ENZYME ACTIVITY WITH THE WIRELESS SPECTROMETER

Enzymes are hyper-specific – often an enzyme interacts with only one substrate. Like catalysts in other chemical reactions, enzymes are not consumed during the reaction as they help turn the substrate into the final product. Notice in the following reaction that the enzyme is present before and after the reaction.

$$\text{enzyme(l)} + 2H_2O_2\,(l) \rightarrow 2H_2O(l) + O_2\,(g) + \text{enzyme(l)}$$

Hydrogen peroxide (H_2O_2) is a byproduct of aerobic respiration in cells and is used in cell signaling and apoptosis. Hydrogen peroxide is highly reactive and can produce free radicals that damage nucleic acids, so cells must carefully regulate its concentration. To remove excess hydrogen peroxide, cells produce an enzyme (called catalase in animal cells and peroxidase in plant cells) which breaks down H_2O_2 into oxygen (O_2) and water (H_2O), as shown above. Guaiacol will also be present in this investigation, changing from brown to yellow when oxygen binds to it, serving as an indicator for the reaction.

Driving Question:

How is the catalyzed decomposition rate of hydrogen peroxide affected by enzyme concentration?

AP Connections: The concepts covered in this lab align to the "AP Biology Curriculum Framework."
Essential Knowledge 1.B.1, 2.D.1, 4.A.s, 4.B.1 / Science Practices 4.2, 5.1, 6.1, 6.4, 7.2 / Learning Objectives1.9, 1.16, 2.24, 4.3, 4.17

Materials

- Spectrometer
- 1mL graduated diposable pipette (4)
- Graduated cylinder, or syringe, or adjustable micro-pipet, 10-mL
- Enzyme Solution, 5-mL
- pH 7 Buffer Solution, 10-mL
- 3 brown bottles
- Cuvettes (5)
- Guaiacol Solution, 5-mL
- Distilled water
- 0.1% H_2O_2, 10-mL

Investigate

Complete the following lab procedure and analysis. Record observations, data, and explanations in your lab notebook.

1. Put on your safety goggles and gloves.
2. Turn on the spectrometer and connect it to your computing device.
3. Use the Analyze Solution tab to calibrate the spectrometer for light and dark.
   a. With the sample well empty press the calibrate dark icon.
   b. Prepare a blank with 2.0 mL of buffer, 0.5mL of guaiacol, and 1.0mL of hydrogen peroxide in a cuvette.
   c. Cap and invert the cuvette 3 times to mix.
   d. Place the blank into the sample well and press the calibrate light icon.
4. With the blank still in the spectrometer collect some data and set 470nm as the target wavelength using the coordinate tool.
5. Proceed to the time tab.
6. Read through this step (a-d) entirely before proceeding:
   a. Add the following in a cuvette:
   1.0mL of pH 7 buffer
   0.5mL of H_2O_2 substrate
   0.25mL of guaiacol
   b. Cap and invert the cuvette 3 times to mix.
   c. Add 0.25mL of enzyme extract, cap and invert cuvette to mix.
   d. Quickly place cuvette in the spectrometer and begin recording data for 2 minutes.
7. Using the linear fit tool determine the rate of reaction in absorbance-per-second. Record rate in a data table.
8. Compare results with the other lab groups. Determine the class average, minimum, and maximum. What variables could contribute to the observed variation?
9. If the enzyme concentration is halved what will happen to the rate of reaction?

Explain

10. Repeat steps 6 and 7, using half the concentration of enzyme. Be sure to maintain the sample volume.
11. Record the results in your notebook and compare the rate of reaction. Was your hypothesis in question 9 correct?


CALIBRATE DARK


CALIBRATE REFERENCE


START RECORDING


STOP RECORDING

ANALYZE SOLUTION

CONCENTRATION

TIME


SCALE TO FIT


ADD COORDINATE


SHOW LINEAR FIT


EXPERIMENT OPTIONS


QUICKCALCS

enzyme teacher tips

1. Guaiacol Preparation (Day before the lab)

   Guaiacol can be ordered in concentrate from many chemical suppliers. Dilute 1.5mL with 500 mL of distilled water. Store the Guaiacol in brown bottles and keep refrigerated. The solution should last for years if stored properly. Wear gloves and goggles when handling Guaiacol as it can be a skin irritant. Solution should be prepared in a fume hood or well ventilated area.

2. Enzyme Preparation

   Numerous peroxidase or catalase sources will work (yeast, liver, root vegetables, commercial extract, etc.). The lab was tested with turnip extract which can be prepared as follows. Peel a turnip and cut into small pieces, blend to liquefy ~20g in 500mL of distilled water. Filter using cheese cloth then a coffee filter. Extract should stored in brown bottles and refrigerated.

   Test before providing to students. If enzyme is too concentrated the reaction may proceed before students can get the cuvette into the spectrometer. If the reaction starts to plateau within 2min, cut the concentration in half and test again.

3. Substrate Preparation

   Hydrogen peroxide (3%) is widely available at grocery and drug stores. Prepare a 0.1% solution by adding 15mL of 3% H_2O_2 to 435mL of distilled water. Store in brown bottles and keep refrigerated.

4. pH Buffers

   Buffers referenced in the lab were prepared using pHydrion capsules (PASCO Part # SC-2321, contains 4, 7, 10). Dissolve capsule in 100mL distilled water. Additional buffers can be purchased from chemical suppliers.

5. Other

   Volumes for this lab were optimized to fit in a standard 3.5mL cuvette. If you would prefer to run the lab with larger volumes a standard 13mm test tube will fit in the wireless spectrometer and enable a solution volume up to 8mL.

   Make sure students do not mix pipettes between solutions. Contamination can significantly skew the results. Set up stations for each solution and label the appropriate pipets to reduce the chance of mixing.

   For inquiry investigations students can readily test the impact of pH, temperature, enzyme, and substrate concentration with a few additional supplies.


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