**Instructor   
Information**

**Enzyme Action:  
Temperature of Denaturation**

1. This experiment can be done with the following O2 gas sensors sold by Vernier: Go Direct Oxygen Gas (order code: GDX-O2) and O2 Gas Sensor (order code: O2-BTA). The O2-BTA requires an interface, such as LabQuest 2.
2. Test the experiment before the students begin. Depending on the type of enzyme you use, the activity will vary greatly; you may need to dilute the enzyme solution or make a new solution to get the ideal reaction rate.
3. This experiment may take a single group two lab periods to complete. A good breaking point is after the completion of Part I, when students have tested the enzyme at room temperature. Alternatively, if time is limited, different groups can be assigned different exposure temperatures. The data can then be aggregated and shared.
4. Your hot tap water will likely be in the range of 50–55°C. You may need to supply pre-warmed temperature baths for Part II, where students need to maintain water baths at or above this temperature range. Warn students not to touch the hot water.
5. We recommend purchasing purified catalase enzyme from Flinn Scientific, Ward’s Natural Science, or Sigma-Aldrich. The concentration of enzyme varies from 2000–5000 units/mg and depends on the bottle. Store the catalase powder as instructed. Enzyme activity may decrease from year to year, but will remain viable for up to three years.
6. Use the following instructions to prepare an enzyme solution:

**Purified catalase**

* + 1. Make a stock solution of 1000 units/mL.
    2. Dilute the stock solution to 200 units/mL for use by the students.

Yeast suspension

* + 1. Dissolve 1 package (7 g) of dried yeast per 100 mL of 2% sugar solution. To prepare a 2% sugar solution, add 20 grams of sugar to make one liter of solution.
    2. Incubate the suspension in 37–40°C water for at least 10 minutes to activate the yeast.
    3. To ensure a uniform yeast concentration, make the suspension available on a magnetic stirrer and instruct your students to withdraw their samples from the center as the suspension is being stirred.
    4. The yeast may need to be diluted if the reaction occurs too rapidly.

Liver suspension

* + 1. Homogenize 0.5 to 1.5 g of beef liver in 100 mL of cold water.
    2. Keep the suspension on ice until it is to be used.
    3. Dilute the suspension as needed based on reaction rate.

1. You can purchase 3% H2O2 at any supermarket. If refrigerated, bring it to room temperature before starting the experiment. Dilute the 3% H2O2 by 50% to create a 1.5% solution.
2. All Vernier O2 gas sensors should always be used and stored upright. Do not get the sensor wet.
3. This experiment works best using a Stir Station, micropipettes, and microtubes. If you do not have access to these materials, you can use beral pipettes and test tubes. Incubate 1 mL of enzyme suspension in a test tube in the desired water bath. Then use 5 drops of enzyme suspension to catalyze the reaction instead of 100 µL. Add the drops to a test tube filled with 10 mL of 1.5% H2O2 . Cover the opening of the test tube with a finger and gently invert the test tube two times. Then pour the contents of the test tube into the 250 mL Nalgene bottle. Place the O2 gas sensor into the bottle as shown in Figure 1 in the student experiment. Wait 10 seconds and then start data collection.

If you are using Go Direct sensors, see [**www.vernier.com/start/go-direct**](http://www.vernier.com/start/go-direct) for information about how to connect your sensor.

For additional information about the Vernier probeware used in this experiment, including tips and product specifications, visit [**www.vernier.com/manuals**](http://www.vernier.com/manuals) and download the appropriate user manual.

