Enzymes and Their Functions

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Abstract

This lesson focuses on enzymes, their importance in biological processes and how their functions are affected by different factors, such as temperature, pH and concentration. Following an introductory activity and lecture on the topic, the students will conduct an experiment using amylase (enzyme) and starch (substrate) as an example. In this experiment, the production of monosaccharides by the enzyme activity is quantified in time. An inquiry-based activity will follow in which students will develop their own experiment based on different factors affecting enzyme activity. Student will develop a problem statement and hypothesis, collect and interpret data, reach to their own conclusions based on the data and complete a lab report.

General Objectives

1. Understand the concept of enzymes, chemical reactions, catalysts and substrates
2. Understand how different environmental factors affect enzyme activity
3. Develop an experimental design based on enzyme activity
4. Develop a problem statement and hypothesis, collect and analyze data and arrive at conclusions

Appropriate level: Life Science, high school

Student's pre-requisites: introduction to diffusion

Time required: 7 class periods of 45 minutes and 2 class periods of 90 minutes (see time-line below)

Time-line for inquiry-based lesson plan

Day	Time (min)	Objective
Day 1	45	Lock-and-key model: Activity to introduce the concept of enzymes
Day 2	45	Lecture on enzymes and their functions
Day 3	45	Answer questions from lecture on enzymes and their functions
Day 4	45	Introductory experiment on enzymes (NYS required activity)
Day 5	90	Inquiry-based activity with enzymes: Collecting the data
Day 6	45	Inquiry-based activity with enzymes: Analyzing the data
Day 7	45	Inquiry-based activity with enzymes: Designing your own experiment (factors affecting enzyme's activity)
Day 8	90	Inquiry-based activity with enzymes: Carrying out your own experiment (factors affecting enzyme's activity)
Day 9	45	Inquiry-based activity with enzymes: Finishing analyzing data and lab report (factors affecting enzyme's activity)

National Science Education Standards

Science Content Standards

- Science as Inquiry
    - Abilities necessary to do scientific inquiry
    - Understanding about scientific inquiry

Standard 1

Students will use mathematical analysis, scientific inquiry, and engineering designs, as appropriate, to pose questions, seek answers, and develop solutions.

- Key Idea 1: The central purpose of scientific inquiry is to develop explanations of natural phenomena in a continuing and creative process.
- Key Idea 2: Beyond the use of reasoning and consensus, scientific inquiry involves the testing of proposed explanations involving the use of conventional techniques and procedures and usually requiring considerable ingenuity.
- Key Idea 3: The Observations made while testing proposed explanations, when analyzed using conventional and invented methods, provide new insights into natural phenomena.

Standard 4

Students will understand and apply scientific concepts, principles, and theories pertaining to the physical setting and living environment and recognize the historical development of ideas in science.

- Key Idea 1: Living things are both similar to and different from each other and from nonliving things.
- Key Idea 5: Organisms maintain a dynamic equilibrium that sustains life.

Performance Indicator

1.2: Describe and explain the structures and functions of the human body at different organizational levels (e.g. systems, tissues, cells, organelles).

- 1.2h: Many organic and inorganic substances dissolved in cells allow necessary chemical reactions to take place in order to maintain life. Large organic food molecules such as proteins and starches must initially be broken down (digested to amino acids and simple sugars respectively), in order to enter cells. Once nutrients enter a cell, the cell will use them as building blocks in the synthesis of compounds necessary for life.

5.1: Explain the basic biochemical processes in living organisms and their importance in maintaining dynamic equilibrium.

- 5.1f: Biochemical Processes, both breakdown and synthesis, are made possible by a large set of biological catalysts called enzymes. Enzymes can affect the rates of chemical

change. The rate at which enzymes work can be influenced by internal environmental factors such as pH and temperature.

- 5.1g: Enzymes and other molecules, such as hormones, receptor molecules, and antibodies, have specific shapes that influence both how they function and how they interact with other molecules.

Background – Enzymes

Enzymes are compounds that facilitate chemical reactions. These compounds are mostly proteins found in living organisms and are very important for cells to live and function. For example, the food that you eat is broken down into smaller pieces by different enzymes known as the digestive enzymes. Some examples are proteases, lipases and carbohydrases.

Enzymes are catalysts. Catalysts are compounds that accelerate a reaction without being changed. Enzymes are not destroyed or changed, but rather reused in the same chemical reaction over and over. The compounds that enzymes act upon are known as substrates. Enzymes bind to an active site in the substrate and lower the energy needed for the reaction to occur making it faster. The energy required for a chemical reaction to occur is known as the activation energy. The substrates are changes to form a product. The name of the enzyme usually ends in *–ase* and is derived from the substrate that is affected by it. For example, enzymes that break down proteins are called proteases.

