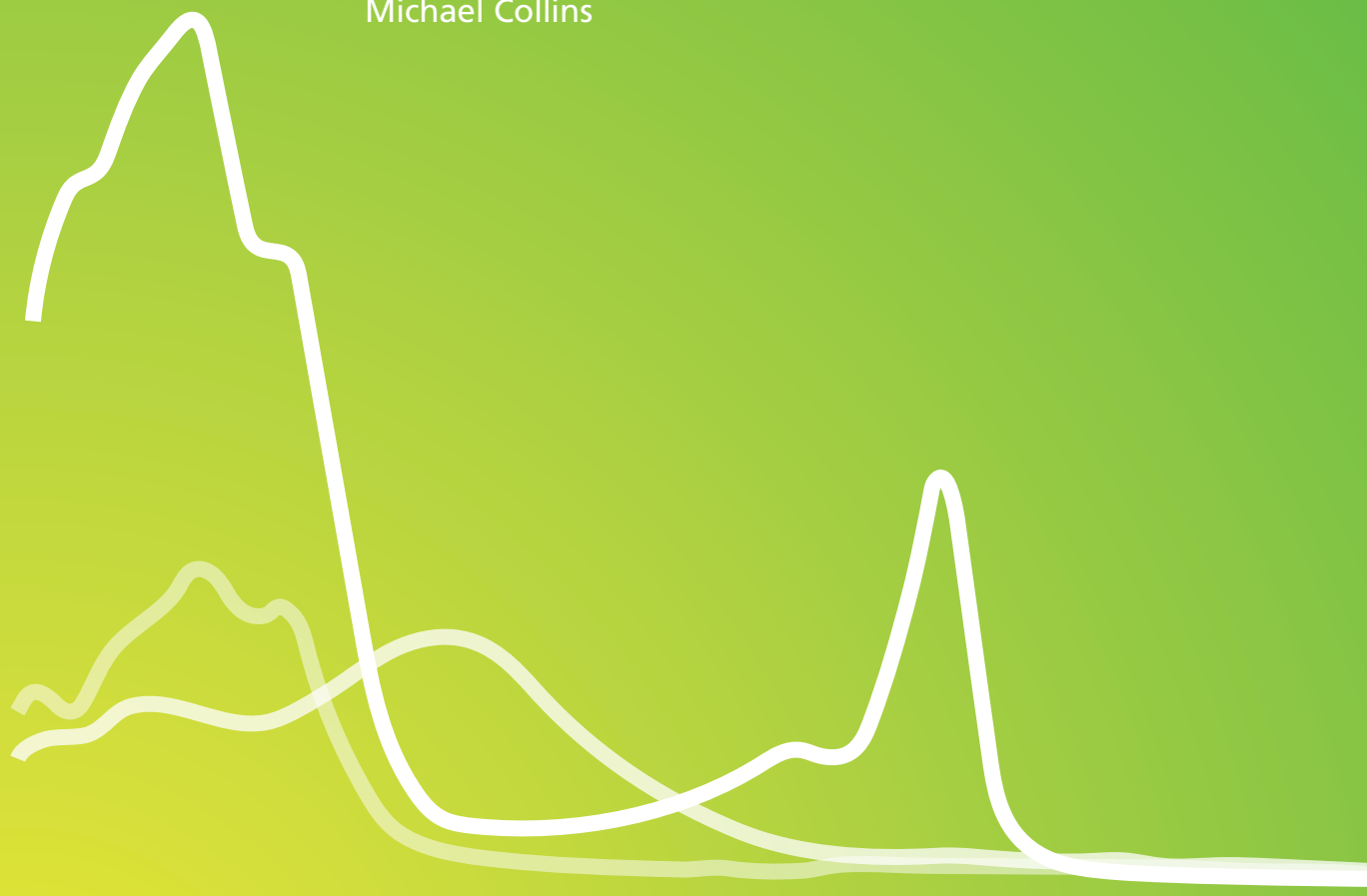
Investigating Biology through Inquiry

Experiments Using Open and Guided Inquiry Approaches

4th Edition

By
Johnathan M. Melville
Donald L. Volz
Michael Collins



BIO-I

Vernier

Contents

Experiments

Appendices

Equipment Used in Experiments

		Sensors										Kits				
		pH	Conductivity	Gas Pressure	Spectrophotmeter	Oxygen Gas	Carbon Dioxide Gas	Temperature	Heart Rate	Dissolved Oxygen	Ethanol	Got Protien? Kit*	Biofuel Enzyme Kit*	Comparative Proteomics Kit I*	Peroxidase Enzyme Activity^	Evolution of Yeast with a Carbon Dioxide Gas Sensor^
1	Investigating Buffers	X														
2	Diffusion		X													
3	Investigating Osmosis			X												
4	Chemistry of Membranes				X											
5	Investigating Protein				X							X				
6A	Testing Enzyme Activity (Oxygen Gas)					X										
6B	Testing Enzyme Activity (Gas Pressure)			X												
6C	Testing Enzyme Activity (Spectrometer)				X										X	
7	Introduction to Biofuels: Enzyme Action				X								X			
8	Analysis of Enzymes using Tyrosinase				X											
9	Cellular Respiration						X									
10A	Sugar Metabolism with Yeast (Carbon Dioxide Gas)						X	X								
10B	Sugar Metabolism with Yeast (Ethanol)										X					
11	Fermentation with Yeast			X				X								
12	Photosynthesis by Chloroplasts				X											
13	Transpiration of Plants			X												
14	Plant Pigments				X											
15	Heart Rate								X							
16	Investigating Dissolved Oxygen									X						
17	Investigating Primary Productvity									X						
18	Modeling Population Dynamics	No sensor used														
19	Water Monitoring	X	X							X						
20	Evolution of Cellobiase in Fungi				X								X			
21	Introduction to Molecular Evolution	No sensor used												X		
22	Evolution of Yeast						X									X

* Kit available from Bio-Rad Laboratories

^ Kit available from Flinn Scientific

PRELIMINARY ACTIVITY FOR

Testing Enzyme Activity

(Oxygen Gas Sensor)

Open Inquiry Version

Many organisms can decompose hydrogen peroxide (H_2O_2) enzymatically. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as *catalysts*, substances that speed up chemical reactions without being destroyed or altered during the process. Enzymes are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second.

