Investigating the effect of ph of hydrogen peroxide upon the activity of catalase...

Variables involved:

Different pH levels will affect the Catalase enzyme by determining whether the enzyme activity will increase, decrease or if it will denature altogether, so the pH level of the Hydrogen Peroxide, which Catalase breaks down into oxygen and water, will be our independent variable.

The temperature of the Hydrogen Peroxide will affect the rate of reaction with the Catalase, thus this will be a controlled variable and will need to be kept constant.

The substrate concentration can also affect enzyme activity in regards to its rate of reaction, so this will also need to be kept constant.

The amount or volume of the chicken liver needed to get the Catalase enzyme can also affect the rate of reaction; therefore this will also be needed to be kept constant.

I will also be using buffer solutions to change the pH of the Hydrogen Peroxide, thus the amount or volume used will also be kept constant.

All these variables can affect the rate at which oxygen is broken down from Hydrogen Peroxide by the enzyme Catalase, so the measurement of how much oxygen is diffused will be our dependent variable.

How to deal with the variables:

The pH level of the Hydrogen Peroxide is the independent variable and so must be adjusted. This can be achieved by the use of buffer solutions that are able to change the pH levels. Thus in this experiment, they will be used

to change the pH level to the pH’s 1, 4, 10 and 14. A pH probe will be used to show this occurring.

The temperature of the Hydrogen Peroxide will be kept constant as it will be placed in a water bath that has its temperature level set to 30. This will accurately and effectively ensure that the Hydrogen Peroxide is all at the same temperature.

The substrate concentration will be also be regulated by using a measuring cylinder to measure the amount so that it is consistent in every experiment. We measure this by the readings at the side of the measuring cylinder. For this experiment, 5ml of the substrate concentration will be used.

To prevent the Catalase enzyme from having too much of a significant affect on the rate of reaction, this will also be kept constant. The chicken liver will be measured to 1cm and weighing 0. 5g for every test-tube of an experiment.

The volume of the buffer solutions used shall also be regulated so that it is constant. Again, a measuring cylinder will be used to measure the amount used to 5ml before it is added to the Hydrogen Peroxide.

To measure the amount of oxygen that is diffused after the Catalase enzyme has reacted with the Hydrogen Peroxide, a gas syringe will be utilised to measure this.

Apparatus:

-Hydrogen Peroxide (10%) -Chicken Liver (Catalase)

-Buffer solutions (pH 1, 4, 10, 14) -water bath

-Test-tubes/beaker -measuring cylinders

-pH probe -Test-tube stand

-gas syringe -scalpel

-cutting board -ruler

-paper -pencil

-electronic weighing machine -computer with scientific software

-bunsen burner -tripod stand/thermometer

Method:

First, the Catalase from the chicken liver will be obtained. It will be cut up with a scalpel and measured by a ruler to 5 pieces of 1cm and weighed with the weighing machine so that it weighs 0. 5g. Then, Hydrogen Peroxide will be measured to 25 ml in a measuring cylinder and 5 ml will be poured into 5 separate test tubes, thus there will be five different measurements. Then, buffer solutions will be added to the Hydrogen Peroxide solutions in the test tubes. Four of the test tubes will be given different buffer solutions with different pH levels, and these include pH 1, 4, 10 and 14. The buffer solutions will first be measured with measuring cylinders so that there is 5 ml to be added to each of the four test tubes. The last of the test tubes will not have the Hydrogen Peroxide change in pH level (through my preliminary work, I

have found out that the pH level of the Hydrogen Peroxide, 10 % to be approximately pH 7. 5.