Background – Diffusion

When there is a difference in concentration or a concentration gradient of a specific particle, these particles will tend to 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 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 travel through the meshes of the membrane. However, the rice will stay inside the tubing because it is too large to travel through the meshes of the membrane. The kidneys are similar to a dialysis membrane. As blood passes through the kidneys, small molecules and small proteins will be filtered though and ejected from the body as waste.

The following activities will require dialysis membranes to study the enzyme activity of amylase. Amylase is an enzyme that converts polysaccharides into monosaccharides. A polysaccharide is a long chain of sugars attached together. In the case of starch (a polysaccharide), amylase will break it down to form glucose. If amylase and starch are placed inside the dialysis tubing, as the glucose forms from the enzyme activity it will diffuse out from the membrane because it is small enough. However, the amylase and the starch will stay inside the tubing. By detecting the amount of glucose outside the dialysis tubing in time, we can then study the rate (how fast or how slow) the enzyme is working.

Day 1: 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.

Materials

1. Locks and keys
    a. Total locks and keys: 24 sets (part 1) – 6 red, 6 blue, 6 green and 6 yellow
    b. Total locks and keys: 24 sets (part 2) – 12 red, 6 blue, 6 green

Procedure – Part 1:

1. Divide students in groups of 2.
2. To each group of students, provide 4 sets of locks and keys. *(each lock has a unique color and each key will go with only one lock)*
3. Ask each group to make 5 observations about the locks and keys – *time: 10 minutes. (i.e. one key for one lock, reusable, locks/ keys have different shape, key makes locks open)*

Observations:
1. ___________________________________
2. ___________________________________
3. ___________________________________
4. ___________________________________
5. ___________________________________

4. Ask the students to share their observations and write them on the blackboard.
5. Collect the set of locks and keys and continue with Part 2.

Procedure – Part 2:

1. Ask each group of students to make 3 predictions (from previous observations) about a new set of keys – *time: 5 minutes.*

Predictions:
1. ___________________________________
2. ___________________________________
3. ___________________________________

2. To each group provide a new set of 4 locks and keys. *(in this case, 1 key will open 2 locks, 1 key will not open any locks and 1 key open only 1 lock)*
3. Ask the students to test their predictions and to say if whether or not each prediction was valid based on their results.

Were your predictions made for the new set of locks and keys valid?
____

4. Ask the students to make 3 additional observations.

Observations:
1. ______________________________
2. ______________________________
3. ______________________________

5. Ask the students to find the lock that matches their key that did not open any locks from their set – *time: 5 minutes.*

Day 2-3: Lecture on Enzymes

Objective

The objective of this lecture is to give further information on enzymes and their functions, and be able to correlate the lock-and-key model introduced in Day 1 with enzymes.

Procedure

1. Give lecture on enzymes.
2. Ask the students to make comparisons between enzymes/substrates and keys/locks (3 similarities and 3 differences – *time: 5 minutes*).

Similarities:
1. ______________________________
2. ______________________________
3. ______________________________

Differences:

1. ____
2. ____
3. ____

3. Ask the students to share their ideas and write them on the blackboard.
4. Allow the students to complete the following questions.

Enzymes and Their Functions (Assignment)

1. Match the following words with their definitions

__e___ Product
__d___ Active Site
__f___ Enzymes
__b___ Catalyst
__c___ Substrate
__a___ Activation Energy

a. amount of energy required for a chemical reaction to occur
b. substances that bring about a chemical reaction without being changed itself
c. substance that an enzyme act upon
d. regions on surfaces of enzymes that fit the substrate
e. substance formed from the substrate at the end of a chemical reaction with an enzyme
f. proteins that speed up chemical reaction

