LABORATORY INVESTIGATION

The Breakdown of Hydrogen Peroxide by Catalase

Teacher Instructions

In this investigation students study the chemical breakdown of hydrogen peroxide by the enzyme catalase and acquire practice using the methods of science. Both experiences are enriched by microcomputer based data collection. Teams of 3 students are preferable.

Time
One double lab period to run the experiment and to print out the data for analysis outside of the laboratory.

Vernier Equipment
Pressure Sensor and LabPro interface

Materials per Class
safety goggles, 5 beakers (50 ml.), 1 liter of 1.5% hydrogen peroxide (ph 7), 1/4 lb. chopped liver, 1 apple sliced, 1/4 lb. ground hamburger, 1 pkg. of splints, 5 packages of safety matches, 5 16-mm. X 150-mm. test tubes, test tube brush (5), syringe 10 ml. (5), 500 ml. of (400 unit / ml.) catalase solution, syringe 1 ml. (5), 125 ml Erlenmeyer flask (5), 1 liter of distilled water, 500 ml. of buffer pH 7.

Safety
It is necessary for students to wear safety goggles always. The catalase - hydrogen peroxide reaction generates considerable pressure in the reaction vessel. This vessel should never be charged with more than the recommended volume of hydrogen peroxide. Also, do not use concentrations of hydrogen peroxide above 1.5%. The usual precautions when using matches and splints should be followed.

Preparations
1. Catalase can be purchased as a dessicated powder from biological supply companies (See Appendix A). Store the substance, as received, in a freezer until used. The catalase enzyme stock solution is prepared on the day of the laboratory using the following general formula:

\[(\text{concentration needed}) \times (\text{volume needed}) / (\text{# units / mg.}) = X \text{ mg. catalase}\]

\[(400 \text{ unit / ml.}) \times (200 \text{ ml.}) / (\text{from bottle label}) = X \text{ mg. catalase}\]

2. Dissolve the X mg. of catalase powder in 200 ml. of pH 7 buffer. This stock solution is the 100% enzyme concentration used in the investigation. Store the stock solution in a refrigerator when not being used. Distribute 50 ml. of stock solution to each team at the beginning of the laboratory period.

3. Fresh 1.5% hydrogen peroxide is needed for this investigation. Three percent concentrations of hydrogen peroxide can be purchased close to the time of use from any local pharmacy. Dilute
500 ml. of 3% hydrogen peroxide with 500 ml. of buffer (pH 7) to make 1 liter of 1.5% solution.

4. A common source of error is incomplete mixing of the enzyme and the hydrogen peroxide solution. Remind the students to follow carefully the procedural steps as given in the laboratory. To ensure thorough mixing, the reaction chamber should be swirled slowly during the data recording period. The chamber should not be shaken as some solution could get into the rubber hose leading to the pressure sensor.

5. Another error is introduced if the temperature of the gas in the reaction chamber is increased or decreased. Tell the students to hold only the top of the flask when it is being swirled. They should not cover the flask with their hand when swirling it.

6. After the students have printed their graphs, it will be important to help them firm up in their minds the chain of events that are represented by the line on the graph. They will need to associate mentally the graph’s line with the increasing pressure, and the increasing pressure with the release of oxygen from the hydrogen peroxide. In addition, the slope of the line must be seen to represent the rapidity (rate) of the enzyme’s action on the hydrogen peroxide. They should observe that the rate is constant and the slope of the line linear, only during a short initial period. As time progresses, they should see that the line of the graph flattens out and that this shows the enzyme is working less efficiently. Student discussion of limiting factors and identification of the limiting factor in this chemical reaction should then be undertaken to complete the data analysis. In advanced classes, the teacher may want to help the students find the initial rate of the reaction by dividing the rise of the linear portion of the graph by its run. This rate value allows the student to make a quantitative comparison of the different experimental runs of the investigation.

7. It is recommended that each class also complete item 2 of the Going Further section of the investigation. This added investigation will show the importance of pH in enzymatic regulated reactions. To complete this part of the investigation prepare buffers of pH 3, 5, 7, 9 and 11. Use these buffers to dilute the 3% hydrogen peroxide to 1.5% solutions.

8. Working enzyme solutions of pH 3,5,7,9, and 11 are made from a 800 unit / ml stock solution. Make the stock solution using distilled water. Working solutions of the enzyme are prepared by diluting the stock solution with equal volumes of a specific buffer.

9. The reaction chamber is made from a 125 ml. Erlenmeyer flask (See figure 1). The flask is fitted with a #5 two hole rubber stopper with two Luer-lock adaptors. A 2-way valve is connected to one of the adaptors. The second adaptor is jointed to a section of plastic tubing with two Luer-lock connectors One connector is jointed to the rubber stopper and the other end is jointed to the pressure sensor. A discarded plastic or metal culture tube cap serves as the enzyme vessel.

10. The program, Logger Pro, collects the data samples, stores them and plots them. For this experiment it will be necessary to change the graph X-Y scales. Start by clicking on the Logger Pro icon to load the program. This brings up the chart and graph with default scaling. Set the scale of the Y axis by double clicking on one of the numbers alone the Y axis. This step opens the Y-axis option dialog box. Move the pointer to the autoscale button, click and drag to the manual choice, release. Two boxes appear containing default values for the Y-axis scale. Highlight the top value (100) and type in the new scale value (120). Move the pointer to the bottom box. Highlight the value and type in the new choice (100). Next, click OK to complete the Y-axis changes.

11. Set the scale of the X-axis (time). Double click on one of the numbers along the X-axis. The X-axis dialog box opens. Move the pointer to the autoscale button. Click and drag to the manual
choice and release. Highlight the number in the right scale box. Type in the new value (200). Close the X-axis option box by clicking OK.

12. Make a test run of the investigation following preparation of the experiment’s reagent. Use 1 ml of enzyme and 10 ml of 1.5% hydrogen peroxide for the test run. The pressure curve should rise rapidly and level off near the top of the computer graph.

13. If the curve is too steep or too flat change the range of the Y-axis. Compressing the range increases the slope of the graph. Increasing the range decreases the slope of the graph. The test run above (step 14) should produce the greatest rate of change (100% conc). Collect data for the remaining tests using the new range. Readings for the remaining concentrations should all fall within the new Y axis range of values.

14. Quantifying the rate of the reaction by finding the slope of the linear portion of each graph is a useful application of mathematics. Demonstrate the following steps to the class. First, construct a straight “best fit” line along the linear section of the graph (See figure 2 below). Next, select two points, one near each end of the linear section. Draw a line through the first point that is parallel to the X axis. Draw a line through the second point that is parallel to the Y axis. These lines should intersect at a right angle. Measure the distance from the second point to the point of intersection. Call this distance the rise. Measure the distance from the first point to the point of intersection. Call this distance the run. Find the slope of the linear section of the line by dividing the rise by the run. This slope value is an estimate of the true rate of the reaction when limiting factors are absent.

15. To save time and to provide a single focus for this laboratory you might want to complete step 16 of the procedure as either a demonstration or as a separate laboratory activity.

16. Sample test run data for enzyme concentrations of 100%, 75%, 50%, 25% and 0% follow. The reaction rates are calculated from the slope of the linear portion of each pressure graph.
Table 1 Catalase Reaction Rate

<table>
<thead>
<tr>
<th>Run</th>
<th>Enzyme Conc.%</th>
<th>Reaction Rate (kPa/Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1.25</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>2.98</td>
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<tr>
<td>4</td>
<td>75</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Reaction Rate Change with Enzyme Concentration
50% Catalase, Change of Pressure with Time

Pressure (kPa)

Time (s)

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