The Effects of Varying Enzyme Concentration, Substrate Concentration, pH, and Temperature on Fungal Amylase Activity

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Abstract

The questions investigated in this experiment regard the macromolecule known as enzymes. Enzymes are specialized proteins that help catalyze reactions within a cell. The experiment tested the effect of varying enzyme concentration, substrate concentration, pH, and temperature on the speed of enzymatic reactions.

The results of the experiment testing the effect of varying enzyme concentration on the speed of enzymatic reactions showed that the higher the enzyme concentration, the faster the reaction occurs. The conclusion that the speed of an enzymatic reaction is increased when there is high enzyme concentration can be drawn from this experiment.

The results of the experiment testing the effect of varying substrate concentration on the speed of enzymatic reactions showed that the higher the concentration of substrate, the slower the reaction occurs. The conclusion that increased substrate concentration slows enzymatic reaction completion time can be drawn.

The results of the experiment testing the effect of varying pH on the speed of enzymatic reactions showed that the enzyme functioned best at a certain pH. The conclusion can be made that enzymes function at an optimal pH and any deviation from that pH hinders the speed of reaction from these results.

The results of the experiment testing the effect of varying temperature on the speed of enzymatic reactions showed that the enzyme functioned best at an optimal temperature. The conclusion that enzymes function at an optimal temperature and any deviation from this temperature hinders the speed of the reaction can be made from these results.

Introduction

Enzymes are generally proteins. Being proteins, they are composed of amino acids linked together by peptide bonds in their primary structure. They are given shape by the alpha helices and beta-pleated sheets in their secondary structure and 3D shape by the interactions between R groups in their tertiary structure. Some proteins contain quaternary structure, multiple polypeptide chains, but not all do.

With proteins, shape determines function. The shape of a protein determines its function in a cell, and any disruption to that shape also disrupts the biological activity of that protein. The addition of ions or salts, a change in pH or temperature, or a change in the primary structure of a protein can greatly alter that protein's shape, thereby altering its function, or, if the change in temperature, salt or pH is drastic enough, even completely denature it, rendering it useless.

An enzyme is a special kind of protein; it helps carry on all of the complex reactions necessary for the continuation of life. Enzymes act as catalysts for most chemical reactions in a cell. Chemical reactions consist of the breaking and formation of covalent bonds between atoms in a cell. In order to break and reform the bonds, molecules must have an input of energy known as the energy of activation. Once a bond breaks and new bonds are formed, that energy is either given off as heat or is stored in the bond. The entire purpose of an enzyme is to lower this activation energy so a cell does not have to expend extra energy on the breaking and reforming of bonds. With less of an energy input, the cell is also able to catalyze more reactions in less time than it would without enzymes.

Because enzymes have a structure-function relationship, they only are able to catalyze one type of reaction. The enzyme has an indentation on its surface known as an active site. The active site is where the substrate, the reactant, binds to the enzyme. After binding with the enzyme, both the enzyme and substrate undergo a conformational change in order to align them in the proper position for the reaction to take place. Once the reaction has occurred, the enzyme releases the products and the enzyme can start to catalyze another reaction because the enzyme is not destroyed as a result of the reaction (Shefferly 2011).