2. Properties of enzymes

a. Name 2 properties of enzymes

1. __reusable________
2. __specific_________

This is important because ____
____

3. Naming enzymes

a. Enzymes names end with___-ase____

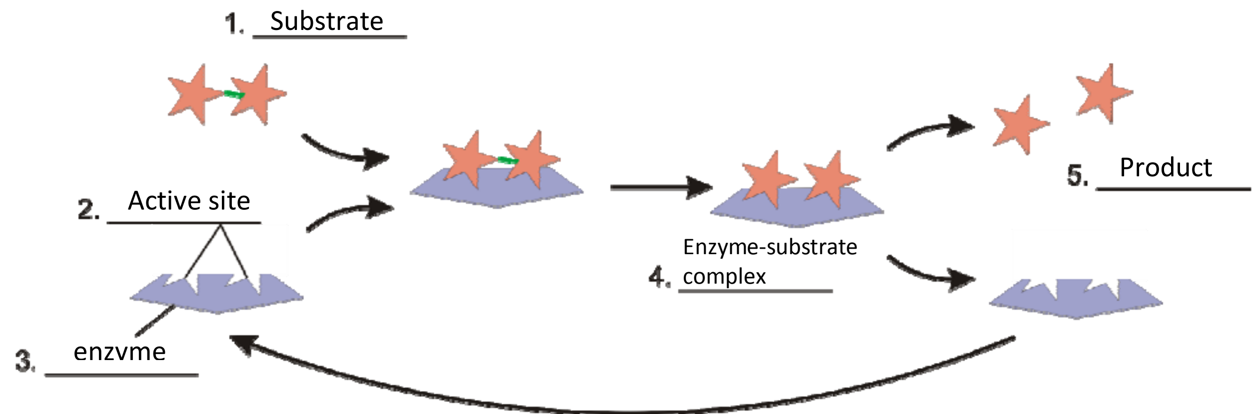
Examples___proteases, amylase, lipases, carbohydrases_____________________________

b. Enzymes are named after____substrate___ ___________________________________

Examples ___proteases, lipases, carbohydrases ________________________________

4. Lock-and-key model

Fill in the blanks with the appropriate name.



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

5. Comparisons between substrate/enzymes and locks/keys

List 3 similarities and 3 differences between substrates/enzymes and locks/keys (based on previous activity)

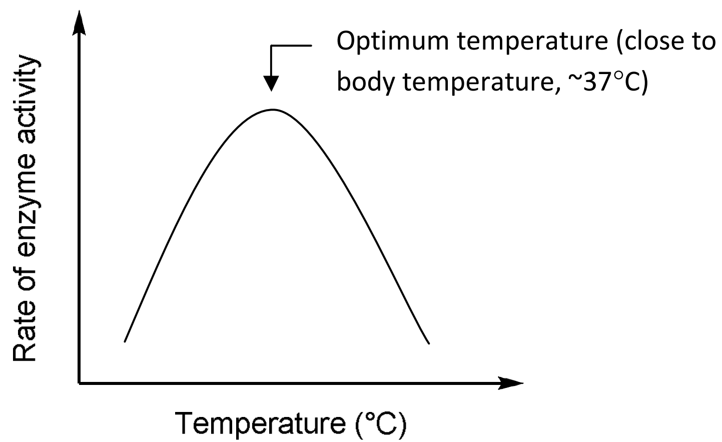
Similarities:	Differences
1. ____________________	1. ____________________
2. ____________________	2. ____________________
3. ____________________	3. ____________________

6. Factors affecting Enzyme Activity

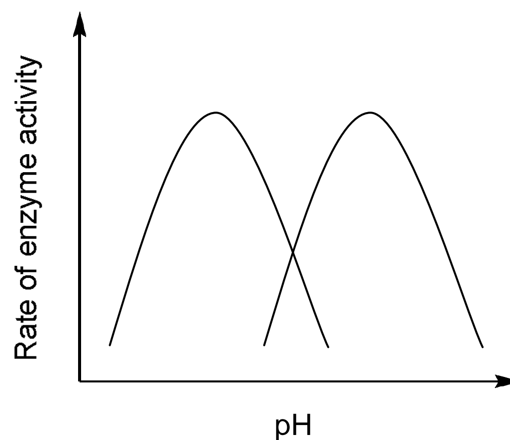
Enzymes are affected by

1. __temperature____
2. __pH____
3. __enzyme/substrate concentration____

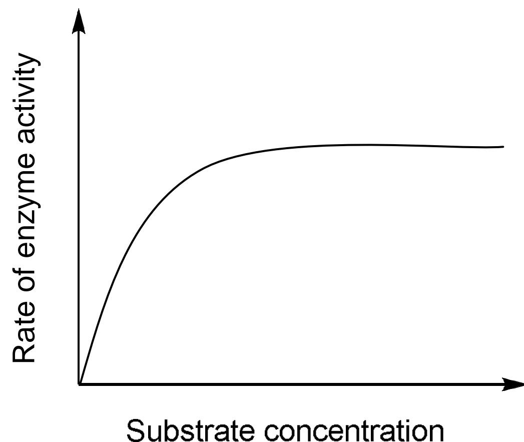
a. The effect of temperature on enzyme activity

