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AN EXPERIMENT INVESTIGATING THE EFFECT OF PH ON ENZYME ACTIVITY: HOW DOES PH AFFECT THE RATE OF CATALYTIC REACTION OF CATALASE BREAKING DOWN HYDROGEN PEROXIDE?

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Scientific Report

Title

An experiment investigating the effect of pH on enzyme activity: How does pH affect the rate of catalytic reaction of catalase breaking down hydrogen peroxide?

Research Question

How does pH affect the rate of catalytic reaction of catalase breaking down hydrogen peroxide?

Background information/Introduction

What are enzymes?

Enzymes are biological catalysts that speed up biochemical reactions by lowering their activation energy **(Figure 1)**. They play an important role in cellular metabolism. They either split a substrate apart or bind a substrate together. Most enzymes are wholly or partially made of proteins. Types of enzymes include catalase, diastase, pectinase and so on. Enzymes can be used repeatedly because they are not consumed during the reaction. Enzymes increase the rate of reaction but have no effect on the concentrations of reactants and products at equilibrium.

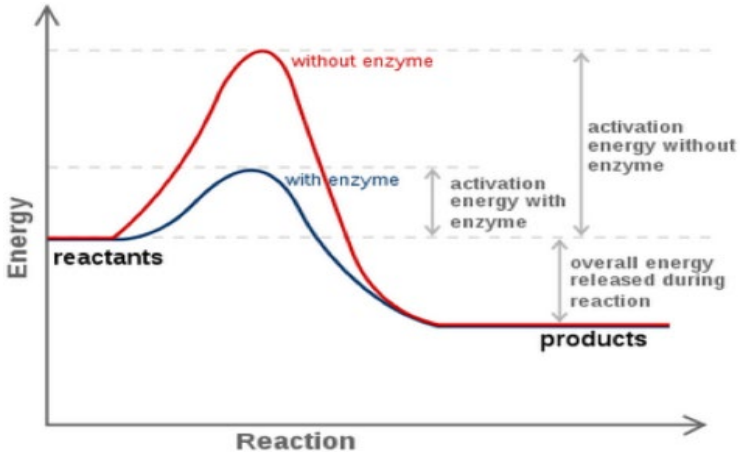


Figure 1. Differences in activation energy between reactions with and without enzyme
Reference: https://www2.nau.edu/lrm22/lessons/enzymes/enzymes.html

Additionally, enzymes are very specific for their substrates in vivo, for example, hydrogen peroxide only reacts with catalase. The active site of an enzyme is the location on the enzyme where substrate binds. Induced fit model explains that this complementary binding where both enzyme and substrate change shape a little bit so that they bind together at its maximum strength at the transition state of a catalytic reaction **(Table 1)**.

Table 1. Stages of enzyme catalysis.

Stages of enzyme catalysis

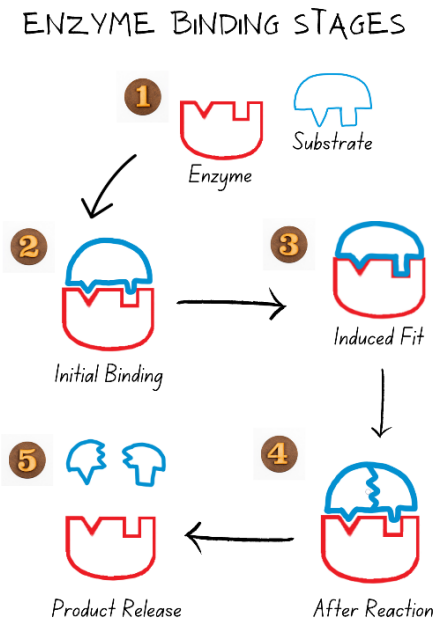
Stage 1:
First thing that happen in an enzyme-substrate reaction. The substrate is yet to come into contact.

Stage 2:
The enzyme binds with the substrate, but this binding is not perfect. The forces holding the enzyme and the substrate is strong, but they are not in their maximum strength yet. They do not fit like puzzle pieces.

Stage 3:
Both enzyme and the substrate change shape a little bit and binds together at its maximum strength. The enzyme is catalysing at its full force.

