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Peroxidase Enzyme Activity

AP* Biology Big Idea 4, Investigation 13
An Advanced Inquiry Lab

Introduction

Enzyme activity—how the rate of an enzyme-catalyzed reaction depends on concentration, pH, temperature, metal ions, etc.—provides the central basis for understanding how enzymes function. Among the thousands of different enzymes in a single cell, peroxidases are among the most active and the most widely distributed. Peroxidases protect plants and animals against cell damage by catalyzing the breakdown of hydrogen peroxide, a natural but toxic by-product of aerobic respiration. Investigate the activity of turnip peroxidase by measuring its rate of reaction with hydrogen peroxide and a natural reducing agent called guaiacol.

Concepts

- Enzyme structure and function
- Enzyme–substrate binding
- Kinetics and rate laws
- Active site

Background

Enzymes are the catalysts of biological systems. Enzymes and catalysts increase the rates of biological or chemical reactions by decreasing the activation energy required for a reaction and providing a lower energy pathway from reactants to products. Like all catalysts, enzymes are also not "consumed" during a typical reaction. Most enzymes are globular proteins that are able to bind reactant molecules, called substrates, at their active sites. Although it enters into the reaction pathway, the free enzyme is restored at the end of the reaction when the product is released from the binding site.

The relationship between the structure and function of enzymes is responsible for three unique features that differentiate them from other common catalysts. The first noteworthy feature of enzymes is their immense catalytic power. Enzymes are the most efficient catalysts known, giving rise to rates 10^3 to 10^7 faster than uncatalyzed reactions. Enzymes are also highly specific with respect to both the reactions they catalyze (reaction specificity) and the choice of reactants (substrate specificity). Finally, the activity of enzymes can be regulated—activated or inhibited—by interacting or binding with various small molecules and other proteins, as well as by environmental factors. Enzyme regulation allows an organism to control its metabolism and also to respond to external stimuli and changes in the environment.

The optimum activity of many enzymes depends on the presence or cooperation of nonprotein cofactors. Metal ions, especially Fe^{3+}, Zn^{2+}, and Mg^{2+}, are common inorganic cofactors. The role of metal ions in regulating enzyme activity reflects their importance as essential nutrients. Water-soluble vitamins are the source of organic cofactors, also called coenzymes, for enzymatic reactions. Examples include NAD^+, which is derived from niacin, and FAD, which comes from riboflavin or Vitamin B2. Both NAD^+ and FAD are involved in energy production during metabolism.

Peroxidase enzymes are widely distributed in animals, plants, and bacteria, to protect cells against the effects of oxidative stress and cell damage due to hydrogen peroxide. Peroxidases are easily extracted from turnips and other root vegetables and provide a model enzyme for studying enzyme activity—how the rate of an enzyme-catalyzed reaction depends on biotic and abiotic factors, such as enzyme and substrate concentration, pH, temperature, and the presence of inhibitors and activators. Enzyme activity studies, in turn, reflect the structure and function of enzymes and provide a foundation for understanding the mechanism or theory of enzyme action.

Various naming systems are currently in use for identifying enzymes. Many enzymes, such as the digestive enzymes pepsin and trypsin, may have medical, consumer or commercial applications and are known by common names. Recommended scientific names for enzymes generally consist of two words, the name of the substrate followed by the type of reaction that is catalyzed. The enzyme alcohol dehydrogenase, for example, catalyzes the oxidation—also known as dehydrogenation, because two hydrogen

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atoms are removed—of alcohol. In 1972 a systematic way of naming enzymes was adopted to prevent confusion and to show the relationships among different enzymes. According to this system, enzymes are classified into six groups based on the general type of chemical reaction that is catalyzed. Classes of enzymes and the reactions they catalyze include:

Oxidoreductases—oxidation/reduction reactions

Transferases—transfer of a functional group from one substrate to another

Hydrolases—hydrolysis reactions (reaction of water with substrates)

Lyases—elimination reactions of substrate molecules to generate products containing a double bond

Isomerases—isomerization reactions

Ligases—bond-forming reactions

The term peroxidase refers to both a class of oxidoreductase enzymes and to specific enzymes within that class. As a general class of enzymes, peroxidases catalyze the oxidation–reduction decomposition reaction of hydrogen peroxide. There are two general types of peroxidases—catalase and peroxidase. Catalase catalyzes the disproportionation reaction of hydrogen peroxide to water and oxygen gas (Equation 1). In reactions mediated by catalase, hydrogen peroxide substrate molecules act as both the oxidizing agent (electron acceptor) and reducing agent (electron donor). Peroxidase acts differently, requiring the presence of other naturally occurring organic reducing agents, such as ascorbic acid and glutathione, to catalyze the decomposition of hydrogen peroxide. These organic reducing agents, abbreviated AH_2, transfer hydrogen atoms and electrons to hydrogen peroxide, resulting in the formation of water and an oxidized organic substrate, represented by A_2 in Equation 2.

