TEACHER INFORMATION

Enzyme Action: Testing Catalase Activity

1. Editable Microsoft Word versions of the student pages and pre-configured TI-Nspire files can be found on the CD that accompanies this book. See *Appendix A* for more information.

2. This experiment may take a single group several lab periods to complete. A good breaking point is after the completion of Part I, when students have tested the effect of different enzyme concentrations. Alternatively, if time is limited, different groups can be assigned one of the three tests and the data can be shared.

3. Your hot tap water may be in the range of 50–55°C for the hot-water bath. If not, you may want to supply pre-warmed temperature baths for Step 21, where students need to maintain very warm water. Warn students not to touch the hot water.

4. Many different organisms may be used as a source of catalase in this experiment. If enzymes from an animal, a protist, and a plant are used by different teams in the same class, it will be possible to compare the similarities and differences among those organisms. Often beef liver, beef blood, or living yeast are used.

5. To prepare the yeast solution, dissolve 7 g (1 package) of dried yeast per 100 mL of 2% glucose solution. Incubate the suspension in 37–40°C water for at least 10 minutes to activate the yeast. Test the experiment before the students begin. The yeast may need to be diluted if the reaction occurs too rapidly. The reaction in Step 14, with 3 mL of 3% hydrogen peroxide, 3 mL of water, and 2 drops of suspension should produce a pressure of 130 kPa in 40 to 60 seconds.

6. To prepare a 2% sugar solution, add 20 grams of sugar to make one liter of solution (100 mL per group is needed).

7. To prepare a liver suspension, homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water. You will need to test the suspension before use, as its activity varies greatly depending on its freshness. Dilute the suspension until the reaction in Step 14, with 3 mL of 3% hydrogen peroxide, 3 mL of water, and 2 drops of suspension produces a pressure of 130 kPa in 40 to 60 seconds. The color of the suspension will be a faint pink. Keep the suspension on ice until used in an experiment.

8. You can purchase 3% H_2O_2 from any supermarket. If refrigerated, bring it to room temperature before starting the experiment.

9. Emphasize to your students the importance of providing an airtight fit with all plastic-tubing connections and when closing valves or twisting the stopper into a test tube.

10. The accessory items used in this experiment are the #1 single hole stopper fitted with a tapered valve connector and the section of plastic tubing fitted with Luer-lock connectors.

11. The length of plastic tubing connecting the rubber stopper assemblies to each gas pressure sensor must be the same for all groups. It is best to keep the length of tubing reasonably small to keep the volume of gas in the test tube low. **Note:** If pressure changes during data collection are too small, you may need to decrease the total gas volume in the system. Shortening the length of tubing used will help to decrease the volume.

12. Vernier Software & Technology sells a pH buffer package for preparing buffer solutions with pH values of 4, 7, and 10 (order code PHB). Simply add the capsule contents to 100 mL of distilled water.

13. You can also prepare pH buffers using the following recipes:
    - pH 4: Add 2.0 mL of 0.1 M HCl to 1000 mL of 0.1 M potassium hydrogen phthalate.
    - pH 7: Add 582 mL of 0.1 M NaOH to 1000 mL of 0.1 M potassium dihydrogen phosphate.
    - pH 10: Add 214 mL of 0.1 M NaOH to 1000 mL of 0.05 M sodium bicarbonate.

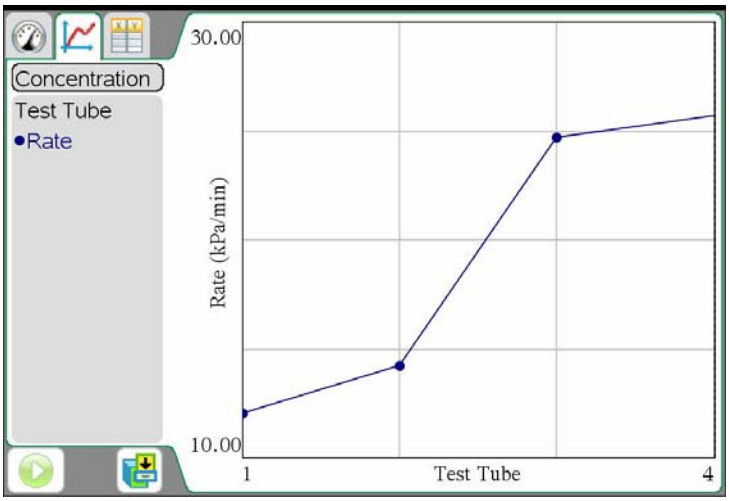
14. You may need to let students know that at pH values above 10, enzymes will become denatured and the rate of activity will drop. If you have pH buffers higher than 10, have students perform an experimental run using them.

