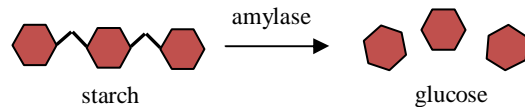


Name: _____ Date: _____

Activity Sheets

Enzymes and Their Functions



What are Enzymes?

Enzymes are compounds that assist chemical reactions by increasing the rate at which they occur. For example, the food that you eat is broken down by digestive enzymes into tiny pieces that are small enough to travel through your blood stream and enter cells. Enzymes are proteins that are found in all living organisms. Without enzymes, most chemical reactions within cells would occur so slowly that cells would not be able to work properly. Enzymes function as catalysts. **Catalysts** accelerate the rate of a chemical reaction without being destroyed or changed. They can be reused for the same chemical reaction over and over, just like a key can be reused to open a door many times. Enzymes are generally named after the substrate affected, and their names usually end in -ase. For example, enzymes that break down proteins are called proteases. While lipases break down lipids, carbohydrases break down carbohydrates.

The compounds that enzymes act upon are known as **substrates**. The substrate can bind to a specific place in the enzyme called the **active site**. By temporarily binding to the substrate, an enzyme can lower the energy needed for a reaction to occur, thus making this reaction faster. The energy required for a chemical reaction to occur is known as the **activation energy**. Once the reaction between an enzyme and a substrate is complete, the substrate is changed to a **product** while the enzyme remains unchanged. The rate of the reaction between an enzyme and a substrate can be affected by different factors. Some of the factors that can affect enzyme activity are temperature, pH, concentration of the enzyme and concentration of the substrate. In living organisms, enzymes work best at certain temperatures and pH values depending on the type of enzyme.

1. What are enzymes? _____
2. What is a catalyst? _____
2. How do enzymes work? _____
3. An example of an enzyme: _____

A Little More Information on Diffusion and Dialysis Membranes...

Molecules are in constant motion. So when there is a difference in concentration, or a concentration gradient of a specific particle, the particles will move toward the lower concentration in order to maintain an evenly distribution across the whole area. This movement of particles from higher concentration to lower concentration is known as diffusion.

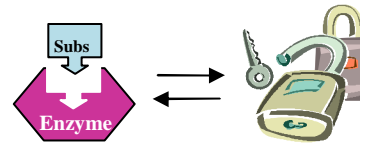
A dialysis membrane is a mesh-like material that will only allow certain particles of a specific size or smaller to pass or diffuse through. For example, if inside a dialysis tubing we place grains of rice and salt, the salt will diffuse out the tubing because it is small enough to move through the mesh of the membrane. The rice, however, will stay inside the tubing because it is too large to move through the mesh of the membrane. Kidneys are similar to a dialysis membrane. As blood passes through the kidneys, small molecules will be filtered out and eliminated from the body as urine.

1. What is diffusion? _____
2. What is a concentration gradient? _____
3. What is a dialysis membrane? _____

Name: _____

Date: _____

Activity Sheets – Part 1 (A)
Enzymes and Their Functions: Lock-and-Key Activity



A. Lock-and-Key Model

Objective: The objective of this activity is to introduce the concept of enzymes and their functions through a lock-and-key model by using real locks and keys as an analogy.

Procedure - Part 1.1:

1. Set 1 of locks and keys will be provided by your teacher.
2. Try all keys with all locks and answer the following questions about Set 1 of locks/keys.
 - a. Were you able to open all locks? _____
 - b. Do all keys open all locks? _____
 - c. Can 1 key open more than 1 lock? _____
 - d. Can you open the same lock with the same key more than once? _____
 - e. Do all keys have the same shape? _____

Procedure - Part 1.2:

1. A new set of locks/keys (Set 2) will be provided by your teacher.
2. From the previous observations, make 3 predictions about Set 2 of locks/keys. *Hint: Think about the specificity and reusability of the keys, and about the shape of the locks/keys.*

PREDICTIONS:

- a. _____
 - b. _____
 - c. _____
3. Test your predictions and say if whether or not each prediction was valid based on the results.