Catalase-catalyzed reaction $2H_2O_2 \rightarrow 2H_2O + O_2$ *Equation 1*

Peroxidase-catalyzed reaction $2H_2O_2 + 2AH_2 \rightarrow 4H_2O + A_2$ *Equation 2*

The differences in the two equations shown above provide a basis for studying the enzyme activity of turnip peroxidase in this guided-inquiry laboratory investigation. Many endogenous organic compounds may be used as reducing agents in Equation 2. One of the most common and convenient reducing agents for this purpose is guaiacol, a colorless compound having the formula $C_7H_8O_2$. Oxidation of guaiacol according to Equation 2 converts it to a dark orange compound called tetraguaiacol. The rate of the reaction may be followed by measuring the absorbance or color intensity of the orange product as a function of time.

The mechanism of enzyme activity, that is, how an enzyme catalyzes a reaction, is important from a practical viewpoint to learn about the metabolic basis of many diseases, and from a theoretical viewpoint in providing a model for how enzymes function. The mechanism of a reaction is a step-by-step description of the course of the reaction at a molecular level. Enzyme-catalyzed reaction mechanisms are generally determined through a deductive process by analyzing enzyme kinetics. The rate of an enzymatic reaction is studied under a variety of conditions to see how different factors affect the rate, giving rise to a general model of enzyme activity. The model should be consistent with the kinetic evidence and should explain the unique properties of enzymes, including their immense catalytic power, substrate specificity, and regulatory control.

Experimental Overview

This advanced inquiry lab begins with a baseline activity measuring the absorbance or color intensity of the orange product formed from guaiacol and hydrogen peroxide as a function of time for three different concentrations of turnip peroxidase. (The enzyme is extracted from turnip roots using pH 7 phosphate buffer.) Graphical analysis of absorbance versus time gives the rate of the reaction—and the first clue for a model of enzyme action. Using this general procedure students continue in the *Opportunities for Inquiry* portion of the lab to design experiments in search of additional evidence that may support or refute the mechanism of enzyme action.

Materials

Buffer, pH 5, 16 mL

Guaiacol, $C_7H_8O_2$, 0.2% solution in isopropyl alcohol, 4 mL

Hydrogen peroxide, H_2O_2, 0.02% solution, 8 mL

Phosphate buffer, pH 7, 8 mL

Peroxidase enzyme extract in phosphate buffer, 6 mL

Pipet bulb

Pipets, serological, 2-mL, 5

Spectrophotometer

Test tubes, 13 × 100 mm, 7

Test tube rack

Timer, seconds

Safety Precautions

Guaiacol is toxic by ingestion. The guaiacol solution is prepared in isopropyl alcohol and has an aromatic, creosote-like odor that may be irritating to the nose and throat. Isopropyl rubbing alcohol (70%) is a flammable liquid. Keep away from heat, flames, and other sources of ignition. Dilute hydrogen peroxide solution may be irritating to the eyes and skin. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and wash hands thoroughly with soap and water before leaving the laboratory. Please follow all normal laboratory safety guidelines.

Baseline Activity

1. Read the entire procedure before beginning. Pay special attention to the requirements for mixing the contents of the substrate and enzyme tubes and timing the reaction. Use a dedicated serological pipet for each solution. Precise volume measurements and accurate timing are crucial for rate studies.
2. Turn on the spectrophotometer, adjust the wavelength setting to 500 nm, and allow the instrument to warm up for 15–20 minutes.
3. Prepare a "blank" by combining 4 mL pH 5 buffer, 2 mL 0.02% hydrogen peroxide, 1 mL 0.2% guaiacol, and 2 mL phosphate buffer in a 13 × 100 mm test tube.
4. Zero the spectrophotometer (zero absorbance, 100% transmittance) at 500 nm using the blank solution.
5. Prepare three series of separate 13 × 100 mm test tubes containing substrates (tube S) and the enzyme (tube E) with buffers as shown in the table below. Note that the enzyme concentration is varied in Trials 1–3 but that the concentration of substrate and the total volumes are held constant. The pH 5 buffer provides pH control, while the presence of the phosphate extraction buffer in tube E makes it possible to vary the enzyme concentration while maintaining the overall buffer composition constant.