This was found out through the use of the pH probe). Once the buffer solutions have been added, it will be stirred so that the solution mixes with the Hydrogen Peroxide. Then the pH probe will be used to check whether the pH level of the Hydrogen Peroxide in the 4 different test tubes have changed before proceeding to put the test tubes in the water bath that has its temperature set to 30?. They will be left heated for 5 minutes to ensure that they reach the temperature before the experiment continues. After 5 minutes, one at a time, one of the cut up pieces of chicken liver will be added to a test tube and quickly a gas syringe will be connected to that test tube to measure how much oxygen is produced from the reaction between the Hydrogen Peroxide and the Catalase in the chicken liver. The measurement on the gas syringe (how much oxygen produced) and the pH level of the Hydrogen Peroxide in the test tube will be recorded after 5 minutes before the next Catalase is added to another test tube, and this will be repeated until all five test tubes have been covered. Once I have all the recordings of all five test tubes, the equipment will be cleaned and I will do the experiment again. I will do the experiment 3 times, so therefore I shall have 3 sets of 5 measurements.

Predictions:

Each enzyme has its own optimum pH, and I can deduce from my discovery of Hydrogen Peroxide being pH 7. 5 during my preliminary work that the enzyme Catalase would have an optimum pH level of around that figure and

this would show that it would work in slightly alkaline solutions. Therefore, I would predict that the Catalase enzyme would denature when it is put into the Hydrogen Peroxide solutions that have been mixed with the more acidic buffer solutions (pH 1 and 4) because if enzymes are put into pH's that are significantly lower or above from their optimum pH, then they would denature. I would predict as well that the enzyme would still work for the Hydrogen Peroxide solutions that are pH's 10 and 14 as they are only slightly above the optimum pH, and so the enzyme would still be working, but probably the enzyme activity or rate would have decreased.

There are a few anomalies/uncertainties that I have considered as I began, as well as after, the collection of my data. First of all, I used 5 ml of Hydrogen Peroxide in each individual experiment and measured it with a measuring cylinder. However, the table on which I placed the measuring cylinder so that I could read a stable reading of the measurement and my reading of the measurements itself could still be slightly off. Furthermore, after measuring it to 5 ml, I transferred it to a test tube and this could affect the amount of Hydrogen Peroxide used in that not all of it could have transferred, as probably little pints or drops of the Hydrogen Peroxide could still be left behind. Another uncertainty could also be when I measured the amount of the pH buffers to be used. I performed a similar routine as with my measurement of Hydrogen Peroxide, and so there could have been similar uncertainties in terms of the measurement of the amount of pH buffers used and my reading of the measurements.

Moreover, I had to mix 5 test tubes of 5 ml Hydrogen Peroxide with each of the 5 different pH buffers that I was using measuring 5 ml as well (in my

experiment, as mentioned before, I used 5 test tubes in each experiment). However, the total amount of 10 ml could be slightly off, because of the uncertainties mentioned earlier, and so the data collected could be slightly incorrect, so therefore I have included an uncertainty level of (ml +/- 0. 05) in the table above for the amount of Hydrogen Peroxide and pH buffers used. Another uncertainty could be the way I measured the amount of the Catalase enzyme I would use, which came from the chicken liver I used. I cut out pieces of chicken liver and weighed them on an electronic weighing machine so that they were 0. 5 kg. However, there is still a small chance that they could be either 1 kg higher or lower, so therefore I have added an uncertainty level of (kg +/- 0. 1) in the table for amount of Catalase enzyme used.

Also, I did my experiment over a couple of days and so for the first two experiments, I used the same chicken liver, however, for the third experiment I used fresh chicken liver to get the Catalase enzyme and so therefore this could also be an uncertainty in that it could affect the quality or activity of the Catalase used. Another uncertainty could be with the pH probe that I used to ensure that the pH level of the Hydrogen Peroxide solutions had changed after they were mixed with the buffer solutions. I used an electronic pH probe that was linked to a computer and where I could read what pH the solution was on a computer software. After I used it on one solution in a test tube, I quickly rinsed it over with water so that when I use it to read another test tube with a different pH, it would give me a more accurate answer without any of the previous solution still present on it. However, there is still a small chance that there could be a little of the

previous solution left on the pH probe as I used it to read the pH over another test tube, thus this could possibly alter the pH reading a bit and so this has to be taken into account.