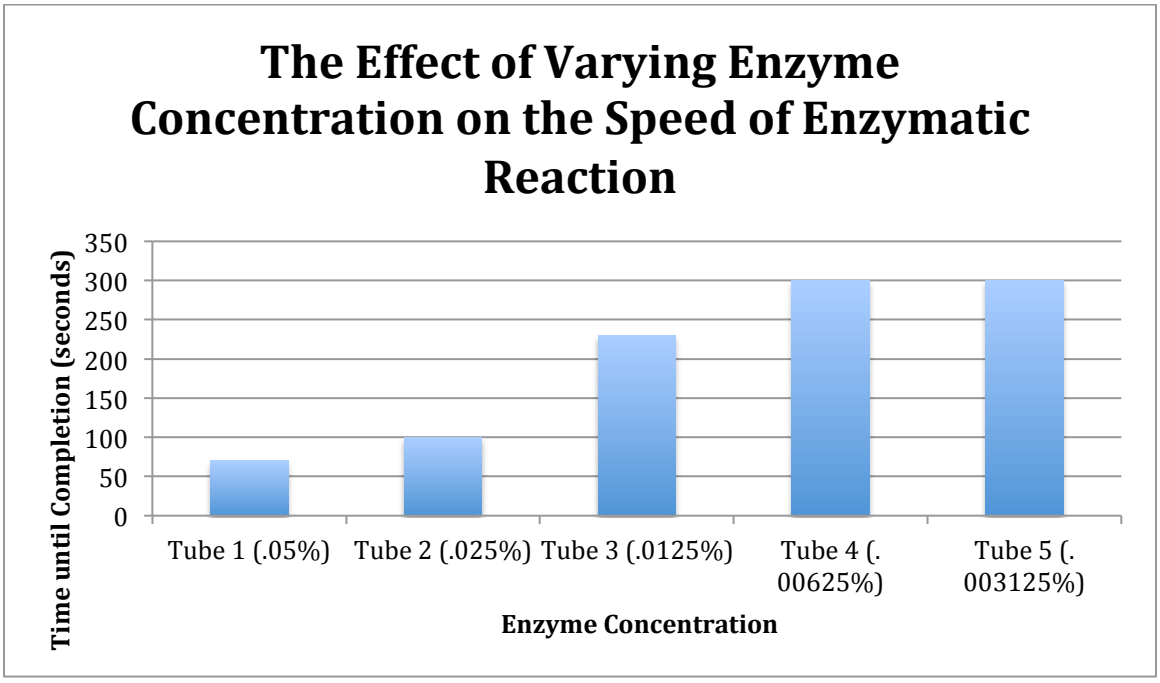
In one of the experiments performed, the effect of enzyme concentration of the enzyme fungal amylase was tested to see how concentration affected the time for reaction completion. The hypothesis for this test was if the enzyme concentration was increased, then the speed of the reaction would decrease. The second experiment tested the effect of substrate concentration on the speed of the enzymatic reaction. The hypothesis for this test was that if the substrate concentration was increased, then the speed of the enzymatic reaction would increase. The third test performed tested the effect of pH on amylase activity. The hypothesis was that if the pH was changed beyond its optimal pH then the speed of the reaction would decrease. The final experiment tested the effect of temperature on enzyme activity. The hypothesis was if the temperature was changed from the optimal temperature, then the speed of the enzymatic reaction would decrease.

Results

In the experiment, we set out to test the effects of varying enzyme concentration, substrate concentration, pH change, and temperature change on the speed of an enzymatic reaction. In regards to concentration, the test tube with the highest concentration of

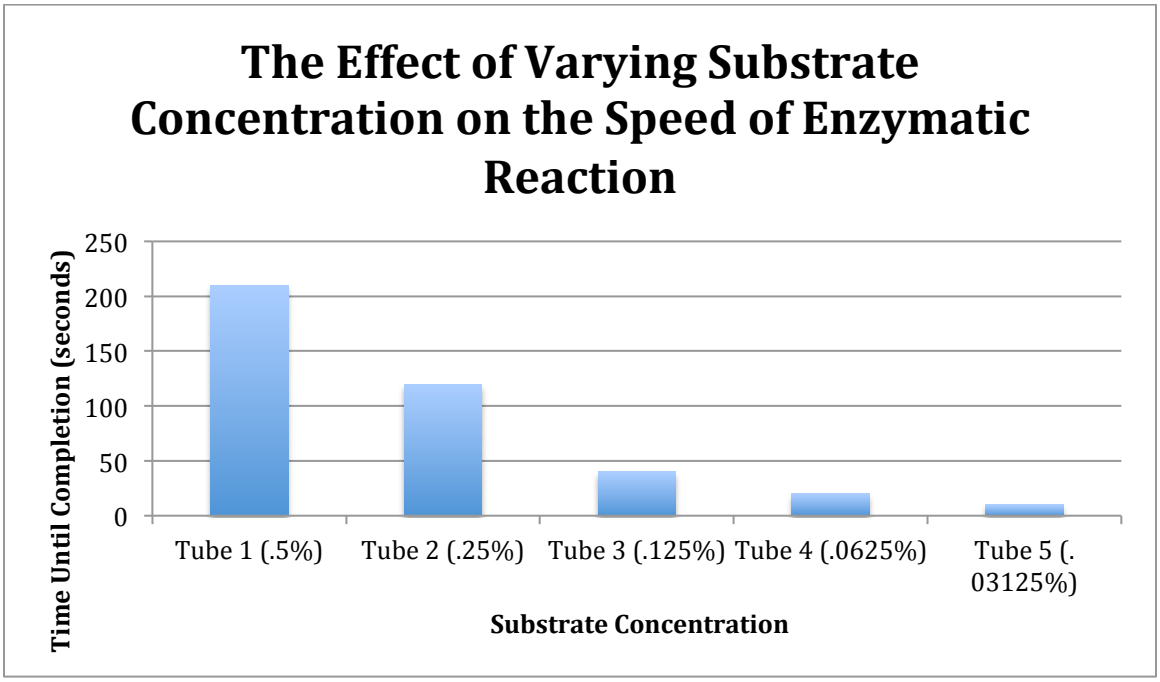
enzyme had the highest enzymatic reaction speed. Tube one was the tube with the highest enzyme concentration at .05% amylase. The tubes after tube number one had a decrease in enzyme concentration and therefore a decrease in the enzymatic reaction speed. The results of this experiment are shown by figure one. (NOTE: In tube 4 and tube 5, after the 300 second mark was reached, the experiment was stopped.)

