Biology 2B 10/10/10

Enzyme Lab

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Aim: Investigate the effect of a factor that influences enzyme activity

Background: Scientists attended a Pre Lab discussion about enzymes reviewing the many factors that should commonly affect enzymes such as temperature, poison, pH, number of enzymes and solubility. The role of enzymes was also reviewed in order for students to develop their own lab. Enzymes are proteins which serve as a catalysts, a chemical agent that changes the rate of a reaction without being consumed by the reaction. Every reaction before starting has a hill of energy that it must first get over before it can begin its reaction known as the Energy of Activation. This energy of activation is much higher without the enzyme present and with the enzyme is lowered to the point where reactants can turn into products more readily. In regards to temperature, higher temperatures speed up all reactions but at a certain temperature enzymes begin to become denatured(deactivated) and at even higher temperatures 60 □ or more they only continue to become less effective.

Research Question: At what temperature does the catalase of lived produce the most Oxygen in a reaction of hydrogen peroxide?

Hypothesis: If the catalase's temperature is changed by both heating and cooling then the catalase will be most effective in terms of amount of Oxygen produced (ml) when cooled.

Independent Variable: Temperature of enzyme liver

-Increased the liver temperature by 22□C from room temperature of the catalase solution to correlate a high temperature at 44□C

-Decreasing the liver temperature by 22□C from room temperature of the catalase solution to correlate a low temperature 0□C

-Increase is by 22□C because in order to reach 0□C it must be decreased by 22□C from room temperature

-Begin with room temperature

Dependent Variable: Amount of Oxygen produced from the liver, Hydrogen Peroxide reaction (ml)

-Calculated by the amount of water displaced by Oxygen

Control Variable: Room temperature of the Liver

Materials:

-15 test tubes
-3 Thermometers
-3 Pipets
-Blender
- 1 stopper w/ tub
-Hydrogen Peroxide
-1 plastic tub
-Ice Bath tub
-Tongs
-Weightboat
- Liver Container
- Clock
-Hot Water Tub
-1 100ml beaker
- 2 10ml graduated cylinders
-Scalpel
-Water

Data Table:

	Amount of Water displaced (ml), Oxygen Produced				
Temperature (□C)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
low temperature 0□C	5.0	5.0	8.5	3.0	4.5
Room temperature 22□C	19.5	19.8	35.0	15.0	26.0
high temperature 44 □C	12.0	15.0	9.0	14.0	7.0

Calculation Table:

example
Avg Amount of Water displaced = Avg
Avg= Sum of trials 1-5/3 of Trials
Avg= 5.0ml+5.0ml+8.5ml+3.0ml+4.5ml/5
Avg=5.2ml

Temperature	**Average amount of Water Displaced(ml), Oxygen Produced**	**One Standard Deviation**
low temperature 0□C	5.2ml	2.03

Room temperature 22□C	23.06ml	7.74
high temperature 44 □C	11.4ml	3.36

Procedure:

1. Retrieve Lab Materials from Supplies Cart in Center of the Lab
2. Construct a Data a Table for recording data collected
    a. Label Column One Temperature (□C)
    b. Label Columns 2-6 Trial followed by numbers 1-5
    c. Label row 1 column 1 0□C
    d. Label row 2 column 1 Room Temperature leaving space to add calculated temperature
    e. Leave row 3 column 1 44□C
3. Retrieve Liver for Liver Solution
    a. Gather weightboat, scalpel, electronic balance and Liver Container
    b. Turn Electronic balance on after plugging in
    c. Place weightboat on electronic balance
    d. Press reset button on electronic balance zeroing out the balance
    e. Open liver Container
    f. Using Scalpel, make an incision on the liver cutting a small piece
    g. Place liver on weightboat
    h. Continue to add/remove level by making incisions with scalpels until 2.5g is gathered
    i. Place liver by Blender
4. Retrieve Water for Liver Solution
    a. Turn cold water tap halfway
    b. Place 100ml beaker under water
    c. Use Pipet to adjust amount of water in beaker to exactly to 100ml.
5. Make Liver Solution
    a. Plug Blender in
    b. Tighten removable bottom securely
    c. Pour 100ml beaker of water in
    d. Let liver drop into blender using scalpel to remove as much liver as possible from weightboat
    e. Secure Lid onto Blender
    f. Press Frappe Button
    g. Blend until liver solution no longer has visible chunks and is a light brown color.
    h. Pour Liver Solution from blender back into 100ml beaker
6. Fill Water Tub
    a. Place Tub under tap
    b. Turn Water on Halfway
    c. Wait until tub is ¾ full of water
    d. Place Water tub on table