	Trial 1		**Trial 2**		**Trial 3**	
Tube	**S1**	**E1**	**S2**	**E2**	**S3**	**E3**
pH 5 buffer	1 mL	2 mL	1 mL	2 mL	1 mL	2 mL
0.02% H_2O_2	2 mL		2 mL		2 mL	
0.2% Guaiacol	1 mL		1 mL		1 mL	
Enzyme Extract		0.5 mL		1 mL		0.25 mL
Phosphate Buffer		1.5 mL		1 mL		1.75 mL

6. When ready to begin Trial 1 for the kinetics run, carefully pour the contents of tube S1 into tube E1 and ***immediately start timing***. Pour the combined contents back into tube S1, wipe the outside of the tube with lab tissue, and place the test tube in the spectrophotometer tube holder.
7. Measure and record the absorbance as a function of time *every 20 seconds* over a total period of 240–300 seconds. Since initial rate data corresponding to the first 10% of reaction completion is more accurate than longer term rate data, it is crucial to obtain accurate measurements as early as possible. **The elapsed time between mixing the tubes and recording the first absorbance measurement should be no greater than 20–40 seconds!**
8. Repeat steps 5 and 6 for the remaining two trials with different concentrations of the enzyme.
9. Graph absorbance versus time for each trial to determine the rate of the reaction. Consider the enzyme concentration in Trial 1 to be a "normalized" concentration equal to 1x and compare the enzyme concentration and rate of reaction for each trial to that of Trial 1.

Opportunities for Inquiry

1. Consider the following questions while reflecting upon your knowledge of enzyme structure, reaction rates, and how interactions among proteins as well as biotic and abiotic factors may influence the rate of an enzyme-catalyzed reaction.
   a. What evidence from the effect of enzyme concentration on the reaction rate supports a dynamic theory for biological reactions?
   b. Does the substrate concentration influence the rate of the reaction?
   c. How might a model of enzyme–substrate binding be reflected in the shape of the curve for substrate concentration versus reaction rate?
   d. Does enzyme activity depend on pH? Is there an optimum pH for the reaction?
   e. How does the pH profile for an enzymatic reaction demonstrate the acidic and basic properties of proteins and enzymes?
   f. Do metal ions activate or inhibit the rate of enzyme-catalyzed decomposition of hydrogen peroxide?
   g. What factors might predict the effect of temperature on the rate of an enzyme-catalyzed reaction?
   h. Do peroxidase enzymes from different vegetables have similar activity?
2. Plan, discuss, execute, and evaluate an experiment to test a question regarding the rate of an enzyme-catalyzed reaction and the model of enzyme activity.
   a. Decide upon one question that your group would like to explore.
   b. Develop a testable hypothesis.
   c. Discuss and design a controlled experiment to test the hypothesis.
   d. List any safety concerns and the precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experiment.
   e. Determine what and how you will collect and record the data.
   f. How will you analyze the data to test your hypothesis?
   g. Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
   h. Once the experiment is complete, analyze the data and evaluate whether the experimental evidence supports, refutes or provides no information concerning the hypothesis.
   i. Explain the results in terms of the mechanism of enzyme action, structure–function relationships involving proteins, and metabolic control of biological reactions.
   j. Make suggestions for additional experiments to modify or revise the hypothesis.