FIGURE ONE



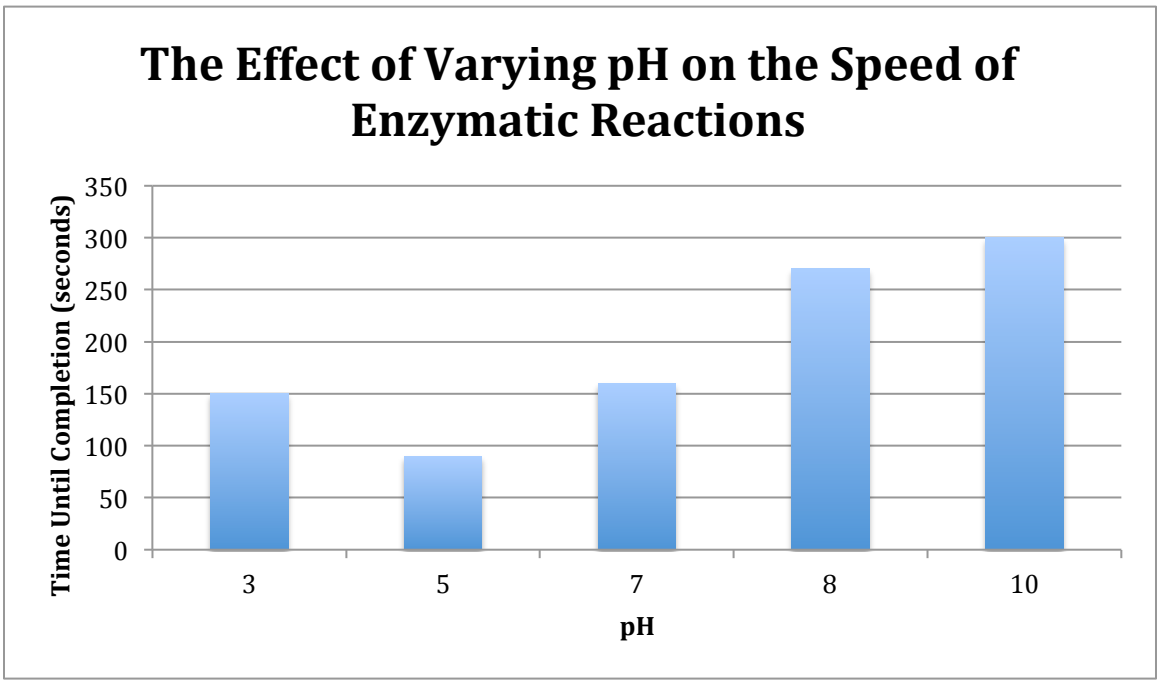
In regards to concentration of the substrate, the tube with the lowest amount of substrate concentration (Test Tube 5) had the highest speed of enzymatic reaction. The tube had a concentration of .03125%. The tubes with a higher substrate concentration showed an increase in the time needed to complete the enzymatic reaction. The results of this experiment are shown in figure two on the next page.