Another uncertainty could be that possibly the pH buffers used were not exactly the pH that they are said to be, probably just slightly higher or lower and so this could also be an uncertainty. Another uncertainty could be that each experiment that I did could not have had exactly the same pH levels for each of the Hydrogen Peroxide solutions with the altered pH, like for example the pH level for the Hydrogen Peroxide that has been altered to pH 1 might not be exactly the same in experiment 1, 2 or 3 and so therefore this is also taken into account as it can affect the outcome of the investigation. Another uncertainty could be with the water bath that I used to make the temperature of the Hydrogen Peroxide solutions to 30?

While the water bath that was used is electronically controlled and was set to 30?, there is still a small chance that it could be either slightly higher or lower so this is also acknowledged. Furthermore, for my third set of experiment, I had used a water bath that was heated via a Bunsen burner as there was no available electronic water bath that was set to 30? on that day, so therefore I measured the temperature with a thermometer and had the water bath in a beaker. This is much less accurate than an electronic water bath, so this is acknowledged as an uncertainty. Another uncertainty could be that my reading on the gas syringe for the result of the amount of oxygen produced might not be accurate, and so therefore could also affect my results. Furthermore, the time limit of recording the reaction between the Catalase and the Hydrogen Peroxide solutions could have affected the

outcome in that I only recorded for 5 minutes, but maybe the results would have been different if I had extended the time limit and let the experiment go for longer, so this is also considered as an uncertainty.

Analysis:

Mean

Oxygen given off by Hydrogen Peroxide (pH 1)

0. 3

Oxygen given off by Hydrogen Peroxide (pH 4)

2. 3

Oxygen given off by Hydrogen Peroxide (pH 7)

27

Oxygen given off by Hydrogen Peroxide (pH 10)

14

Oxygen given off by Hydrogen Peroxide (pH 14)

0

The above table displays the mean of the different Hydrogen Peroxide solutions with different pH levels. The following indicates the standard deviation of both year groups, i. e. taking individual values and comparing how far away they are from their mean value:

Standard Deviation

SD (Hydrogen Peroxide, pH 1)

0. 47

SD (Hydrogen Peroxide, pH 4)

1. 7

SD (Hydrogen Peroxide, pH 7. 5)

1. 6

SD (Hydrogen Peroxide, pH 10)

1. 2

SD (Hydrogen Peroxide, pH 14)

0

The standard deviation values above are quite low. Low standard deviation indicates that the data shows less variation from the mean value, while high standard deviation indicates the data being a wider variation from the mean. By definition, this would mean that the data is quite reliable.

Significant difference in the oxygen levels given off by Hydrogen Peroxide pH 1

and pH 4:

t Stat

1. 6

t Critical two-tail

2. 8

T-Test: Two-Sample Assuming Equal Variances

In my data processing of the oxygen levels given off by the Hydrogen Peroxide solutions with pH 1 and pH 4, my value for t has a value which is less than my critical value, as is shown above. Therefore, I must actually accept my null hypothesis and conclude that that there is no significant difference between the oxygen levels produced between the two pH levels. However, there could be an uncertainty here in that probably the results were not extensive enough or that there was not enough results to make an accurate judgement. I would think that there would most probably be a significant difference had there been more results of each to compare with, because from the current results, there is actually a rise in two of the experiments for the pH 4 solution (4 and 3), which shows quite a difference when compared to the results of the pH 1 solution, which had zero and one in its results.

Significant differences in the oxygen levels given off by Hydrogen Peroxide pH 4 and pH 7:

T-Test: Two-Sample Assuming Equal Variances

t Stat

15

t Critical two-tail

2. 8

In my assessment of the oxygen levels between the Hydrogen Peroxide solutions with pH’s 4 and 7, however, my value for t is exceedingly greater than the critical value, thus I can reject my null hypothesis and deduce from this that there is a significant difference between the oxygen levels produced between the two pH levels. I think that the reason for this test producing such a different result from the previous test is easily explained; while the results collected in all the experiments were equal (3 pieces of results) the difference is shown here because of the overwhelming difference in the levels of oxygen produced between these two groups (pH 4 solution had results below 5 while the pH 7 solution had results that were all greater than 20).

