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Lab 2: Enzymes

Introduction:

Three enzymes will be studied in today's lab: **Invertase**, **Amylase**, and **Pepsin**. These three enzymes have been widely studied and their properties are well known.

Invertase catalyzes the following reaction: Sucrose → glucose + fructose

The activity of the enzyme can be detected by using a reagent that can detect reducing sugars (both glucose and fructose are reducing sugars). One reagent commonly used to measure invertase activity in industrial procedures is Dinitrosalicylate (DNS). Reducing sugars are produced by the action of invertase on sucrose; these reducing sugars reduce DNS to aminonitrosalicylate (ANS). This can be observed as a color change from yellow/orange to red/brown.

Amylase hydrolyzes starch, liberating glucose. The activity of amylase can also be detected by using DNS reagent.

Pepsin (a protease) hydrolyzes proteins and releases small peptides and amino acids. Pepsin activity can be detected by measuring pH changes that accompany protein degradation.

Safety:

Some of the chemicals used in the following test are hazardous/poisonous. They are safe to use as long as proper caution and safe handling techniques are applied in the laboratory.

- Do not allow chemicals to enter mouth or small cuts or scratches on the hands.
- Do not inhale the vapors or powders or allow them to blow around.
- Always wash your hands carefully after an experiment and follow directions of instructor regarding safety precautions.

Dispose of chemicals and results AS DIRECTED!!!

REMEMBER TO CLEAN WORK AREAS WITH VESPHENE WHEN FINISHED!

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Experiment 1: Demonstrating Invertase Activity

Materials:

- 1) Three test tubes
- 2) Buffer 1
- 3) Invertase
- 4) Sucrose
- 5) DNS (Dinitrosalicylate)

Procedure:

- 1) Make sure all solutions are at room temperature before proceeding.
- 2) Obtain three test tubes and label them #1, #2, and #3.
- 3) First add substrate (sucrose) and Buffer 1 as shown in the table below (No sucrose in tube #2).
- 4) Add the enzyme (invertase) to the tubes as shown in the table below (No invertase in tube #1).
- 5) Make sure to add enzyme to appropriate tubes with as little time delay between each as possible.
- 6) Mix the contents.
- 7) Set aside for 15 minutes.
- 8) Add 1 ml of DNS to each tube with as little time delay between each as possible.
- 9) Mix well.
- 10) Place tubes in boiling water bath for 10 minutes, then remove promptly to cool.

	Test Tube #1	Test Tube #2	Test Tube #3
Sucrose (ml)	1.0	<i>NONE</i>	1.0
Invertase (ml)	<i>NONE</i>	0.5	0.5
Buffer 1 (ml)	1.0	1.5	0.5

Interpretation:

Describe the color of tube contents: **(0.5 POINTS for completing table)**

	Before DNS is added	After DNS is added	After boiling
Test Tube #1			
Test Tube #2			
Test Tube #3			

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Questions:

(0.5 POINTS for each question)

- 1) Which tube(s) did you expect to display a color change after boiling? Is that what actually happened?

- 2) Briefly explain what was demonstrated by this experiment.

Experiment 2: Demonstrating Amylase Activity

Materials:

- 1) Six test tubes
- 2) Buffer 2
- 3) Amylase
- 4) Starch

Procedure:

- 1) Set up six test tubes- Label each test tube with the corresponding time interval as indicated below.

	Tube 1 (blank)	Tube 2 (1 min.)	Tube 3 (2 min.)	Tube 4 (4 min.)	Tube 5 (8 min.)	Tube 6 (16 min.)
Starch	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Amylase	None	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml

- 2) Note the time as soon as amylase is added to the tubes! Start a timer!
- 3) To the test tube 1 (blank) add the following reagents at the same time so no reaction can occur:

	Tube 1 (blank)
DNS	1.0 ml
Amylase	1.0 ml

This tube represents a “time zero” condition, before the reaction between enzyme and substrate has had a chance to occur.

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- 4) Leave the tubes at room temperature
- 5) Using the intervals of 1, 2, 4, 8 and 16 minutes (test tubes should be labeled accordingly) add 1.0 ml of DNS reagent according to those intervals.
 - a. Ex: Tube 2- After 1 min. has elapsed add 1.0 ml of DNS
 - b. Ex: Tube 3- after 2 min. have elapsed add 1.0 ml of DNS.
 - c. Continue this process for the remaining tubes.
- 6) Once DNS has been added to all of the tubes, boil them in a water bath for ten minutes. (This step is used to develop the color).

Interpretation:

Describe the color of test tube contents after boiling: **(0.5 POINTS for completing table)**

	Tube 0	Tube 1	Tube 2	Tube 4	Tube 8	Tube 16
Color						

Questions: (0.5 POINTS for answering question)

- 1) Did the time that the enzyme was in contact with the substrate influence the color observed in the tube?

- 2) What is DNS and why is it used in the experiment (see introduction)?

Experiment 3: Demonstrating Pepsin Activity

Materials:

- 1) 2 test tubes
- 2) 0.01 M HCl
- 3) Water
- 4) Pepsin Solution
- 5) Universal Indicator
- 6) Bovine Hemoglobin

Procedure:

- 1) Obtain 2 test tubes
- 2) Add 2.0 ml of 0.01M HCl to both tubes
- 3) Add 2.0 ml water to tube #1
- 4) Add 2.0 ml Pepsin solution to tube #2
- 5) Add 2 drops of Universal indicator to both tubes. NOTE THE COLOR OF EACH.
- 6) Add a few grains of Bovine hemoglobin to both tubes
- 7) After 30 minutes note the color of each test tube

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Interpretation:

(0.5 POINTS for completing table)

Describe color changes:

	Color when indicator added	Color 30 minutes after hemoglobin was added
Test Tube #1		
Test Tube #2		

Questions:

(1.25 POINTS for each question)

1) Did the pH of the sample Increase or decrease?

2) Is that more basic or more acidic?