Determining the optimal temperature and ph of barley amylase assignment

Determining the Optimal Temperature and PH of Barley Amylase Abstract The purpose of this experiment was to find the optimal temperature and pH of barley alpha-amylase. I hypothesize that the optimal temperature would be 55 degrees Celsius and the optimal pH would be 5. 5. In this experiment, the starch is used as a substrate to examine the optimum temperature and pH for the reaction of alpha amylase. It is known that the measuring of disappearance (absorbance) of the substrate starch with iodine using spectrophotometer will show the concentration of the substrate which will also reflect on the reaction rate.

Once the reaction rates are figured out, the optimal temperature and pH can be determined. The result concluded that the optimal temperature was at 50 degrees Celsius and the optimal pH was at 5. 0. I have evaluated that my hypothesis was not supported through this experiment however, it has been clarified that the optimal temperature and pH of barley alpha-amylase turns out to be around 50-55degrees Celsius, and the pH of 5. 0-5. 5 based on the research of MacGregor in 1978. Introduction The purpose of this experiment is to determine the optimal temperature and pH of barley amylase with starch using the absorbance rate of alpha-amylase.

When reactants undergo a reaction, enzymes, proteins that serve as chemical agents without being consumed by the reaction, are used to speed up the reaction. (Campbell, Reece, 2005) Every enzyme has its own optimal temperature and pH which they can be most efficiently active. Thus, in order for an enzyme to be most efficiently active, the environmental factors, such as temperature and pH, must support the reaction. The increase in temperature instantaneously increases the rate of enzymatic reaction by

causing the molecules to move rapidly which alludes the substrates to collide with active sites more frequently.

However, because the substrate binds to the active site by weak interactions, such as hydrogen bonds and ionic bonds, temperatures above certain degrees causes the disruption of the weak bonds and ends up with the denatured protein molecule. (Campbell, Reece, 2005) This works same for the optimal pH value for an enzyme. An example of the importance of the relationship between the pH and enzyme would be found in the activities and the functions of pepsin. Pepsin is a digestive enzyme in the stomach that works best at pH 2. If pepsin was located in the area where pH is 6-8, the pepsin would be denatured.

Therefore in a reaction, the greatest molecular collisions and the fastest conversions of the reactants to product molecules are at the optimal temperature and pH. (Vliet, 2008) In this experiment, the starch is used as a substrate to examine the properties of the alpha amylase-enzyme’s, harvested commercially form germinating barley seeds, optimum temperature and pH for the reaction of alpha amylase. (Vliet, 2008) Based on the research of MacGregor in 1978, I hypothesize that the optimal temperature of the temperature will be at 55 degrees Celsius and the optimal pH will be at 5. . According to MacGregor (1978), found the optimum temperature of malted barley alpha-amylase to be 55 degrees Celsius and 5. 5 optimum pH. Materials and Methods I followed the basic procedure for the temperature lab and the pH lab for the experiment of determining the optimal temperature and pH of barley amylase. First, I set the spectrophotometers to the lmax which determines for the starch/I2KI

solution (560 nm). Then, in an Erlenmeyer, add 35 ml starch and 35 ml of distilled water. Then I thoroughly mixed starch before drawing volume.

This flask was used as my reaction flask. Then I put I2KI indicator (0. 1 ml = " 100" on eppendorf) in each of 12 cuvettes. After that, I made the blank by putting 5 mls of water (5 ml glass pipette) into one of these cuvettes. Then, I took the initial reading pipette 5 mls from Erlenmeyer (5 ml glass pipette) into a cuvette. Then took an absorbance reading and recorded it on the data sheet. For the temperature experiment, five different water baths were prepared at five different temperatures, 15, 30, 45, 55, 60, and 70 degrees Celsius.

In each water bath, there are 35 ml of distilled water and 35ml of starch in separate flasks. When these reactions reached the appropriate temperature, a T= 0 reading was taken and I added enzyme. Then I followed the basic step. For the pH experiment, six different starch solutions were prepared by mixing 35ml of stock starch solution with 35ml of water buffered at the pHs of 4. 0, 4. 5, 5. 0, 5. 5, 6. 0 and 6. 5. All these starch solutions are in the separate reaction flask. Then the basic procedure was followed. After obtaining all the data, the reaction rate was ound using the following formula: Reaction rate = (1/2 delta Absorbance)/ (Time that Ai -1/2delta Absorbance Took) (Vliet, 2008) Results The results for the absorbance readings over time of reactions at different temperature turned out to be a negative slope, bowed-in graphs. Couple of the points from different temperatures overlapped but generally the graph showed consistence. From initial point to around 2-5 minutes, most of the graphs showed drastic drops

in the absorbance readings but as the time moves on from 4-20 minutes, there were only minor changes causing the graph to shape bowed-in.

The same result was shown for the pH graph as well. There were drastic changes within 0minutes to 10 minutes, but the rest of the 10 minutes only showed very minor changes, resulting in bowed-in shape graph. For the Reaction rate vs. temperature graph, the graph fluctuated up and down but at 55 degree Celsius, the reaction rate recorded highest, 0. 327. However, at 70degree Celsius, the reaction rate drastically raised but did not reach as far as 0. 327. For the reaction rate vs. pH graph, the graph is shaped like a bell curve except at the pH of 6. , the reaction rate dropped drastically and went back up at 6. 5 but did not reach up to the highest point, which was at the pH of 5. 0, resulting in the reaction rate of 0. 329. Discussion My results did not support my hypothesis that the optimal temperature of barley alpha-amylase would be 55 degrees Celsius and the optimal pH would be at 5. 5. My results showed that the optimal temperature was 50 degrees Celsius and the optimal pH would be at 5. 0. However, my results support that the actual optimal temperature and pH is around 50 to 55 and 5. 0 to 5. 5.

The general trends in my graphs for absorbance readings over time of reactions at different temperature and pH was that all the graphs turned out to be a bowed-in shape. This tells that there are big drops of absorbance readings from initial time to under 10 minutes but the rest of the times, the absorbance did not change much at all. For the graphs for reaction rate vs. temperature or pH showed a general trend for the bell curve. However, the graphs did not turn out to be the perfect bell curves. On the reaction rate vs.

pH, the reaction rate for the pH 6. 0 seems to be a miscalculation or human experimental error.

The miscalculation may have been made while drawing a best-fit curve on the absorbance readings over time of reactions at different pH graph. Also there is a great possibility of experimental error. If there are changes to be made on the procedure to obtain more accurate result, the method of using the best-fit curve graph on the absorbance reading over time graphs should change. Because for this specific lab, it was not allowed to use mechanical devices to aid the graph of the best-fit curve graph, there must have been great consequences in dealing with finding the closest optimal temperature or pH on the reaction rate graph.

Also, the slight mistake that was made on the best-fit curve may have affected the reaction rate graph majorly. Literature Cited Campbell, N. A. and J. B Reece. 2005. Biology 7th edition. San Francisco. Benjamin Cummings. MacGregor, A. W. 1978. Alpha-Amylase I from Malted Barley—Physical Properties and Action Pattern on Amylose. Journal of Cereal Chemistry 55: 754 – 765. Vliet, Kent A. 2008. A Lab Manual For Integrated Principles of Biology Part One-BSC2010L third edition. Pearson Custom Publishing. United States of America