Significant differences in the oxygen levels given off by Hydrogen Peroxide pH 7 and pH 10:

T-Test: Two-Sample Assuming Equal Variances

t Stat

8. 7

t Critical two-tail

2. 8

The value for t is also greater than the critical value for my data processing of oxygen levels between Hydrogen Peroxide solutions with pH 7 and 10, so therefore I can reject my null hypothesis and prove that there is a significant difference between the oxygen produced between these two pH levels. The uncertainty here could only be that the amount of Catalase enzyme used or the amount of the solution used might not have been exactly the same as indicated in the explanation of my uncertainties in page 4, though I have made the method for this test and my execution of it as accurate as possible to avoid huge anomalies.

Significant differences in the oxygen levels given off by Hydrogen Peroxide pH 10 and pH 14:

T-Test: Two-Sample Assuming Equal Variances

t Stat

16

t Critical two-tail

2. 8

The value for t once again is also greater than the critical value here, thus I am able to conclude that I can reject my null hypothesis and that there is a significant difference between the oxygen levels produced between these two pH levels. The uncertainty here is similar to the one mentioned above.

Significant differences in the oxygen levels given off between Hydrogen Peroxide pH 1 and pH 7 and compared to the significant differences in the oxygen levels given off between Hydrogen Peroxide pH 14 and pH 7:

T-Test: Two-Sample Assuming Equal Variances

t Stat

22

t Critical two-tail

2. 8

t Stat

23

t Critical two-tail

2. 8

The first table above shows the results concerning the comparison of Hydrogen Peroxide solutions of pH levels of 1 and 7 while the second one shows the results concerning the comparison of Hydrogen Peroxide solutions of pH levels of 14 and 7. Both t values are greater than their respective critical values and so we can reject our null hypothesis and prove there is a significant difference in both findings. What is interesting to note is that the second table has a higher result than the first in terms of higher t value in

comparison to the critical value. This significance will be explained in my analysis section.

Significant differences in the oxygen levels given off between Hydrogen Peroxide pH 4 and pH 7 and compared to the significant differences in the oxygen levels given off between Hydrogen Peroxide pH 10 and pH 7:

T-Test: Two-Sample Assuming Equal Variances

t Stat

15

t Critical two-tail

2. 8

t Stat

8. 7

t Critical two-tail

2. 8

The first table above shows the results concerning the comparison of Hydrogen Peroxide solutions of pH levels of 4 and 7 while the second one shows the results concerning the comparison of Hydrogen Peroxide solutions of pH levels of 10 and 7. Both t values are once again greater than their respective critical values and so we can reject our null hypothesis and prove

there is a significant difference in both findings. This will also be further explained in my analysis section.

Conclusion:

In my prediction before I began my experiment, I predicted that Catalase had an optimum pH 7. 5 and so the reaction would work best at this pH, and this prediction was backed up with the preliminary work I did prior to the experiments. With the results obtained from my experiment, I can further conclude that my experiment got the results that I had predicted. My chart on the mean values of Hydrogen Peroxide levels show a normal distribution, which shows that my data had not had an affect on the other and so this would make it more accurate. It also proves that the Catalase does work best at a pH level of 7. 5. Furthermore, my graph showing the optimum pH level for the Catalase enzyme shows the relationship between the pH levels and the mean rate of reaction; as the level of pH increases (my independent variable) the rate of reaction (my dependent variable) also increases to a certain level where it reaches its highest (the optimum pH level) and then the rate of reaction starts to fall. This can be explained in that the optimum pH is the neutral pH level in which Catalase normally reacts in, and so this is where the enzyme works the fastest. This means that more oxygen is released from the Catalase reaction with this pH level of Hydrogen Peroxide, which is pH 7. 5, and this is shown in the two graphs just mentioned.

