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| **Project 3.2.4: The Amylase Investigation** |

Introduction

In Project 3.2.2 or Project 3.2.3, you investigated the basic structure of the digestive system as well as the importance of enzymes in the chemical breakdown of food. Our diet supplies us with the raw materials needed to synthesize the energy compound adenosine triphosphate (ATP), an amazing molecule that provides power to the human machine. However, just as raw petroleum out of the ground must be processed before it can be used in automobiles; food must be broken down into much smaller molecules before it can be used efficiently by the body.

As part of the digestive process, chemical reactions break polymers in our food into the monomers that can be used by the body for energy production and for growth and repair. These chemical reactions are catalyzed by enzymes, proteins that increase the rate of reaction without being changed or used up. In PBS, you built a model to explore the structure, function and action of enzymes. In this activity, you will look closer at factors that have an impact on how these important proteins operate. Even though all enzymes are specific to a given substrate, many behave in similar ways under extreme environmental conditions.

Amylase is an enzyme that has the ability to break down starch, a polysaccharide, into smaller disaccharides. In the last project, you saw that two types of amylase function in the human digestive system, salivary amylase and pancreatic amylase. Each enzyme functions in a different area of the body, but both work to harness the power in carbohydrates and break these complex molecules into simple sugars. In this project, you and a partner will design and carry out experiments to investigate the way in which factors such as temperature and pH can affect the action of amylase. Enzymes are specific to the reaction they catalyze and each one functions under unique environmental conditions. Your team will investigate one of the following questions:

* Question #1: How do changes in pH impact an enzyme’s rate of reaction?
* Question #2: How do changes in temperature impact an enzyme’s rate of reaction?
* Question #3: Does the percent concentration of an enzyme impact the rate of reaction?

Equipment

* Computer with Internet access
* Laboratory journal
* *Biomedical Sciences Experimental Design* handout
* *How to Write a Scientific Laboratory Research Report* handout
* Science Laboratory Report rubric
* Amylase enzyme, 5% solution in water
* Lugol’s iodine solution
* HCl (aq) pH3
* HCl (aq) pH6
* NaOH(aq) pH8
* NaOH(aq) pH11
* Starch solution
* Water (distilled)
* Balance (triple beam or electronic)
* Beaker (250mL)
* Beaker (500mL)
* Graduated cylinder (25mL)
* Hot plate
* Ice
* pH paper
* Disposable 1mL pipettes
* Test tubes
* Test tube rack
* Thermometer
* Safety glasses
* Lab apron
* Stirring rod
* Stop watch
* Calculator

Note: The list of equipment is meant to be a guide for ideas. If your design requires something not listed, check with the teacher to see if it is available.

Procedure

**Part I: Standard Reaction**

In this part of the project, you will run a standard reaction to investigate the action of amylase and an indicator that can be used to monitor reaction progress.

1. As a starting point, do some research on amylase including where it is found in the body, the reaction it catalyzes, and the ideal chemical and physical conditions of the location where it is used.
2. Remember that as an enzyme works, it interacts with its substrate(s) and converts the substrate(s) to product(s).

**Enzyme**

Substrate(s) ------------🡪 Product(s)

1. In your laboratory journal, write the simple equation that describes the amylase reaction that occurs in our bodies. What is/are the substrate(s)? What is/are the product(s)?
2. Put on all safety equipment.
3. Place 5 mL of starch solution in a test tube. Add 3 drops of iodine to the starch solution.
4. Add 0.5 mL amylase solution to the test tube and immediately start the timer. Stop the timer when the solution has lost most of its blue-black color. Note: the reaction may take a few minutes. Record this time.
5. Note that the rate of reaction is defined by the amount of reactant used up per second, or the amount of product produced per second. This means that you can measure the rate of reaction of amylase by determining how long it takes for the starch to be broken down into sugar.
   * + You will be using 5 mL of the 50 mg/mL starch solution. This means you will use 250 mg of starch in each reaction. (5 mL x 50 mg/mL = 250 mg)
     + In order to calculate the reaction rate, divide 250 mg by the number of seconds it took for the starch to break down.

250 mg starch ÷ # seconds = reaction rate

* For example, if the starch took 60 seconds to break down, 250 mg divided by 60 seconds = 4.17 mg/sec

1. Calculate the rate of reaction using the information in the above step. Record the results in your laboratory journal. Clean the test tube.
2. Share your results and discuss your findings with the class.

Part II- Experimental Design

In this project, you and a partner will investigate how a specific environmental factor influences enzyme function. You will design and carry out your own investigation.

1. Work with a partner on the investigation question assigned by your teacher. Record all of your work in your laboratory journal.
2. Discuss with your partner an experimental design. Follow the steps outlined in the Biomedical Sciences Experimental Design handout.
3. Keep in mind all aspects of valid experimental design, including controls.
4. Use the following guidelines to help you with your design:
   * Do not put a test tube directly on the hot plate or on ice. Place the test tube in a beaker with ice or in a beaker with water on the hot plate.
   * Each time you run the experiment, you will need one tube of starch and iodine and one tube of amylase solution.
   * Any adjustments to pH, temperature, and percent concentration should be done to the amylase solution and not to the starch/iodine solution.
   * Heat alone will make the starch break down above 50˚C. If you add hot enzyme solution to starch and the color change is rapid, you won't know whether the breakdown of starch is due to the elevated temperature or to the rate of the enzymatic reaction. So if you test any temperature above 50˚C make sure to bring it back down below 50˚C before combining it with the starch solution. (You are not testing the enzyme at the hot temperature, just whether the enzyme was denatured at that temperature or not.)
5. Write a draft of your procedure on loose leaf paper; include experimental design, possible data tables and graphs that will be used to summarize the data. Explain safety precautions that must be taken when carrying out the procedure. When the draft is completed, meet with the teacher for approval.
6. Make any additions or corrections suggested by the teacher and write a formal, detailed lab procedure in your laboratory journal.
7. Create the data table you will use.
8. Proceed with the actual experiment when given approval.
9. Clean all tools and equipment and return supplies to the designated area.
10. Share your findings with the class.
11. Show your laboratory journal with the completed project to the teacher before proceeding.
12. Obtain the *How to Write a Science Lab Report* handout and the Science Laboratory rubric from your teacher.
13. Using the handout, create a written report which will be submitted to the teacher for evaluation. This report should follow the structure outlined on the document and will be scored according to the Science Laboratory Report rubric. Write the report for one of your experiments (not both).
14. Answer the Conclusion questions.

Conclusion

1. Based upon your experimental results, write a paragraph discussing the relationship between environmental conditions and enzyme function.

Temperatures that are high cause the enzymes to denature or unfold. Colder temperatures affect the enzymes activity but is less likely for the enzyme to denature.

1. High fever can be a very dangerous condition for anyone, as it is an indicator of a serious infection. Based on this project, suggest a possible explanation for this medical concern.

A high fever can overheat the body and cause enzymes in the body to not operate well.

1. Salivary amylase and pancreatic amylase are released by two accessory organs of the digestive system. In what structures do the enzymes actually chemically digest food?

Amylase digests starch in the mouth. and the rest of the digestion occurs in the stomach and small intestine.

1. Based on the information you have learned about the digestive system, describe the optimal pH for the action of pancreatic amylase. Do classroom experimental results seem to support this finding? Why might there be differences?

Denaturization is the main affect of ph on amylase. The enzyme will change its shape and basically stop working.