Teacher's Notes

Peroxidase Enzyme Activity

Materials Included in Kit (for 8 groups of students)

Buffer envelopes, pH 3–6, and 8, 1 each
Guaiacol, $C_7H_8O_2$, 0.2% solution in isopropyl alcohol, 100 mL
Hydrogen peroxide, H_2O_2, 3%, 100 mL
Sodium phosphate, dibasic, solution, Na_2HPO_4, 0.2 M, 300 mL
Sodium phosphate, monobasic, solution, NaH_2PO_4, 0.2 M, 300 mL
Pipets, serological, 2-mL, 5

Additional Materials Needed (for each lab group)

Distilled or deionized water
Ice
Pipet bulb
Spectrophotometer or colorimeter
Test tubes, 13 × 100 mm, 6, and rack
Thermometer (for inquiry investigations)
Timer, seconds

Additional Materials Needed (for *Pre-Lab Preparation*)

Turnip (root/tuber)
Blender
Erlenmeyer flask, 500-mL
Filter paper and funnel
Graduated cylinder, 10-mL
Knife, paring

Pre-Lab Preparation

Phosphate Extraction Buffer: Prepare one liter of pH 7 phosphate buffer by mixing equal volumes, 250 mL each, of 0.2 M sodium phosphate monobasic and sodium phosphate dibasic solutions. *Dilute to one liter with distilled or deionized water.*

Reaction Buffers: Dissolve one each pH 3–8 buffer envelopes in 500 mL distilled or deionized water according to packet instructions. Prepare separate buffer solution for each desired pH.

Dilute Hydrogen Peroxide, 0.02%: Dilute 3 mL of 3% hydrogen peroxide to a final volume of 500 mL using distilled or deionized water. Store in a cool, dark area protected from heat and light.

Enzyme Extraction: Peel and cut a turnip root into small cubes, about 1 cm on each side. Measure approximately 2 g (about 2 pieces) in a weighing dish. Place 500 mL of pH 7 phosphate extraction buffer and the turnip root pieces in a blender. Pulse the turnip root in 1–3 min bursts three times, with 2 minutes rest between pulses, to homogenize and extract the enzymes. Filter the turnip enzyme extract through filter paper and store the extract over ice or in the refrigerator. Use within one week. Check the enzyme activity of the turnip peroxidase extract before doing the baseline activity. It is important to pre-test the extract so that the absorbance (color) change occurs at a convenient rate that can be accurately measured, that is, neither too fast nor too slow. Absorbance values greater than one correspond to very low transmittance and are less accurate.

Safety Precautions

Guaiacol is toxic by ingestion. It has an aromatic, creosote-like odor and may be irritating to the nose and throat. Isopropyl rubbing alcohol (70%) is a flammable liquid. Keep away from heat, flames, and other sources of ignition. Dilute hydrogen peroxide solution (3%) may be irritating to the eyes and skin. Exercise care when using a knife to peel and cut the turnip. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and remind students to wash hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Buffers and leftover isopropyl alcohol solutions may be rinsed down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Alignment with AP Biology Concepts and Curriculum Framework

Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce, and to maintain dynamic homeostasis.

Enduring Understandings and Essential Knowledge

2D1: All biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

Big Idea 4: Biological systems interact, and these systems and their interactions possess complex properties.

Enduring Understandings and Essential Knowledge

4A1: The subcomponents of biological molecules and their sequence determine the properties of that molecule.

4B1: Interactions between molecules affect their structure and function.

Learning Objectives

- The student is able to design a plan for collecting data to show that all biological systems are affected by complex biotic and abiotic interactions (2D1 and SP 4.2, 7.2).
- The student is able to use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule (4A1 and SP 6.1, 6.4).
- The student is able to analyze data to identify how molecular interactions affect structure and function (4B1 and SP 5.1).

Science Practices

4.2 The student can design a plan for collecting data to answer a particular scientific question.

5.1 The student can analyze data to identify patterns or relationships.

6.1 The student can justify claims with evidence.

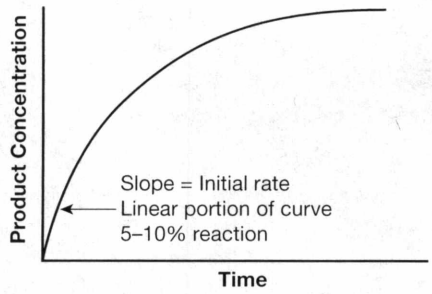
6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models.

7.2 The student can connect concepts in and across domains to generalize or extrapolate in and/or across enduring understandings and/or big ideas.