Were your predictions made for Set 2 of locks/keys valid?

4. Make 3 observations of Set 2 of locks/keys.

OBSERVATIONS:

- a. _____
b. _____
c. _____

5. Make comparisons between enzymes/substrates and keys/locks (3 similarities and 3 differences).

SIMILARITIES:

- a. _____
b. _____
c. _____

DIFFERENCES:

- a. _____
b. _____
c. _____

6. Share your similarities/differences with the classroom (your teacher will keep track of these in the blackboard).
7. Complete the following questions (Activity Sheet – Part 1 (B)).

Activity Sheets – Part 1 (B)

Enzymes and Their Functions: Lock-and-Key Activity

B. Enzymes and Their Functions – Questions

1. Match the following words with their definitions.

- | | |
|-------------------------|--|
| _____ Product | a. Amount of energy required for a chemical reaction to occur. |
| _____ Active site | b. Substances that bring about a chemical reaction without being changed itself. |
| _____ Enzymes | c. Substance that enzymes act upon. |
| _____ Catalyst | d. Regions on the surface of enzymes that fit the substrate. |
| _____ Substrate | e. Substance formed from the substrate at the end of a chemical reaction with an enzyme. |
| _____ Activation energy | f. Proteins that speed up chemical reactions. |

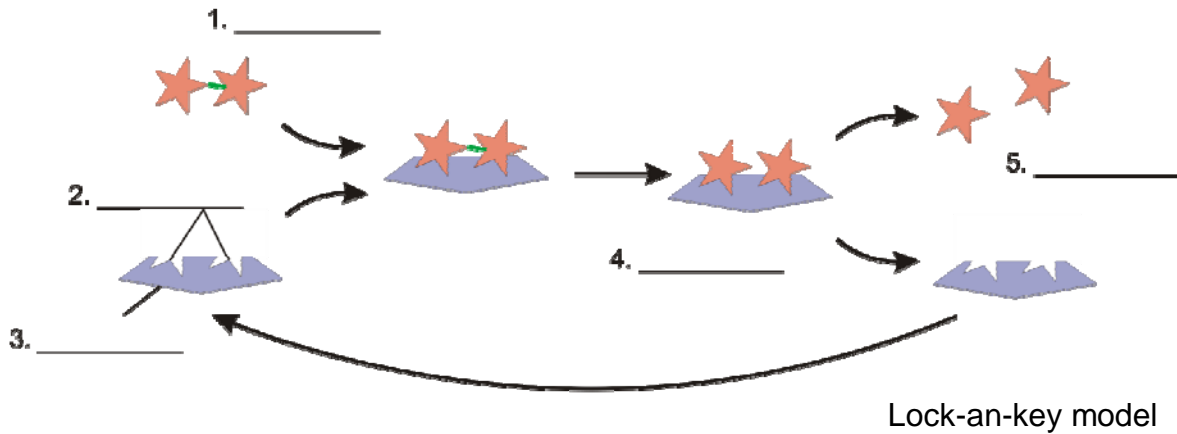
2. Characteristics of enzymes

- a. One characteristic of enzymes is that they are reusable. This is important because _____
- b. Name other 3 characteristics of enzymes:
1. _____
 2. _____
 3. _____

3. Naming enzymes

- a. Enzymes names end with _____
Examples _____
- b. Enzymes are named after _____
Examples _____

4. Fill in the blanks with the appropriate name.



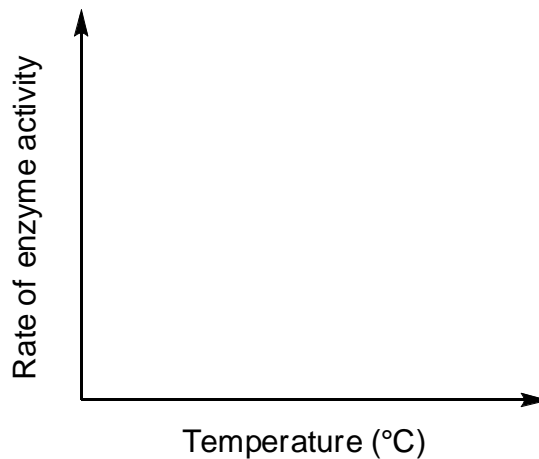
Names: product, active site, enzyme, substrate, enzyme-substrate complex.