Stage 4:
The enzyme is separating the substrate into two parts.

Stage 5:
Products of the reaction are released from the enzyme and the enzyme is returned to its initial state as in stage 1.

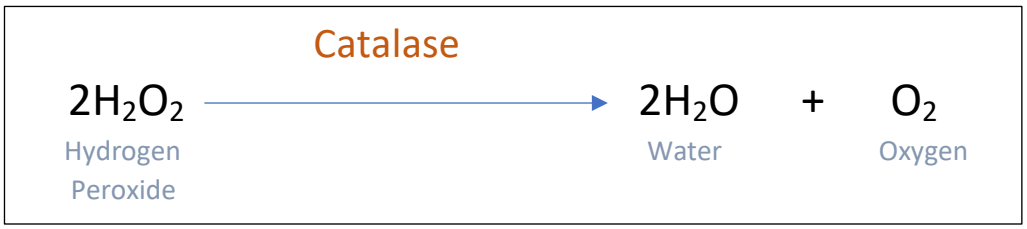


Factors affecting enzyme activity

The factors that affect the enzyme activity on a given substrate are potential hydrogen (pH), temperature, enzyme and substrate concentrations and presence of enzyme-specific inhibitors or activators. Enzymes are highly sensitive and function optimally at their favourable pH, temperature, enzyme and substrate concentrations, and in the absence of enzyme-specific inhibitors. PH is a scale measure whether a chemical is acidic or basic and describes how much ionic hydrogen (H+) or hydroxide (OH) it contains. In this experiment, pH will be investigated on the rate of enzyme-catalysed reaction.

What is hydrogen peroxide?

The substrate used in this experiment is hydrogen peroxide which is a pale blue liquid with a chemical formula of H_2O_2. It has antiseptic properties and can be used as an oxidising and bleaching agent. It is also a poisonous by-product of cellular metabolism produced by living cells. The enzyme catalase splits hydrogen peroxide apart to produce oxygen and water.



Aim

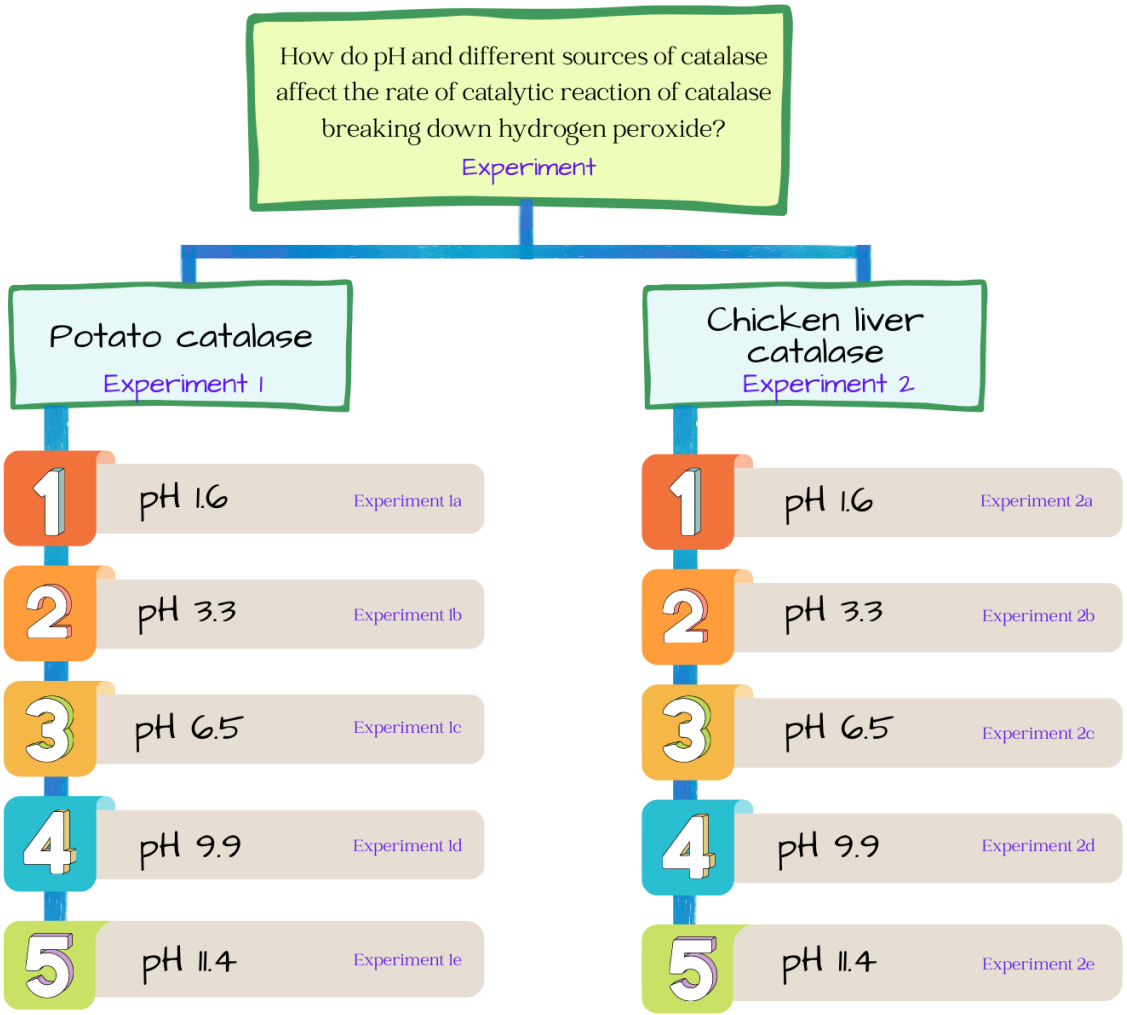
This experiment investigates the effect of pH on catalase activity and observes the catalytic action of two sources of catalase on hydrogen peroxide.

