



Cornell Institute for Biology Teachers

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Lab review date: 6/23/08

Title:

The Properties of Enzymes: A Study of Catalase

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**Appropriate
Level:**

Life Science, High School, Honors, or Advanced Placement Biology

**Living
Environment**

1-Inquiry, analysis, design: 3-Analysis of results: 3.1a; **4-Content:** 1-Living things: 1.2h; 2-Inheritance: 2.1g,i,; ,5-Dynamic Equilibrium: 5.1f,g. 5.3a,b

Abstract:

The structure and function of enzymes is a central theme in cellular and molecular biology. In this laboratory exercise, a crude cell extract is prepared from potatoes. Activity of the enzyme, catalase [which catalyzes the reaction $2\text{H}_2\text{O}_2(\text{l}) \rightarrow 2\text{H}_2\text{O}(\text{l}) + \text{O}_2(\text{g})$], is then studied using a simple assay for O_2 . To conduct the assay, a filter is soaked in crude potato extract, then transferred to the bottom of a beaker containing hydrogen peroxide. Catalase causes O_2 to collect in the filter which in turn causes the filter to rise. Students are able to explore the effect of enzyme and/or substrate concentration and pH on the amount of product formed by measuring the time taken for each filter to collect enough oxygen to rise. Students average their results, calculate the inverse of the “time to rise,” and pool the data in order to plot the characteristic curve showing the dependence of enzyme activity on substrate and enzyme concentration.

Time Required:

1 period to prep students and demonstrate preparation of potato extract.
1 period to collect data.
1 period to compile class data and analyze results.

Teacher Information

Objectives:

This experiment allows students to collect data on enzyme activity using a protocol that is relatively fun and an enzyme preparation that is cheap and easy to prepare. The data can be plotted and analyzed, and conclusions about the affects of enzyme and substrate concentration on product formation can be drawn.

Level of Course:

As written, the exercise is appropriate for Regents students. It can be made more challenging for Honors or AP students by having them design the range of substrate and/or enzyme concentrations to be tested, or modify the basic lab to test the effects of temperature and/or pH on enzyme activity.

Information with Which Students Must Be Familiar:

This experiment reinforces the Regents syllabus concepts regarding the characteristics of enzymes.

Time Required:

In class: The experiment can be carried out in one 45-minute class period. Pooling of data and discussion may require some additional time.

Before class: Teacher preparation (allow 1-2 periods) consists mainly of obtaining and distributing the supplies (hydrogen peroxide, potatoes, blender, stop watches, etc) and glassware (many beakers, graduated cylinders). Making the optional phosphate buffer will require ten minutes.

Materials:

per team of students:

- 100-ml graduated cylinder (1)
- glass stirring rod
- hole punch
- small beakers (3), one of which must be 50-ml in volume and transparent
- phosphate buffer, pH 7 (optional); allow ~150 ml per team
- stop watch or clear view of a clock with a second hand
- Whatman 2.4 cm GF/A Glass microfibre filters (available from Fischer Scientific catalog #987412 BX for a box of 100) or use “holes” punched from standard 20# bond copier paper - allow at least 6 per team
- hydrogen peroxide (as sold, 3% solution; allow ~ 50 ml per team)
- graph paper
- forceps
- marking pens

per class:

- blender (1)
- 1000 ml graduated cylinder (1)
- potatoes (about 1 large potato for 24 students)
- cheese cloth from a science supply company (DON'T use grocery store cheese cloth)
- large funnel (1)
- knife to cut the potato
- boiled potato

for Part 3 (per group or for class demo):

- test tubes (5)
- pH solutions (3, 5, 7, 9, 11)
- metric ruler
- catalase solution (60% solution from Part 1)
- test tube rack
- hydrogen peroxide (3% solution)
- graph paper

