Fermentation of Sucrose in *S. cerevisiae*

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*Based on a lab developed at the University of Missouri - St. Louis by Don Cohn and his team*

**Objective:** To investigate rates of respiration in yeast.

**Background:**  
Yeast are unicellular eukaryotic fungi. Like all living things, they require energy to maintain their complex structures, grow, and reproduce. Yeast cells will obtain this energy by breaking down biomolecules using cellular respiration. In the presence of oxygen, yeast will use aerobic respiration to completely break down sugars into CO$_2$ and water. When given the choice, yeast will do aerobic respiration because the ATP output is much higher. However, when oxygen is not present, yeast can utilize an anaerobic pathway known as alcohol fermentation in which they partially break down sugars and produce CO$_2$ and ethyl alcohol (C$_2$H$_5$OH).

The process of fermentation involves two main phases: an energy producing phase, and an NAD+ recycling phase. In phase one, the cell breaks down a molecule of glucose (C$_6$H$_{12}$O$_6$) to produce two molecules of pyruvate (C$_3$H$_4$O$_3$) and two molecules of ATP. The cell also reduces two molecules of NAD$, forming NADH.

![Diagram of Fermentation](http://www.emc.maricopa.edu/faculty/farabee/biobk/biobookglyc.html)
NAD+ is essential for the breakdown of glucose into pyruvate. If the cell is to continue producing ATP in this way, it must find a way to recycle its NADH back into NAD+. This brings us to phase two of fermentation. The cell will convert its two pyruvate molecules into CO₂ and ethanol and, in the process, oxidize NADH to NAD+. Now the process can begin again.

The byproduct of yeast fermentation is ethanol which can become poisonous to the cells in high concentrations. Most yeast can tolerate between 10 and 15% alcohol depending on the yeast strain and environmental conditions (Alba-Lois). Therefore, yeast will do alcoholic fermentation when oxygen is not present and switch back to aerobic fermentation when oxygen becomes available. As yeast carry out fermentation, the CO₂ they release reacts with the surrounding water, forming carbonic acid. H₂O + CO₂ → H₂CO₃

As yeast continue to carry out this process, the surrounding solution becomes more and more acidic. We are able to detect this pH change using an indicator. This experiment uses bromthymol blue, which will be blue in alkaline and neutral solutions, but turn green and then yellow in the presence of a weak acid. By measuring the time required for the color to change from blue to green, you can observe how fast the yeast are releasing CO₂.

In this lab, you will mix different amounts of a 2% sucrose solution into each of your test tubes and record the time it takes before you see a color change.

**Pre-Lab Questions**

1. Develop a hypothesis for your experiment.
   
   ____________________________
   ____________________________
   ____________________________

2. Write the chemical equation for alcohol fermentation.

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3. Draw your experimental set-up in the space below.
Materials:
6 small test tubes  2% sucrose solution  droppers  test tube rack
graduated cylinder  yeast mixture

Procedure:
**Note**: The conditions used in this experiment provide only a small surface area exposed to oxygen. The volume of liquid below the surface quickly becomes anaerobic, resulting in fermentation becoming the predominant process that yeast will be using to obtain energy.
1. Label each tube with the number of drops of sucrose: 0 drops, 2 drops, 4 drops, 6 drops, 8 drops or 10 drops.
2. To each tube, add 5 mls of the yeast mixture.
3. Carefully, add the appropriate number of drops of 2% sucrose to each tube. Stopper the tubes.
4. **Record the start time** when you add the sucrose solution drops.
5. Carefully mix the contents of each tube thoroughly.
6. After each minute, mix the contents of each tube and look for a color change to green. Compare the color to the control tube. Observe the tubes and once you detect a color change for each tube, **record the ending time**.

Data Table:
Prepare a data table below to record your data and the class average data.
Graph:
What is the independent variable in the experiment? _________________
What is the dependent variable in the experiment? _________________
Prepare a line graph (on a separate piece of paper) of your data and the class average data. Graph both lines on the same graph.

