, six different glucose solutions must be prepared.

This laboratory exercise can be scheduled in various ways. Conducting the basic reaction and discussing the results require about 2 hours. Conducting the follow-up experiment—either student-designed or instructor-designed—and discussing the results require about 3 hours.

The Student Outline provides directions for the instructor-presented version of Part II that studies the effect of using various glucose concentrations as substrate.

Student Outline

Fermentation uses NADH + H^+ formed in glycolysis to reduce pyruvate and recycle the coenzyme. In alcoholic fermentation, this is a two-step process, resulting in the formation of carbon dioxide and ethanol. In this study of fermentation, the metabolic pathway is performed by *Saccharomyces cerevisiae*, a single-celled fungus (yeast) that contains the cellular machinery capable of breaking down glucose by alcoholic fermentation. You will first develop a procedure to

measure the rate of alcoholic fermentation. Then you will apply that method to a more specific study of fermentation.

Part I: The basic fermentation reaction

Prepare a 40°C water bath in a 1000-mL beaker and maintain the temperature within a 38-42°C range**.** Place the nutrient solution(s), the water and the yeast suspension in the large class 40°C water bath. Label two conical centrifuge tubes that will be used as fermentation tubes: control tube and experimental tube.

Preparing the control tube: Fill the tube to the 8ml line with distilled water. Swirl the flask of 7% yeast suspension and then fill the remainder of the fermentation tube with yeast suspension, extending the fluid meniscus above the lip of the tube. Screw on the cap. A few drops of the contents should spurt from the hole. Invert the tube a few times to mix the contents. Remove the cap and dip a DiaScreen test strip into the tube. After 60 seconds, compare the reagent side of the strip with the color chart. Record the value in Table 1. Replace the cap. Check to make sure there are no sizable air bubbles. If there is a space at the tip of the tube, mark the fluid line with a permanent fine point marker. Slide a metal nut over the tube and put the tube aside.

Preparing the experimental tube: Fill the experimental tube to the 8ml line with 1.0 % glucose solution. Then continue as you did for the control tube.

Running the experiment: Place the two tubes into the 40°C water bath and set the stopwatch. Every 2 minutes, remove both tubes from the water bath. Hold each tube with the cap at the bottom and the conical portion at the top. Mark the fluid level with a permanent fine point marker. Invert the tube twice to mix the contents. Remove the cap and dip a fresh Diascreen strip into the contents. After 60 seconds compare the reagent side of the strip with the color chart. Record the values in Table 1. Replace the cap and place the tubes back in the water bath. When the glucose reading reaches 0mg/dL, remove the two tubes from the water bath. Record in Table 1 the values that you marked on the fermentation tubes at each time interval.

Converting data: To convert the markings on the tubes to total volume of air space, subtract the starting value of the fluid level from every other marked level (Figure 1). Do this for both the control and the

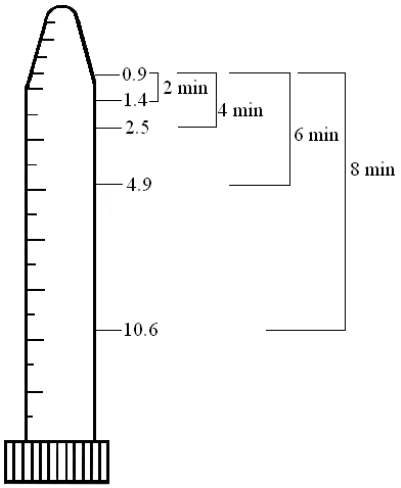

Figure 1. Conversion of marks to carbon dioxide volume.

experimental tubes. To determine change in volume in air space due to fermentation and not to leakage through the hole in the cap of the tube, subtract values for the control tube from values for the experimental tube. Convert the glucose units from mg/dL (a common unit used in the medical setting for which DiaScreen was originally designed) to mg/mL (a more common laboratory unit).

Table 1. Individual group data for changes in carbon dioxide and glucose in fermentation tubes

Time (min)	Control Tube			Experimental Tube		
	Level of air space in tube (ml)	Volume of air space (ml)	Glucose concentration (mg/dL)	Level of air space in tube (ml)	Volume of air space (ml)	Glucose concentration (mg/dL)
0						
2						
4						
6						
8						
10						
12						

Copy the class average for the control and experimental data and record that information in Table 2.

Table 2. Class data for volume of air space and concentration of glucose in control and experimental tubes.