Tips for the Teacher

- A fun way to introduce this lab (and characteristics of proteins in general) is to make use of the catalase in egg white. You may wish to have the students record observations, then discuss them after the demonstration. Start with three small, clear glass beakers, and three room temperature (or body temperature) fresh eggs. Pour Reagent A (or, if you want to share the secret with students) phosphate buffer, pH 7, into one beaker, half filling it. Then break an egg white into it. Pour Reagent B (3% H₂O₂) into the second beaker, and add an egg white to it. Pour Reagent C (distilled white vinegar, 5% acidity or greater) into the third beaker, then add the egg white. Stir each solution gently. You'll observe that nothing much happens to the buffered egg, while the egg white congeals (becomes denatured) in the vinegar (like an egg cooking). Meanwhile, mounds of white foam accumulate in the peroxide solution. After a few minutes, add peroxide to both the egg white in buffer and the egg white in vinegar; this dramatically illustrates that the denatured catalase is inactive. Be sure to have all the reagents relatively warm (but not warmer than body temperature) for the most spectacular demo. Also, try to end up with beakers nearly full; the mass of foam spilling over the top of each beaker is particularly effective (note: put down paper towels first).
- Keep the blender for grinding potatoes at the demonstration desk. Blending is quick and filtration is simple. It is not necessary to skin the potato. The potato puree is filtered thoroughly and quickly by 4-ply cheesecloth which can be obtained from most biological supply houses.
- While this experiment will work without buffering the solutions (using water as diluent), the use of a buffered solution will help to maintain catalase activity for a longer period of time before the enzyme becomes significantly denatured. You may want to use buffer to make the extract and then allow students to use water for dilution. Also, it's typical for biochemistry experiments to be carried out in buffer.

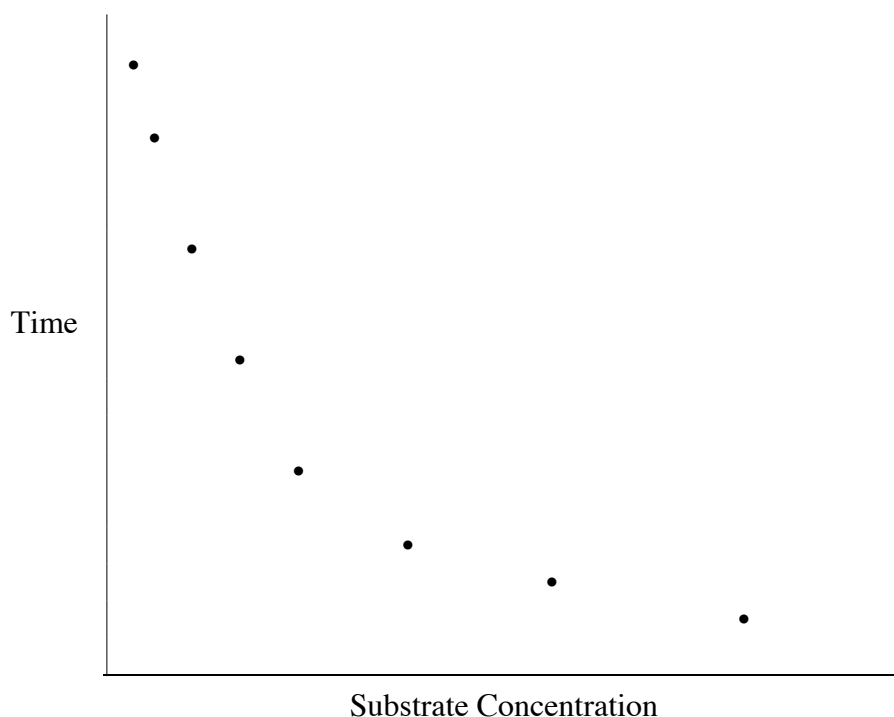
Make the 100 mM phosphate buffer at pH 7 as follows (allow 250-300 ml of buffer per class):

Weigh out 8.70 grams K_2HPO_4 and 6.80 grams KH_2PO_4 and add one liter of distilled water. Mix thoroughly and check pH.

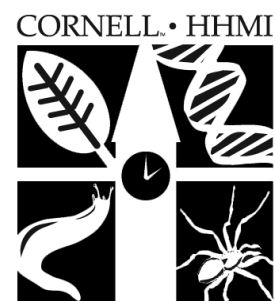
Alternative approach: If you don't have these chemicals, you can order pre-mixed buffer salts from science supply companies, e.g., Carolina Biological Supply, #84-9314 Buffers for pH 4, pH 7, and pH 10 (sufficient for 2-liters buffer).

