

Title of Lab: The effect of concentration on enzyme activity.

Subject: Grade 12 Biology

Student names:

Date:

UOIT Course: CURS 4100 – Biology

Instructor: Shirley Smith

Lab modified by: Lindsey Row, Angela Michalecki, Raymond Luong

Title: The effect of concentration on enzyme activity

Course/Grade/Strand: Grade 12 Biology: Biochemistry

Expectations:

Overall:

- B2. Investigate the chemical structures, functions, and chemical properties of biological molecules involved in some common cellular processes and biochemical reactions;
- B3. Demonstrate an understanding of the structures and functions of biological molecules, and the biochemical reactions required to maintain normal cellular function.

Specific:

- B2.5. Plan and conduct an investigation related to a cellular process (e.g., factors that affect enzyme activity; factors that affect transport of substances across cell membranes), using appropriate laboratory equipment and techniques, and report the results in an appropriate format [IP, PR, C]

Learning Goals:

- Students will examine the enzyme catalase and the effects of enzyme concentration on enzyme activity.
- Students will safely handle laboratory equipment and chemicals.
- Students will represent their data in the form of a graph.

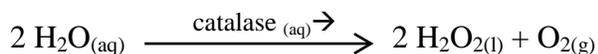
Background Information:

The body uses oxidative reactions to metabolise amino acids and fats. These reactions produce reactive chemical species, such as H_2O_2 , that could damage cellular machinery. Organelles called peroxisomes contain antioxidant enzymes which act to protect cells from this type of damage. One enzyme found in peroxisomes called catalase functions to prevent the accumulation of H_2O_2 in the body by catalyzing its decomposition into water and oxygen.

Introduction:

Enzymes are functional proteins that catalyze specific reactions in living organisms. Most are proteins in tertiary or quaternary structure whose overall shape affects their performance. In this investigation, you will conduct a controlled experiment to examine how different environmental conditions affect enzyme activity.

You will use catalase as the enzyme under study. As discussed, catalase catalyzes the decomposition of hydrogen peroxide, $\text{H}_2\text{O}_{2(\text{aq})}$, into water, $\text{H}_2\text{O}_{(\text{l})}$, and oxygen, $\text{O}_{2(\text{g})}$, according to the following equation:



Purpose:

You will conduct a controlled experiment to examine how enzyme concentration affects the rate of enzyme activity.

Materials:

- Safety glasses
- Safety gloves
- Rubber stopper (for flask) with hole and tubing
- Erlenmeyer flask
- 5 - 50 ml beakers
- Beef liver extract
- 1000mL beaker
- (3%) Hydrogen peroxide
- 100 ml graduated cylinder
- 2 - 25mL graduated cylinders
- 40 filter paper discs
- Stopwatch
- Forceps
- Stir rod
- Retort stand with clamp

Safety and disposal:

- Hydrogen peroxide is corrosive and may cause burns to the respiratory tract, skin and eyes.
- Do not inhale vapours.
- Wear safety glasses and gloves.

READ ALL OF THE INSTRUCTIONS BEFORE YOU START!**Procedure:**

1. In the 50 mL beakers, perform 4 serial dilutions of the full strength beef liver extract by first combining 20ml of full strength beef liver extract with 20ml of distilled water. Repeat 3 times until you have 4 concentrations: full strength, half strength, quarter strength, eighth strength. Read the observation tables to check that you have performed the serial dilutions correctly.

Note: you may need to add tap water to the stock solution to make it easier to pour.

2. Fill a 1000 mL beaker approximately $\frac{3}{4}$ full of water.
3. **For this step refer to Figure 1.** Fill the 100 mL graduated cylinder completely. Cover the top of the graduated cylinder with your hand and invert the cylinder 180°, submerging it directly into the water bath. Try to keep as much water in the cylinder as possible. Clamp the graduated cylinder to the retort stand allowing the graduations to be seen.

(Note: you may need to let some water out of the cylinder so that the initial volume of air is easily measured.)



Figure 1: Experimental set-up. Note that the tubing is stable inside the graduated cylinder, and the graduations are easy to read.

- Record the initial volume of oxygen in the inverted graduated cylinder in your observation table.
- Using a graduated cylinder, measure 5ml of 3% hydrogen peroxide and carefully pour it into the Erlenmeyer flask.
- Note: Dilution solutions may separate. Be sure to stir beakers before this step.**

Using the forceps, dip 4 filter paper discs into the desired dilution (i.e. Dilution A, Dilution B, etc). Be sure that there is a constant amount of beef liver extract on each paper disc.

- Drop the filter paper discs into the Erlenmeyer flask all at the *same* time. **Immediately** put the rubber stopper on the flask. Gently swirl the flask 3 times to ensure that the discs separate. Start the timer from the moment the first bubble enters the graduated cylinder.
- After 15 seconds, remove the rubber stopper from the flask and record the new volume of O_2 in the graduated cylinder in the observation tables. (**Table 1 and Table 2**)
- Clean the flask. Repeat using the other 3 dilutions of beef liver extract and the control.

