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# Enzymatic Explorations: Investigating Solutions for Lactose Intolerance

## Experiment

### Lactase Action

- Go Direct $CO_2$ Gas Sensor

## Workshop Presenter

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# Lactase Action

($CO_2$ Gas Sensor)

Lactose, a disaccharide sugar found naturally in mammalian milk, is utilized by infants as one of their initial sources of energy. During infancy, mother's milk is often the child's sole source of nutrition. This milk sugar, lactose, must undergo an enzymatic reaction that separates the disaccharide molecule into two monosaccharides: glucose and galactose. This action is carried out in the cells lining the small intestine. The enzyme facilitating this reaction is called lactase. After the split, the resulting simple sugar molecules are released and the lactase enzyme is available to react again. Glucose molecules are absorbed and transported to the liver while galactose molecules undergo another enzymatic reaction converting them to glucose.

Human utilization of milk as a food source varies across the globe. The adaptive production of sufficient lactase is a trait expressed in cultures that relied on dairy products over the generations. People from cultures lacking reliance on dairy products are prone to lactose intolerance, missing the level of lactase production necessary to metabolize the lactose molecule from milk. When dietary lactose escapes lactase action, the molecule proceeds to the large intestine where it is subjected to bacterial fermentation. As increased amounts of lactose pass through the small intestine without conversion, anaerobic bacteria in the colon increase fermentative gas production and discomfort, typical symptoms of lactose intolerance.

In this lab, you will assess the functioning of lactase. One way is to determine if the enzyme is converting the disaccharide into glucose and galactose by measuring the amount of glucose produced. You can use glucose test strips, originally made for diabetics to detect glucose levels. The test strip turns a range of colors to indicate the sugar's concentration in solution.

An alternative test for lactase activity measures the production of $CO_2$ gas by yeast. Presumably, yeast are unable to digest lactose. Yeast metabolize glucose aerobically during respiration, according to the equation

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

Water is produced and $CO_2$ is released as the sugar is broken down in glycolysis. By monitoring the production of $CO_2$, we can use yeast to indicate lactase activity.

## OBJECTIVES

- Test the action of lactase.
- Use glucose test strips to monitor the presence of glucose.
- Determine if yeast can metabolize glucose, lactose, or galactose.

## MATERIALS

Chromebook, computer, **or** mobile device
Graphical Analysis app
Go Direct $CO_2$ Gas
lactase solution
5% galactose solution
5% glucose solution
5% lactose solution
10 mL graduated cylinder
600 mL beaker (for water bath)
Beral pipet
three 18 × 150 mm test tubes
test-tube rack
hot and cold water
stopwatch
Tes-Tape or other glucose test paper
thermometer
yeast suspension
goggles

*Figure 1*

## PROCEDURE

### Part I Testing for the production of glucose

1. Obtain two test tubes and label them test tube 1 and test tube 2.

2. Obtain the lactose sugar solution. Add 2.5 mL of the sugar solution to both test tubes, as listed in Table 1. **Note**: Do not add the lactase to the test tube until Step 7.

Table 1

| Test tube | Lactose sugar solution (mL) | Lactase (drops) |
|---|---|---|
| 1 | 2.5 | 2 |
| 2 | 2.5 | none |

3. Prepare a water bath for the sugar solutions. The water level in the tank should cover 3/4 or more of the test tube while maintaining a temperature between 35°C and 37°C. If an automated water bath is not available, combine some warm and cool water in the 600 mL beaker to establish and maintain a 35–37°C bath. **Note**: If you need to add more hot or cold water to maintain a constant temperature, first remove about as much water as you will be adding or the beaker may overflow.

4. Measure the glucose concentration.
   a. If the test paper is supplied in a continuous strip, tear off a small piece (0.5 cm) of glucose test paper. Otherwise, obtain one test strip.
   b. Using a clean pipette, withdraw a drop or two of sugar solution from test tube 1.
   c. Place one drop of sugar solution onto the glucose test paper.
   d. Follow the instructions on the glucose test paper package to develop the test paper. This usually requires a 30 or 60 second wait before you compare the color of the tape to the supplied color chart.
   e. Record the approximate concentration of glucose in Table 3.
   f. Discard any sugar solution remaining in the dropper. Rinse the dropper by taking up clean water and expelling it into a waste beaker.
5. Repeat Step 4 for the second test tube.
6. Place 2 drops of lactase solution into test tube 1 only. Gently mix the contents of the tube.
7. Set both test tubes in the water bath and start timing. Be sure to keep the temperature of the water bath in the 35–37°C range.
8. Incubate the test tubes for 10 minutes, taking a glucose test once every minute for 10 minutes. Repeat Step 4 and record the concentrations of glucose in Table 3 once every minute.