Time (min)	Control Tube		Experimental Tube	
	Volume of air space (ml)	Glucose Concentration (mg/dL)	Volume of air space (ml)	Glucose Concentration (mg/dL)
0				
2				
4				
6				
8				
10				
12				

Table 3. Class data for volume of carbon dioxide produced and concentration of glucose in experimental tube.

Time (min)	Volume of CO_2 produced (ml)	Glucose concentration (mg/mL)
0		
2		
4		
6		
8		
10		
12		

Part II: Studying the effect of increasing substrate concentration

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You will work with six different glucose concentrations in this part of the experiment: 0.25%, 0.5%, 1.0%, 2.0%, 4.0%, and 6.0%. You will conduct the experiment as you did in Part I, but you will only use experimental tubes and will omit the DiaScreen strips.

As you did in Part I, preheat all solutions before you place them in the fermentation tubes. Then prepare four experimental tubes of the same glucose concentration using the same procedure as specified in Part I. Place the tubes in the 1000-mL 40°C water bath. Every 2 minutes, remove the tubes from the water bath and mark the bottom of the air space for each tube as you did in Part I. Continue for the agreed on experimental time. Convert the marks on the tubes to volume of CO_2 produced, and record the values in the table below. Determine the average.

Table 3. Results of trials with glucose concentration of _____ %.

Time (min)	Tube 1		Tube 2		Tube 3		Tube 4		Average CO_2 produced (ml)
	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	
0									
2									
4									
6									
8									
10									

Repeat the procedure with the second glucose concentration that you have been assigned. Record the data in the table below.

Table 4. Results of trials with glucose concentration of _____ %.

Time (min)	Tube 1		Tube 2		Tube 3		Tube 4		Average CO_2 produced (ml)
	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	Level of air space (ml)	Volume of CO_2 produced (ml)	
0									
2									
4									
6									
8									
10									

Compare your group results for each glucose concentration with the other group that used the same concentration. Determine the average of your results and record those values in the table at the board. Then copy the compete set of class averages into Table 5 below.

Table 5. Average volume of carbon dioxide produced at various glucose concentrations.

Time (min)	CO_2 Produced at Different Concentrations of Glucose (ml)					
	0.25% [glucose]	0.5% [glucose]	1.0% [glucose]	2.0% [glucose]	4.0% [glucose]	6.0% [glucose]
0						
2						
4						
6						
8						
10						

Part III: Data Analysis

Using Excel, prepare the following figures:

1. Relationship between glucose concentration and CO_2 produced over increasing time in basic reaction
2. Relationship between CO_2 produced over time at varying glucose concentrations
3. Relationship between reaction rate and glucose concentration. Select a time at which the reaction rate is linear for all the reactions. Calculate the velocity of the reaction (ml CO_2/min) for each glucose concentration at that time. Graph these values against glucose concentration.

Materials

Materials for exercise as described in Student Outline:

Equipment per section

Several large water baths set to 40°C

Test tube racks to accommodate 15 x 130 test tubes; several in each water bath

Computer with Excel; presenter

Equipment per group

Length of bench paper

Label tape

Scissors

Test tube rack to accommodate tubes

15-mL conical centrifuge tubes with caps; about 8 for each part of the experiment

Test tubes (15 x 130); about 8 per group for

heating solutions/suspension
Hex nuts (6)
Transfer pipettes
Ultra-fine permanent marker
50-ml Erlenmeyer flask for yeast suspension
Thermometer
1000-mL beaker (used as group water bath)
Large culture dish (for overflow of water bath)
100-mL beaker to "bail" and replenish small water bath
Lab timer
Kimwipes
Computer with Excel

Supplies per section:
1000 mL 7% yeast suspension

Glucose solutions (0.25%, 0.5%, 1.0%, 2.0%, 4.0%, 6.0%); in 125 mL plastic squirt bottles

Supplies per group:
Bottle of DiaScreen 1G glucose test strips with color panel
50 mL of 1% glucose in squirt bottle
50 mL of dH_2O in squirt bottle

Additional materials for student-designed Part II
Digital balances
Equipment to prepare various other solutions
Additional water baths
Buffers; pH meters
Fructose, maltose, lactose, sucrose; Lactaid® and Beano®

Notes for the Instructor

Reinking et al. (1994) address the problem presented by the loss of yeast/sugar solution from the fermentation tubes as the experiment progresses. They suggest that this loss can be corrected by calculating CO_2 production produced in a given time period by current volume of yeast/sugar solution. They also provide evidence that this correction is not necessary. You could certainly bring up this concern with your students and ask them how to address it. In our experience, only a small number of students have ever expressed concern over this source of potential error. We do not use Reinking et al.'s "correction" when we conduct the experiment.

