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Sodium Alginate Respiration

Student Laboratory Kit

Introduction

Discover the effects of food type on respiration using yeast encapsulated in sodium alginate. When carbon dioxide is produced the pH of the aqueous solution decreases because carbon dioxide reacts with water to make carbonic acid. Measuring the changes in pH is an indirect way to measure the rate of respiration.

Concepts

- Respiration
- Rate of reaction
- Productivity

Background

A novel method of controlling the amounts of organisms during laboratory testing is by encapsulating yeast in sodium alginate. Sodium alginate is a polysaccharide extracted from brown seaweed. When it comes in contact with a solution containing calcium ions, it forms a gel. By dropping a mixture of sodium alginate and yeast into the calcium solution using a syringe, small spheres are made in a process called spherification. These spheres are permeable to small molecules, including the reactants and products of respiration. The yeast remain alive throughout this process and can continue with respiration.

Alcoholic fermentation is a form of anaerobic respiration, which is yeast's preferred method of metabolizing glucose. The yeast get energy for metabolic processes when glucose ($C_6H_{12}O_6$) is broken down into carbon dioxide (CO_2) and ethanol (C_2H_5OH).

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

During respiration, all organisms, including yeast, release carbon dioxide into their environment. The addition of carbon dioxide to aquatic environments results in an overall decrease in pH because aqueous carbon dioxide reacts with water to form carbonic acid (H_2CO_3) as shown in the following reaction. When carbon dioxide is added to the water, a proportional amount of carbon dioxide reacts to form carbonic acid to maintain equilibrium.

$$CO_2\,(aq) + H_2O \rightarrow H_2CO_3\,(aq)$$

Because of this, pH is a good indicator for measuring the relative rate of respiration. In a laboratory experiment, you can isolate one type of organism, such as yeast, to see how the rate of respiration changes when the conditions in the environment are changed.

Experiment Overview

The purpose of this experiment is to measure the pH of a solution containing yeast encapsulated in sodium alginate when the yeast are fed different concentrations of sucrose.

Pre-Lab Questions

1. How do the sodium alginate and yeast spheres model an animal cell?
2. What are the reactants and products for alcoholic fermentation?
3. How does dissolved carbon dioxide change the pH of the solution?

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Materials

Bromthymol blue indicator solution, 0.005% 20 mL
Calcium chloride, 0.3 M, 30 mL
Sodium alginate and yeast mixture, 15 mL
Sucrose, 1 g
Balance
Beakers, 50 mL, 2
Cheesecloth, 20 cm × 20 cm square
Funnel or strainer
Graduated cylinder, 50 mL
Support stand with clamp
Syringe, 20 mL
Test tubes with screw caps, 2
Weighing dishes, 2

Safety Precautions

All materials are nonhazardous. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Procedure

Part A. Preparing the Yeast Spheres

1. Add 30 mL of calcium chloride solution to a 50-mL beaker.
2. Draw up 15 mL of sodium alginate and yeast mixture into the syringe.
3. Secure the syringe with a clamp attached to a support stand, and place the calcium chloride solution beneath the syringe as shown in the Figure 1.

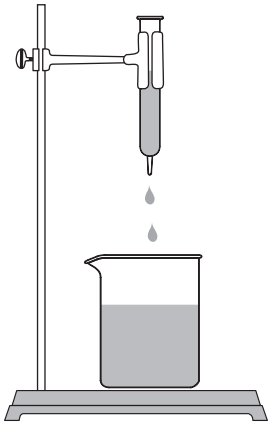

Figure 1.

4. Allow the sodium alginate and yeast mixture to drip into the beaker until approximately 50 spheres of equal size have been made. You may need to apply a slight amount of pressure to the plunger.
5. Fit a cheesecloth in a funnel, or use a strainer with a fine mesh.
6. Pour the yeast spheres into the funnel, and strain the calcium chloride solution into a clean, empty beaker.
7. Pour 20 yeast spheres into a weighing dish, and record the mass values of the spheres. It should be close to one gram.
8. Repeat step 7 with a second weighing dish, and ensure the mass values of the spheres in the two dishes are the same within 0.1 g, and the spheres are of uniform size. Record the mass in the appropriate data table on the *Sodium Alginate Photosynthesis Worksheet.*

