

# **Exercise 1: Enzyme Specificity**

**Table 1: Microscopy Magnification** 

Well	Content	Glucose Concentration (mmol/L)
Α	3 drops 5% sucrose + 3 drops dH <sub>2</sub> O	Negative
В	3 drops 5% lactose + 3 drops dH <sub>2</sub> O	Negative
С	3 drops 5% sucrose + 3 drops lactase	Negative
D	3 drops 5% lactose + 3 drops lactase	60
E	3 drops 20% glucose + 3 drops dH <sub>2</sub> O	110

Photo 1: Glucose Testing Results



#### Question 1

Explain why a glucose test strip may be used to determine the catalysis of lactose and sucrose.

Lactose and sucrose are disaccharides consisting of one glucose molecule and one galactose molecule (lactose) or one fructose molecule (sucrose). The breakdown of each disaccharide results in glucose molecules that are detected by the glucose test strip.

## Question 2

Did the lactase enzyme show substrate specificity? Explain your answer by referencing your results in Data Table 1 and Photo 1.

The lactase enzyme did show substrate specificity by catabolizing lactose but not sucrose. The lactose-lactase solution in well D tested positive for glucose (60 mmol/L), a product of catabolism, while the sucrose-lactase solution in well C tested negative for glucose as shown in Data Table 1 and Photo 1. By reacting with only one of the two disaccharides, the enzyme demonstrated substrate specificity.

# Exercise 2: Enzymes, Temperature, and pH

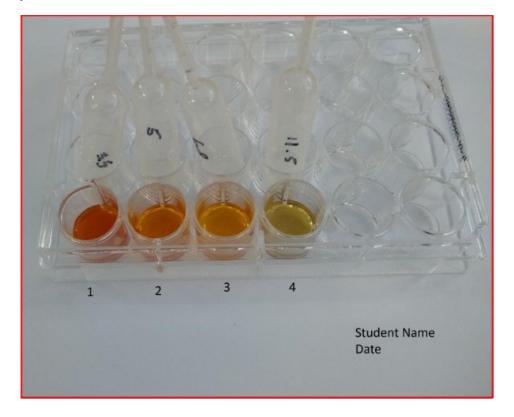
**Data Table 2: Enzymes and Temperature** 

Water Bath	Temperature (°C)	Initial Solution Color	Starch Present (Y/N)	Final Solution Color	Starch Present – Final (Y/N)
Hot	91	Black	Υ	Black	Υ
37°C	37	Amber	Ν	Amber	N
Ice	1	Amber-Brown	Υ	Amber	N

Data Table 3: Enzymes and pH

рН	Color	Starch Present (Y/N)
3.5	Orange	Y
5.0	Light Orange	Y
6.8	Yellow	N
11.5	Grey	Y

Photo 2: pH Results



#### Question 1

Describe the relationship between temperature and the enzymatic activity of  $\alpha$ -amylase.

- a. Of the three temperatures tested, which is the optimal temperature for enzyme activity?
- b. Did any of the temperatures cause the enzyme to denature?

Explain your answer by referencing your results in Data Table 2.

a. Of the three temperatures tested, the optimal temperature for enzymatic activity of  $\alpha$ -amylase is 37°C. When the temperature of the enzyme was reduced to 1°C, the reaction slowed and resulted in a brown solution when tested with IKI, indicating starch remained in the solution as reported in Data Table 2. When the lactase was heated to 90°C it became inactive and resulted in a black solution when tested with IKI, indicating a high level of starch in the solution. At 37°C, no starch was detected in the solution, suggesting that the enzyme has completely catabolized the starch molecules.

b. The 90°C treatment caused the enzyme to denature, but not the 1°C, as the 90°C solution tested positive for starch after 10 minutes at room temperature. Conversely, the 1°C solution tested negative for starch after 10 minutes at room temperature indicating that the enzyme was functioning to break down the starch molecules.

#### **Question 2**

What is the pH range for  $\alpha$ -amylase activity? Did any of the pH levels cause the enzyme to denature? Explain your answer by referencing your results in Data Table 3 and Photo 2.

The pH range for  $\alpha$ -amylase activity is 3.5-6.8, although some starch remained in the 3.5 and 5.0 solutions as shown by the orange colors in Photo 3. Very little starch was detected at 6.8 and indicated by the mostly yellow solution color. The 11.5 pH solution showed high levels of starch, as shown by the gray tinted solution in Photo 3, and suggests that pH values this low denature the enzyme.

#### Question 3

Do your results for the effects of both temperature and pH on  $\alpha$ -amylase activity support digestion of starch occurring in the mouth and intestines but not the stomach of humans? Explain your results by referencing your data and the temperature and pH of the mouth, stomach, and small intestines.

Yes, both temperature and pH results support that the digestion of starch occurs in the mouth and small intestines, but not the stomach of humans. The internal temperature of the human body is  $37^{\circ}$ C and this temperature resulted in the catabolism of all starch in the test as reported in Data Table 2. When testing for pH, almost all starch was catabolized at pH 6.8, and less starch catabolized and pH 5 and pH 3.5. The pH level of the stomach is 2-3 and is below the optimum range of  $\alpha$ -amylase activity. Conversely, the pH level of the mouth and small intestines is 6.5-7.5 and includes the pH value of 6.8 that resulted in the most complete catabolism of starch in this exercise as reported in Data Table 3 and Photo 2. The pH value of 11.5 is beyond the range of values found in the digestive tract and caused the enzyme to denature.

## **Extension Question**

John is lactose intolerant and adds a lactase enzyme tablet to cold beverages when consuming dairy products. Last night, John decided to make a cup of hot cocoa and added a lactase tablet to the hot beverage. After consuming the cocoa, John experience a reaction to the beverage indicating it contained lactose when consumed.

Apply your knowledge of enzymes, temperature, pH, and specificity to explain to John why the lactase pill added to the beverage did not function and how he could enjoy this dairy treat in the future.

The hot temperature of the beverage most likely denatured the proteins of the enzyme so that it was no longer functional. To remedy this, John should consume the tablet with lukewarm or cooler liquid immediately before drinking the hot cocoa, so that the protein structure of the enzyme remains intact.