Prior to the laboratory session
Preparation of students
1. Students have already learned about the glycolytic pathway in lecture.

2. Students are assigned the section of their textbook that explains fermentation. Their "ticket" into lab is to be able to explain in general terms how an experiment could be designed to measure the rate of fermentation.

Preparation of equipment

1. Choose a type of centrifuge tube that has the units of volume on the length of the tube clearly printed, not merely embossed. Values at the tip are generally not printed on the tubes, but are embossed. We use Falcon 35-2099 tubes. Tubes can be reused multiple times until the numbers are illegible. The tubes are packed sterilely. Sterility is not an issue in conducting the exercise, however.
2. Prepare the centrifuge tubes by perforating the cap with a hat pin or sturdy needle. It's easier to do this off-center because the plastic is thinner. Reinking et al. (1994) specify four holes. We make only one perforation in each cap.
3. Hex nuts (3/4- inch standard hex nuts) can be obtained at a hardware store. They cost about $0.70 apiece.
4. We set up two large water baths in the lab for use by the six groups. These are only used to bring the experiment contents up to 40^{o}C, not to run the actual experiment. Student groups use small water baths instead of a large water bath so the water can be easily changed. Because CO_2 pushes out the contents of the fermentation tubes, the water in the bath becomes quickly fouled.
5. We use large culture dishes as "overflow" containers for the 1000-mL water baths. When students reach into the beakers to remove/replace tubes, water frequently is pushed out.
6. We have good success with SARSTEDT fine point markers (No./REF 95.954). They hold up better with the task of writing on a not-so-dry surface.

Preparation of supplies

1. We have had the best success with Red Star powdered yeast. Prepare the yeast suspension about an hour before the laboratory session begins. Because the yeast easily falls of out suspension, make sure to frequently resuspend the yeast before distributing it to students. Also, remind students of the need to resuspend the yeast before they measure it out. In the course in which this experiment is used, students work in groups. A 6-group laboratory class needs about 500 mL of suspension for each part of the experiment. We make 1000 mL for each section, however, to allow for student error, accidents, and the occasional need for a group to repeat the experiment. Freshly made yeast suspension doesn't yield the same results as an older suspension.
2. Whether you have students make their own carbohydrate solutions depends on their skills in this area and the amount of time you want to devote to this aspect of the experiment. While students certainly benefit from the experience of preparing their own solutions, we prefer to give them prepared solutions and spend more of their laboratory time discussing results.
3. We use a 1.0% glucose solution in Part I of the experiment because that matches the upper limit of the DiaScreen sensitivity range.
4. We use DiaScreen 1G test strips to conduct the experiment. These are available from Hypoguard/ARKRAY http://www.arkrayusa.com/con_diascreen_reagent_strips.html. The catalog number is D11100 / 08317-1110-00. These strips are also available as an OTC purchase from many drug stores and medical suppliers. A bottle of 100 strips costs about $7.00.

The expiration date is not a concern because they are not being used clinically. We have used strips that are several years old with no unfavorable results. These strips are not used in Part II because the glucose concentrations exceed the upper sensitivity limits of the strips. The experiment can be conducted using alternate brands of glucose test strips. Be aware, however, that different types of strip require different wait times and may measure glucose concentration in different units of measure.