H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water as follows:

$$2\ H_2O_2(aq) \rightarrow 2\ H_2O + O_2(g)$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: *catalase*, found in animals and protists, and *peroxidase*, found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this Preliminary Activity, you will use catalase in yeast to catalytically decompose hydrogen peroxide. You will use an O_2 Gas Sensor to determine the rate of catalase activity by measuring oxygen gas produced as H_2O_2 is decomposed.

At the start of the reaction, there is no product, and the O_2 concentration is the same as the atmosphere. Shortly after data collection begins, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the initial rate. In this investigation, we will refer to this as the rate of catalase activity. As the peroxide is decomposed, less of it is available to react and the O_2 is produced at lower rates. When no more peroxide is left, O_2 is no longer produced. When data collection is complete, you will perform a linear fit on the resultant graph to determine catalase activity.

After completing the Preliminary Activity, you will use reference sources to find out more about catalase, enzymes, and enzyme activity, and then you will choose and investigate a researchable question dealing with catalase activity. Some topics to consider in your reference search include the following:

- catalyst
- enzyme
- catalase
- hydrogen peroxide
- collision theory
- reaction rate

PROCEDURE

1. Obtain and wear goggles.
2. Start the data-collection program
3. Connect the O_2 Gas Sensor to your Chromebook, computer, or mobile device. Use an interface if necessary.
4. Prepare to initiate the catalase catalyzed reaction.
   a. Use a utility clamp to fasten an O_2 Gas Sensor to the Stir Station.
   b. Place 10.0 mL of 1.5% H_2O_2 into a clean 250 mL Nalgene bottle. Take care to minimize depositing drops on the sides of the bottle.
   c. Place a stir bar into the bottle.

