

# Alcoholic Fermentation in Yeast -- Teacher Preparation Notes

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## Teaching Points

- If sugar is available but oxygen is not, yeast uses glycolysis to metabolize the sugar and produce ATP, followed by alcoholic fermentation which results in the production of alcohol and carbon dioxide.
- When more sugar is available, yeast has a faster rate of alcoholic fermentation.
- Experimental method (e.g. need for controls, replicates, change only one variable at a time)

## Equipment and Supplies

### Needed per Student Group for Experiment I

15 mL test tubes (4) (If you have larger test tubes and balloons to fit them, you will need to adjust the amounts of sucrose solution and yeast.)

Test tube rack or plastic cup to hold the test tubes (1)

Method for labeling test tubes (1)

Fast-rising highly active Baker's yeast (sometimes called breadmaker yeast) (1 tsp) (Make sure that the yeast has not reached its expiration date)

10 mL measure for sucrose solutions

1/2 mL or 1/8 teaspoon measure for yeast (1/2 mL = 1/10 teaspoon, but 1/8 teaspoon measures are readily available and sufficiently accurate for this experiment)

Water balloons that fits snugly on your test tubes (4)

Ruler to measure millimeters (1)

Warm sucrose solution, 1%, 5%, 10% (10 mL of each concentration + extra for spillage)

To make sucrose solution:

1%: add 1 g of sugar to every 99 mL of water

5%: add 5 g of sugar to every 95 mL of water

10%: add 10 g of sugar to every 90 mL of water

Warm tap water (~ 20 mL)

Optional: tub of hot water to keep your sucrose solutions and water warm

### Needed for Experiment II (The exact quantities needed vary depending on students' questions)

Test tubes, yeast, water balloons, etc. and 10% sucrose solution, as described above

Salt

Oil

Egg or egg substitute

Flavoring and additives such as cinnamon and raisins

Some way to maintain test tubes at different temperatures during the experiment

**Needed per group for optional activity to make bread** (If you want to make bread, you probably should have students carry out Experiment II in larger test tubes with 25 mL of sucrose solution and 1/4 teaspoon of yeast in each test tube; quantities of ingredients listed below are estimated for this larger amount of sucrose solution.)

Flour (4 Tbsp)

Bowl to mix bread in (1)

Something to mix bread with (plastic knife, spoon, etc.)

Metal baking cup (1)

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<sup>1</sup> These teacher preparation notes and the related student handout are available at [http://serendip.brynmawr.edu/sci\\_edu/waldron/](http://serendip.brynmawr.edu/sci_edu/waldron/).

## Instructional Suggestions

The flow of this activity is as follows:

1. Students learn basic information about aerobic cellular respiration and alcoholic fermentation.
2. Students carry out an experiment to test whether sucrose concentration affects the rate of alcoholic fermentation in yeast and analyze the results.
3. Optional Experiment II: Students design an experiment to test the effects of other bread ingredients or temperature on the rate of alcoholic fermentation in yeast. Instead of water as their control, they will use the 10% sucrose solution without the test ingredient or at the temperature used in the first experiment as their control.
4. Optional Bread Baking: The students can use the yeast solution from the treatment with the most CO<sub>2</sub> bubbles to make a roll of bread.

You may want to do the review of metabolism on page 1 of the Student Handout, as well as the introduction to the experimental design on page 2 of the Student Handout on the day before your lab period to ensure that your students will have enough time to complete the experiment during the lab period. See page 3 of these notes for additional information on metabolism.

We recommend that you warm the water and sucrose solutions before the lab period by placing them in a tub of hot water in order to assure that the yeast metabolism will be rapid enough to produce good results in the 20 minute observation period. The rate of fermentation will be measured by assessing the amount of CO<sub>2</sub> produced by two methods: measuring the depth of the layer of bubbles trapped in the foam on top of the yeast solution and observing balloons capping the test tubes which catch the CO<sub>2</sub> produced and get bigger. Do not leave the test tubes with balloons overnight since the pressure of accumulated CO<sub>2</sub> may pop off the balloons and create a mess.

For students to see the general trends and variability in the results in different replications, we recommend that you collect the data on the depth of CO<sub>2</sub> bubbles at 20 minutes from each student group and display these data in a graph with sucrose concentration (0%, 1%, 5% and 10%) on the X axis and depth of bubbles in millimeters on the Y axis; use a different symbol for the data from each group and for the class average at each sucrose concentration. In a class discussion of these data, you may want to ask students if they are aware of any differences in methods that could have resulted in points that deviate from the general trend; this may help students realize the importance of precision in experimental methods to achieve accurate results.

Another question that we suggest you include in your introduction to this activity or on page 4 of the student handout is "What do yeast need ATP for?" It will help your students to know that yeast are single cell fungi which absorb nutrients from their environment (e.g. bread dough, grapes, tree bark). This question will give you the opportunity to review a variety of cellular processes that use ATP including synthesis of macromolecules such as proteins and pumping ions across the cell membrane.

