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School Code: 0682

*Measuring and comparing the decomposition of H2O2 by the catalytic activity of ‘bos primeginus liver’ depending on the pH level range of 1-14 of the substrate with the addition of sodium hydroxide (NaOH) as an base, and hydrochloric acid (HCl, as an acid.*

During this lab I measured how the pH level of the substrate hydrogen peroxide affected the catalytic activity of the enzyme ‘bos primeginus liver’. The initial and final pressure during the catalytic reaction is measured through various trial in which sodium hydroxide (NaOH), a base, and hydrochloric acid (HCl), an acid, are added to H2OH in order for the pH level to increase/decrease from a range of 1 to 13. Moreover, we were able to discover how an enzyme is active if it is between its optimum pH ranges where if not, enzyme activity is highly reduced or, even so, denatured.

**Criterion 1: Personal engagement**

This lab allowed me to compare the rates of reaction that an enzyme has in relation with the pH level of the substrate, that the enzyme is in. The rates of reaction over a period of time, measured by a pressure sensor, allowed me to understand how the oxygen released when hydrogen peroxide is destroyed through the catalyst reaction varies depending on certain factors, such as the ph. Moreover, the investigation allowed me to gain deeper understanding on the importance of maintaining such elements, such as pH, temperature and concentration substance, in an equilibrium range where enzymes are not denatured and the body can maintain its normal function carried out by proteins.

The reaction was measured by pressure sensors, which recorded the change in pressure through out the catalyst reaction. Moreover, the pressure sensors allowed me to understand how the enzymes destroy the production of oxygen, from the substrate hydrogen peroxide, at different rates because of the pH alteration.

**Criterion 2: Exploration**

Research Question: How does the pH level (1.01,1.44, 6.8, 13.68, ±.05) of the substrate hydrogen peroxide (H2O2) affect the catalyst activity in the enzyme ‘bos primeginus liver’, measured by a pressure sensor, when the volume, temperature and substrate concentration is kept constant?

Background Research Information:

A catalytic reaction is when there is an increase in the rate of a chemical reaction of an enzyme. Moreover, when a piece of liver, acting as the enzyme, is added to the hydrogen peroxide it will cause a decomposition of more stable elements giving a product of water and oxygen. (2) (Explaining the bubbles and solution as a results of the reaction). Thus as hydrogen peroxide (and in some cases with the addition of a base and an acid) reacts with an enzyme, the oxygen that is produced through the catalytic reaction is measured by the oxygen produced measured through a pressure sensor.

2 H2O2 → 2 H2O + O2

Yet the rate of reaction depends on various factors that might affect the rate in which the hydrogen peroxide reacts with the enzyme, one of them being the pH level of the substrate. To see the effects that pH had on a catalytic reaction, Hydrochloric Acid was added to the substrate Hydrogen Peroxide, causing hydrochloric acid dissociate into H+ and Cl- thus causing the contribution to Hydrogen ions once it is added to H2O2 and therefor making the pH decrease. (7)

On the other hand, to increase the pH Sodium Hydroxide is added to split it apart into Na+ and OH-, making the hydroxide ions to combine with the hydrogen ions from the H2O2 to form more water molecules. (6)

Consequently, by adding a base or and acid, the substrate used through a catalytic reaction in this lab will increase or decrease its pH. This alters the optimum pH of an enzyme thus making intermolecular bonds to break

Variables:

**Table 1: Variables Through Out the Lab**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable Type** | **Variable** | **Units & Uncertainties**  | **Description of how variables manipulated** |
| Independent | pH level | mol/liter ±.05 | pH was changed through the addition of Sodium Hydroxide, to raise the pH level, and Hydrochloric Acid, to lower the pH level |
| Dependent  | Pressure Change | (N/m2) ±.0.2 | A pressure sensor was used in order to obtained the initial pressure and the final pressure over a defined period of time. The pressure change was then obtained through the subtraction of the final - initial pressure  |
| Control | Volume | mL | The volume was kept the same for all trials by adding water (H2O) to reach the same amount of substrate on all trials |
| Time | S | A 10 second time frame was given for each reaction in order to obtain the pressure change in the same amount of time  |
| Temp | C | Room Temp was maintained through out the experiment |
| Size of liver | cm | The liver, being a 1x1 cm square, was estimated to be the same for all trials since it was the enzyme component which defined how much reaction had to occur |
| Concentration of Hydrogen Peroxide | mol | The substrate hydrogen peroxide is added to all tubes since it is the pressure from the oxygen being released from that substrate which defines the catalyst reaction |