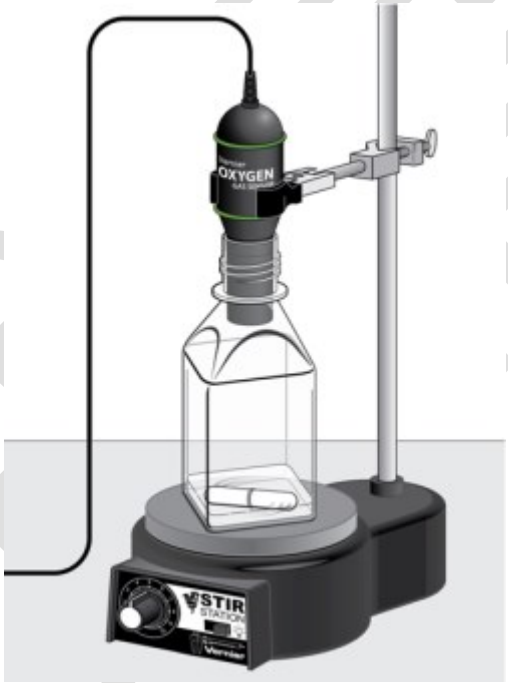


Figure 1

5. Initiate the enzyme catalyzed reaction and start data collection. **Note**: The next steps should be completed as rapidly as possible.
   a. Using micropipette, add 100 µL of enzyme suspension to the contents of the Nalgene bottle.
   b. Swirl the contents of the bottle for 2–3 seconds to ensure thorough mixing.
   c. Place the O_2 Gas Sensor into the bottle as shown in Figure 1. Gently push the bottle up onto the sensor until it stops. **Note**: The sensor is designed to seal the bottle with minimal force.
   d. Position the O_2 Gas Sensor and Nalgene bottle assembly on the Stir Station.
   e. Start the magnetic stirrer, and adjust it to a medium speed.

f. Start data collection.

6. When 200 seconds have elapsed, stop data collection.

7. Remove the O_2 Gas Sensor from the Nalgene bottle. Rinse the bottle with water and dry it with a soft paper towel.

8. Perform a linear fit on the 30–200 s portion of the graph. Record the slope of the line, *m*, as the rate of catalase activity, in % O_2/s.

QUESTIONS

1. What was the rate of catalase activity?

2. Why is it important that cells contain catalase?

3. List three factors that could possibly affect catalase activity.

4. List at least one researchable question concerning catalase activity.

PRELIMINARY ACTIVITY FOR

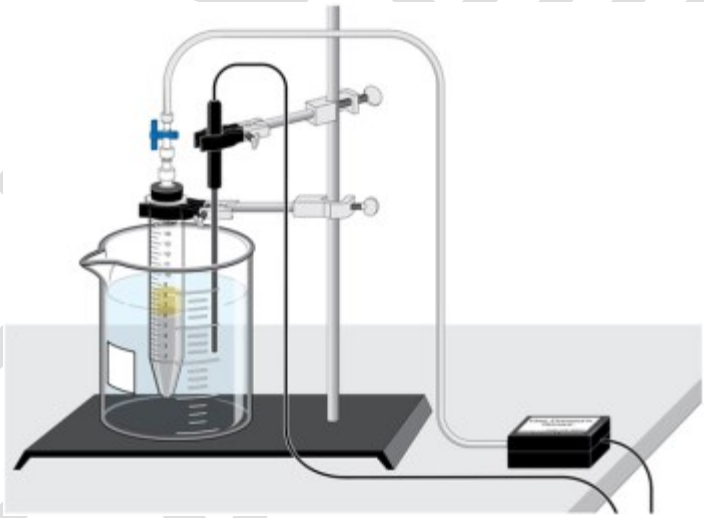
Fermentation with Yeast

Open Inquiry Version

Yeast can metabolize sugar in two ways, *aerobically*, with the aid of oxygen, or *anaerobically*, without oxygen. When yeast metabolizes a sugar under anaerobic conditions, ethanol (CH_3CH_2OH) and carbon dioxide (CO_2) gas are produced. An equation for the fermentation of the simple sugar glucose ($C_6H_{12}O_6$) is:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 + \text{energy}$$

The metabolic activity of yeast can be determined by the measurement of gas pressure inside a fermentation vessel.