- Show the students how to handle the filters to ensure uniformity in the procedure. Point out that they must place the moistened disk at the bottom of a beaker of hydrogen peroxide and be consistent in stopping timing as soon as disks have completely "lifted off." Students should observe that some filters partially rise before completely lifting off. Discuss this in the context of experimental "error"; it's an unavoidable source of variability.
- Technically, students should use a fresh preparation of H_2O_2 for each trial. This becomes very expensive, so instead have students use the forceps to remove the disk as soon as it rises in order to preserve the H_2O_2 at roughly the same concentration for the next trial. Check to see whether the time varied significantly over 3 trials (another source of experimental error).
- A scheme is provided for dividing the class into five different teams for Part 1 and six teams for Part 2, each testing a single enzyme concentration with 1% H_2O_2 , and a single substrate concentration with 60% potato extract. If there are more than six teams, the teacher may add more enzyme/substrate concentrations or have two groups duplicate the assigned enzyme/substrate concentrations.
- For the boiled potato control, take a 10 ml sample of the extract. Place it in a test tube in a boiling water bath for several minutes.
- The graph of the results will not look like the graph of enzyme activity found in the Regent's Syllabus. If the students plot their data directly, the x-axis is "hydrogen peroxide concentration" and the y-axis is "time." The resulting graph (see the following figure) will be a curve going from infinity down to a short period of time. **If you want to have a curve similar to the curve found in the Regent's Syllabus, you will have to graph Rate = 1/time vs enzyme (or substrate) concentration.**

Graph showing 1/[enzyme activity] vs. concentration



- Using the potato extract and reagents at hand, the students can also carry out a mini-experiment about heat of reaction. The students should make a 1% H_2O_2 solution, and place 5 ml into each of two test tubes. Then, add 3 ml of concentrated potato extract to one tube and measure the temperature change over time. Add 3 ml of diluted potato extract to the other tube and measure its temperature change. The students should observe a difference in the appearance of the tubes and a temperature difference of about 3 degrees.
- For Honors or AP classes, the teacher could give a less structured protocol, allowing the students to design their own experiments to test the relationship between O_2 production rate and substrate concentration, enzyme concentration, pH, and/or temperature. Catalase activity should show a nice dependence on any of these factors as long as students plan their experiment to test a reasonable range of variables. The teacher must stress the importance of the controls and replicates in the students' designs.
- Answers to questions in the pre-lab: The reason for testing the amount of O_2 produced from H_2O_2 by an enzyme free filter is to show that O_2 production is catalyzed only in the presence of the extract. The second control, the boiled potato extract, is used to show that the “catalytic something” in the extract is a protein, as this is the component of the extract which is most heat-susceptible. The teacher may wish to point out that these two controls are performed by **every** biochemist working with **any** enzyme; these are critical controls, even if they seem trivial to the students.



Catalytic Activity of Enzymes

Introduction

Enzymes are biological molecules that catalyze (speed up) chemical reactions. You could call enzymes the “Builders and Do-ers” in the cell; without them, life could not occur. Every cell makes hundreds of different enzymes to carry out the reactions necessary for life. Fortunately for the cell, enzymes are not used up when they catalyze a reaction, but can be used over and over.

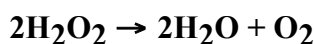
The DNA in each cell encodes all the information needed to make its many different enzymes. Enzymes are relatively large molecules of protein. They are produced whenever the cell “senses” a need for that particular enzyme; that is, whenever a job needs to be done in the cell which only that enzyme can do it.

The molecule (or molecules) on which an enzyme acts is called its **substrate**. Enzymes are said to be very “specific,” meaning that they recognize only one substrate (or a few closely related substrates) and convert it into a specific product. You could say that each enzyme can do only one type of job. Each enzyme is specific because it is folded into a particular three-dimensional shape. Within the folds of each enzyme is the **active site**, the place where the substrate fits and where the chemical reaction takes place.