**Table 2:** Materials

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| --- | --- | --- | --- |
| **Item** | **Quant. Used** | **Size** | **Units of measurement & Uncertainty**  |
| Test Tubes | 25 | Appropriate for the pressure sensor lid to fit in | mL (± 0.1 cm) |
| Beaker | 7 | 30 mL | mL (± 0.1 cm) |
| Pippet | 1 | - | - |
| Vernier Pressure Sensor | 1 | - | - |
| Vernier pH level indicator | 1 | - | - |
| Hydrogen Peroxide | 25  | - | - |
| Sodium Hydroxide | 10  | - | - |
| Hydrochloric Acid | 10  | - | - |
| Water | 15 | - | - |
| bos primeginus liver | 25 | 1 x 1 cm | cm (± 1 cm) |
| Goggles | 1  | - | - |
| Knife | 1 | - | - |
| Cutting Board  | 1 | - | - |

Methods of Data Collection:

The pH of the substrate changed by the addition of Hydrochloric Acid and Sodium Hydroxide to the Hydrogen Peroxide. Both solutions were added to either increase or decrease the pH level through the alteration of the Hydrogen ions of H2OH. Thereby, the volume of each trial was kept the same through the addition of water.

*Procedure:*

1. Materials were gathered on a clean desk
2. Pressure sensor was turned on by plugging it in
3. Temp was kept constant throughout the lab by working in a closed room with a temp of 23 C
4. 5 test tubes where labeled A1, A2, A3, A4, A5
5. Step #3 was repeated with new beakers by labeling them B1, B2, B3, B4, B5, C1 ….. C5, D1…..D5, E1…...E5.
6. Liver was placed on the cutting board and with the help of the knife and a ruler 25 pieces of 1x1 cm squares were cut
7. 1 mL of hydrogen peroxide were poured to all tubes
8. 1 mL of water was added to all tubes labeled A# (1-5)
9. 5 mL of Sodium Hydroxide was mixed with 5 mL of water.
10. 1 mL of the substance Sodium Hydroxide with was poured to each beaker labeled B1, B2, B3, B4, B5
11. 15 ml of Sodium Hydroxide was mixed with 5 mL of Water
12. 1 mL of the substance Sodium Hydroxide with was poured to each beaker labeled C# (1-5)
13. Steps 8-11 where repeated by using Hydrochloric Acid instead of Sodium Hydroxide

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| Table 3: Estimating the Pressure of an Enzyme Through a Catalytic Reaction. |
| pH (mol/liter ±.05) | Initial Pressure (kPA ±.02) | Final Pressure (kPA ±.02) |
| Trials | Trials |
| Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 1.01 | 102.17 | 99.56 | 101.38 | 102.51 | 102.34 | 101.15 | 100.58 | 102.29 | 102.68 | 103.14 |
| 1.44 | 100.20 | 100.35 | 100.81 | 101.09 | 100.47 | 100.39 | 100.52 | 100.98 | 101.95 | 101.06 |
| 6.80 | 101.49 | 99.61 | 99.61 | 99.56 | 99.59 | 106.38 | 125.38 | 125.50 | 124.47 | 125.36 |
| 13.68 | 102.17 | 99.56 | 101.38 | 102.51 | 102.34 | 101.15 | 100.58 | 102.29 | 102.68 | 103.14 |
| 13.68 | 99.61 | 99.67 | 99.56 | 99.61 | 101.01 | 100.75 | 102.80 | 102.00 | 101.03 | 103.82 |
| All data was collected at Room Temperature (≈23 decrees C) under standardized pressure. Carla Frias & Katerina De La Borda collected all data on Sepember 19th. Liver samples where placed under 25 test tubes (CV) with different pH levels (IV) which where changed through the addition of Sodium Hydroxide and Hydrochloric Acid. This will allow us to understand effects that pH level has on a catalyst activity in an enzyme. pH measured through a pH sensor. |
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1. A 1x1cm piece of liver was placed on the top part of a A1 tube avoiding it to be in contact with the substrate.
2. The lid of the Pressure Sensor was placed on the tube still avoiding the contact of the liver with the substrate
3. The tube was then shaken until bubbles started to formulate
4. Using the information given by The Pressure Sensor, all data was recorded
5. Steps 11-14 where repeated to the rest of the tubes

**Safety, Environmental & Ethical Considerations**

* Goggles were used through out this lab in order to maintain safety because of the addition of acids and bases.