5. Factors affecting enzyme activity (use a textbook for part a-c)

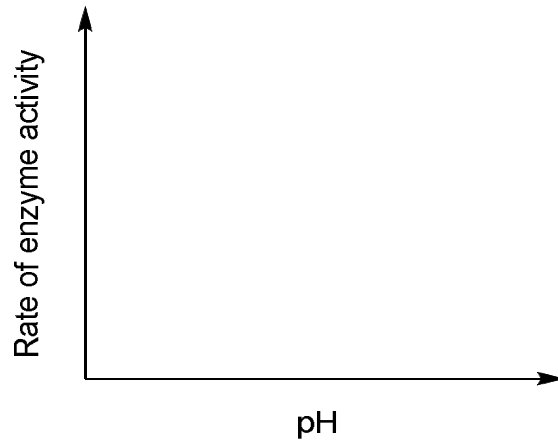
Enzyme activity can be affected by:

- a. _____
- b. _____
- c. _____

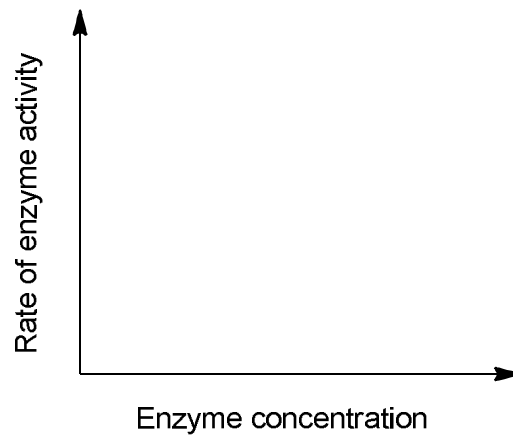
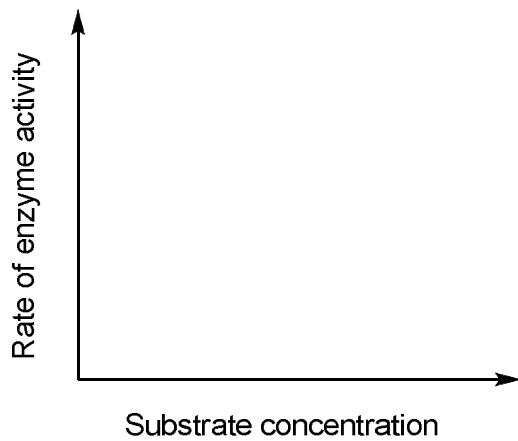
a. The effect of temperature and pH on enzyme activity



b. The effect of pH on enzyme activity



c. The effect of enzyme and substrate concentrations on enzyme activity



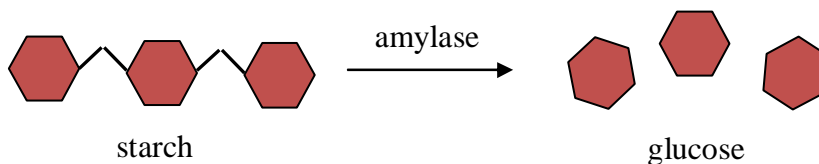
Name: _____ Date: _____

Activity Sheets – Part 2

Enzyme Action: The Breakdown of Starch into Glucose

Introduction

To study how the enzymes act upon the substrates, we will use amylase and starch as an enzyme and a substrate, respectively. Amylase converts polysaccharides into monosaccharides. Polysaccharides are long chains of sugars attached together, while monosaccharides are single sugar molecules. In the case of starch (a polysaccharide), amylase will break it down into glucose (monosaccharide) as illustrated below.