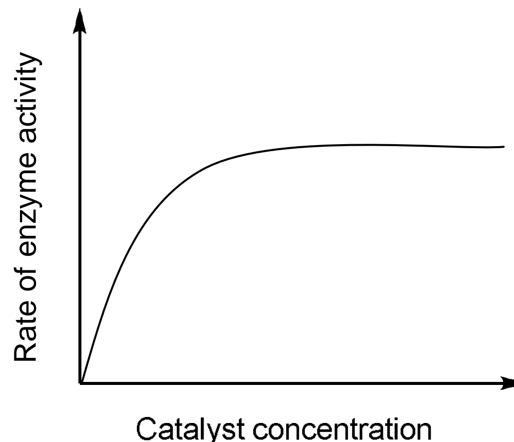


b. The effect of pH on enzyme activity



c. The effect of enzyme and substrate concentrations





7. Review:

1. What are enzymes?
2. What are 2 properties of enzymes?
3. How are enzymes named?
4. What are 3 factors that affect enzymes?

Homework: page 56, question 1 – 4

Day 4: Introductory Experiment to Enzymes

Objective

The objective of this activity is to introduce amylase as an enzyme and monosaccharide colorimetric detection methods, benedicts' solution.

Materials

1. 6 beakers (600 mL)
2. Dialysis tubing
3. 12 Dialysis clips
4. 12 transfer pipettes
5. 6 stir plates
6. 6 magnetic stirrers
7. Corn starch
8. amylase
9. benedicts' solution
10. water bath

Procedure

This experiment will be following the NYS required activities. No details will be given in this lesson.

Day 5-8: Inquiry-Based Activity with Enzymes (Enzyme Lab)

Objective

The objective of this activity is to allow the students to perform an experiment with enzymes and understand the factors that affect enzymes. This day and the following, the experiment to be performed will be explained in detail and students will be allowed to design their own experiment. Students should be divided in groups of 2.

Materials:

a. 6 Beaker (600mL)
b. Dialysis tubing
c. corn starch
d. amylase
e. Distilled water
f. 6 Pippetor (1 mL)
g. Pipette tips (1 mL)
h. 12 Transfer pipettes
i. 36 Eppendorf tubes (2 mL)
j. 6 Timer
k. vortex
l. 6 Floating disk
m. 6 Stir plate
n. 6 Stir bar
o. 2 Graduated cylinder (500 mL)
p. 6 stir/heating plates
q. Benedicts' solution (~15 mL)
r. Small centrifuge
s. Spectrophotometer
t. 36 plastic cuvettes
u. Cuvette holders
v. 6 eppendorf tube racks
w. pH meter

Pre-lab (performed by instructor prior to lab with students)

A. Glucose calibration curve

Facilitator should perform a calibration curve of glucose concentration with absorbance. This is done by preparing various solutions of different concentrations of glucose with fixed amount of benedicts' solution and detecting its correspondent absorbance using a spectrophotometer.
Alternatively, if time is a constraint, the values of m = -0.8125 and b = 0.6452 can be used.

Procedure:

1. Start a stirring water bath at 90°C using a heating/stir plate.
2. Weigh 2 mg of glucose and place it in a eppendorf tube or vial.
3. Dissolve the glucose with 2 mL of distilled water to make a 1 mg/mL solution.
4. Label 7 eppendorf tubes (2 mL) with numbers from 1 to 7.
5. Add the following to each tube with the corresponding number:

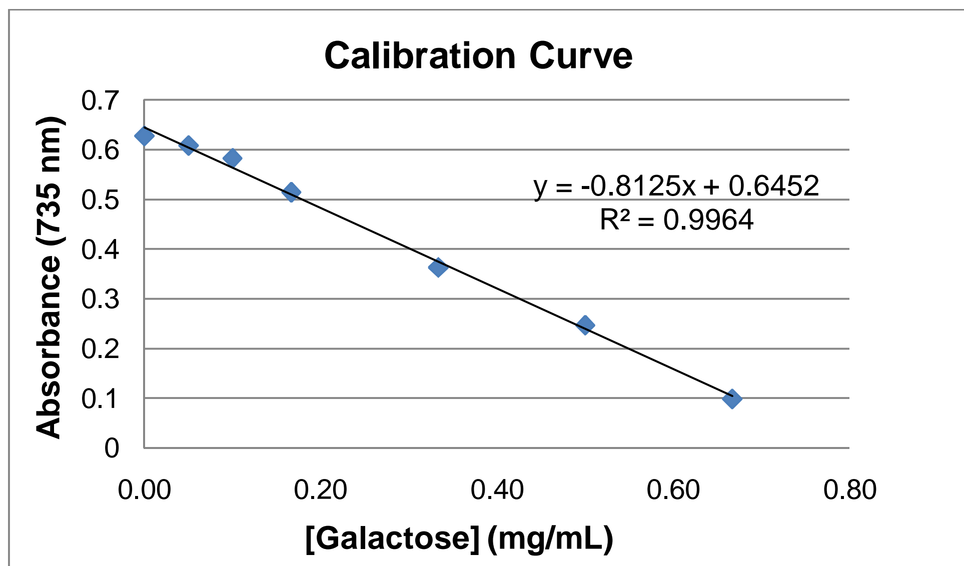
Tube #	Monosaccharide stock solution, 1 mg/mL (µL)	Distilled water (mL)	Benedicts' solution (µL)	Final concentration (mg/mL)
1	0	1.50	300	0.00
2	75	1.425	300	0.05
3	150	1.35	300	0.10
4	250	1.25	300	0.17
5	500	1.0	300	0.33
6	750	0.75	300	0.50
7	1000	0.50	300	0.67