Lab Hints

- Enough materials are provided in this kit for eight groups of students to complete the baseline activity as written. Buffers and substrate solutions are also provided for individual groups of students to each perform one additional series of guided-inquiry investigations testing one variable, such as substrate concentration, pH, and temperature, on the rate of reaction of enzyme peroxidase. See the *Sample Results* and *Additional Sample Results* sections for possible inquiry investigations.
- If the extraction will be performed by students, this step can be incorporated into the *Opportunities for Inquiry.* Enzyme activity will depend on the source of the vegetable and its freshness. Adjust the amount of turnip used, as well as the volume of buffer solution, to obtain a convenient rate (increase in absorbance of 0.3–0.6 units over a 3-minute period for Trial 1).
- The enzyme extract should be stored in the refrigerator and used within one week of preparation. Students should run a new baseline for each series of inquiry experiments.
- A variety of plants may be used as sources of peroxidase and may be incorporated into the *Opportunities for Inquiry.* Students may research suitable examples. The options are abundant—turnip, horseradish, radish, lettuce, tomatoes, spinach, legumes, etc. Fish peroxidases have also been widely studied.

- Peroxidases from different sources will likely have different pH and temperature profiles. In addition, peroxidase extracts from a single source normally contain a mixture of enzymatic forms, called isoenzymes or isozymes, which will also have different optimum values for pH and temperature stability.
- The College Board has stipulated knowledge and laboratory skill prerequsities for AP Biology in both math and chemistry content areas. Chemistry prerequisities that are relevant to this laboratory investigation include knowing how to measure volume and temperature, determine the rate of a chemical reaction, and calculate solute concentrations. Graphing skills are also listed as a relevant math prerequisite. We recommend that teachers provide a worksheet or activity for students to review the concepts of kinetics and reaction rates with students before beginning this laboratory investigation. A short primer is provided in the *Teaching Tips* section.
- Initial rates are generally used to compare reaction rates for different concentrations of enzyme or substrate, and for determining optimum pH and temperature values. The initial rate is calculated from the slope or linear portion of a graph of product concentration (absorbance) versus time, corresponding to approximately 5–10% of reaction completion. This is done because a graph of product concentration versus time for a chemical reaction begins to curve or level off as the reaction proceeds. Rate is proportional to reactant concentration, so as the reactants are depleted the rate decreases.



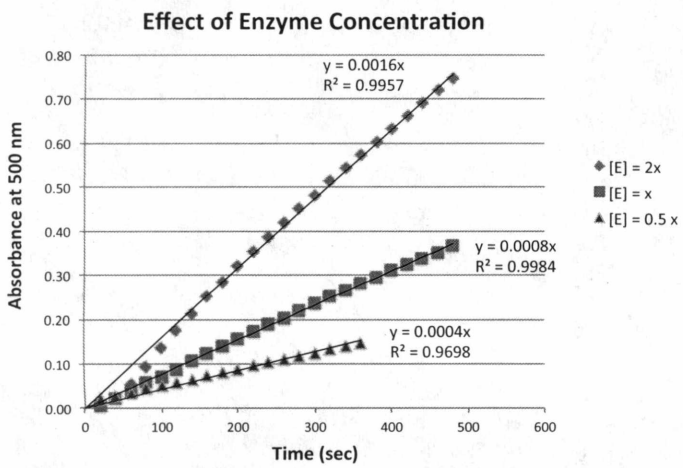
- The procedure for the baseline activity calls for pouring the contents of the substrate tube into the enzyme tube, and then back into the substrate tube, prior to beginning absorbance measurements. This is done to ensure adequate mixing of the contents and to obtain accurate reaction rates. Students should begin timing with the first pour, however, as soon as the enzyme and substrate are combined.
- Students must remember to keep the total volume of the enzyme and substrate solutions (test tubes) constant when they design their own inquiry experiments in Part B. This is done by varying the amount of enzyme or hydrogen peroxide solution and then adjusting the volume of buffer to accommodate the difference. See the baseline activity for an example.
- The effect of substrate concentration on reaction rate gives rise to a characteristic hyperbolic or "saturation" kinetics curve (see the graph in the *Additional Sample Results* section). Understanding the shape of this curve provides insight into the single most important take-home lesson relating the structure and function of enzymes, namely, formation of the enzyme–substrate complex. At low substrate concentrations, the rate of the reaction increases almost linearly as the concentration increases. At higher concentrations of hydrogen peroxide, the rate of the reaction behaves differently than a typical chemical reaction. The rate increase becomes more gradual and eventually levels off and reaches a maximum or saturation velocity. Saturation kinetics is observed because once all of the enzyme in solution (or in a cell) is bound with substrate at its active site, adding further substrate cannot and does not increase the rate of reaction.
- The concept of saturation kinetics discussed above may make it challenging for students to identify suitable substrate concentrations that will illustrate the "desired" results. If the substrate concentration in the baseline activity is already near the saturation level for the amount of enzyme (which depends on the activity of the extract and other factors), increasing or decreasing the substrate amount by a factor of two will not change the rate much at all. See the graphs in the *Additional Sample Results* section for concentrations that worked in our independent lab testing. Students must indeed experiment to find concentrations that will work under their laboratory conditions. Remind students that they should change only the volume of substrate, and adjust the volume of buffer to keep the total volume of the contents of the "S" tube constant.
- If a spectrophotometer or colorimeter is not available, the concentration of colored product after a specific time interval can be estimated by color comparison with a series of standard solutions. Prepare a concentrated stock solution of the orange product by mixing 15 mL of pH 5 buffer, 10 mL of 0.02% H_2O_2, 5 mL of 0.2% guaiacol, and 10 mL enzyme. Call the concentration of the stock solution x and then dilute it to obtain solutions that have concentrations equal to 0.1x, 0.2x, 0.4x, 0.5x, 0.6x, and 0.8x. *When comparing the color of a test solution versus the standards, it is essential that the path*

