**LAB – OBSERVING ENZYME ACTIVITY AND HYDROLYSIS**

**DESIGN**

**Background:**

Enzymes are proteins which help to speed up chemical reactions. Without enzymes many chemical reactions would take place at such a slow rate the organism may die during the process. Enzymes may aid chemical reactions which break down substances (polymers 🡪 monomers) and chemical reactions which build molecules (monomers 🡪 polymers). Enzymes may be affected by environmental factors such as temperature, pH, or substrate concentration.

If the concentration of the substrate is increased then more reactions will be able to take place until a certain point. Unless the concentration of enzymes is also increased then the enzyme activity will level off, after all there are only so many active sites so if they’re full, they’re full. One way to increase enzyme activity without actually increasing the substrate or enzyme concentration is by exposing more of the enzymes. Increasing the surface area of the tissue will do this.



**Aim:**

To be able to recognize the presence and function of enzymes in commercial products and evaluate which one works best due to the substrate concentrations of each.

**Hypothesis: ­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

 **Explanation of Hypothesis: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Variables:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Type** | **Variable** | **Control** | **Impact on results** |
| **Independent** |  |  |  |
| **Dependent** | The amount of hydrolization will be measured. | After the reaction, the liquid, or what was hydrolocized, will be measured in mL’s.  | If the amount of liquid is not read accurately the results could be skewed. The amount of liquid resulting from the reaction will indicate how much hydrolysis took place.  |
| **Control**  | Mass of detergent |  | If the mass of the detergent is not controlled then one sample may have a higher concentration than another making the results inaccurate.  |
| Volume of water |  |  |
| Volume of gelatin |  |  |
| Time | About 48 hours |  |
| Temperature | All samples will be tested in the same lab environment, which is at about 25˚C.  |  |
| Other |  |  |

**Materials:**

**Methods:**

Day 1

1. Obtain several samples of laundry detergent. List the ingredients on the label. Note the mention of the word enzyme in the ingredients list or on the label.
2. Place 1.8 g of instant gelatin or 1.8 g of sugar free gelatin in a beaker. Slowly add 50 mL of boiling water and mix, using a stirring rod. To this hot gelatin solution, very slowly add 0.7 g of washing soda (Na2CO3) while stirring.
3. Pour 5 mL of the liquid gelatin-soap mixture into 5 test tubes placed in a test tube rack. Remove the bubbles on the top of each tube with a Pasteur pipette. Cover the tubes tightly with plastic wrap and secure with tape. Allow these to cool at room temperature or in a refrigerator until day 2.

Day 2

1. Mark each test tube 1 - 5. Also, mark each test tube with the height of the gelatin with a straight line. It is from this point that you will measure the breakdown, or hydrolysis, of the gelatin from day to day.
2. Prepare a 10% solution of 4 detergents. To do this you will mix 1 cm3 of detergent with 9 mL of distilled water. Remember 1 cm3 = 1 mL.
3. Add about 1 mL or 30 drops of the first detergent solution to the gelatin surface of the first test tube. Repeat for each of the other samples. Reseal the test tubes and place it them in the test tube rack for each observation.
4. To the 5th test tube add 1mL or 30 drops of distilled water. This will be your control group.
5. Put the test tubes aside for 48 hours at room temperature.

Day 3

1. After 48 hours at room temperature, draw another straight line at the top of the gelatin layer. Measure the distance in mm between the first line and the second line. This indicates the amount of hydrolysis of the protein (gelatin) by the enzymes in the detergent. Record your data in the chart.

**DATA COLLECTION AND PRESENTATION**

**Data Collection:**

* This data collection table is incomplete. Please make sure it is complete before you submit it.

|  |  |  |
| --- | --- | --- |
| **Detergent****Type** | **Amount of Hydrolysis mm after 48 hours****(+/- 1 mm)** | **Rate of Hydrolysis mm/min****(+/- 1 mm/min)** |
| None - water |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

**Data Processing:**

* Calculate the number of minutes in 48 hours.
* Find the rate of hydrolysis for each samples using mm/hour.
* Show your work and explain what you are calculating.

**Data Presentation:**

Present, in a bar graph, the rate of hydrolysis for each sample.

**CONCLUSION AND EVALUATION**

* This conclusion and evaluation is not in the formal format. You will not need to write a formal conclusion and evaluation. The only requirement for this section is to answer the following questions:

Questions

1. What is an enzyme? What is a substrate?

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1. What environmental factors influence enzyme activity? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. Why was it important for you to include a 5th test tube to which you added water? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. Why did you observe a difference in gelatin levels among the different test tubes from day 2 to day 3? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. Why are enzymes added to many laundry detergents? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
5. Do you think detergent enzymes are stable at temperatures found during a hot water cycle in a washing machine? Are enzymes in detergent stable in the presence of bleach? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
6. What kind of stains do you think detergent enzymes could most effectively remove? Explain. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
7. Many enzymes are named after their substrates. What kind of substrate might the enzymes known as proteases act upon? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
8. How do you think changes in room temperature could affect your results? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
9. How do you think changes in pH could affect your results? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_