6. Vortex each tube for 5 seconds.
7. Place all the tubes in a floating disk.
8. Place the floating disk in the heating water bath for 10 minutes.
9. Remove the tubes from the water bath and allow them to cool down to room temperature (~ 10 minutes).
10. Centrifuge the tubes for 3 minutes.
11. Without disturbing the solution in your sample, use a pippetor to remove 1mL of the liquid and place it in the plastic cuvette with the same number. *Note: Try not to touch the bottom of the tube where the red precipitate is, if any.*
12. Set the spectrophotometer to 735 nm and obtain the absorbance of each sample.
13. Record the data and plot glucose concentration vs. absorbance (a linear plot with a negative slope should be observed).
14. Perform a linear trend (in excel) and obtain the slope (m) and y-intercept (b) values.

Tube #	Final glucose concentration (mg/mL)	Absorbance at 735 nm
1	0.00	
2	0.05	
3	0.10	
4	0.17	
5	0.33	
6	0.50	
7	0.67	

Slope (m) = _________________________________
y-intercept (b) = ______________________________

**** The following plot of glucose concentration with absorbance is expected (in this case, galactose was used as the monosaccharide):***



B. Materials to be prepared prior to lab day

1. In a vial, weigh 3g of starch (one per group)
2. In a vial, weigh 1g of amylase (one per group)
   Note: If amylase is not going to be used immediately, place in fridge after weighing and do not dissolve in water until ready for the experiment.
3. Cut 15 cm of dialysis tubing (one per group)

Enzymes Lab

Procedure – Part 1: Collecting the data

Each group will set up the same control experiment (6 time points) and prepare a graph of glucose concentration in time.

Note: A negative control experiment can be performed where only starch is placed in the dialysis tubing (no amylase) and benedicts' solution is used to detect for the presence of glucose at 45 minutes.

1. Provide the following materials to students (per group):

a. 1 Beaker (600mL)
b. Dialysis tubing (pre-cut)
c. Pre-weighted starch (3g)
d. Pre-weighted amylase (1g)
e. Distilled water (~510 mL)
f. 1 Pippetor (1 mL)
g. Pipette tips (1 mL)
h. 2 Transfer pipettes
i. 6 eppendorf tubes (2 mL)
j. 1 eppendorf tubes rack
k. Timer
l. vortex
m. Floating disk
n. Stir plate
o. Stir bar
p. Graduated cylinder (500 mL)

2. Label 6 vials with numbers from 1 to 6 and your initials.
3. Using a graduated cylinder, measure 500 mL of water and place it in the beaker.
4. Dissolve 3 g of starch with 5 mL of distilled water.
   Note: a white cloudy solution should be seen.
5. Stir or vortex the starch solution.
6. Dissolve 1 g of amylase with 5 mL of distilled water.
   Note: A brown solution should be seen.
7. Stir or vortex the amylase solution.
8. Seal one of the ends of the dialysis tubing by wrapping a stir bar and clipping the end using a dialysis clip.
9. Use a transfer pipette to add all of the starch solution in the dialysis tubing.
10. Use another transfer pipette to add all of the amylase solution in the dialysis tubing.
11. Clip the other end of the dialysis tubing with a dialysis clip.
12. Insert the sealed dialysis tubing in a beaker with water (this is time 0 minutes).
13. Start the timer.
14. At each specific time (see Table 1), remove 1.5 mL of the water from the beaker and place it in an eppendorf tube labeled with a number corresponding to that time.
    Note 1: if pippettors are not available, use a small graduated cylinder to measure the sample.