7. Fill graduated cylinder with water
    a. Turn tap water on a quarter of the way
    b. Wait until water is completely full of water and then turn tap water off\
    c. Have a Lab Partner hand fully over the graduate cylinder
    d. Place cylinder in water and turn upside down and remain so until ready for data collection
8. Prepare Hydrogen Peroxide and place in beakers
    a. Open Hydrogen Peroxide container
    b. Pour 10ml into a 10ml graduated cylinder
    c. Use a Pipet to adjust amount of Hydrogen Peroxide to exactly 10ml
    d. Pour 10ml of Hydrogen peroxide into test tube
    e. Repeat until all 15 test tubes are full
9. Calculate temperature of liver to establish room temperature of the Liver solution(control)
    a. Place thermometer into 100ml beaker
    b. Wait about 2 minutes making sure the thermometer is no longer changing temperature
    c. Record temperature of thermometer onto Row 2 Column 1 next to Room Temperature
10. Conduct Data
    a. Pour 10ml of Liver solution into 10ml graduated cylinder
    b. Use Pipet to gather 1ml of liver solution changing the concentration in the graudated cylinder from 10ml to 9ml
    c. Place a Hydrogen Peroxide filled test tube in the hand of the partner 2 who is holding the graduated cylinder underwater
    d. Have the other Partner 1 hold stopper w/ tube and the Pipet filled with 1ml of Liver solution
    e. Have partner 2 place tube inside 100ml graduated cylinder
        i. With graduated cylinder underwater lift about 2 inches
        ii. Push tube inside cylinder
        iii. Slowly lower 100ml graduated cylinder down until tube Is secured in cylinder
    f. Have partner 1 note starting time by looking at the clock
    g. In the same second squirt Pipet filled with 1ml of Liver solution into test tube with 10ml of Hydrogen Peroxide
    h. As quickly as possible stop the test tube with the stopper
    i. Have partner 1 count out 20 seconds by looking at the clock
    j. After 20seconds record amount of amount of oxygen produced in graduated cylinder. (this will be the amount of water that escaped outside of the cylinder noted by the empty space in the graduated cylinder)
11. Repeat step (10) 4 more times
12. Cool Liver solution to 0□C
    a. Pour 10ml of Liver solution into 10ml graduated cylinder
    b. Remove bottom piece of graduated cylinder leaving only the cylinder left
    c. Place thermometer inside cylinder

d. Dig small space in ice tub with enough space for 10ml cylinder
e. Place 10ml liver solution into ice
f. When thermometer reads 0□C repeat step 10 b-j 4 more times making sure to note that the 10ml graduated cylinder will be the one filled with 0□C liver solution
g. Between each trial place the 10ml graduated cylinder will be the one filled with 0□C liver solution back into ice tub

13. Heat up Liver to 44□C
    a. Fill hot water tub ¾ full with water
    b. Plug hot water tub in
    c. Turn knob clockwise until halfway heat
    d. Pour 10ml of Liver solution into 10ml graduated cylinder
    e. Remove bottom piece of graduated cylinder leaving only the cylinder left
    f. Place Thermometer inside cylinder
    g. With tongs grab 10ml graduated cylinder
    h. Place cylinder in hot water tub until temperature reaches 44□C
    i. At 44□C run back to lab table with graduated cylinder
    j. Repeat step 10 b-j note that the 10ml graduated cylinder will be the one filled with 44□C liver solution
    k. Between each trail repeat step 13 a-j 4 more times.

