The factors affecting enzyme activity

Title: The Factors Affecting Enzyme Activity Speeds Problem: How do the factors of temperature, pH level, substrate concentration and enzyme concentration specifically and significantly alter the rate of enzymatic reactions? Background Information: Enzymes are critical to the function of human life. They are the controllers of all the chemical reactions within our bodies – they catalyze relatively unreactive metabolic reactions, making them reactive. With very rare exception, enzymes are proteins; more importantly, they are catalysts (chemical agents). The function of catalysts is to accelerate the rate of a reaction without being consumed by the reaction. They accomplish this by binding to the reacting molecules, called the substrate, which forms an enzyme-substrate complex. Thus, enzymes are substrate-specific.

Of extreme importance to the enzyme’s function is its active site; it is a pocket of sorts, an indentation whose shape is absolutely critical – the substrate must fit it perfectly, or the enzyme cannot bind to it, and thus it will remain unreactive. Furthermore, the enzyme can have an induced fit, which brings chemical groups of the active site into positions that enhance their ability to catalyze the chemical reaction (usually stressing a bond reducing activation energy). That activation energy is supplied by the enzyme and is the initial investment of energy required for starting a reaction; it triggers the more reactive transition state of the substrate. Enzymes, like anything else biological, are not perfect. They rely heavily on their tertiary shape to function correctly – denaturation (via structural change, which could likely occur through factors taken to extremes, some of which we will be testing) will destroy them.

In addition to denaturation, there are inhibitors which can be affective in counteracting the effects of an enzyme. For example, the allosteric site of an enzyme acts as an on/off switch for the enzyme. Allosteric regulation occurs when the enzyme binds to the allosteric site, which either stabilizes conformation or stabilizes the nactive form of the substrate. In other words, the substrate is completed with enough of the product that it does not require any more. Another form of inhibition is feedback inhibition, which is the switching off of a metabolic pathway by its end-product, which acts as an inhibitor of the enzyme within that pathway.

All of these characteristics and potentialities of enzymes are critical in predicting the affect various factors might upon the enzymatic reaction (in this case, the hydrolysis of starch). In this particular reaction, amylase (the enzyme) breaks the bond between adjacent glucose units and inserts water molecule ions, thus breaking the glucose down into monosaccharides called maltose. Enzymes have a preferred range of values for any of the variables in this experiment – a range of optimal conditions. When these optimal conditions are exceeded or failed to be met, the enzyme will be much less effective.

Thus, we will be testing for the optimal conditions of amylase, and how drastically or not drastically the four independent variables affect amylase’s efficiency. Hypotheses: If the temperature is increased, than the enzymatic rate will also increase. If the pH level is increased, than the enzymatic rate will decrease. If the substrate concentration is increased, than the enzymatic rate will increase. If the enzyme concentration is increased, than the enzymatic rate will decrease.

Procedure: The general procedure to be used to observe the enzymatic reaction (the hydrolysis of starch) will be the detection of starch in the substance. If there is no starch remaining, it has all been hydrolyzed, and the amylase was successful. The rate it takes for that to occur is the efficiency of the enzyme, and will vary as we vary the independent variables. The dependent variable in all the reactions is time, or the rate of hydrolysis. The experimental group for all the reactions will be the groups tested at varying levels of the independent variable (ideally, these would be the groups that only possess the independent variable, but we will find it difficult to create a group possessing no temperature or no pH, for example). In this context, the control group for all the reactions will be the general procedure for detecting starch; the levels of the independent variables in that procedure will serve as our constants to be compared against.

Thus, for the first test, the independent variable is temperature. Using four flasks, four separate temperatures will be gauged with a constant amount of amylase (. 5 mL) at simultaneous time intervals (one minute, and then every 2 minute interval until the starch is completely hydrolyzed). The varying rates will thus (hopefully) be a direct result of the varying temperatures (5, 20, 37, 80), being the only intentionally and notably changed factor among the four flasks. In the second test, the independent variable is pH.

It is exactly like the first test, except the four flasks vary in pH, rather than in temperature. The third procedure tests the independent variable of substrate concentration. It does this by consistently increasing the concentration of substrate in each flask. The first flask begins with 20 mL of starch (50% dilution) and is mixed thoroughly. 0 mL of that mixture is passed on to the

second flask, which repeats the procedure (25% dilution), and passes on 20 mL of its mixture to the next (12.

5% dilution), and so on to the fourth (6. 25% dilution). Finally, . 1 mL of amylase is added to each flask and mixed, and tbecause they now vary in dilution only, the time difference it takes for the complete hydrolysis will be a result of the varying dilutions. The last test is exactly like the third test, but uses enzyme concentration rather than substrate concentration as the independent variable.