length for viewing the solution colors be identical. This means that all the solutions must be in the same size test tube, filled to the same depth or volume, and the color must be viewed in the same direction, either vertically down the length of the test tube or horizontally across the tube. As an example of this technique, imagine that in Trial 1 the color of the mixed enzyme-substrate solution after three minutes most closely matches the 0.4x color standard. When the enzyme concentration is doubled in Trial 2, the color of the solution after the same length of time (three minutes) might be expected to match the 0.8x color standard. This technique will require considerable trial-and-error experimentation to prepare a color series in the same general concentration range as the rate trials will produce.

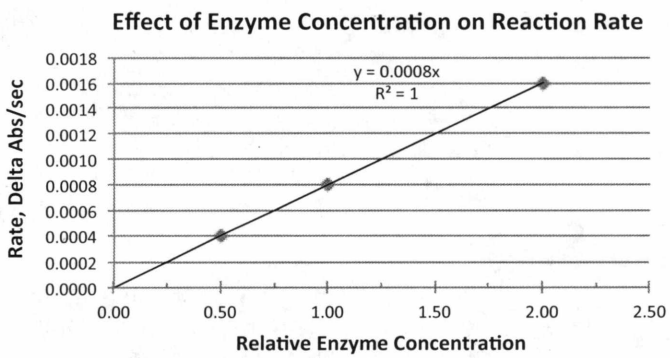
Teaching Tips

- Kinetics is the study of the rate of chemical reactions, which describes how fast a reaction occurs. The greater the rate of a reaction, the less time that is needed for reactants to be converted to products. Reaction rates are therefore inversely proportional to time. In general the rate of a reaction increases as the concentration of reactants increases in accord with the dynamic or collision theory of a chemical reaction. In order for a reaction to occur, reacting molecules must first collide. Any factor that changes the total number of collisions, the average energy or favorable orientation of the colliding molecules will affect the reaction rate.
- As reactants are transformed into products in a chemical reaction, the amount of reactants will decrease and the amount of products will increase. The rate of the reaction is determined by measuring the concentration of reactants or products as a function of time. In the case of the peroxidase-catalyzed decomposition of hydrogen peroxide, the formation of an orange-colored product from guaiacol provides a simple visual clue to determine the reaction rate based on the time it takes for the color to appear.
- The purpose of this technology-based experiment is to use spectrophotometry or colorimetry and graphical analysis to determine the reaction rate. A spectrophotometer measures the absorbance or transmittance of light. The absorbance of the solution is measured at specific time intervals. Since the absorbance is directly proportional to the concentration of the colored product, a graph of absorbance versus time has the same characteristics as a graph of concentration versus time. The rate of the reaction is obtained from the slope of the linear portion of the graph.
- Enzyme kinetics is the basis for important medical tests called enzyme assays to determine the concentration of an enzyme in blood, cells or tissue. Enzyme assays are useful in clinical diagnosis of disease. In an enzyme assay the amount of product formed in a specific time period is measured to determine the reaction rate. This rate is then compared against a reference or calibration curve of enzyme activity versus enzyme concentration to determine the actual enzyme concentration in the sample.