Note 2: The amylase will form a brown layer on the top while the starch will form a white layer on the bottom. As time passes, the brown layer (amylase) will increase as the white layer (starch) will decrease.

Note 3: The samples collected by the students can be stored overnight in a fridge. However, if longer storage times are required, store samples in a freezer.

Procedure – Part 2: Analyzing the data

1. Provide the following materials to students (per group):

a. 1 beaker (600 mL)
b. 1 stir/heating plate
c. 1 magnetic stir bar
d. Benedicts' solution (~ 2 mL)
e. water (~300 mL)
f. Small centrifuge
g. Spectrophotometer
h. 6 plastic Cuvettes
i. Cuvette holder
j. Timer
k. Vortex
l. Floating disk
m. 1 Pippettor (1mL)
n. Pipette tips

2. Start a stirring water bath at ~90°C using a heating/stir plate.
3. To this sample (1-6), add 300 µL of Benedicts' solution.
4. Vortex each tube for 5 seconds.
5. Place all the tubes in a floating disk.
6. Place the floating disk in the heating water bath for 10 minutes.
7. Remove the tubes from the water bath and allow them to cool down to room temperature (~10 minutes).
8. Centrifuge the tubes for 3 minutes.
9. Label 6 plastic cuvettes with numbers from 1 to 6 and your initials.
10. Without disturbing the solution in your sample, use a pippetor to remove 1mL of the liquid and place it in the plastic cuvette with the same number. *Note: Try not to touch the bottom of the tube where the red precipitate is, if any.*
11. Set the spectrophotometer to 735 nm and obtain the absorbance of each sample.
12. Write down the absorbance of each sample in Table 1.
13. Obtain the 'm' and 'b' values from your instructor, determine the glucose concentration for each sample and complete Table 2.

    Note: Glucose concentration is calculated with: $x = \frac{y-b}{m}$

    'y' *is the absorbance*

    'm' *and* 'b' *are from pre-lab (calibration curve)*

TABLE 1

vial #	Time (minutes)	Absorbance (735nm)
1	0	
2	5	
3	10	
4	20	
5	30	
6	45	

Calculating glucose concentration

a. Obtain the values of m and b from your instructor
   m = ___________________________
   b = ____________________________

b. Absorbance at 735 nm (y) = ___________________________

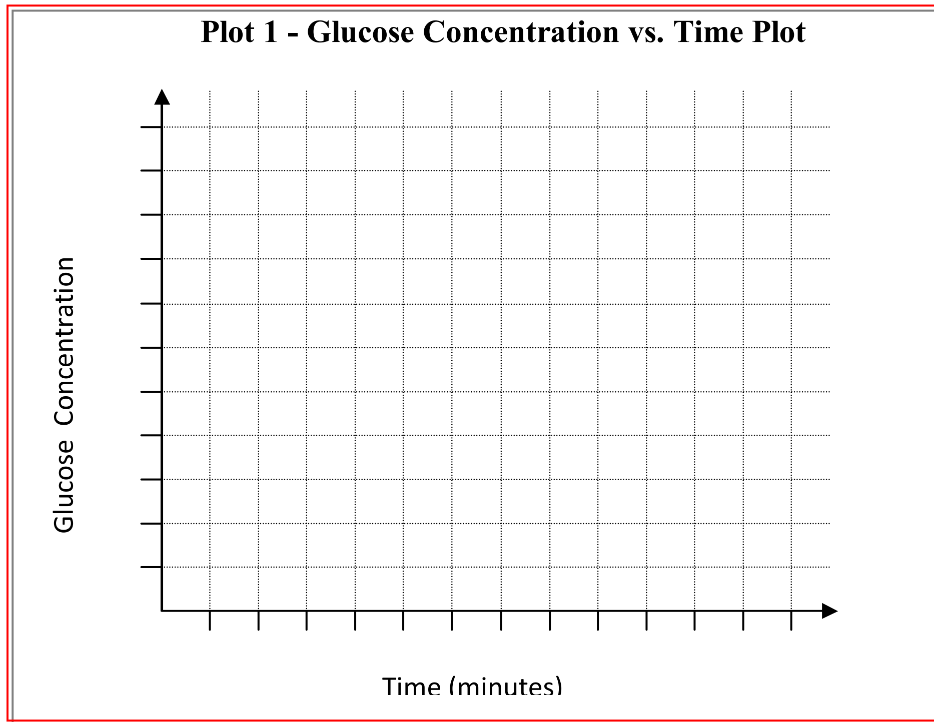
c. glucose concentration $= \frac{y-b}{m}$