While the rate of reaction shown for pH levels 4 and 10 are not as reactive as the optimum pH, they still show an increase in the rate of reaction, which shows that Catalase can still react at these levels even though both are not

quite close to the optimum pH. The results also show that between pH 7. 5 and pH 10 there is a difference of 2. 5 pH, and that there is a difference of 13 between them in the mean amount of oxygen given off in a reaction while between pH 7. 5 and pH 4, there is a difference of 3. 5 pH and between them they show a difference of 24. 7, and this proves another point that I had made in my prediction. This shows that I was right in predicting that the enzyme would work less effectively in a more acidic level and this proves my point as pH 4 is acidic. I also said that it would work better in pH 10 compared to pH 4 and this also proves it. This is because the difference between the optimum pH and pH 10 is not as wide as the one between the optimum pH and pH 4, so naturally the closer the pH is to the optimum pH, the more the rate of reaction would be. This is shown on my graph for Oxygen produced in Hydrogen Peroxide with pH's 4, 7. 5 and 10.

The results for pH's 1 and 14 show that that was barely a significant increase in the rate of reaction as a result of the Hydrogen Peroxide being either too acidic or too basic. We can relate this to the presence of ions within these pH level substances. Ions will pull on other molecules within the substance because of their charge, and so if there are a lot of molecules then the enzyme will not work, it will become denatured. This causes the enzyme, and its active site, to change in shape and lose its properties and thus substrates are not able to be broken down. However, in my experiment I obtained a slight rate of reaction in oxygen level given off of 1 for one of the experiments that I did for the pH 1 Hydrogen Peroxide solution (see table). This is an anomaly to my hypothesis as I had suggested that the enzyme would not work at such low levels from the optimum pH, and this also

contradicts the point I had made earlier that the enzyme works less in more acidic levels as there is no reaction for the pH 14 solution, but the result is too small to take into heavy consideration, and so it could either be an uncertainty or I might have made a mistake somewhere through the experiment.

To conclude my experiment and investigation, I can say that my results overall prove my hypothesis that for each different pH level of the Hydrogen Peroxide solution, the rate of reaction will be entirely different from one another. I also predicted that the Catalase would work best in pH 7. 5 as I found out through my preliminary work that that was the optimum pH level of the Catalase enzyme and so would have the highest rate of reaction and I was correct. I reasoned this prediction on my knowledge that pH 7. 5 is close to where acid and base substances are neutral. The other pH levels that I used showed a slightly lesser increase in the rate of reaction (pH 4 and pH 10), or very little or no increase at all (pH 1 and pH 14). This is because when the substance is not neutral, which is the optimum pH for Catalase, the enzyme and its active site modifies or changes its shape because it cannot work as effectively in different pH levels from its optimum, and thus this reduces the rate and amount of substrates that are able to be broken down.

Evaluation:

Out of the entire experiment, there were a few problems that I encountered. One of the weakness of my experiment was that for my third set of experiment, I had to use a Bunsen burner and a beaker to heat a water bath to 30? as there were no available water baths that were set for my specified

temperature level on the day I did my third set of experiments, thus this could be argued that my third set of results might not be as accurate as the other sets. Another weakness could be that my decision to only record 5 minutes of the rate of reaction once the Catalase has been added to the Hydrogen Peroxide solutions could have hindered any further possible chances of oxygen given off being recorded. Also, the Catalase enzyme that was used had been taken from chicken liver. For the first two sets of my experiments, the Catalase was taken from the same chicken liver, however, for my third set of experiment the chicken liver was changed to a fresh new chicken and this could have affected my results and could be taken into consideration as an error.

There were also limitations to the experiment. I did my experiment with several other people who were doing theirs, and between us there were only 4 measuring cylinders to be shared and so this slowed down my experimentation process for having to wait in turn to use the equipment. We also had to share a limited amount of the chicken liver and the Hydrogen Peroxide solutions. Also, there were only two electronic water baths that catered to many people that wanted many different temperatures for the water bath. Thus, this limited my options in using the electronic water bath and made me use a Bunsen burner and beaker for a water bath in my third set of experiment.

To improve this experiment, I could have also done one where I looked at the other factors that affected the enzyme activity of Catalase, like temperature or substrate concentration for example if I had more time. Then I could have compared the results between the factors, and see the similarities and

differences. The levels of pH that I used could have also been improved, in that I could do another experiment of a similar kind but focus on closer pH levels to the optimum pH level, so that I get a more accurate account of the enzyme activity .