Enzymes work very quickly, often catalyzing thousands of reactions per minute. The rate at which an enzyme works is influenced by many factors including temperature and pH. Enzymes have a temperature and pH at which they work best, and if an enzyme is exposed to extremes of heat or pH it won't work at all! The interactions that hold the protein in its particular shape become disrupted under these extreme conditions, and the 3-dimensional structure unfolds. In this case, the enzyme is said to be **denatured**. Other important factors that influence enzyme activity are the concentration of substrate and the concentration of enzyme. Up to a point, the more substrate that is present, the faster the reaction. However, when the substrate concentration is so high that an enzyme is working as fast as it can, further increases of substrate concentration will have no effect on the rate of product formation.

Background

The enzyme that you will study in this experiment is called “catalase.” Its job is to break down its substrate hydrogen peroxide (H_2O_2), which is a naturally occurring poison. Without catalase, H_2O_2 could kill the cell. The reaction catalyzed by catalase is:



The products remaining after catalase does its job are oxygen gas and water; two very non-poisonous molecules.

In the home and hospital, hydrogen peroxide is used as an antiseptic to clean out wounds. Have you ever noticed that when hydrogen peroxide is swabbed on a cut it bubbles? This is because enzymes in the cut from your body and from infecting bacteria catalyze the rapid degradation of hydrogen peroxide into water and oxygen. The bubbles are oxygen.

Catalases are very common. They are found in almost all cells that grow in oxygen, including potato tubers. In this experiment, a blender is used to grind up a potato in water to release the catalase from the potato cells. The ground-up potato is filtered through cheesecloth to separate potato skin and cell debris from the liquid which contains most of the cell's enzymes, including catalase. To actually measure the catalase activity, small disks are dipped into the potato cell extract. When this enzyme-containing disk is placed in a solution of hydrogen peroxide, the enzyme begins to work. As the catalysis occurs, oxygen is produced, and bubbles of the gas become trapped in the fibers of the disk. When there are enough O₂ bubbles, they lift the filter to the surface. The speed with which the O₂ is produced depends both upon how much enzyme is present and on the concentration of the hydrogen peroxide. The more enzyme, the faster the product (O₂) is made. Similarly, the higher the concentration of the substrate, hydrogen peroxide, the faster the product is made. You can see what happens when you vary either the concentration of enzyme or the concentration of the hydrogen peroxide.

To do this experiment, five of the teams of students will carry out one version of the experiment using low, medium, and high enzyme concentration and a constant level of substrate, 1.0% H₂O₂. Then, the other six teams will do another version of the experiment using low, medium, and high H₂O₂ concentration with cell extract diluted to 60% of its original concentration. At the end of the lab, experimental results will be pooled and the class as a whole will generate data showing the relationship between catalase activity and both enzyme and substrate concentrations.

A third experiment dealing with the effect of pH is provided. This portion could be performed easily by one or two teams of students. It also lends itself nicely to a teacher demonstration for the entire class.

For an experiment to be meaningful, there must be controls. Three controls important to this lab will be demonstrated by your teacher:

Control #1: A paper disc that **has not** had potato extract added to it is dipped in H₂O₂.

Control #2: A paper disc that **has** been dipped in potato extract is placed in a beaker of water.

Control #3: A paper disc that **has first** been dipped in boiled potato extract **and then** placed into a beaker of H₂O₂.

Observe and record on page 8 what occurs as each control experiment is demonstrated.

Why is each control important?

What does each control experiment show you?

Materials

Your team will need the following supplies:

- potato extract (prepared by your teacher)
- 1000 ml flask with distilled/deionized/dechlorinated water
- 1 - 250 ml beaker for potato extract
- 200 ml 1 % H₂O₂ solution for the first part of the experiment
- 3% H₂O₂ solution to dilute for second part of the experiment
- 100 ml graduated cylinder
- 8 - 100 ml beakers
- forceps
- 40 filter paper disks
- paper towels
- stopwatch, if available
- 60% catalase
- calculator

Experimental Procedure for Teams Working with Various Catalase Concentrations

1. Making the potato extract:

- Watch your teacher prepare the potato extract as follows:
- Cut clean potatoes into chunks (allow one potato per team of students)
- Place the potato chunks in the blender and add 200 ml of buffer per potato.
- Puree in the blender.
- Pour potato puree through four layers of cheesecloth placed in funnel. Collect as much fluid as possible. This fluid contains the enzyme **catalase**, among many other things that were stored inside the cells of the potato.
- Add enough distilled water to bring the final volume to 200 ml per potato. Swirl the flask to mix the solution. This will be arbitrarily designated as “100%” catalase extract. (Each team should have a 250 ml beaker containing 200 ml of 100% catalase.)