TABLE 2

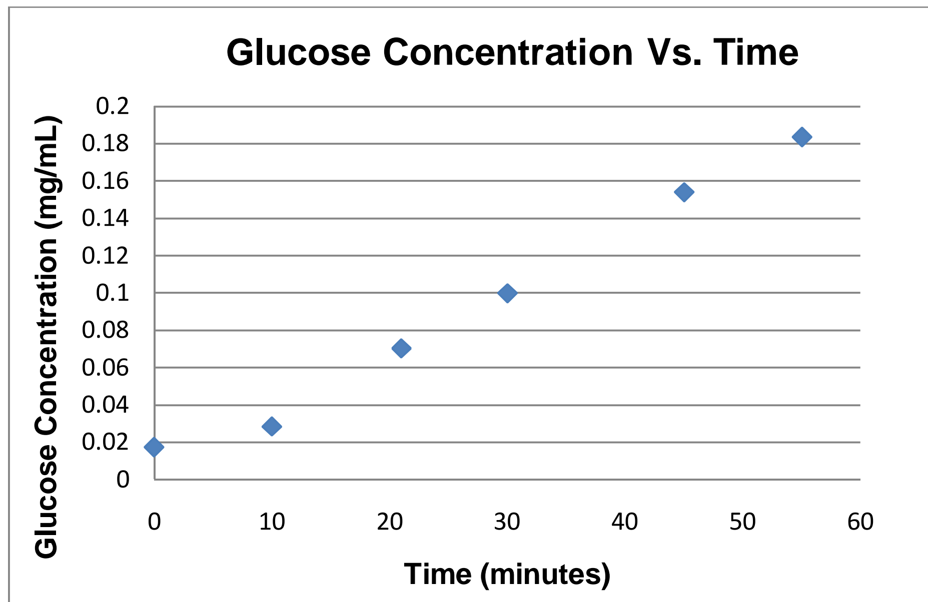
Independent Variable / **Dependent Variable**

Entry	Time (minutes)	Glucose concentration calculation $\left(\frac{y-b}{m}\right)$	Glucose concentration (mg/mL)
1	0	= -----------------	
2	5	= -----------------	
3	10	= -----------------	
4	20	= -----------------	
5	30	= -----------------	
6	45	= -----------------	

14. With the data in Table 2, plot the time (x-axis) with the absorbance (y-axis)



The following plot of glucose concentration vs. time should be expected:



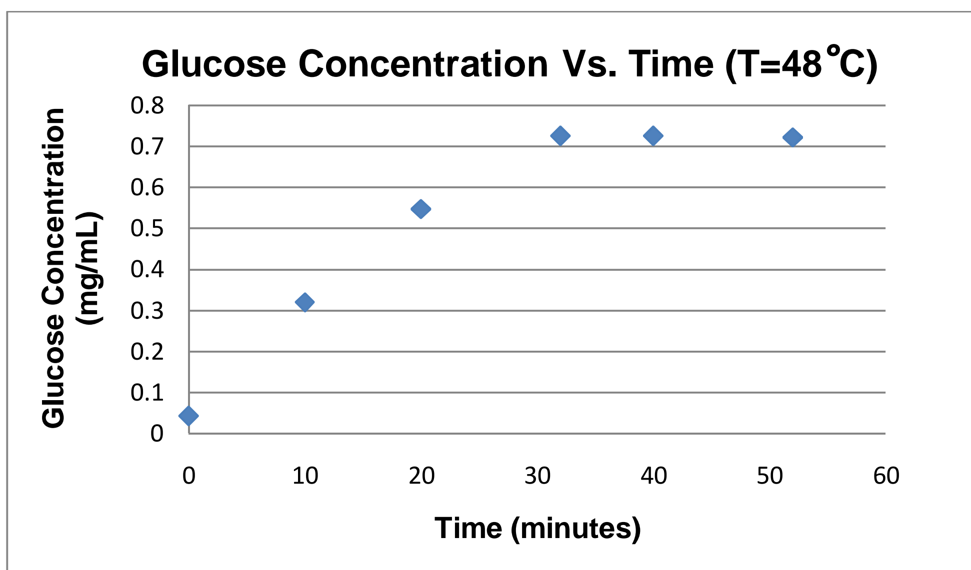
Procedure – Part 3: Designing an experiment
*** Note: Lab report sample is on page 20-22**