Part B. Testing the Impact of Sugar Concentration on Respiration

9. Add 0.1 g of sucrose to 10 mL of bromthymol blue solution.
10. Unscrew the lid from a test tube, and add 20 yeast spheres.
11. Using a pipet, fill the test tube with the bromthymol blue and sucrose solution to the 10-mL mark, and screw on the cap.
12. Repeat steps 9–11 with the bromthymol blue solution without sugar in the second test tube.
13. Record the color and corresponding pH of the solution in each vial in the data table.
14. Record the color of the solution and note bubble formation in the tube every five minutes for up to 30 minutes, or as time allows in the data table.
15. Calculate the change in pH.
16. Answer all Post-lab analysis and calculations questions.

Disposal

Consult your instructor for appropriate disposal procedures. The calcium chloride solution can be reused many times.

Name: _____

Sodium Alginate Respiration Worksheet

Data Table: Mass

Sample	Mass of Spheres
Sucrose	
No Sucrose	

Data Table: Change in pH

Sample		Initial	5 min	10 min	15 min	20 min	25 min	Final
Sucrose	pH							
	Color							
No Sucrose	pH							
	Color							

Post-Lab Analysis and Calculations

1. What was the change in pH of each of the samples?

2. Which sample experienced a higher rate of respiration? Provide evidence to support your answer.

3. At what point in time did you begin to see changes in the color of each sample?

4. Sucrose is a disaccharide consisting of one monomer of glucose and one monomer of fructose. Respiration requires glucose. Enzymes break the disaccharide into glucose and fructose and additional enzymes help convert fructose to glucose. Predict whether the rate of respiration will be higher, lower or the same if glucose is used instead of fructose. Provide evidence to support your answer.

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Teacher's Notes

Sodium Alginate Respiration

Materials Included in Kit *(for 15 pairs of students)*

Bromthymol blue indicator solution, 0.04%, 50 mL
Calcium chloride, $CaCl_2$, 0.3 M, 500 mL
Sodium alginate, 10 g*
Sucrose, 40 g
Yeast, 7 g, 1 pkg*
Cheesecloth, 1 sq. yd.
Pipets, extra-large, disposable, 15
Syringes, 20 mL, 15
Test tubes with screw tops, 30
Weighing dishes, 30

*For *Pre-Lab Preparation*

Additional Materials Required *(for each lab group)*

Water, distilled*
Beakers, 50 mL, 2
Beaker, 250 mL*
Beaker, 600 mL*
Funnel or strainer
Stir plate with stir bar*

*For *Pre-Lab Preparation*

Pre-Lab Preparation

Part A. Preparing Sodium Alginate with Yeast

1. Add 3 g of sodium alginate to a 600-mL beaker containing 300 mL of distilled water, and stir until all the sodium alginate is incorporated and the solution is thick and uniform. This may take more than one hour. *Note:* This step may be done ahead of time. Cover the beaker and store in a laboratory refrigerator for up to two weeks.
2. Just before lab, add 5 g of yeast to 100 mL of warm distilled water. Stir on low until yeast is suspended.
3. Add yeast suspension to the sodium alginate solution, and stir until evenly combined. At this point you may choose to divide this stock sample into 20-mL quantities for each lab group, or each lab group may use their syringe to remove a 15-mL portion from the stock.

Part B. Preparing Bromthymol Blue Indicator Solution.

1. Add 25 mL of 0.04% bromthymol blue to 175 mL of distilled water to make 0.005% bromthymol blue indicator solution.
2. Adjust the pH using a dilute base to achieve a blue starting color. This corresponds to a pH of about 8.

Safety Precautions

Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

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 leftover calcium chloride solution may be reused or rinsed down the drain with excess water according to Flinn Suggested Disposal Method #26b. The sodium alginate spheres and excess sodium alginate may be placed in the trash according to Flinn Suggested Disposal Method #26a. The bromthymol blue solution may be neutralized with sodium hydroxide according to Flinn Suggested Disposal Method #24b.

Teacher's Notes *continued*

Lab Hints

- Enough materials are provided in this kit for 30 students working in pairs, or for 15 groups of students. This laboratory activity can reasonably be completed in one 50-minute class period.
- The pre-lab questions may be completed before coming to lab, and the *Post-Lab Analysis and Calculations* may be completed the day after the lab.
- A number of factors will affect the pH rate of change. A noticeable change in pH may take place in as little as five minutes, but it may take longer.
- Rather than using bromthymol blue, a pH meter can be used to measure the pH throughout the experiment. In this situation, a container other than a test tube will be needed.