**Criterion 3: Analysis**

**Raw Data Table:**

**Sample Calculations/Data Processing:**

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| Table 4: Estimating the catalytic reaction of an enzyme measured by the Pressure Change in relation with of the substrates pH level |
| pH (mol/liter ±.05) | Change of Pressure (kPA ±.02) | Rate of Pressure Change (kPA ±.02) |
| Trials | Average | Trials | Average | Standard Deviation |
| Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
| 1.01 | -1.02 | 1.02 | 0.91 | 0.17 | 0.80 | 0.38 | 0.10 | 0.10 | 0.09 | 0.02 | 0.08 | 0.08 | 0.04 |
| 1.44 | 0.37 | 0.17 | 0.10 | 0.86 | 0.59 | 0.42 | 0.04 | 0.02 | 0.01 | 0.09 | 0.06 | 0.04 | 0.03 |
| 6.8 | 4.89 | 25.77 | 25.89 | 24.91 | 25.77 | 21.45 | 0.49 | 2.58 | 2.59 | 2.49 | 2.58 | 2.14 | 0.93 |
| 13.68 | 1.39 | 3.13 | 2.44 | 1.42 | 2.79 | 2.23 | 0.14 | 0.31 | 0.24 | 0.14 | 0.28 | 0.22 | 0.08 |
| 13.68 | 1.14 | 3.13 | 2.44 | 1.42 | 2.79 | 2.18 | 0.11 | 0.31 | 0.24 | 0.14 | 0.27 | 0.22 | 0.08 |
| All data was used from Table 1 where its different pH level had an effect on the 'Change in Pressure' and overall 'Rate of Reaction' during a catalytic reaction. Al data was collected at room temp (≈23 decrees C) under standardized pressure. Pressure was recorded from a Pressure Sensor through out the liver samples used for the 25 test tubes (containing different pH levels). |
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*Calculations*

1. Change of Pressure: Final Pressure – Initial Pressure

Example: 106.38 - 101.49 = 4.89

1. Rate of Pressure Change: Change of Pressure/time

Example: 4.89 / 10 = 0.48

**Graph:**

Graph 1:

**Trend:** There is a very weak positive correlation between the pressure change and the pH level because these two variables depend on denaturing the enzyme or reaching its optimum level. Thus as pH increases it does not necessarily mean that the rate of reaction will also increase; This explains why the enzyme tolerates pH changes, meaning that the trend line slowly increases/decreases, until reaching a point of denaturing the enzyme and causing non or partial rate of reaction, which justifies the straight lines in the points that had between 0-1 kPA total rate of reaction.

**Criterion 4: Evaluation**

During this lab we found out that the enzyme catalytic reacted depended hugely on the pH level of hydrogen peroxide being too far or relatively close from its optimum. Through the addition of a base (sodium hydroxide) or an acid (hydrochloric acid), then the enzyme reached a point of denature due to such high or low pH level.

        By taking a look at Graph 1, when the enzyme was added to the solute of hydrogen peroxide with water, having a pH of 6.80, the pressure change was of 21.45 (kPA). Then, when 1 mL of Hydrochloric acid was added to the hydrogen peroxide, the pH level decreased and gave a pH of 1.01. Thus as the pressure change was measured, results were of 0.38 (kPA), giving effective results of comparing the pressure change of a solute with a pH of 6.80 and a pH of 1.01. Thenceforward, when the pH of hydrogen peroxide was increased to 13.86, by the addition of 1 mL of Sodium Hydroxide, the pressure change was 2.23 (kPA). Moreover, a comparison between an optimum pH, being 6.8, and the pH level which denatured the catalytic reaction of the enzyme, being 1.01 or 13.68, could be discovered and proved through data collection.

        The catalytic reaction that occurred in this lab through the addition of the subtract hydrogen peroxide and a piece of liver underwent the decomposition of intermolecular bonds of the substrate as well as the production of oxygen through the alteration of the pH level. We noticed that the pH, which had the greatest pressure change, was the substrate with the pH of 6.8. This explains how once enzymes reaches its optimum pH level, being mainly around 7 (1), it endures its highest chemical reaction (enzyme activity). Moreover, as the liver reacted with the substrate who had an optimum pH level of 6.8, then a greater amount of decomposition was performed in such period of time and thus caused greater amount of oxygen from H2O2 to push/pressure from inside of the test tube and be measured by the pressure sensor.

On the other hand, when Hydrochloric Acid (HCl) was added to water, it made the positive Hydrogen ions bind with the water’s Hydrogen ions and cause the substrate to decrease its pH level. (3) Then, when it was added to hydrogen peroxide, the overall pH of the substrate of where the enzyme reactin In decreased significantly. Furthermore, when Sodium Hydroxide (NaOH), a base, was added to the hydrogen peroxide, it underwent the same process where the hydroxide ions (OH) of the base combined with the hydrogen ions of the water, causing a greater amount of water and the overall pH of the substrate to increase. (6) So as the substrate was more, or less, acidic, it caused the pH to leave its optimum pH level of 7 and the hydrogen bonds of the enzymes environments to be disturbed and denatured, decreasing the amount of reaction that occurred. (4)

        Additionally, when the pH of the substrate hydrogen peroxide rapidly increased/ decreased, the shape of the enzyme was being affected. The pH was altering the ionization of the amino acids in the protein (the bonding of Hydrogen molecules), causing the ionic bond that helps determining the shape to be altered. (5) This change in shape caused the function of the enzyme to be distorted by not allowing the substrate to bind to the active site and undergo the catalytic reaction, explaining such low rate of reactions from the 4 of 5 pHs which where off from the optimum level.

