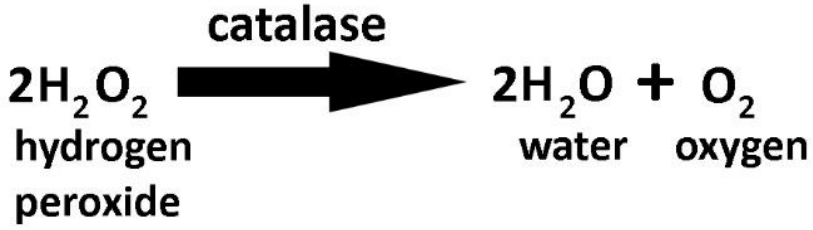
Effect of pH on Enzyme Activity: Catalase

As you recall from reading the section on naming and preparation of enzymes, catalase is an enzyme found in nearly every organism. It breaks down hydrogen peroxide to produce water and oxygen. If you haven't read the section on naming and preparing enzymes, please read it now before proceeding with the lab.



In today's activity you will be examining the effect of pH on enzyme activity using pH buffers, the enzyme catalase and hydrogen peroxide. pH can have a dramatic effect on protein structure and enzyme function. Acid and base functional groups compete with hydrogen bonds and alter the ionization of R-groups that are critical to protein structure. By competing with established bonds within the molecules and altering existing bonds, pH changes can potentially change the shape of the active site and therefore potentially change the activity of the enzyme. Protein structure involves at least three levels of structure and in some cases a fourth level of structure. Primary structure is a listing of the amino acids in the peptide chain. Secondary structure (alpha helix, beta pleated sheet) is created by the interaction of polar entities within the individual amino acids and the formation of hydrogen bonds. Tertiary structure results from the interaction of amino acid R-groups.

Enzymes have evolved to function optimally under specific conditions of pH, temperature and concentration. The activities in today's lab are designed to exemplify these characteristics. You will be measuring the activity of catalase from either potato or liver extract in various buffered pH solutions (2, 4, 7, 10, and 12). Write a hypothesis for your experiment and the response of catalase to various pH conditions here:

_____

_____

Materials

Test tubes – 5	pH 4 buffer	Test tube rack
Pipettes	pH 7 buffer	1 Small beaker - 15 mL
Catalase extract	pH 10 buffer	Ruler
Hydrogen peroxide	pH 12 buffer	Graduated cylinder
pH 2 buffer	Sharpie	Stopwatch

Procedure

1. Pick up 5 test tubes and a test tube rack from the supply table.
2. Label the test tubes as follows: pH 2, pH 4, pH 7, pH 10, and pH 12.

3. Mark each test tube 1 cm and 4 cm from the bottom of the tube.

4. Fill each tube to the 1 cm line with the appropriate buffer.

5. Add 20 drops of catalase extract to each tube. Swirl the tubes. Allow each tube to sit for 5 minutes swirling occasionally.

6. Add hydrogen peroxide to the 4 cm line of the pH 2 test tube and start the timer. After 45 seconds measure the height of the bubbles. Record the height here. _____ mms.

7. Add hydrogen peroxide to the 4 cm line of the pH 4 test tube and start the timer. After 45 seconds measure the height of the bubbles. Record the height here. _____ mms.

8. Add hydrogen peroxide to the 4 cm line of the pH 7 test tube and start the timer. After 45 seconds measure the height of the bubbles. Record the height here. _____ mms.

9. Add hydrogen peroxide to the 4 cm line of the pH 10 test tube and start the timer. After 45 seconds measure the height of the bubbles. Record the height here. _____ mms.

10. Add hydrogen peroxide to the 4 cm line of the pH 12 test tube and start the timer. After 45 seconds measure the height of the bubbles. Record the height here. _____ mms.

	mms of bubbles	**Was catalase active?**
pH 2		
pH 4		
pH 7		
pH 10		
pH 12		

1. How do you explain your results?

2. Was your hypothesis supported?

3. What was your dependent variable?

4. What was/were your independent variables?

5. Graph your results.

6. What is the pH optimum for your enzyme?

7. Name the enzyme.

8. Name the products of the enzyme.

9. Name the substrate of the enzyme.