1. Ask the students to write down in the lab report two questions about what they think will affect enzyme activity (based on previous lecture).
2. Allow the students to discuss the questions among their group and pick one question.
3. Ask the students to design an experiment with their partner that will answer the question and fill in the rest of Page 1 of the lab report. Examples can be the effect of temperature (higher or lower), pH (acid or basic) or enzyme/substrate concentration on enzyme activity. *(make sure that you approve the experiment to be performed)*

Procedure – Part 4: Inquiry-based activity

1. Provide materials to students. This will depend on their experimental design. (*Basic materials are those from part 1 and 2 of Enzyme Lab).*
2. Allow the students to set up, run their experiments, collect data and discuss results. If required, several days
   a. *Set up – 15 minutes*
   b. *Collect data – 40 minutes*
   c. *Clean up – 15 minutes*
   d. *Process data and discuss conclusions – 20 minutes*
3. If needed, an extra day will be given to finish processing data, discuss results and complete the lab report.

**** An example of temperature effect on enzyme activity is as following (T=48°C)***



Enzymes Lab Report

Group member names ____
Date of experiment ____

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

1. ____
2. ____

B. Designing an experiment

1. What factor are you planning on analyzing?___________________________________
2. What is you independent variable? _______________________________________
3. What is your dependent variable? ____
4. How long will your experiment last? ______________________________________
5. How many data points will you collect? ___________________________________
6. What is a good problem statement for your experiment?
   ____
   ____
7. What is your hypothesis?
   ____
   ____

Approval from instructor ____

C. Collecting Data

TABLE 3

Entry	Time (minutes)	Absorbance (735 nm)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Calculating glucose concentration

a. Obtain the values of m and b from your instructor
m = ________________________
b = _________________________
y = Absorbance at 735 nm (from Table 1 – dependent variable)

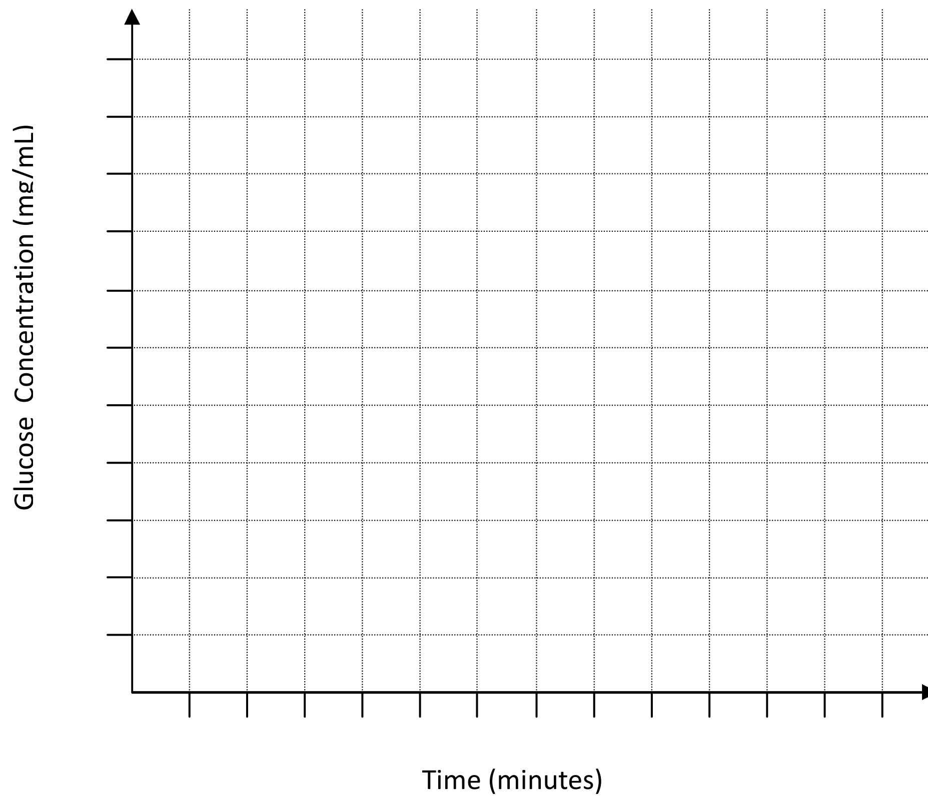
b. glucose concentration $= \frac{y-b}{m}$

TABLE 4

	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		= -----------------	
7		= -----------------	
8		= -----------------	
9		= -----------------	
10		= -----------------	

D. Results - Plotting your data

Glucose Concentration vs. Time Plot



a. What trend for glucose concentration with time was observed with 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

____

____

____