2. Together with your partners, prepare your enzyme concentrations in the beakers.

- Label the beakers with tape and pen: 20%, 40%, 60%, 80%, and 100%.
- Make the appropriate dilutions. For example, if you are doing test #1(20%), measure 8.0 ml of the potato extract using the graduated cylinder and pour into the beaker.
- Rinse the graduated cylinder, then add 32.0 ml of distilled H₂O and stir well with the stirring rod.

- Make the rest of the enzyme solutions using the chart below as a guide.

Test	Extract Concentration	Volume of Enzyme	Volume of Water
#1	20%	8 ml	32 ml
#2	40%	16 ml	24 ml
#3	60%	24 ml	16 ml
#4	80%	32 ml	8 ml
#5	100%	40 ml	0 ml

- Obtain the flask of 1% hydrogen peroxide if it is not at your table. This is the substrate for this part of the lab.
- Now you are ready to begin measuring the effects of enzyme concentration on enzyme activity.
 - Pour 30 ml of the 1% H₂O₂ solution into a clean beaker, labeled “reaction beaker.”
 - Pick up a paper disk with a clean forceps. Using the forceps, dunk the disk in your enzyme extract for 5 seconds, until the disk is uniformly moistened but not beaded with shiny drops of liquid.
 - Drain it on a piece of paper towel for 5 seconds to remove excess enzyme from the disk.
 - The reaction is now ready to be started and timed.
 - Using forceps, place the filter disk (containing the enzymes) onto the **bottom** of the “reaction beaker” containing 1% hydrogen peroxide.
 - One person should watch the clock/stopwatch, another watch the rising disk. Stop timing as soon as disks have completely ‘lifted off’ the bottom of the reaction beaker.
 - Watch the filter disk. You should see tiny bubbles of oxygen being released as the hydrogen peroxide is broken into water and oxygen by the catalase.
 - Record the time in seconds for each trial on the chart. Be precise in your timing and recording.
 - Remove the disk and discard it.
 - Obtain another disk and repeat steps 3-4 exactly as done above.
 - Repeat the experiment a third time exactly as above: now you have “triplicate” measurements of the rate of oxygen production in 1% H₂O₂ at each particular enzyme concentration. Average these 3 values and record in the chart below. Repeat this procedure for all the concentrations of enzyme.

Data Table / Enzyme Concentration

Test Number	Trial 1	Trial 2	Trial 3	Team Average	Class Average
1 20%					
2 40%					
3 60%					
4 80%					
5 100%					

7. Clean up all materials!

Experimental Procedure for Teams Working with Various Hydrogen Peroxide Concentrations

1. Label the clean beakers with the percent hydrogen peroxide that will be used in this part of the experiment: 2%, 1.5%, 1.0%, 0.8%, 0.6% and 0.3%. Together with your partner, prepare your particular substrate concentrations in each of the labeled beakers. For example, for test #1, measure 20.0 ml of the concentrated H_2O_2 using the graduated cylinder and pour into the beaker. Add 10 ml of distilled water to the graduated cylinder and pour into the hydrogen peroxide. Stir well with the stirring rod. Rinse the graduated cylinder. See chart for proportions of hydrogen peroxide and water to mix for each dilution. Place all of the beakers on the table in front of you in order from lowest to highest concentration of hydrogen peroxide.

Test #	Substrate Concentration	Volume of H_2O_2	Volume of Water
1	2.0%	20 ml	10 ml
2	1.5%	15 ml	15 ml
3	1.0%	10 ml	20 ml
4	0.8%	8 ml	22 ml
5	0.6 %	6 ml	24 ml
6	0.3 %	3 ml	27 ml

2. Next, obtain your 60% catalase solution.
- 3a. Using forceps, dip a disk in the 60% potato extract for 5 seconds, let it drain on a paper towel for 5 seconds. Then, using forceps, place the filter (containing enzymes) on the bottom of the “reaction” beaker (2.0% H_2O_2).