**ESTIMATED TIME**

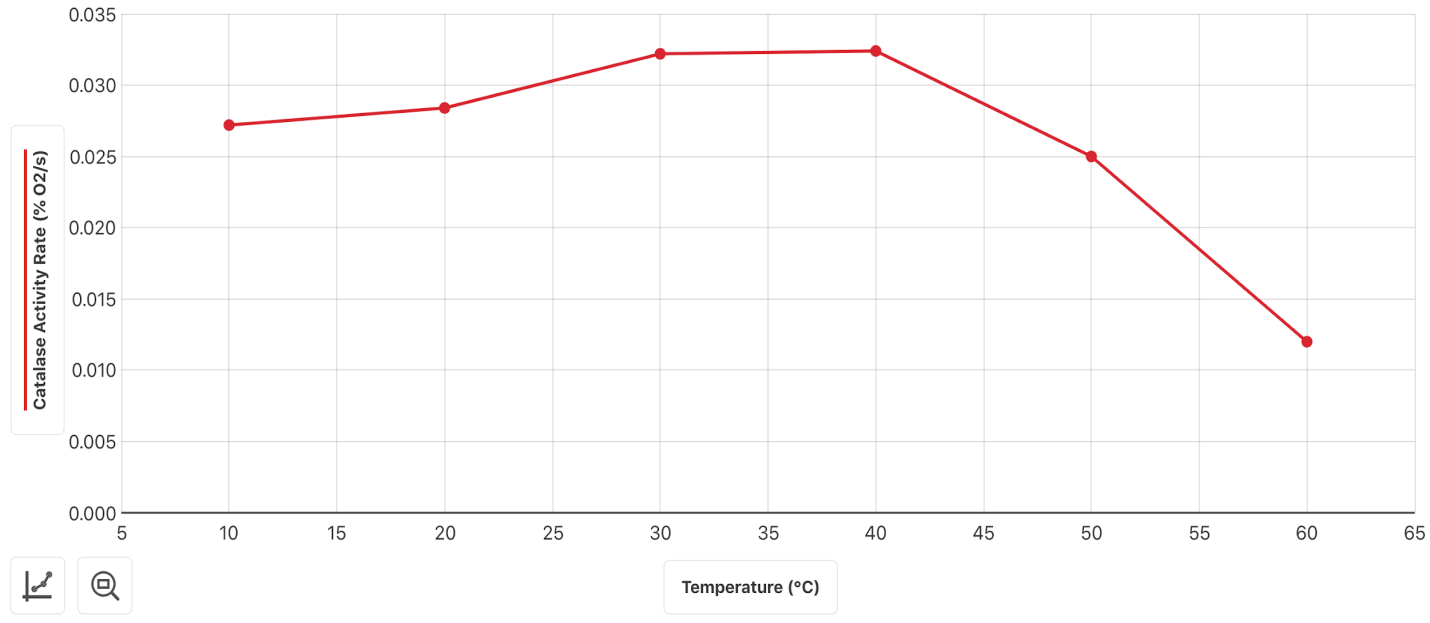
We estimate that setup and data collection can be completed in two 45-minute class periods.

**NEXT GENERATION SCIENCE STANDARDS (NGSS)**

|  |  |  |
| --- | --- | --- |
| Science and Engineering Practices | Disciplinary Core Ideas | Crosscutting Concepts |
| Analyzing and Interpreting Data  Developing and Using Models | LS1.A: Structure and Function | Cause and Effect  Structure and Function |

**SAMPLE RESULTS**

|  |  |
| --- | --- |
| Table 1 | |
| Exposure temperature  (°C) | Reaction rate  (% O2/s) |
| 10–15 | 0.0272 |
| 20–25 | 0.0284 |
| 30–35 | 0.0322 |
| 40–45 | 0.0324 |
| 50–55 | 0.0250 |
| 60–65 | 0.0120 |



*Figure 1 Temperatures above 40℃ appear to denature the enzyme catalase*

**ANSWERS TO QUESTIONS**

1. The exposure temperatures with the highest rate of enzyme activity should be in the 30°C or 40°C range. The lowest rate of enzyme activity should be found at exposure temperatures above 40°C. Lower rates of enzyme activity may be found at exposure temperatures below 20°C
2. Exposure temperature does not seem to alter the enzyme until the temperature is above 40°C. Exposure temperatures above this level appear to cause enzyme activity to decrease.
3. At high temperatures, enzymes lose activity as they are denatured.