Alcohol Fermentation Demonstration

Introduction

Use colorful indicators to demonstrate the products of fermentation.

Concepts

- Fermentation
- Decomposition of glucose
- Acid–base indicators

Background

Interest in fermentation, the breakdown of sugar to alcohol, dates far back in human history. Until 1860, it was believed to be a purely chemical process having nothing to do with living organisms. Then Louis Pasteur showed that fermentation involves a living process carried out by yeast and bacteria. Glucose is fermented according to the following net equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Many different intermediate products can be formed during this reaction and in various concentrations depending upon the conditions. In this demonstration, the production of ethyl alcohol and CO_2 are illustrated. The use of the color indicator, resazurin, further helps to highlight the conditions present in the fermentation chamber.

Materials

Glucose (dextrose), 15 g
Lugol's iodine solution, several drops*
Phenol red solution, 0.01%, 10 mL
Resazurin solution, 0.1%, 3 drops
Sodium hydroxide solution, 1 M, several drops*
Yeast, active dry, 3 g
Water, distilled or deionized
Condenser*
**Optional equipment for Iodoform Test*
Erlenmeyer flask, 250-mL
Glass tube
Hot plate*
Microscope*
Plastic or latex Tygon® tubing, 12″
Stopper, one-hole, to fit flask
Test tube, 13 × 100 mm
Test tube rack
Watch glass*

Safety Precautions

Please consult your current Flinn Scientific Catalog/Reference Manual for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The solution in the Erlenmeyer flask may be rinsed down the drain according to Flinn Suggested Disposal Method #26b. The solution in the test tube will be slightly acidic, hence its yellow color in phenol red. Add a weak base such as sodium carbonate until solution is neutralized, orange in color according to Flinn Suggested Disposal Method #24b.

Procedure

1. Fill a test tube ¾ full with 0.01% phenol red solution and place it in a test tube rack.
2. Place 150 mL of distilled water into a 250-mL Erlenmeyer flask. (A larger flask can be used with proportional increases in other ingredients.)
3. Place 15 g of glucose (or dextrose) into the flask and swirl the contents until all the glucose dissolves.
4. Add 3 g of active dry yeast to the flask and swirl until the yeast is uniformly mixed in the solution.
5. Add three drops of resazurin solution to the solution in the flask.

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6. Use a one-holed stopper, a glass tube, and flexible plastic or latex tubing to complete the fermentation setup as shown in Figure 1.
7. Note the initial color of the solution in both the flask and the test tube.
8. Let the fermentation apparatus sit undisturbed for an extended period of time (even overnight if necessary).
9. Record the color changes of the solutions in both the flask and test tube.
10. Save the solution in the flask for the optional test for alcohol given below.

Extension (Optional—Iodoform Test for Alcohol)

a. When the yeast activity has ceased in the flask (bubbling stops), decant the supernatant fluid into a clean flask.

b. Connect the flask to a condenser and distill the liquid.

c. Catch the first portion of the distillate and test for alcohol as follows:

1) Place 2–3 mL of distillate onto a watch glass.
2) Add several drops of sodium hydroxide solution to the distillate.
3) Add a few drops of Lugol's iodine solution to the distillate.
4) Heat the watch glass gently on a hot plate and note the formation of iodoform crystals.
5) Examine the distinctive yellow crystals under a microscope.

Note: Iodoform is toxic and has a disagreeable, pungent odor. Do not handle the solid.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The solution in the Erlenmeyer flask may be rinsed down the drain according to Flinn Suggested Disposal Method #26b. The solution in the test tube will be slightly acidic, hence its yellow color in phenol red. Add a weak base such as sodium carbonate until solution is neutralized, orange in color according to Flinn Suggested Disposal Method #24b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Constancy, change, and measurement
Content Standards: Grades 5–8
Content Standard B: Physical Science, properties and changes of properties in matter
Content Standard C: Life Science, structure and function in living systems
Content Standard F: Science in Personal and Social Perspectives, science and technology in society
Content Standards: Grades 9–12
Content Standard B: Physical Science, chemical reactions
Content Standard C: Life Science, the cell

Discussion

Resazurin solution is commonly used to show the depletion of oxygen in a solution by living microbes. It is often used to test for the relative number of microbes present in milk. When microbes are present, the resazurin goes through a series of color changes indicating the oxygen condition of the solution. The yeast suspension quickly consumes the available oxygen and the anerobic condition for alcoholic fermentation results. The solution in the flask changes color from an initial deep purple, to pink, peach, orange, and to nearly colorless, indicating the anaerobic conditions in the flask. These changes occur easily within one class period.

As carbon dioxide is produced in the fermentation process, the gas bubbles through the tube and into the phenol red solution in the test tube. As the CO_2 bubbles through the solution, the pH of the solution continually decreases. When the pH gets below a neutral 7.0, the phenol red turns to a yellow color. This bubbling and change in the phenol red is a clear indication of the carbon dioxide produced during fermentation. The optional iodoform test provides a clear demonstration of the other product resulting from yeast fermentation—ethyl alcohol.

Materials for *Alcoholic Fermentation* are available from Flinn Scientific, Inc.

Catalog No.	Description
Y0008	Yeast, Baker's Active, pkg/3
D0002	Dextrose, 500 g
GP3045	Flask, 250-mL
GP9020	Glass Tubing, Soft Glass
R0013	Resazurin Solution, 1%, 20 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.