Teaching Tips

- This laboratory activity fits it well when studying the metabolic processes of photosynthesis and respiration or during the study of enzymes in energy conversion.
- Sodium alginate is an edible polymer used to spherify food and drinks in the practice of gastronomy.
- To convert this to a guided-inquiry activity, allow students to manipulate a different variable. Students could investigate dif- ferent varieties of yeast, different types of sugar, different starting pH values of the solution, different temperatures of the solution, or different sizes or number of spheres. There is enough sodium alginate to make three batches, and the calcium chloride can be reused.
- The following student laboratory kits can be used to further explore respiration and metabolism: *Evolution of Yeast—Guided Inquiry Kit* (Flinn Catalog No. FB2125) and *Lactose Intolerance—Demonstration Kit* (Flinn Catalog No. FB1570).

Answers to Pre-Lab Questions *(Student answers will vary.)*

1. How do the sodium alginate and yeast spheres model a cell?

   The sodium alginate is permeable to gases as are the cell membranes of cells. The yeast contain cellular machinery for respiration and carry out respiration.

2. What are the reactants and products for alcoholic fermentation?

   The reactant is glucose ($C_6H_{12}O_6$), and the products are carbon dioxide (CO_2) and ethanol (C_2H_5OH).

3. How does dissolved carbon dioxide change the pH of the solution?

   A portion of dissolved carbon dioxide reacts with water to form carbonic acid. The carbonic acid decreases the pH of the solution.

Sample Data Table: Change in pH *(Student data will vary.)*

Sample		Initial	5 min	10 min	15 min	20 min	25 min	Final
Sucrose	**pH**	*8.2*	*7.8*	*7.5*	*7.1*	*6.9*	*6.3*	*6.0*
	Color	*Blue*	*Blue*	*Blue-Green*	*Blue-Green*	*Green*	*Light Green*	*Yellow*
No Sucrose	**pH**	*8.7*	*8.2*	*8.0*	*7.8*	*7.7*	*7.5*	*7.3*
	Color	*Blue*	*Blue*	*Blue*	*Blue*	*Blue*	*Blue-Green*	*Blue-Green*

*pH was verified using a pH sensor.

Teacher's Notes *continued*

Answers to Post-Lab Questions and Calculations *(Student answers will vary.)*

1. What was the change in pH of each of the samples?

   Student answers will vary. The sample that contained sucrose had a starting pH of 8.2 and an ending pH of 6.0, for a total decrease of 2.2 in 30 minutes. The sample that didn't contain sucrose had starting pH of 8.1 and an ending pH of 7.3, for a total decrease of 0.8 in 30 minutes.

2. Which sample experienced a higher rate of respiratoin? Provide evidence to support your answer.

   Student answers will vary. The data collected show that the sample with sucrose had a higher rate of respiration. Both samples decreased in pH, indicating that respiration was occurring; however the sample with sucrose had a decrease in pH of 2.2 versus 0.8.

3. At what point in time did you begin to see changes in the color of each sample?

   Student answers will vary. The data collected shows that the sample with sucrose began to change within the first 10 minutes. The sample without sucrose began to change at about 25 minutes.

4. Sucrose is a disaccharide consisting of one monomer of glucose and one monomer of fructose. Respiration requires glucose. Enzymes break the disaccharide into glucose and fructose, and additional enzymes help convert fructose to glucose. Predict whether the rate of respiration will be higher, lower or the same if glucose is used instead of fructose. Provide evidence to support your answer.

   If glucose is used instead of sucrose, the rate of respiration at the beginning will be higher. The glucose would be immediately available for respiration. The lag time seen in the color change with sucrose is due to the enzyme activity needed to convert sucrose to glucose and fructose, then fructose to glucose.

Acknowledgements

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The *Sodium Alginate Respiration—Student Laboratory Kit* is available from Flinn Scientific, Inc.

Catalog No.	Description
FB2124	Sodium Alginate Respiration—Student Laboratory Kit
D0005	Dextrose, Monohydrate, 500 g
Y0008	Yeast, pkg of 3 packets, 7 g ea.

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