SAMPLE RESULTS

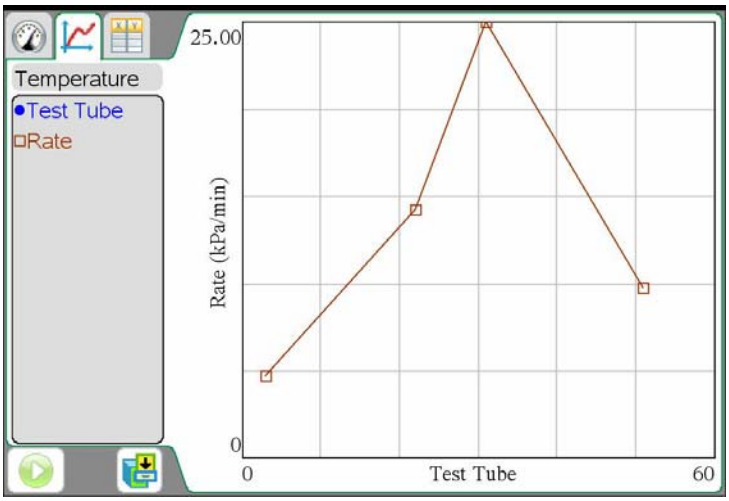
Label	Reaction Rate (kPa/min)
1 drop	12.06
2 drops	14.27
3 drops	24.72
4 drops	25.74

Actual Temperature (°C)	Reaction Rate (kPa/min)
3 °C	4.73
22 °C	14.31
31 °C	25.02
51 °C	9.84

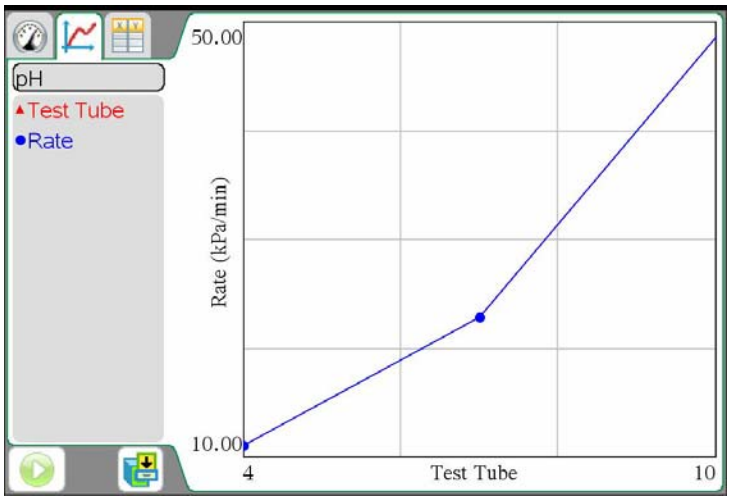
Label	Reaction Rate (kPa/min)
pH 4	11.16
pH 7	22.86
pH 10	48.60



The effect of enzyme concentration on the rate of activity.



The effect of temperature on the rate of enzyme activity



The effect of pH on the rate of enzyme activity

ANSWERS TO QUESTIONS

1. The rate should be highest when the concentration of enzyme is highest. With higher concentration of enzyme, there is a greater chance of an effective collision between the enzyme and H_2O_2 molecule.

2. Roughly, the rate doubles when the concentration of enzyme doubles. Since the data are somewhat linear, the rate is proportional to the concentration. At a concentration of 5 drops, the rate in the above experiment should be about 30.89 kPa/min.

3. The temperature at which the rate of enzyme activity is the highest should be close to 30°C. The lowest rate of enzyme activity should be at 50°C.

4. The rate increases as the temperature increases, until the temperature reaches about 50°C. Above this temperature, the rate decreases.

5. At high temperatures, enzymes lose activity as they are denatured.

6. Student answers may vary. Activity is usually highest at pH 10 and lowest at pH 4.

7. Student answers may vary. Usually, the enzyme activity increases from pH 4 to 10. At low pH values, the protein may denature or change its structure. This may affect the enzyme's ability to recognize a substrate or it may alter its polarity within a cell.