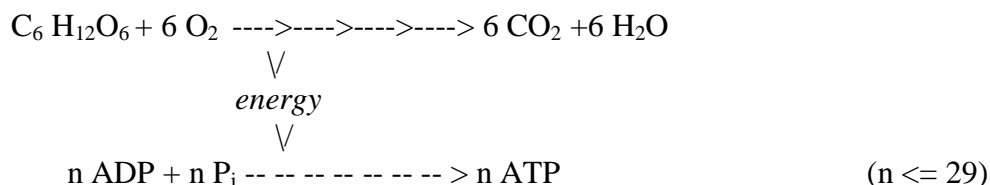
If you are omitting Experiment II, use the Word file for the Student Handout to omit the bottom of page 4 and pages 5-6 from the handout for your students. If you are including Experiment II, see <http://busycooks.about.com/od/bakingscience/a/yeastbreadingredients.htm> for a discussion of what the different ingredients of bread are used for.

The students can use the yeast solution in one of their treatment test tubes to produce a small loaf of bread. For the students to be able to eat their bread after they bake it, it will be necessary to make sure the test tubes and containers that hold the sugar etc. are clean and that students wash

their hands and work areas before they start the experiment. To make bread they will 1) put 3 Tbsp of flour and 2 Tbsp of warm water into a bowl. 2) Add the chosen yeast solution to the bowl. 3) Mix the contents. 4) Knead the dough on a paper plate or a piece of paper, using extra flour as needed. 5) Form a ball and place in a labeled tin muffin cup. 6) Cover with a warm paper towel. Allow at least an hour for the dough to rise before you bake the bread. If you need to leave the bread overnight without baking you should store it in the refrigerator so it doesn't rise too much and collapse. You can bake the cups in a preheated toaster oven in your room or collect all the muffin cups on a large cookie sheet and ask the cafeteria staff to bake them all at once at 360° F until golden brown. The students can then taste their bread the next day.

## Discussion of Metabolism

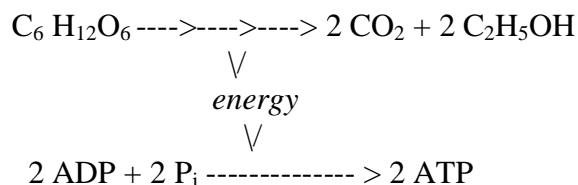
The following coupled equations summarize the overall chemical changes that occur in aerobic cellular respiration of glucose.



In the first equation, the multiple arrows represent the multiple steps of glycolysis, the Krebs cycle and the electron transport chain.

For aerobic cellular respiration, the number of ATP molecules generated for each glucose molecule is variable and less than previously thought. A brief explanation is provided in "Cellular Respiration and Photosynthesis -- Big Concepts, Misconceptions, and Classroom Activities" (available at <http://serendip.brynmawr.edu/exchange/bioactivities/cellrespiration>). These recent findings are interesting as an example of how science progresses by a series of successively more accurate approximations to the truth.

The following coupled reactions summarize the chemical changes in anaerobic metabolism when glycolysis is followed by alcoholic fermentation.



One possible question is why yeast can't use glycolysis without alcoholic fermentation when O<sub>2</sub> is not available. In the process of glycolysis, NAD<sup>+</sup> is reduced to NADH. For glycolysis to continue, NADH must be oxidized back to NAD<sup>+</sup>. When O<sub>2</sub> is available as an electron acceptor, NADH enters the electron transport chain and is oxidized to NAD<sup>+</sup> in a process that contributes to the production of ATP. When oxygen is not available, yeast use alcoholic fermentation in which the pyruvate produced by glycolysis is converted to acetaldehyde (releasing CO<sub>2</sub>) and acetaldehyde reacts with NADH to produce ethanol and NAD<sup>+</sup>.

The yeast which is used to make bread is *Saccharomyces cerevisiae*. This yeast is a facultative anaerobe, which means that when oxygen levels are low or glucose levels are high, sugar is metabolized without using oxygen, resulting in the production of a small amount of ATP, as well as carbon dioxide and ethanol. As the bread bakes, the ethanol evaporates. Bubbles which

contained carbon dioxide provide the fluffy texture of bread. *Saccharomyces cerevisiae* and other members of the same genus are used in making wine and beer, where, obviously, the production of alcohol is a major goal.

## **Related Activities**

- An alternative activity, "Is Yeast Alive?", uses yeast metabolism as one way of testing whether the little brown grains of yeast are alive (available on this website).
- In the worksheet/discussion activity, "Barley & Oat's Brewing Backfire!", students compare aerobic cellular respiration and alcoholic fermentation and then interpret evidence to figure out why a micro-brewer's beer has no alcohol (available at <http://serendip.brynmawr.edu/exchange/bioactivities>).
- The activity, "Taste Test: Can microbes tell the difference?", uses gas production as a measure of rate of yeast metabolism with different foods such as artificial sweeteners and different beverages (available at <http://www.asm.org/Education/index.asp?bid=35292>).
- The activity, "Yeast on the Rise", tests the rate of rising in bread doughs that differ in the concentrations of sugar or other ingredients (available at <http://www.microbeworld.org/resources/experiment/pgs62-65.pdf>).