During the laboratory session

1. Make sure the students monitor the temperature of the 1000-mL water bath. They should maintain the temperature within a 5^{o}C range.
2. We usually have the students carry out a practice run of the experiment to get the "choreography" worked out. They should be aware of minimizing the time that the fermentation tubes spend outside of the water bath.
3. We don't have the students "preheat" the components of the experiment in the "practice" run of the experiment. After the practice trial, we discuss the aspects of the experiment that could contribute to error. In that context, we ask the student why they have to maintain the temperature of their group water bath. Would it be helpful, then, if the components didn't have to heat up to 40^{o}C?
4. Make sure students mark off the initial level of the fluid in the fermentation tubes. The fluid seldom completely fills the tube.
5. Make sure students use the DiaScreen strips correctly. Frequently, students will hold the strip in the fermentation tube for 60 seconds, rather than quickly dipping it into the tube and then reading the color change after 60 seconds. If a strip registers a color half-way between two colors on the chart, students can estimate the average.
6. Make sure students understand how to convert fluid levels to volume of CO_2 produced and how to correct for the "leakage" that occurs through the hole.
7. Using permanent markers on wet tubes can compromise their ability to write. Have a supply of replacement markers available. It also helps to dry off the tube with a Kimwipe first.
8. Students should continue to collect data in Part I until the glucose reading equals 0. This generally takes about 12 minutes.
9. In Part I (and in Part II if all the groups are carrying out the same experiment) it helps to have students graph and analyze the average data rather than group data. This simplifies the discussion because everyone is thinking about the same values. It eliminates the problem of "outlier" data obtained by an individual group. Finally, it provides the students with an opportunity to address data presentation when n>1.
10. If all the groups are carrying out the same experiment, we ask each student group to record their values in a large table on the blackboard.
11. Prior to beginning Part II, we ask the students to predict the results from increasing the glucose concentration. Almost without exception, students predict a constant relationship between the two variables. The figure that they ultimately obtain from this section provides another opportunity for a serious consideration of enzyme activity.
12. In Part II, we try to ensure that at least two groups will use each of the six glucose solutions. If you have groups of varying skill levels, assign the higher concentrations to groups of lower skill and the lower concentrations to groups of higher skill.

13. To determine the duration of the experiment in Part II, help the students decide on a time that will allow for glucose fermentation in the tubes with the highest concentration. The reaction is usually completed in about 12 minutes.

Data analysis

This experiment provides an opportunity for a degree of data analysis with which most first-year students are unfamiliar. Because the procedure results in clearly distinct values, data analysis is quite productive.

1. Using the average data from Part I, students prepare a 2-y axis graph in Excel (Appendix A). This provides an excellent opportunity to interpret data in a figure. Usually, the figure will be similar to Figure 1 below.

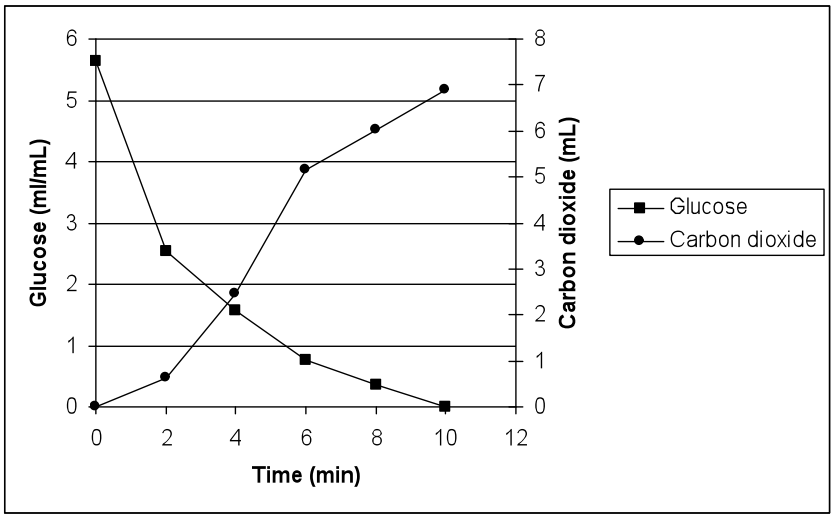

Figure 2. Fermentation by *Saccharomyces cerevisiae*. Experiment was conducted at 40°C. Glucose was measured with DiaScreen strips and CO_2 production was measured by changes in fluid volume of reaction tube (n=12).

2. Students established in Part I that the change in volume due to leakage was quite small. Therefore, we don't adjust the volume of produced gas to account for leakage in Part II.
3. Using the average data from Part II, students prepare a graph of CO_2 production from various glucose concentrations over time. Usually, the figure will be similar to Figure 2 below.

