**ENZYME ACTIVITY\* (adapted from CB lab 13)**

How do abiotic or biotic factors influence the rates of enzymatic reactions?

**BACKGROUND**

Enzymes are the catalysts of biological systems. They speed up chemical reactions in biological systems by lowering the activation energy, the energy needed for molecules to begin reacting with each other. Enzymes do this by forming an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. The enzyme’s active site is very selective, allowing only certain substances to bind. If the shape of an enzyme is changed in any way, or the protein denatured, then the binding site also changes, thus disrupting enzymatic functions.

Enzymes are fundamental to the survival of any living system and are organized into a number of groups depending on their specific activities. Two common groups are catabolic enzymes (“*cata*” or “*kata*-” from the Greek “to break down”) — for instance, amylase breaks complex starches into simple sugars — and anabolic enzymes (“*a*-” or “*an*-” from the Greek “to build up”). (You may know this second word already from stories about athletes who have been caught using anabolic steroids to build muscle.)

Catalytic enzymes, called proteases, break down proteins and are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin. Bromelain often is an ingredient in commercial meat marinades. Papain is an enzyme that comes from papaya and is used in some teeth whiteners to break down the bacterial film on teeth. People who are lactose intolerant cannot digest milk sugar

(lactose); however, they can take supplements containing lactase, the enzyme they are missing. All of these enzymes hydrolyze large, complex molecules into their simpler components; bromelain and papain break proteins down to amino acids, while lactase breaks lactose down to simpler sugars. Anabolic enzymes are equally vital to all living systems. One example is ATP synthase, the enzyme that stores cellular energy in ATP by combining ADP and phosphate. Another example is rubisco, an enzyme involved in the anabolic reactions of building sugar molecules in the Calvin cycle of photosynthesis.

To begin this investigation, you will focus on the enzyme catalase obtained from beef liver, one of numerous sources of this enzyme. Catalase is one of several enzymes that break down peroxide, a toxic metabolic waste product of aerobic respiration. Using catalase, you will develop essential skills to examine your own questions about enzyme function.

Later, you will have an opportunity to select another source of the enzyme, research its properties and mode of reaction, and then design an experiment to explore its function. The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the levels of protein structure, energy transfer, abiotic and biotic influences on molecular structure, entropy and enthalpy, and the role of enzymes in maintaining homeostasis.

**Learning Objectives**

**•** To understand the relationship between enzyme structure and function

**•** To make some generalizations about enzymes by studying just one enzyme in particular

**•** To determine which factors can change the rate of an enzyme reaction

**•** To determine which factors that affect enzyme activity could be biologically important

**General Safety Precautions**

Follow general laboratory safety procedures. Wear proper footwear, safety goggles or glasses, a laboratory coat, and gloves. Use proper pipetting techniques, and use pipette pumps, syringes, or rubber bulbs. Never pipette by mouth! Dispose of any broken glass in the proper container. Since the concentrations of the reactive materials in this laboratory are environmentally friendly (1.5% hydrogen peroxide and food products), they can be rinsed down a standard laboratory drain. The concentrations used here are deemed to be safe by all chemical standards, but recall that any compound has the potentiality of being detrimental to living things and the environment. When you develop your individual investigations you must always consider the toxicity of materials used.

**Key Vocabulary**

**Baseline** is a universal term for most chemical reactions. In this investigation the term is used to establish a standard for a reaction. Thus, when manipulating components of a reaction (in this case, substrate or enzyme), you have a reference to help understand what occurred in the reaction. The baseline may vary with different scenarios pertinent to the design of the experiment, such as altering the environment in which the reaction occurs. In this scenario, different conditions can be compared, and the effects of changing an environmental variable (e.g., pH) can be determined.

**Rate**. In this case, a specific amount of time can be measured, such as mL of oxygen per minute.

**Procedure 1:** Developing a Method for Measuring Peroxidase

in Plant Material and Determining a Baseline

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism.

Catalase is an enzyme that breaks down these peroxides. It is produced by most cells

in their peroxisomes.

The general reaction can be depicted as follows:

Enzyme + Substrate --> Enzyme-Substrate Complex --> Enzyme + Product(s) + ΔG

For this investigation the specific reaction is as follows:

Catalase + Hydrogen Peroxide --> Complex --> Catalase + Water + Oxygen

2H2O2 → 2H2O + O2 (gas)

Notice that the catalase is present at the start and end of the reaction. Like all catalysts, enzymes are not consumed by the reactions. To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In decomposition reaction of peroxide by catalase (as noted in the above formula), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the *actual* volume of oxygen gas released or by using an indicator. In this experiment, oxygen will be measured directly by volume or using a Vernier oxygen probe.

Materials:

Version A: 50 mL graduated cylinder, glass tube with rubber stopper and glass dropper, tray of water, catalase, and 1.5% hydrogen peroxide

Version B: biochamber (500 mL), Vernier oxygen probe with data logger and computer, catalase, and 1.5% hydrogen peroxide

Version A:



1. Fill bin with water
2. Fill graduated cylinder with water by submerging and make sure no bubbles at the top.
3. Fill the glass tube with 10 mL of 1.5% hydrogen peroxide add 1 mL of catalase solution (in ice bucket).
4. Quickly cap the tube with the rubber stopper and submerge and make sure dropper is under the graduated cylinder and that the bubbles are going up the cylinder.
5. Record volume of gas in consistent intervals.

Version B: (see the link below for help setting up Data Logger and Computer wth probe, additional instructions are in the probe box. <http://www.grochbiology.org/GeneralDirectionsforusingtheVernierProbeswithacomputer.docx>)

1. Hook up the Data Logger to the power supply, connect the Data Logger to the USB port on the computer, and connect the probe to channel one on the data logger.
2. Make sure the ratio of 1.5% hydrogen peroxide to catalase is 10:1. (Example: 10 mL of hydrogen peroxide to 1 mL of catalase). Be careful too large of a volume of peroxide to catalase may foam up.



**Designing and Conducting Your Investigation**

You now have the basic information and tools needed to explore enzymes in more depth on your own. In this part of the lab, you will do just that. You will have the chance to develop and test your own hypotheses about enzyme activity. To help you get started, read the following questions, and write your answers in your laboratory notebook.

**•** In Procedure 1, was the limiting factor of your baseline reaction the enzyme or the substrate? How could you modify the procedure you learned to answer this question?

**•** What are three or four factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? How would you modify your basic assay to test your hypothesis?

Design and conduct an experiment to investigate an answer(s) to one of the questions above or another question that might have been raised as you conducted Procedures 1. Remember, the primary objective of the investigation is to explore how biotic and abiotic factors influence the rate of enzymatic reactions. (Hint what can change in the environment?). You are not limited with beef liver; you can use other organ meats, plants, other micro- organisms as a source of catalase (Google????) Make sure you have approval from the teacher before beginning.

Write-up Rubric:

Title

Inquiry Procedure (include materials)

Data Tables

Graph

Power Conclusion <https://docs.google.com/document/pub?id=1L3bwXf5AYMMPSzjdauLLx45haI06295I1000plllG8E>

References APA [www.bibme.org](http://www.bibme.org)