Analysis and Discussion:
Answer the following in an essay form discussion:
• What your hypothesis supported or not by the data? Give evidence to support your answer.
• Describe in detail the process of anaerobic respiration in yeast.
• Why exactly is fermentation necessary to the anaerobic respiration process?
• Compare how NAD+ is recycled in aerobic respiration vs. anaerobic respiration.
• How does the number of drops of sucrose affect the rate of fermentation?
• Using the class data, what sucrose concentration (# of drops/5 ml yeast) gives the highest rate of fermentation?
• Research and explain why alcohol becomes a poison to yeast at high concentrations.
• Include an error analysis.
• Describe another experiment you could conduct to test another factor that would influence the rate of fermentation.

Works Cited:
List the sources you used for this lab in MLA format. Minimum 2 sources.
Teacher Instructions

Stock Solution Preparation:
1) Bromthymol blue, sodium salt (0.5%): Dissolve 0.25 grams bromthymol blue in 50 ml water. If the color is green or blue, the solution can be used. If it is yellow, use 0.1% NaOH to titrate the solution to a dark green or just blue color. Do not add excess NaOH.
2) 1.0% NaOH: Dissolve 1 gram of NaOH in 100 ml water.
3) 0.1% NaOH: 1 ml of 1.0% NaOH into a test tube and add 9 ml water.
4) Sucrose Solution: Dissolve 2 grams sucrose (or table sugar) in water and bring total volume up to 100 ml.

Solution Preparation on the Lab Day:
1) Yeast Suspension: Make a fresh suspension for each class!
This mixture must be used within a few minutes to make the yeast mixture below.
1 gram of dry yeast mixed in 10 ml warm water (30 - 40˚C). Mix thoroughly and allow 5 minutes for yeast to activate.
2) Yeast Mixture:
This mixture should be used within 30 minutes! Make a fresh mixture for each class!
2 mls 0.5% bromthymol blue
2 mls 1% NaOH
10 mls yeast suspension
up to 300 mls with water
Mix thoroughly. The yeast mixture should be royal blue. If it is green-blue, add more 1% NaOH drop wise until the mixture is royal blue. As the yeast mixture sits, the yeast will use glycogen stores and release CO₂, turning the dye green. Therefore, it is very important to make a fresh mixture and use the mixture within 30 minutes.
Helpful Teacher Hints

1. It is important that you add enough NaOH so that the solution is blue and doesn’t turn green too quickly. If too little NaOH is used, the tubes will all change from blue to green at the same time, too quickly for students to measure. On the other hand, you don’t want to use so much NaOH that the color change takes longer than one class period. Do a quick test of your yeast mixture before class: use a straw to blow into a test tube with 5 mL yeast solution. Your breath should turn the solution to green in under a minute.

2. One difficulty students will have with this lab is determining the exact time of the color change. Inform students that it is up to their group to decide when the color change has occurred and how they will be consistent with their measurements. It helps to have them compare their tubes to the control tube.

3. There are many ways to extend this lab. Once students have completed the procedure described in this packet, you may want to have them develop their own procedure and test another question. Students may wish to try

- different numbers of sucrose drops
- different types of sugar (such as glucose, fructose, honey, etc.)
- different volumes of water
- a different indicator.
- leaving tubes uncovered and stirring frequently, providing the yeast with oxygen. Or, a flask with greater surface area.
- another experimental set-up is one where CO₂ is collected from each tube using aquarium tubing. The other end of the tubing can be placed in beakers containing only water and bromthymol blue. The color change may be easier to see in water than in the yeast mixture. The bromthymol blue can then be titrated back to the starting color with 1% NaOH. The drops of NaOH can be counted to provide a way of quantifying the experiment.

http://www.science-projects.com/Fermentation/yeast.htm
4. Activated yeast will metabolize their stores of glycogen when they don’t have sugar. This makes the solution more acidic. Because of this, you should keep the following in mind:
   a. your yeast mixture will be closer to turning green the longer you leave it. You should make a fresh, blue yeast mixture immediately before each class.
   b. students should not test the effect of yeast concentration on rate of respiration because more yeast will simply use up the sugar more quickly and begin digesting glycogen stores.

Works Cited

<www.nature.com/scitable/topicpage/yeast-fermentation-and-the-making-of-beer-14372813>