Conclusion:

After viewing the data collected and noting the ability of the catalse to perform at 3 different levels of temperature it can be concluded that at 22□C the liver solution and hydrogen peroxide reaction produce the most oxygen. This conclusion contradicts the hypothesis which was "If the catalase's temperature is changed by both heating and cooling then the catalase will be most effective in terms of amount of oxygen produced(ml) when cooled." Instead the control is displayed the best ability to produce the most amount of oxygen. This can be observed through comparing the averages of oxygen produced between catalase temperatures. The cold temperature of 0□C produced an average of 5.2ml of oxygen. The room Temperature of 22□C produced an average of 23.06ml of oxygen. The high temperature of 44□C produced an average of 11.4ml. This data supports the conclusion that at room temperature, more oxygen is produced by the reaction.

In regards to the pre-lab discussion the data clearly shows the ability of temperature to affect the performance of the enzyme suggesting that while the lab may not have been conducted perfectly it does in fact hold some credible ground. It can be noticed that room temperature is closest to a human being's body temperature suggesting a reason for how enzymes work in the body of humans as well as how the human body allows for the function of enzymes. First enzymes work most effectively at a temperature that is close to human body temperature and second the human body maintains the temperature of 37(□C) so that enzymes can work efficiently and allow the human body to survive. The previous statement is only a prediction that could only be proven if the experiment tested the performance of the enzyme at 37 (□C).

Weaknesses were mainly within the procedure. Let it first be pointed out that the liver concentration may not be completely accurate. This is due to the fact that when collecting the 2.5g of liver and placing the liver into the blender there was some liver that stuck to the weightboat. This slightly adjusts the liver to water ratio of the liver solution but it does not throw off the collection of data because it applied to all three levels of the Independent variable ensuring precision. Additionally the timing in step 10 h and I was most certainly not kept consistent. This was due to the fact that the pipet did not always squirt all of its liver solution into the test tube in one go combine with the failure to stop the test tube fully in the first capping. This error directly affects the accuracy of the amount of oxygen produced because it allowed some of the oxygen produced in the first few seconds of the reaction to escape into the lab environment instead of travelling into the stopper w/tubing. Not only does it affect accuracy but also precisions because the timing was the always consistent it caused varying amounts of error to occur between each trial. This can be viewed most apparently during Trial 3 for room temperature where 35.0ml of oxygen was produced, an outlier among other trials which affects the average amount of oxygen (ml) produced for room temperature (22□C). This could also explain the reason why the standard deviation was higher for room temperature (22□C) at 7.74 compared to 0□C at 2.03 and 44□C at 3.36. This throws the conclusion slightly off balance because it was error that aided it in displaying the greatest ability to produce the most oxygen. However while it is slightly off balance it is still a viable conclusion supported by outside sources in form of the pre-lab discussion regarding the tendencies of an enzyme to perform "In regards to temperature, higher temperatures speed up all reactions but at a certain temperature enzymes begin to become denatured (deactivated) and at even higher temperatures 60 □ or more they only continue to become less effective."

In spite of the fact that this conclusion is supported by pre-lab discussion there is still room for improvement should this lab be conducted again. First in order to assure the accuracy of liver/water ratio the procedure should be modified in step 5 d to add an additional step to scrape out liver stuck to weightboat so that the proper liver/water ratio can be certain. Second the addition of an additional partner should be introduced into the procedure so that the test tube can be stopped more effectively by a third partner allowing the second partner to focus on holding the test tube and the first partner to focus on emptying the pipet. This in turn will make up for whatever oxygen was lost to the lab environment and instead allow that lost oxygen to be recorded in the data.

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