Alcoholic Fermentation in Yeast¹

I. Introduction

1. What do you think makes the many small holes in a piece of bread? (Hint: Assume the bread dough was made with yeast, and watch the first 1 minute and 25 seconds of the video at https://www.youtube.com/watch?v=5UyaZbNkjP0, through champagne bubbles.)

If sugar is available, living yeast cells will carry out the reactions that are summarized in the graphic to the right. Notice that:

- The sugar glucose is broken down to the alcohol ethanol plus carbon dioxide (CO₂).
- ATP is made from ADP plus P.
- Energy released by the first reaction provides the energy needed for the second reaction.



The pair of curved arrows represents <u>coupled</u> <u>chemical reactions</u>; the top reaction provides energy for the bottom reaction.

2a. These reactions summarize the main process that yeast cells use to make ATP. Why do cells need ATP?

2b. This process is called alcoholic fermentation. Which alcohol is produced?

2c. Bread dough rises because alcoholic fermentation produces a gas and bubbles of gas are trapped in the bread dough. These bubbles are responsible for the many small holes in bread. What gas is produced by alcoholic fermentation?

The graphic above summarizes alcoholic fermentation in two simple coupled chemical reactions. Alcoholic fermentation is actually a complex process that includes twelve different chemical reactions. Each of these twelve chemical reactions requires an **enzyme**.

3a. What are enzymes?

3b. What does it mean to say that a chemical reaction "requires an enzyme"?

4a. Examine the little dry grains of yeast that your teacher will distribute. You will test whether these grains of yeast contain cells that are alive and can carry out alcoholic fermentation. Do you think that these grains of yeast contain cells that are alive _____ or not alive ____?

4b. Explain your reasoning.

¹ By Drs. Ingrid Waldron and Jennifer Doherty, University of Pennsylvania, Biology Department, © 2024. This Student Handout, a longer, more challenging Student Handout, and Teacher Preparation Notes with instructional suggestions and background information are available at https://serendipstudio.org/sci_edu/waldron/#fermentation.

II. Do the grains of yeast contain living cells that can carry out alcoholic fermentation?

You will do an <u>experiment</u> to test whether the dry grains of yeast contain living cells that can carry out alcoholic fermentation. You will need to add:

- water to activate the dormant cells in the dry grains of yeast
- sugar to supply the glucose for alcoholic fermentation. (The sugar you will use is sucrose, the common sugar that people put in their coffee or use for baking. Yeast cells convert sucrose to glucose for alcoholic fermentation.)

5. What would you look for as a sign that the yeast cells are carrying out alcoholic fermentation?

6a. If bubbles are produced in your experiment, the bubbles might be due to a physical or chemical reaction between yeast and water. What <u>control</u> could test for this possibility?

6b. Which combination of results would provide evidence that alcoholic fermentation occurred in the experiment?

a. Bubbles in the experiment and the control

b. No bubbles in the experiment or the control

- c. Bubbles in the experiment but not the control
- d. Bubbles in the control but not the experiment

Explain your reasoning.

For another <u>control</u>, you will use grains of yeast that have been boiled, so the enzymes in the yeast cells have been denatured so they are not active. You will test whether these definitely dead yeast cells produce bubbles when sugar is available.

7a. Can these definitely dead yeast cells carry out alcoholic fermentation of glucose?

yes _____ no ____

7b. Explain why or why not. Include enzymes in your explanation.

8. Use the information on this page (including your answers to questions 5-7) to briefly summarize the planned experiment and controls in the table below.

- Refer to yeast grains that have been <u>boiled</u> as <u>definitely dead</u>.
- Refer to yeast grains that have <u>not</u> been <u>boiled</u> as <u>possibly living</u>.

	Brief Description of What You Will Mix Together	Expected Results if the Possibly Living Grains of Yeast Contain Living Cells that Can Carry Out Alcoholic Fermentation
Experiment		
Control 1		
Control 2		

In your experiment to test for alcoholic fermentation in yeast cells:

- Cup 1 will test for bubble production by possibly living yeast cells in sugar water.
- Cup 2 will test for bubble production by possibly living yeast cells in plain water.
- Cup 3 will test for bubble production by <u>definitely dead</u> yeast cells in <u>sugar water</u>.

Procedure

- A. Label your cups.
- **B.** Weigh the dry ingredients and pour them into the labeled cups.
 - a. For cup 1: 4 g of yeast + 0.5 g of sucrose
 - b. For cup 2: 4 g of yeast
 - c. For cup 3: 0.5 g of sucrose
- C. Add the liquid to the labeled cups
 - a. For cups 1 and 2: 80 mL of 35°C water
 - b. For cup 3: 80 mL of 35°C dead yeast suspension
- **D.** Each person in your group should stir the contents of one of the cups for one minute. Smash any clumps of yeast and, if necessary, use your second spoon to scrape off any yeast that is stuck to the first spoon.
- **E.** Mark the level of the liquid in each cup, so it will be easy to measure the height of the foam layer. Set up the warm water bath with 35°C water, just to the level of liquid in the cups.
- **F.** Put your cups and the thermometer in the warm water bath. Begin timing 10 minutes. Record the beginning temperature of your warm water bath in question 9.
- **G.** Record your observations of any bubbles in the second and third columns of the table in question 9. Do not bump the cups.
- **H.** At minute 9, record the temperature of your warm water bath, and record this ending temperature in question 9.
- I. At the end of 10 minutes, measure the depth of the layer of foam at the edge of each cup. Record your measurements in the last column of the table in question 9. If there is no foam, record the depth of foam as 0.
- J. Report to your teacher the depth of the foam layer in each cup and the beginning and ending temperatures.
- K. Empty and clean the cups and bath. Clean up your workspace.
- **9.** Record your group's data here.

Beginning temperature° C		Ending temperature° C	
	Any bubbles right at the beginning?	Observations during minutes 1-9	Depth of Foam Layer (mm) at 10 minutes
Possibly living			
yeast in			
sugar water (1)			
Possibly living			
yeast in			
plain water (2)			
Definitely dead			
yeast in			
sugar water (3)			

10a. Do your group's results support the conclusion that the possibly living yeast contained living cells that could carry out alcoholic fermentation?

10b. Explain your reasoning.

11a. Your teacher will display the results for all the student groups in your class. Do the class results change your conclusions in your answers to question 10? yes ____ no ____

11b. If yes, summarize your conclusions based on the class results.

12a. In the cups with possibly living yeast in sugar water, how much variation was there in the depth of the foam layer?

12b. What could be the reasons for any differences?

13a. The ingredients for bread include yeast, sugar, water and flour. The gluten protein in the flour traps the CO_2 bubbles produced by the yeast, so the bread dough rises and the bread is fluffy. Propose three ways that a baker could increase the amount that the bread dough rises, so his/her bread would be fluffier. For each proposed change, explain your reasoning.

Change that Could Increase the Amount that the Bread Dough Rises	Explain your reasoning.	

13b. Briefly describe how you could test the effectiveness of the changes that you have proposed in your answers to question 13a.