It thus begins instead with 5 mL of amylase, and follows the above method to pass 5 mL of each mixture on, systematically reducing the dilution of the amylase. Recording procedure is similar to all of the other tests listed. Data Tables: (Time = Time until completely hydrolyzed, in minutes) Effect of Temperature Time Class Avg. T.

Historical Avg Flask 1 – 5° 20 18. 8 16 Flask 2 – 18° 8 6. 4 7. 2 Flask 3 – 37° 6 5. 6 4.

2 Flask 4 – 80° 1 1 2. 1 Effect of pH Time Class Avg. T. Historical Avg Flask 1 – pH 5 1 1. 8 1.

9 Flask 2 – 6 4 3. 8 4. 1 Flask 3 – 7 6 7. 6 9. 2 Flask 4 – 9 hrs.

+ 3 hrs. + 15. 8 Effect of Substrate Concentration Time Class Avg. T.

Historical Avg Flask 1 – 50% – 10 10. 3 Flask 2 – 25% – 6 6. 3 Flask 3 – 12. 5% – 4 3.

4 Flask 4 – 6. 25% – 3 2. 1 Effect of Enzyme Concentration Time Class Avg. T. Historical Avg Flask 1 – 20% 1 1 2.

9 Flask 2 – 4% 4 4 3. 9 Flask 3 – . 8% – – 1 Flask 4 – . 16% – – 4 Analysis: 1. Based on these finds, the optimal temperature for human amylase appears to be 80 degrees Celsius; in this range, the amylase completely hydrolyzes the substance, removing all starch within one minute – a very efficient pace.

In retrospect, it would seem logical for the enzyme to seek optimal conditions at the stable body temperature of the species, but it does not appear to do so. Therefore, it may be possible for the optimal temperatures in oyster, pig or bacterial amylase to not vary all that much. 2. It does not appear to hold true that both temperature extremes slowed the rate of hydrolysis in my experiment, since the high extreme, 80, was the fastest by far.

Perhaps 80 is actually a moderate number, relatively, and a higher extreme needs to/could be chosen for testing. The cold appeared to simply choke and slow down the enzyme molecules to increase the time of hydrolysis significantly. 3. I predicted that as the pH level increased, the enzymatic rate would decrease, leaving the most optimal condition to be at the lowest pH level. That is because the lower the pH, the more acidic the substance; the more acidic the substance, the more reactive I expect to the enzyme to be.

However, this does not take into consideration the internal pH levels of humans as a relative point of necessity, and although the finding confirm my hypothesis, a lower pH is probably necessary to test how long the trend holds true. . At a higher concentration of substrate, hydrolysis, of course,

took longer. This is perfectly logical, because if you have more (or a greater density) of material to be hydrolyzed, the enzyme has more work to do and it will take longer. I predicted these relationships incorrectly in my hypothesis, and I honestly have no idea what I was thinking at the time. 5.

Likewise to question #4, when there is more of the enzyme, it will be able to completely hydrolyze the substance faster than if there were less of the catalyst present. It thus follows that more enzyme did accordingly result in shorter hydrolysis times in this experiment. Conclusion: My hypotheses proved to be half wrong, half right. The half I missed, though, is complete common sense. It is quite obvious that if you have more material, it will take longer to hydrolyze, and if you have more enzyme, it will be a quicker job.

Therefore, I am quick to dismiss that particular part of the hypothesis as temporary dyslexia. Regarding the part of the hypothesis that held true with my experiment, I see in hindsight that they are limited, over-generalized hypotheses to begin with. Suggesting a general trend to providing optimal conditions may hold here, but because all enzymes have an optimal value for performance, the likely scenario is that I simply have not hit the other extreme where the curve falls off into a parabolic shape. Instead of hitting the max, I simply limited my set of data to show a limited relationship. Looking at it from another point of view, though, this particular enzyme may simply like heat, or like very acidic substances – not all enzymes can be expected to require nor prefer moderation. In those terms, perhaps it was a lucky guess based upon a knowledge of enzymes' role as a catalyst.

As far as sources of error, there were definite time constraints that led to rushed experiments and/or unfinished data – a major source of error in the final set of data. Like always, the impossibility of creating and maintaining perfect conditions will be a factor in the source of error; for example, the lab just cannot be humanly timed and executed to perfection, with every drop of iodine dropped perfectly simultaneously and every molecule of water that exact degree it is intended to be. Future studies would probably involve a larger set of data, tested more carefully and with more in-depth research of this particular amylase to suggest why it might like these extremes, or if there is some other paradigm that we are missing in figuring out why it seems to be shooting over or missing that “ climax” or critical/optimal point completely in the data we received.