In this Preliminary Activity, you will use a Gas Pressure Sensor to monitor the pressure inside a conical tube as yeast metabolizes glucose anaerobically. When data collection is complete, you will perform a linear fit on the resultant graph to determine the fermentation rate.

After completing the Preliminary Activity, you will first use reference sources to find out more about sugar fermentation by yeast. You will then choose and investigate a researchable question dealing with fermentation. Some topics to consider in your reference search include the following:

- sugars
- glucose
- monosaccharides
- disaccharides
- fermentation
- anaerobic respiration

- aerobic respiration
- yeast
- enzyme
- substrate
- enzyme inhibitor

PROCEDURE

1. Obtain and wear goggles.
2. Start the data-collection program.
3. Connect the Gas Pressure Sensor and Temperature Probe to your Chromebook, computer, or mobile device. Use an interface if necessary.
4. Connect the plastic tubing to the Gas Pressure Sensor. Connect a 2-way valve to the free end of the plastic tubing. **Note**: Make sure that the valve is in the "open" position.
5. Prepare a water bath for the yeast to ensure that the yeast will remain at a constant and controlled temperature.
   a. To prepare the water bath, obtain some warm and cool water.
   b. Combine the warm and cool water into the 1 L beaker until it reaches 38–40°C. Fill the beaker with 800–900 mL of water.
   c. Secure the Temperature Probe in the water bath with a utility clamp and ring stand as shown in Figure 1.

Figure 2

7. Pipette 3.0 mL of the 0.30 M glucose solution into a clean test tube.
8. Pipette 3.0 mL of yeast suspension into the test test. Gently mix the yeast into the sugar solution. **Important**: The yeast suspension must be removed from the middle of the yeast source that is being stirred by a magnetic stirrer at a constant stirring speed.
9. Place about 1 mL of vegetable oil into the test tube to cover the yeast/glucose mixture as shown in Figure 2. **Note**: Be careful not to get oil on the inside wall of the test tube.
10. Insert the single-holed rubber-stopper into the conical tube. **Note**: Firmly twist the stopper for an airtight fit.
11. Use a utility clamp to fasten the conical tube in the water bath as shown in Figure 1. **Note**: Be sure that most of the test tube is covered by the water in the water bath. The temperature of the gases in the tube must be constant for this experiment to work well.

Figure 3

12. Connect the free end of the plastic tubing to the connector in the rubber stopper as shown in Figure 3.

13. Start data collection. **Note**: Maintain the temperature of the water bath in the 38–40°C range during the course of the experiment. If you need to add more hot or cold water, first remove about as much water as you will be adding, or the beaker may overflow. Use a basting bulb to remove excess water.

14. Monitor the pressure readings displayed during data collection. If the pressure exceeds 130 kPa, the pressure inside the tube will be too great and the rubber stopper is likely to pop off. Disconnect the plastic tubing from the Gas Pressure Sensor if the pressure exceeds 130 kPa.

15. When data collection is complete, disconnect the plastic tubing connector from the rubber stopper. Remove the rubber stopper from the conical tube and discard the contents in a waste beaker.

16. Perform a linear fit on the segment of your graph where the data values are increasing. Record the slope of the line segment, *m*, as the fermentation rate (in kPa/s).

QUESTIONS

1. What was the rate of fermentation?

2. What is the role of the vegetable-oil layer on the surface of the yeast and glucose mixture?

3. List three common sugars, other than glucose.

4. List three factors that could possibly affect fermentation rates.

5. List at least one researchable question concerning sugar fermentation by yeast.