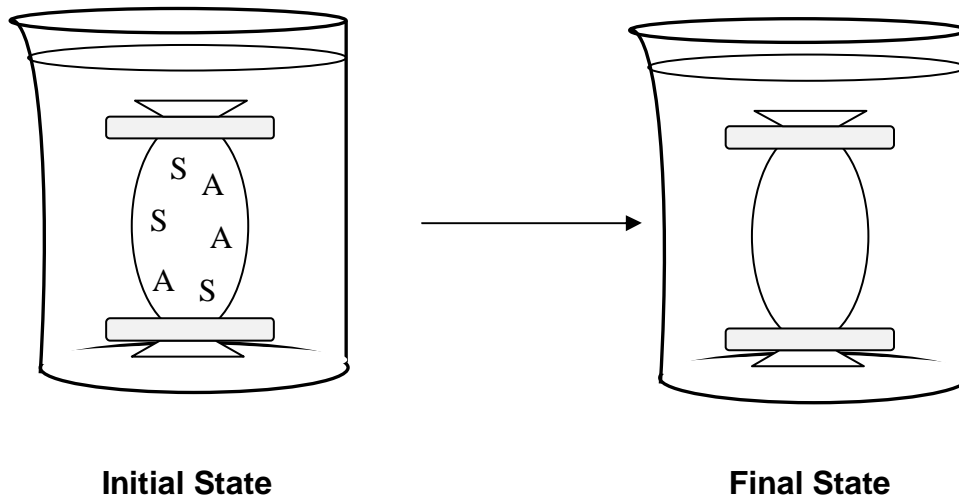
If amylase and starch are placed inside a dialysis tubing, the amylase will immediately begin to break the starch down into glucose. As the glucose forms from the enzyme activity, it will diffuse out through the membrane because it is small enough to fit through the mesh of the membrane. However, the amylase and the starch will stay inside the dialysis tubing because these molecules are too large to fit through the mesh. By detecting the amount of glucose outside the dialysis tubing over a period of time, we can study the rate (how fast or how slow) of the enzyme activity.

Objective: The objective of this activity is to perform an experiment with an enzyme (amylase) and a substrate (starch), and to understand how enzymes work. You will set up the same control experiment (6 time points) for 30-40 minutes (depending on the time available). The amount of glucose produced from the amylase/starch reaction will be quantified in time.

Pre-Lab Questions

1. In the presence of amylase, starch is converted to what smaller molecules?

2. In the initial state, the dialysis tubing will contain starch and amylase. Using the letters "S" for starch, "A" for amylase and "G" for glucose, indicate the areas where each of these molecules will be in the final state.



3. For this experiment our question is: **Does the concentration of glucose changes over time as amylase breaks down starch?**

4. A good hypothesis for this question is:

Procedure – Part 2.1: Collecting Samples

1. Materials will be provided by your teacher.
2. Select the times at which samples will be collected.

The times you will collect samples are (in minutes):

3. Write these times (minutes) in Table 1.
4. Label 7 eppendorf tubes with numbers from 1 to 7 and your initials.
5. Using a graduated cylinder, measure 500 mL of water and place it in the beaker.
6. Remove 1.5 mL of the water from the beaker and place it in tube 1.
7. Dissolve 3 g of starch by adding 5 mL of water to the vial.
Note: a white cloudy solution should be seen.
8. Shake or vortex the starch solution.
9. Dissolve 1 g of amylase by adding 5 mL of water to the vial.
Note: A brown solution should be seen.
10. Shake or vortex the amylase solution.
11. Seal the bottom of the dialysis tubing by wrapping one of the ends around a stir bar and clipping this end using a dialysis clip.
12. Use a transfer pipette to add all of the starch solution into the dialysis tubing.
13. Use another transfer pipette to add all of the amylase solution into the dialysis tubing.
14. Clip the other end (top part) of the dialysis tubing with a dialysis clip.
15. Insert the sealed dialysis tubing in the beaker with water.
16. Start the timer.
17. Set the stir dial in the stir plate to the lowest setting.
18. At each specific time (see Table 1), remove 1.5mL of the water from the beaker and place it in a 2 mL eppendorf tube labeled with the number corresponding to that time (tubes 2 – 6).
19. To tube 7, add 1.5 mL of water.