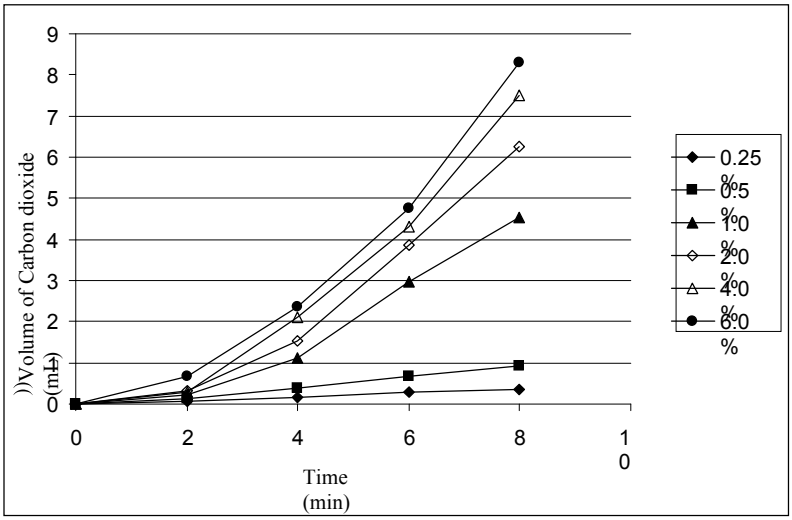

Figure 3. Fermentation by *Saccharomyces cerevisiae* of various glucose concentrations. Experiment was conducted at 40°C. CO_2 production was measured by changes in fluid volume of reaction tube (n=8).

4. Help students to determine the time during which fermentation occurred at a constant rate with all of the glucose concentrations (usually 4 or 6 minutes). Students then plot the CO_2 production at this time against increasing glucose concentration. Students will need help to determine the unit of measure for the y axis. Usually, the figure obtained will be similar to Figure 3 below. This figure provides an opportunity to discuss the rate of an enzymatic reaction.

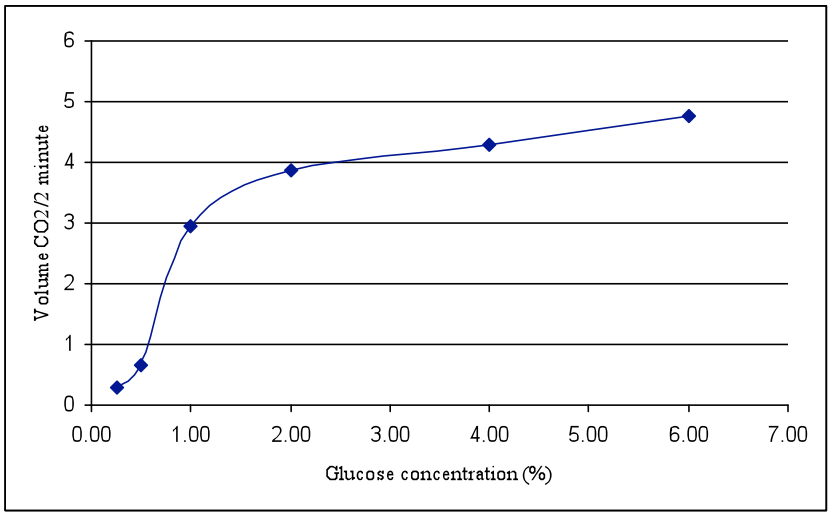

Figure 4. Rate of fermentation by *Saccharomyces cerevisiae* at different glucose concentrations. Experiment was conducted at 40°C. CO_2 was measured by changes in fluid volume of reaction tube (n=8).

After the laboratory session

1. After the experiment, fermentation tubes can be washed and the marks rubbed off with ethanol or acetone.
2. Unused DiaScreen strips can be used past their expiration date.
3. Unused glucose solutions theoretically could be used within a few days if they are refrigerated. Generally, we prepare fresh glucose solutions each day to avoid the possibility of bacterial contamination.
4. Unused yeast suspension should not be used later.

Literature Cited

Reinking, L.N., Reinking, J.L. and K. G. Miller. 1994. Fermentation, respiration, and enzyme specificity: A simple experiment with yeast. The American Biology Teacher,56:164-168.

Appendix A

EXCEL 2001—two y variables on same figure

1. Enter the values for the *x* variable in Column A. Enter the values for the first set of *y* variables in Column B (the ones you want to be associated with the left *y* axis) and the values for the second set in Column C (the ones you want to be associated with the right *y* axis).
2. Highlight all three columns. Click Chart Wizard > xy scatterplot > angular connect > Next.
3. Continue through the steps of Excel until you have completed the graph as a separate sheet. At this stage, both sets of data points will run on the same left-hand *y* axis. If one set of data points is based on a smaller scale than the other, it will appear almost flat.
4. Right click on a data point from the series that you want to be associated with the right *y* axis. Click Format Data Series >Axis > Secondary Axis > OK.
5. Right click anywhere in the graph. Select Chart Options. Fill in the label for the second *y* axis.
6. Right click anywhere in the graph. Select Source Data. Name both of your *y* variables in Series 1 and Series 2.