        The data that was collected throughout this lab did support the scientific context due to the reliability of the data collected. This is because all trials were tested out 5 times, giving repeated analysis on the same sample and experiment, which gave similar results.  This can be proved by the standard deviation which was not greater than 1 for all trial, allowing us to understand that all results where very alike.

        Through out the experiment we came across several strengths, which caused greater results from the lab. We worked at a calm pace, which allowed us to be careful and patient throughout the lab and obtain precise results. Another strength that we faced in this lab was how safe we were. We were always using goggles in order to avoid any dangerous results or events. Another asset from this lab was how Katerina and I worked together when collecting the results in order to help each other and work at a faster pace. We assisted on each other and worked at the same rate in order to obtain results where we could both supervise. Additionally, we also faced some error that caused weakness in our lab. Some of them were:

**Table 5:** Experimental Errors

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| --- | --- | --- |
| **Experimental error** | **Effect on data collected** | **Improvement to design** |
| Hydrogen Peroxide was left unattended for a long period of time instead of sealing the test tube immediately after poured.  | By leaving trials with H2OH unattended, the substrate decomposes since the oxygen escaped. This caused the initial pressure to be altered. | Divide the lab by trials instead of doing the lab all at once. For example, this lab could have been done by testing 5 trials of one pH level then the other 5 with a different pH level and so on.  |
| All materials where not gathered before the lab | The data was performed in a very unplanned manner. This caused a mess on the table thus created chaos and stress | On the future, all materials should be gathered by doing a check list on the materials needed. This will then allow me to perform a fluid lab performance. |
| The equipment used had droplets of water because of rinsing out previous solutions in the beaker/test tube. | Water molecules influenced the precise concentration of the substrate being used to react with the enzyme and thus the pressure measured.  | All material being used must be dried out before used in order to avoid un-wanted material. This could be done with the use of paper towels or test tubes racks.  |
| When pouring different substrates in the test tube (suck as hydrochloric acid, sodium hydroxide or hydrogen peroxide), molecules stuck to the side of the test tubes. Moreover, when the liver was introduced to the test tube it began reacted with those elements stuck on the side of the beaker before the pressure sensor was even placed in order to record all data. | This caused the data collected to lack precision. Pressure change requires the initial rate to be the substrate with out the enzyme, thus if we introduce the liver to the test tube and it begins reacting with the substrates in the sides of the test tube with out the pressure sensor, data is lost.  | Use a specific pipette each solution introduce in the test tube. This will allow the pressure sensor to record all data with delicacy.  |
| pH ranges in the lab did not allow me to gain varied results since all pHs reached by the addition of hydrochloric acid or sodium hydroxide caused the enzyme to be denatured. | The data collected supported scientific information to an extend because I was not able to obtain pH levels which decreased as it left its optimum range.  | Increase the amount of hydrogen peroxide added to all trials and decrease the amount of IV introduced to the test tubes (meaning on trial 1 only 1 mL of NaOH/HC was added, then 3 mL and so on). This will allow the data receive to support scientific information to a deeper level. |

Criterion 5

**Works Cited:**

1. "Introduction to Enzymes." *Effects of PH ()*. N.p., n.d. Web. 06 Dec. 2014.

<http://www.worthington-biochem.com/introbiochem/effectsph.html>

1. "US Peroxide." *Hydrogen Peroxide*. N.p., n.d. Web. 07 Dec. 2014.

<http://www.h2o2.com/products-and-services/us-peroxide-technologies.aspx?pid=112&name=Hydrogen-Peroxide>

1. "US Peroxide." *What Is the PH of H2O2 Solutions?* N.p., n.d. Web. 07 Dec. 2014

<http://www.h2o2.com/faqs/FaqDetail.aspx?fId=26>

1. "Biology for the IB Diploma Coursebook." 2013. 8 Dec. 2014 <<http://tfssbio.pbworks.com/w/file/fetch/54980708/Biology_for_the_IB_Diploma.pdf>>
2. "Solutions to even-numbered problems." 2013. 8 Dec. 2014 <<http://web.calstatela.edu/faculty/jmomand/solutions_to_even_numbered_problems.pdf>>
3. "What Are Acids, Bases, and pH All About, Anyway? - For ..." 2009. 8 Dec. 2014 <<http://www.dummies.com/how-to/content/what-are-acids-bases-and-ph-all-about-anyway.html>>
4. "Acids, Bases, and pH - Wiley." 2005. 8 Dec. 2014 <<http://www.wiley.com/college/pratt/0471393878/student/review/acid_base/7_buffers.html>>