Procedure – Part 2.2: Analyzing the Data

1. Materials will be provided by your teacher.
2. Start a stirring water bath at ~80 – 90°C using a heating/stir plate.
Note: *Do not allow water to boil.*
3. Turn on the spectrophotometer. *Follow the instructions given by your teacher to turn on and set the spectrophotometer.*
4. Label 7 plastic cuvettes with numbers from 1 to 7.
5. To each sample (1 – 6), **except tube 7**, add 0.3 mL of Benedict's solution.
6. Shake or vortex each tube for 5 seconds.
7. Place all the tubes in a floating eppendorf tube holder.
8. Place the floating eppendorf tube holder in the heating water bath.
9. Start the timer.
10. Place a stir bar in the water bath and set the stir dial in the plate to the lowest setting.
11. After 10 minutes, remove the samples from the water bath.
Note: *Caution! Floating eppendorf tube holder and tubes will be very hot. Remove the tube holder using the tab that is above the water level. If possible, use heat resistant gloves.*
12. Allow the samples to cool down to room temperature (~ 3 minutes).
13. Centrifuge the tubes for 3 minutes.
14. Gently remove your samples from the centrifuge and place them in the tube rack. Do not shake them.
15. Without disturbing the solution in your sample, use a pipettor to remove 1 mL of the liquid and place it in the plastic cuvette with the same number.
Note: *Do not touch the bottom of the tube where the red precipitate may be present.*
16. Set the spectrophotometer to 735 nm. *Follow the instructions given by your teacher.*
17. Use cuvette 7 (only water) to set a reference for the spectrophotometer.
18. Obtain the absorbance of each sample (cuvettes 1 – 6).
19. Write down the absorbance of each sample (1 – 6) in Table 1.

TABLE 1

vial #	Time (minutes)	Absorbance (735nm) (y)
1		
2		
3		
4		
5		
6		

20. Obtain the 'm' and 'b' values from your teacher.
 21. Determine the glucose concentration for each sample with the equation given below, and complete Table 2.

Calculating Glucose Concentration

- a. Obtain the values of m and b from your instructor

$$m = \underline{\hspace{4cm}}$$

$$b = \underline{\hspace{4cm}}$$

- b. $y =$ Absorbance at 735 nm

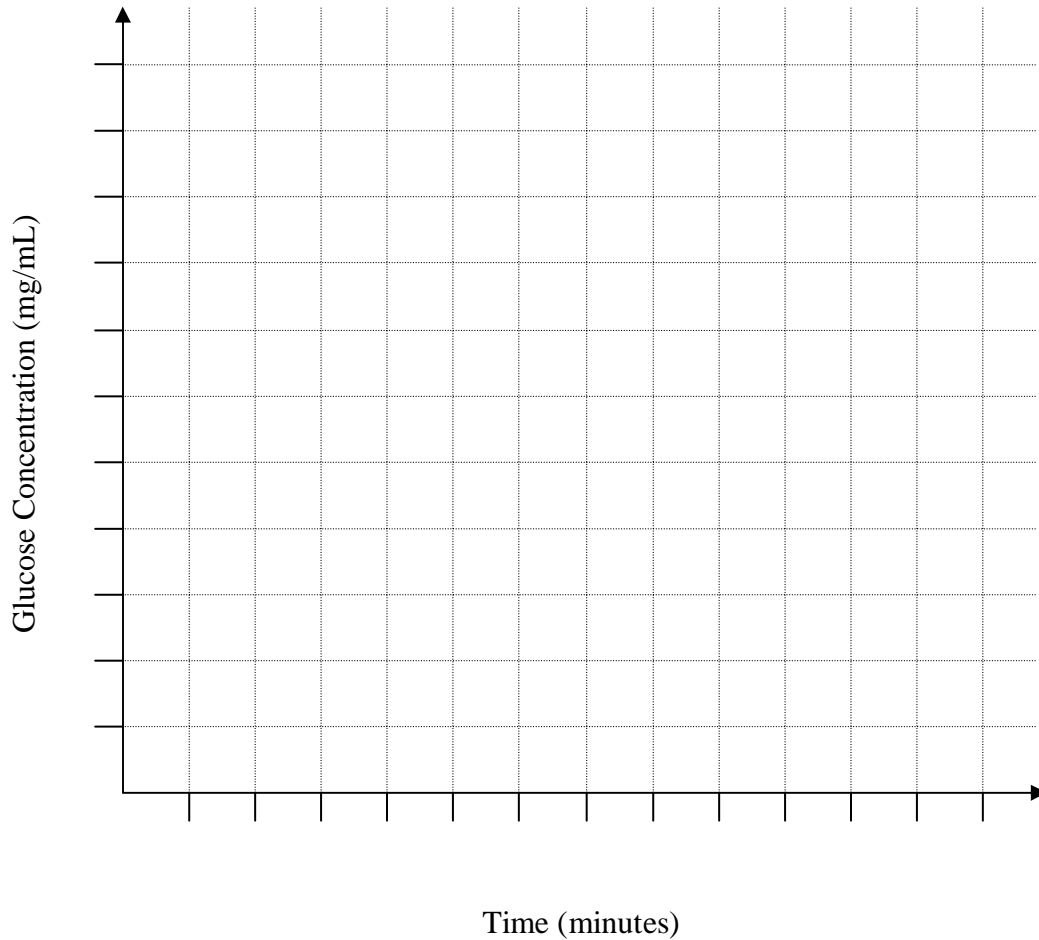
c. glucose concentration $= \frac{y-b}{m}$