**Part II Testing for the ability of yeast to metabolize sugars**

9. Launch Graphical Analysis. Connect the $CO_2$ Gas Sensor to your Chromebook, computer, or mobile device.
10. Set up the data-collection mode.
    a. Click or tap Mode to open Data Collection Settings.
    b. Change End Collection to 240 s. Click or tap Done.
11. You will perform one of the five tests outlined in Table 2 and obtain the results of the other tests from your classmates. Ask your instructor which test you will be performing and record the test number in Table 4.

Table 2

| Test | Sugar | Volume of sugar (mL) | Volume of yeast (mL) | Enzyme |
|---|---|---|---|---|
| 1 | lactose | 2.5 | 2.5 | lactase (8 drops) |
| 2 | lactose | 2.5 | 2.5 | none |
| 3 | glucose | 2.5 | 2.5 | none |
| 4 | galactose | 2.5 | 2.5 | none |
| 5 | none (water only) | none | 2.5 | none |

12. Prepare the sugar/yeast solution.
    a. Place 2.5 mL of the assigned sugar solution into a clean test tube.
    b. Obtain the yeast suspension. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Add 2.5 mL of yeast to the test tube and mix the solution.
    c. If you are performing test 1, add 8 drops of enzyme.
13. Transfer all of the sugar/yeast solution in the test tube to the 250 mL respiration chamber.
14. Quickly place the shaft of the $CO_2$ Gas Sensor in the opening of the respiration chamber. Gently push the sensor down into the bottle until it fits snugly.
15. Click or tap Collect to start data collection.
16. Data collection will end after 4 minutes. Remove the $CO_2$ Gas Sensor from the respiration chamber.
17. Fill the respiration chamber with water and then empty it. Make sure that all yeast have been removed from the respiration chamber. Thoroughly dry the inside of the respiration chamber with a paper towel.
18. If time permits, repeat Steps 12–17 for another one of the solutions.

## PROCESSING THE DATA

1. Determine the rate of respiration.
    a. Examine the graph. Identify and select the data in the most linear region of the graph.
    b. Click or tap Graph Tools, , and choose Apply Curve Fit.
    c. Select Linear as the curve fit. Click or tap Apply.
    d. Record the slope of the line, *m*, as the respiration rate in Table 4.
    e. Dismiss the Linear curve fit box.
2. Place your test tube number and rate value on the class data table for the other lab groups to record.
3. Average the rate values for each of the five tests performed by the class and record these five values in Table 5.
4. Make a graph of respiration rate *vs*. sugar/enzyme combination. Place the sugar/enzyme combinations along the x-axis and the respiration rates along the y-axis.

## DATA

| Table 3: Glucose Concentrations | | |
|---|---|---|
| Time (minutes) | Lactose + lactase | Lactose only |
| 0 | | |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |
| 10 | | |

| Table 4: Your Results | |
|---|---|
| Test | Respiration rate (ppm/s) |
| | |

| Table 5 | | |
|---|---|---|
| Test | Type of sugar / enzyme | Average Respiration rate (ppm/s) |
| 1 | Lactose + lactase | |
| 2 | Lactose only | |
| 3 | Glucose | |
| 4 | Galactose | |
| 5 | None (water only) | |

## QUESTIONS

1. From the results of this experiment, how does lactase function? What is your evidence?
2. Considering the results of this experiment, can yeast utilize all of the sugars equally well? Explain.
3. Hypothesize why some sugars are not utilized by yeast while other sugars are metabolized.
4. How did the results of testing lactase's activity using glucose test paper compare with the results of using yeast as an indicator of activity? What is your evidence?
5. Which test tube served as a control in this experiment? What did you conclude from the control? How did this affect the interpretation of data in this experiment?

## EXTENSIONS

1. Design an experiment to test the activity of Beano® on the sugar melibiose. From the results of this experiment, how does Beano function? What is your evidence?
2. Design an experiment to test whether Beano has any effect on the sugar lactose.
3. Design and carry out an experiment to determine the optimal pH range of activity for lactase.
4. Design and carry out an experiment to determine the functional relationship between rate and lactase concentration.