- b. Time how long it takes the disk to rise from the bottom of the beaker to the top of the liquid. Be sure that the disk is placed at the bottom of the hydrogen peroxide before you start to time the experiment.
- c. Record the time in seconds in the appropriate space on the chart that follows.
4. Obtain another disc and repeat steps 3a - 3c exactly as before.
5. Repeat the experiment a third time. Now you have triplicate measurements of the rate of oxygen production. Average these three values and record on the chart.
6. Repeat this procedure for all of the concentrations of H₂O₂.

Data Table / Substrate Concentration

Test Number	Trial1	Trial 2	Trial 3	Team Average	Class Average
1 20%					
2 40%					
3 60%					
4 80%					
5 100%					

7. Clean up all materials!

After the Experiment

Controls

A. What is the function of a control?

For control #1, a filter paper saturated with water rather than potato extract was placed in a beaker of 1% H₂O₂. How long does it take for the filter to lift off? _____ Explain the significance of the result:

For control #2, a piece of filter paper was saturated with potato extract and then placed in distilled water. How long did it take for the filter to lift off? _____ Explain the significance of the results:

For control #3, 100% catalase was boiled. A filter paper was then saturated with this extract. The disk containing the extract was then placed in a beaker of 1% H₂O₂. How long did it take for the filter to lift off? _____ Explain significance of the results:

B1. Pool your results with those of the rest of the class, record below and fill in the class average portion of the table for your experiment and also for the other experiment.

Data Table for Enzyme Concentration
(mean for each test)

test	team 1	team 2	team 3	team 4	team 5	team 6	team 7	team 8
1: 20%								
2: 40%								
3: 60%								
4: 80%								
5: 100%								

Data Table for Substrate Concentration
(mean for each test)

test	team 1	team 2	team 3	team 4	team 5	team 6	team 7	team 8
1: 2.0%								
2: 1.5%								
3: 1.0%								
4: 0.8%								
5: 0.6%								
6: 0.3%								

- Plot both the team and the class averages on graph paper. The first graphs should be “concentration of enzyme vs. time of reaction.” The x -axis is designated concentration, starting at point 0. The y -axis is $1/t$, so you have to do the math on this before you graph your data. The second graph should be “concentration of substrate vs. time of reaction.” The x -axis is designated concentration, starting at point 0. The y -axis is again $1/t$, so do the math first.

3. Discuss **three** factors that influence the rate of enzyme action:

4. Why did you do the experiment in triplicate?



pH Influence on Enzyme Activity

Materials

- five test tubes
- pH solutions of 3-5-7-9-11
- 60% solution catalase
- graph paper
- test tube rack
- 3% hydrogen peroxide
- metric ruler

Procedure

1. Obtain five test tubes and a test tube rack. Label them pH 3, pH 5, pH 7, pH 9, pH 11.
2. Using a pipettor and a 5-ml pipette, measure 4 ml of each of the pH solutions provided and transfer into the corresponding test tube. To avoid contamination use a different pipettor for each solution.
3. To each of the test tubes, add 2 ml of 60% catalase solution, using the 5-ml pipette and pipettor. Swirl the test tube to help mix the pH solution with the catalase. Allow to sit for 5 minutes.
4. With a 1 ml pipette and the pipettor, measure 1 ml of 3% hydrogen peroxide into each of the test tubes. Allow to react for 5 minutes. Foam should form on the top of the solutions.
5. Measure the distance from the bottom of the test tube to the top of the foam in millimeters and record below.

pH	3	5	7	9	11
height (mm)					

6. In a separate sheet of graph paper graph your results, using the *x*-axis for “pH” and the *y*-axis for “height of solution/foam (mm)”. Describe your results thoroughly and explain the effect of pH on enzyme activity.

Using the information you have learned and your knowledge of the human digestive system, answer the following questions:

1. What is the approximate pH of the stomach?
2. What is the approximate pH of the beginning section of the small intestines?
3. Would the same enzymes that began the digestion of proteins in the stomach work as efficiently in the small intestines? Explain your answer.