TABLE 2

Independent Variable		Dependent Variable	
Entry	Time (minutes)	Glucose concentration calculation $= \left(\frac{y-b}{m} \right)$	Glucose concentration (mg/mL)
1		=	
2		=	
3		=	
4		=	
5		=	
6		=	

22. With the data in Table 2, plot glucose concentration (y-axis) vs. time (x-axis) in the graph below.

Plot 1 - Glucose Concentration vs. Time Plot



Post-Lab Questions:

a. What trend for glucose concentration with time was observed with your data?

b. Write down a conclusion for this experiment (include whether your results does or does not support your hypothesis).

c. Any possible sources of error?

d. Ideas for future research?

Name: _____ Date: _____

Activity Sheets – Part 3

Inquiry-Based Activity on Enzymes



Objective: The objective of this activity is to understand how different factors can affect enzyme activity. You will select an environmental factor that you would like to analyze, and you will design your own experiment using the knowledge gained from the previous activities. This experiment will answer a question based on the effect of the selected factor on enzyme activity.

Procedure – Part 3.1: Designing an Experiment

1. On your *Lab Report*, write two factors that you think will affect enzyme activity (*hint: review the previous notes on enzymes*).
2. Discuss these factors with your partner(s) and pick **one** factor to study.
3. Design an experiment with your partner that will answer a question based on the selected factor and enzyme activity, and complete Part A and B of your *Lab Report*. (*make sure that the instructor approves the experiment to be performed*)

Note: Follow the basic procedure in Part 2.1 and 2.2 with adjustments depending on your experimental design.

Procedure – Part 3.2: Inquiry-Based Activity

1. Depending on your experimental setup, materials will be provided by your teacher.
2. Set up and run the experiment, collect data and discuss your results.
 - a. Set up and collect data – 45 minutes
 - b. Clean up – 15 minutes
 - c. Process data and discuss conclusions – 30 minutes

Note: You can use the data from the experiment in Part 2 to compare to your new results.

3. Complete the rest of the *Lab Report*.

Enzymes and Their Functions – Lab Report

Group member names _____

Date of experiment _____

Based on the previous lecture, what are **2 factors** that affect enzyme function?