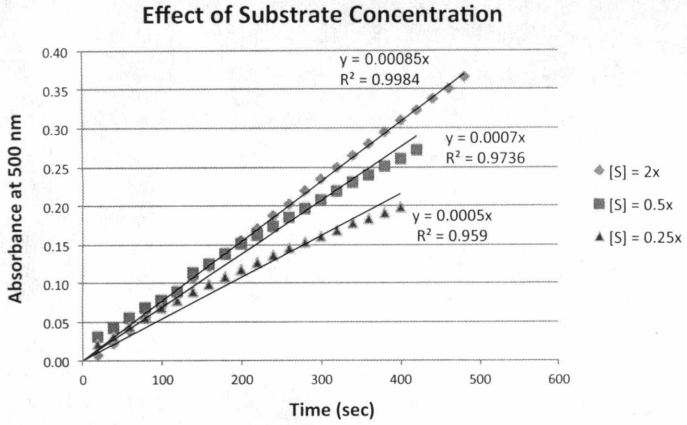
Sample Results



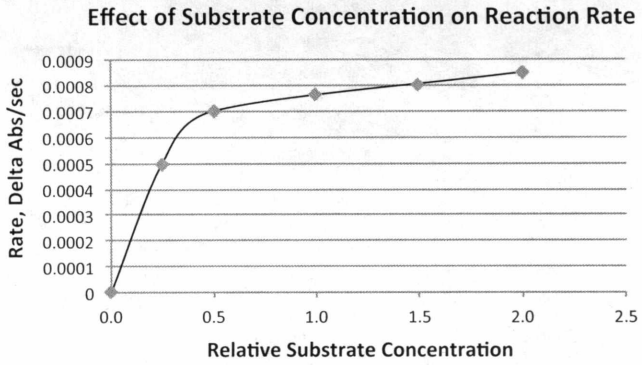
Trial 1: [E] = x = 0.5 mL

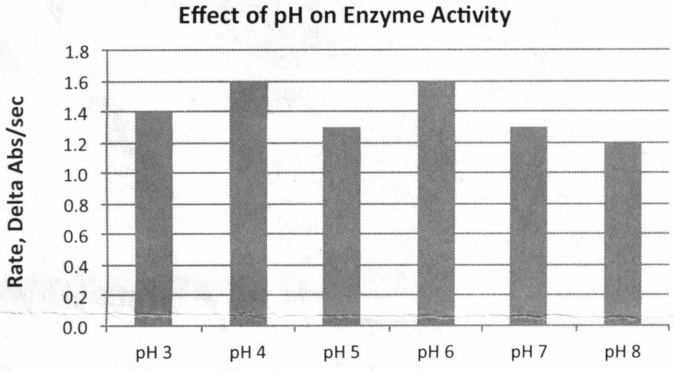


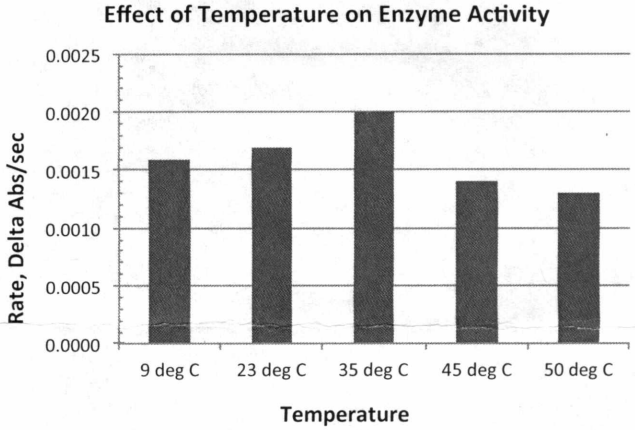
Additional Sample Results



[S] = 1 mL = x







Reference

AP Biology Investigative Labs: An Inquiry-Based Approach. College Entrance Examination Board: New York, 2012.

Peroxidase Enzyme Activity and supporting supplies are available from Flinn Scientific, Inc.

Catalog No.	Description
FB2039	Peroxidase Enzyme Activity
AP8477	Blender, Two-Speed
AP7026	Flinn Scientific Spectrophotometer
GP6063	Culture Tubes, 13 × 100 mm, Borosilicate Glass
AP1677	Test Tube Rack, Polypropylene, Submersible
AP8874	Timer, Count Up/Count Down, with Clock
AP8606	Pipet Bulb, Rubber, 15-mL Capacity

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