

AP Bio – Enzyme Activity

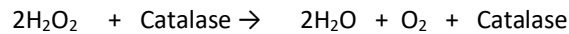
This activity is an alternative to the proposed enzyme lab found in the AP Biology Lab Manual (old – lab #2; new - lab #13).

Introduction

Refer to enzyme background found in the “Revised” AP Biology Lab Manual (2011) – lab #13 and / or the “Old” AP Biology Lab Manual (2009) – lab #2.

The Activity

In this exercise you will study the enzyme catalase, which accelerates the breakdown of hydrogen peroxide (a common product of oxidative metabolism) into water and oxygen, according to the summary reaction:



This catalase-mediated reaction is extremely important in the cell because it prevents the accumulation of hydrogen peroxide, a strong oxidizing agent that tends to disrupt the delicate balance of chemistry.

Catalase is found in animal and plant tissues, and is especially abundant in plant storage organs such as potato tubers, corns, and in the fleshy parts of fruits. You will isolate catalase from potato tubers and measure its rate of activity under different conditions. A disc of filter paper will be immersed in the enzyme solution, and then placed in the hydrogen peroxide substrate. The oxygen produced from the subsequent reaction will become trapped in the disc, thus giving it buoyancy. The time measured from the moment the disc touches the substrate until it reaches the surface of the solution is a measure of the rate of the enzyme activity. (The disc should be flat on top of the solution at the end.)

Materials

100 mL graduated cylinders	Filter Paper
Hole Punch	
50 mL beakers	Blender
Cheesecloth	Distilled Water
Forceps	Hydrogen Peroxide
Ice	Potatoes

Procedure

- A. Extraction of Catalase (this will be done for you on Day 1 of the lab – you will do it yourself on Day 2)
1. Peel a fresh potato tuber and cut the tissue into small cubes
 2. Weigh out 50 grams of tissue.
 3. Place the tissue, 50mL of cold distilled water, and a small amount of crushed ice in a prechilled blender
 4. Homogenize for 30 seconds at high speed

From this point on, THE ENZYME PREPARATION MUST BE CARRIED OUT IN AN ICE BATH!

5. Filter the potato extract, using cheese cloth
6. Pour the filtrate into a 100 mL graduated cylinder and add cold distilled water to bring the final volume up to 100 mL.

NOTE

This extract will arbitrarily be labeled 100 units of enzymes per mL, or 100 units/mL, and will be used in the tests. Before considering the factors that affect enzyme reactions, it is important to demonstrate that the enzyme assay shows that the enzyme actually follows the accepted chemical principles. One way to demonstrate this is by determining the effect of enzyme concentration on the rate of activity, while using a substrate concentration that is in excess.

B. Effect of Catalase Concentration

1. Label five 50 mL beakers as follows:

100%	100 units/mL
75%	75 units / mL
50%	50 units / mL
25%	25 units / mL
0%	0 units / mL

- Prepare 40 mL of enzyme for each of the above concentrations in the following manner: The stock solution for the class is 100%. Distilled water is 0%. Add equal amounts of catalase and water and you have 50%. Add equal amounts of the 50% and 100% and you have 75%. Likewise, mix equal amounts of 50% and 0% (water) and you have 25%. Make sure your mixing volumes are equal. This table may help:

Enzyme Concentration	mL of original enzyme		mL of cold distilled H ₂ O		Units per mL
100%	40	Add	0	=	100
75%	30	Add	10	=	75
50%	20	Add	20	=	50
25%	10	Add	30	=	25
0%	0	Add	40	=	0

KEEP ALL CATALASE PREPARATIONS IN THE ICE BATH!

- Label an identical set of beakers for the substrate. Into each of these beakers, measure out 40 mL of 1.5% hydrogen peroxide solution.
- Punch out 25 discs from the filter paper using the hole punch. Using forceps, immerse a 2.1 cm filter paper disc into the prepared catalase solution for 5 seconds.
- Remove the disc and drain for 10 seconds on a paper towel.
- Place the disc at the bottom of the first substrate solution. The oxygen produced from the breakdown of hydrogen peroxide by catalase becomes trapped in the fibers of the disc, thereby causing the disc to float to the surface of the solution.
- Measure (using a stopwatch) the reaction time for the amount of time from when the disc was placed at the bottom of the beaker until the disc floats on top of the solution (rate will be measured in seconds).

The rate (R) of the reaction is calculated as $R = 1/t$

- Repeat this procedure five times for each enzyme concentration and average the results.
- Record your results in your lab notebook.
- Plot your data on a graph in your lab notebook. Remember the independent variable goes on the X axis and the dependent variable goes on the y axis.

C. Inquiry

- In your lab group discuss factors that could affect the rate of this reaction. Record the factors that you discuss in your lab notebook. Select a factor (1st come 1st serve – one lab group per factor) and claim it on the board.
- Design an experiment that will determine how the factor impacts the rate of the reaction.
- Conduct the experiment – be sure to document your experimental design, materials, procedure, data tables and graphs in your lab notebook (each person should have a lab notebook)
- Create a miniposter with your group using the miniposter rubric.
- Present your experiment to the class.

D. DISCUSSION and/or QUESTIONS for REPORT

- How does enzyme activity vary with enzyme concentration?
- How is the rate of enzyme activity affected by increasing the concentration of the substrate?
- What do you think would happen if you increased the substrate concentration to 40.0% hydrogen peroxide?
- How does changing the substrate concentration compare to changing the enzyme concentration in this experiment?
- From the temperature data, what can you conclude about how temperature affects enzyme activity? How would you explain the results?
- What is meant by optimum temperature? According to the class data, what is the optimum data for the potato catalase and why?
- What would you predict if you had first mixed the catalase (100%) in serial solutions of varying pHs of 3, 5, 7, 9, 11? What would you predict would be optimum pH?
- Design a controlled experiment to test the effect of varying pH, temperature, or enzyme concentration.

DATA TABLES

Name _____

Date _____

Lab partner(s) _____

Table 1 – Effect of Enzyme Concentration on Rate of Activity

Enzyme Concentration %	Enzyme Concentration (units/mL)	Time to Float Disc (in seconds)			Rate	Class Average
		Trial 1	Trial 2	Average		
100	100					
75	75					
50	50					
25	25					
0	0					

Table 2 – Effect of Substrate Concentration on Enzyme Activity

Substrate Concentration (from serial dilutions)	Time to Float Disc (in seconds)			Rate	Class Average
	Trial 1	Trial 2	Average		
0.0 % H ₂ O ₂					
0.75% H ₂ O ₂					
1.5% H ₂ O ₂					
2.25% H ₂ O ₂					
3.0% H ₂ O ₂					

Table 3 – Effect of Temperature on Rate of Enzyme Activity

Temperature (degrees C)	Time to Float Disc (in seconds)			Rate	Class Average
	Trial 1	Trial 2	Average		
4					
10					
22					
40					
65+					

Table 4 – Effect of an Denaturing on Enzyme Activity

Enzyme Concentration (units/mL)	Time to Float Disc (in seconds)			Rate	Class Average
	Trial 1	Trial 2	Average		
Control					
Hydroxylamine					

While you only worked on one of the variables, you are responsible for understanding all the factors manipulated (enzyme concentration, substrate concentration, temperature, – if done – inhibitor, and pH) and how that affects enzyme function.