1. _____
2. _____

A. Designing an Experiment

1. What factor are you planning on analyzing? (*pick only one*)

2. What is the question for your experiment? _____

3. What is your hypothesis? _____

4. What is your independent variable? _____
5. What is your dependent variable? _____
6. What will remain constant in your experiment? _____
7. How long will your experiment last? _____
8. How many data points will you collect? _____

Approval from instructor _____

B. Experimental Procedure

List the steps for this experiment:

C. Collecting Data

TABLE 3

Independent Variable

Entry		Absorbance (735 nm) (y)
1		
2		
3		
4		
5		
6		
7		
8		

D. Calculating Glucose Concentration

- a. Obtain the values of m and b from your instructor

$m =$ _____

$b =$ _____

$y =$ Absorbance at 735 nm (from Table 3)

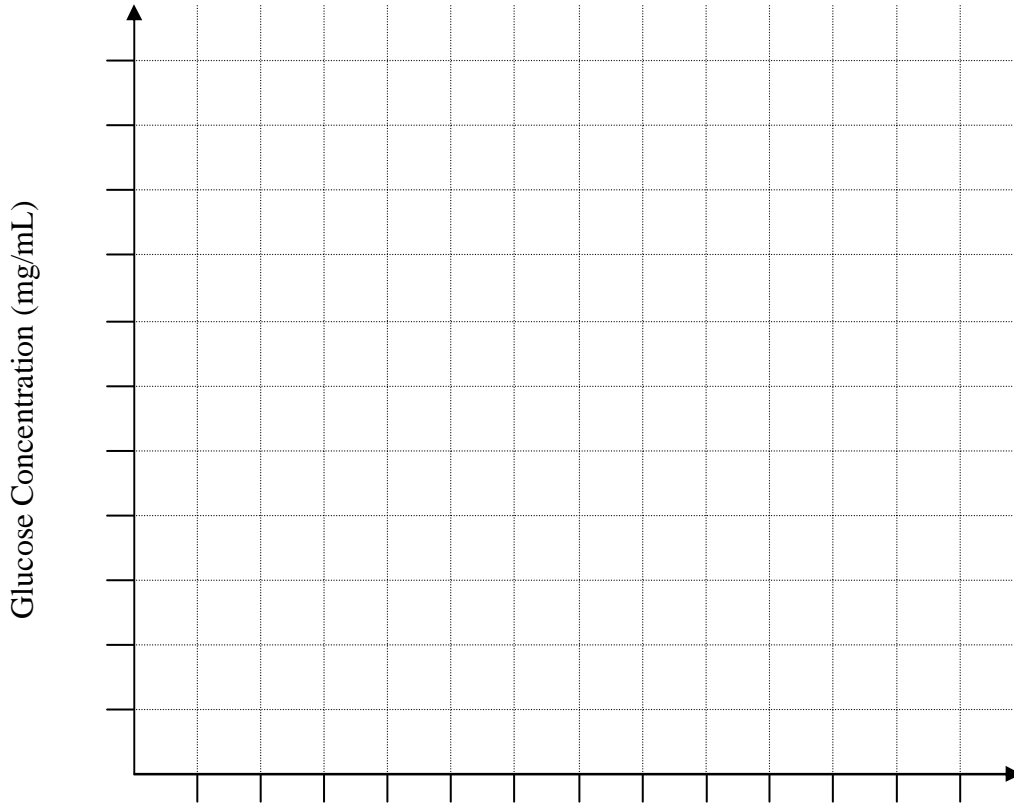
b. glucose concentration $= \frac{y-b}{m}$

TABLE 4

Independent Variable		Dependent Variable	
Entry		Glucose concentration calculation $= \left(\frac{y-b}{m} \right)$	Glucose concentration (mg/mL)
1		=	
2		=	
3		=	
4		=	
5		=	
6		=	
7		=	
8		=	

F. Results - Plotting your Data (*Remember to label the x-axis*)

Glucose Concentration vs. Time Plot



Post-Lab Questions:

a. What trend for glucose concentration with the independent variable was observed in your data?

b. Compared to the control experiment (Plot 1), what effect had your experimental factor on enzyme activity (i.e. higher or lower reaction rate)? Why?

c. Write down a conclusion for this experiment (include whether your results does or does not support your hypothesis).

d. Any possible sources of error?

e. Ideas for future research?