Results:**Table 1.** Volume of oxygen produced by reaction after 15 seconds when mixing various dilutions (1:1, 1:2, 1:4, 1:8, control) beef liver extract enzyme and hydrogen peroxide (3%).

	Dilution A: Undiluted (25 mL beef liver + 25 mL water)	Dilution B: (25 mL Dilution A + 25 mL water)	Dilution C: (25 mL Dilution B + 25 mL water)	Dilution D: (25 mL Dilution C + 25 mL water)	Dilution E: (50 mL water)
Initial volume of O₂ (mL)	20.5	64	70	76	77
Volume of O₂ after 15 seconds (mL)	64	70	76	77	77
Observations					
Comments					

Table 2. Volume of O₂ produced by reaction after 15 seconds when mixing various dilutions (1:1, 1:2, 1:4, 1:8, control) beef liver extract enzyme and hydrogen peroxide (3%).

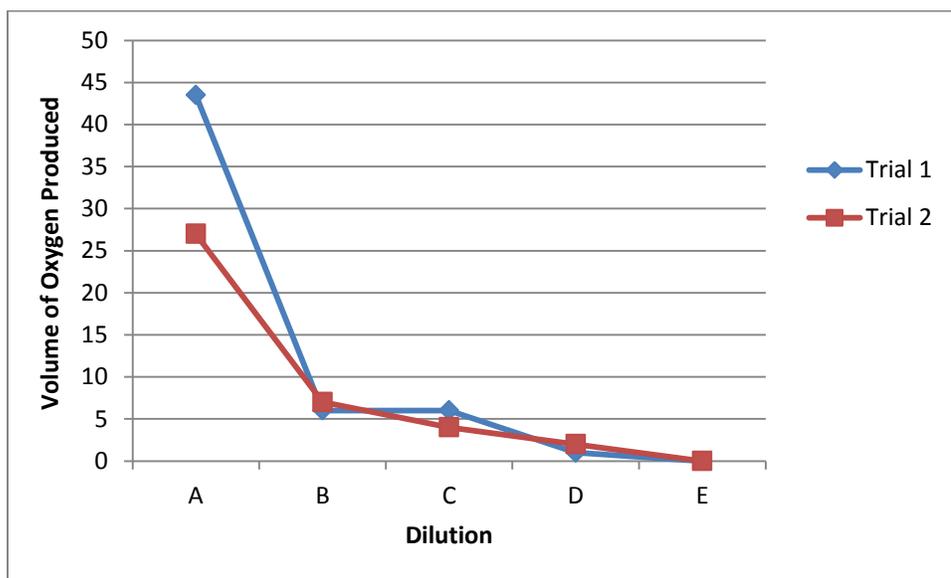
	Dilution A: Undiluted (25 mL beef liver + 25 mL water)	Dilution B: (25 mL Dilution A + 25 mL water)	Dilution C: (25 mL Dilution B + 25 mL water)	Dilution D: (25 mL Dilution C + 25 mL water)	Dilution E: (50 mL water)
Initial volume of O₂ (mL)	10	38	45	49	51
Volume of O₂ after 15 seconds (mL)	37	45	49	51	51
Observations					
Comments					

Analysis Questions:**K&U**

/4

1. Plot a graph of volume of O₂ versus enzyme concentration on the given graph paper. Discuss the trends below. Provide reasoning.

(2 marks for the graph and 2 marks for the trends)



- The more diluted the beef liver extract solution, the less oxygen was produced.
- A greater concentration of a reactant in a system will produce a larger volume of product compared to a lower concentration, with a constant amount of time.

T&I

/3

2. What are possible sources of error? Provide at least 3 ideas.

- Difficult to separate paper discs, so the amount of catalase may not have been constant in each trial.
- If the stopper did not seal the flask properly, oxygen could escape.
- If there are any holes in the tubing oxygen could escape.

T&I

/3

3. Describe how you could improve your experimental methods. Provide at least 3 ideas.

- Inspect tubing prior to lab.
- Perform additional trials
- Ensure that a constant amount of extract gets on the paper discs

A

/4

4. Suggest other experiments you could perform to extend your knowledge of enzyme activity. Provide at least one and describe how you would set up the experiment.
- Could investigate other factors affecting the rate of enzyme activity, such as
 - Temperature
 - pH
 - Saline

Post-lab Discussion:

Enzyme activity is proportional to substrate concentration at low substrate concentration. When there is a higher substrate concentration there are more collisions involving the substrate and active site. In the case of high substrate concentrations, since the active sites are already taken up, an increase in substrate concentration would have a small effect.

Teacher Notes:

- Be sure to review how to perform serial dilutions during the class before the lab; can be difficult to remember
- Demonstrate the set-up of the lab apparatus at the front of the class
- Lab questions will be assigned as homework as the lab will take the full 75 minutes to complete
- If students are running low on time they can use the Trial #2 results from another group. If this does occur, the data must be cited.