FIGURE TWO



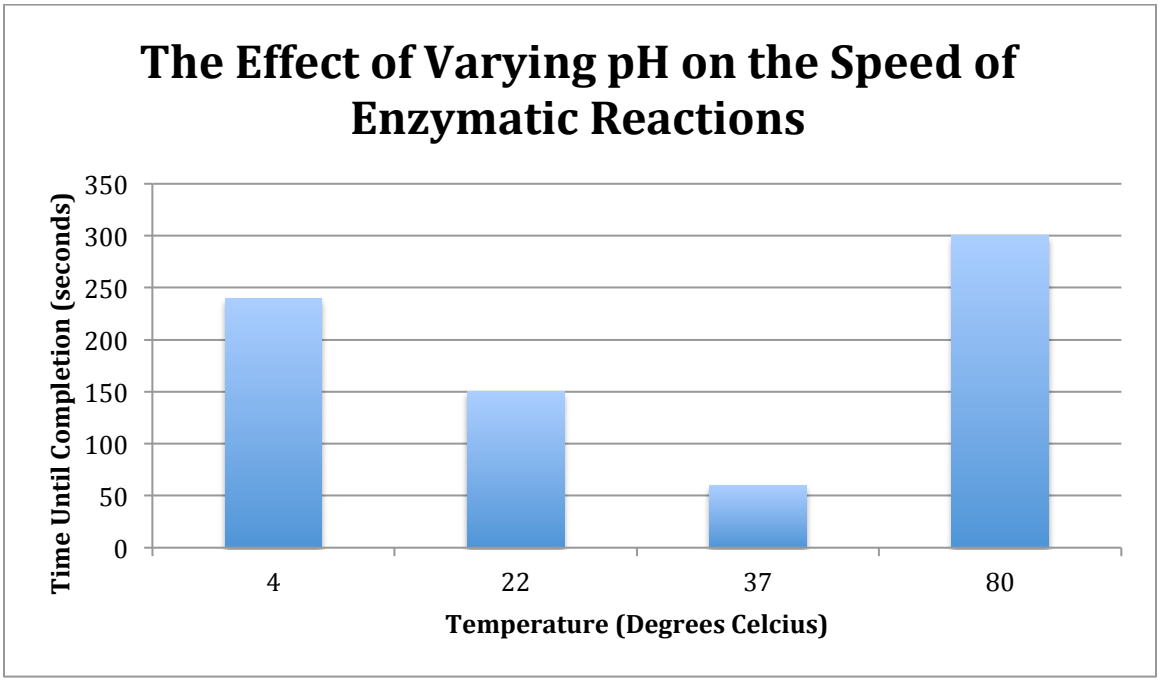
In regards to pH, test tube 2, with a pH of 5, had the highest rate of enzymatic reaction. The other test tubes with varying pHs showed a decrease in enzymatic activity. The results of this experiment are shown in figure 3 on the next page. (NOTE: In test tube 5, with a pH of 10, the experiment was stopped after 5 minutes.

FIGURE THREE



Finally, in regards to temperature, the test tube with the highest rate of reaction was test tube 3 at 37 degrees Celcuis. The other varying temperatures, like pH, showed an increase in amount of time to complete the reaction. The results of this experiment are shown in figure four on the following page. (NOTE: In test tube 4 (80 degrees Celsius) the test was stopped at 5 minutes.)

FIGURE FOUR



Discussion/Conclusions

For all four tests in the experiment, the data supported the hypotheses. In the first test, which tested the effect of enzyme concentration on the speed of completion of enzymatic reactions, the data showed that the higher the enzyme concentration, the greater the speed of the reaction. The conclusion can be drawn that the higher the enzyme concentration, the higher the speed of the reaction will be. This conclusion makes sense because enzymes are reused in reactions, and if there are more enzymes, then the reaction can occur more rapidly. In the second test, which tested the effect of varying substrate concentration on the speed of completion of enzymatic reactions, the results showed that the higher substrate concentration slowed the speed that the enzymatic reaction took to come to completion. These results would make sense because an increased amount of substrate would give the limited amount of enzyme more work to do and more reactions to catalyze for the reaction to come to completion. For the third test, which tested the

effect of varying pH on the speed of enzymatic reaction, the fastest reaction completion speed was with the pH of 5. The other pHs' reaction times were extremely long compared with pH 5. This would make sense because an enzyme has an optimal pH at which it functions the best. Different pHs can hinder enzyme function or even denature it and render it completely useless. For the fourth and final test, which tested the effect of varying temperatures on the speed of enzymatic reaction completion, the fastest reaction completion time was the trial with a temperature of 37 degrees Celsius. The other trials had a much longer time in comparison to the trial with 37 degrees Celsius. These results would make sense because enzymes have an optimal temperature at which they function best. Deviating from this optimal temperature can hinder the speed of the reaction or completely denature the protein if the temperature is high enough.

Works Cited

Shefferly, Nancy. *BSC 115/118 Lab Manual*. Tuscaloosa: University Printing, 2012. Print.