Hypotheses

Hypothesis 1: If the pH is below or above the optimum pH, the rate of the catalytic action of catalase will decrease.

Hypothesis 2: Animal cells contain more catalase than plant cells. Chicken liver that contains higher catalase concentration per unit volume has a faster rate of catalytical reaction than potato.

Experiment flowchart



Variables

Independent Variables

The pH of the hydrogen peroxide solutions and the source of catalases are the independent variable of this experiment.

Dependant Variable

The amount of gas pressure exerted by oxygen produced is the dependant variable of this experiment. The rates of reaction of decomposition of hydrogen peroxide can be compared by measuring the gas pressure using PASCO PASPORT Chemistry Sensor. The higher the gas pressure exerted by oxygen produced at a given time interval, the faster the catalytic reaction is.

Controlled Variables

Controlled variables are to ensure that this experiment is a 'fair test' **(Table 2)**.

Table 2. Controlled variables.

Controlled variables	Method of Control	Reason
Number and volume of potato and chicken liver	The potato and chicken liver cubes are measured with a plastic ruler and cut to a volume of 1 cm^3 using a sharp knife. 1 potato or chicken liver cube is placed in the conical flask of each pH solution.	The potato and chicken liver are cut to the same volume to compare and determine which source of catalase has higher concentration per unit volume. Different number of potato or chicken liver cubes can affect the rate of reaction.
Concentration and volume of hydrogen peroxide in each pH solution	Each pH solution contains the same amount of substrate, i.e., 30 ml of 6% hydrogen peroxide.	Different concentration and volume of hydrogen peroxide will affect the rate of the catalase activity. The amount of substrate must be the same across experiments for comparison.
The same potato is used	The potato cubes used in this experiment are cut from the same potato.	Different potatoes may have different concentration of catalase.
Measuring time for gas pressure	The data is collected at the specific times, i.e., 30 sec, 60 sec, 90 sec and 120 sec.	The data collected must be measured at the same time for comparison.
PH conditions of the substrate	The pH conditions of the substrate for both chicken liver and potato cubes are the same, i.e., pH 1.6, 3.3, 6.5, 9.9 and 11.4.	The same pH conditions are compared across both chicken liver and potato catalases.
Size of conical flask	The 100 ml conical flasks of the same size are used.	Conical flasks must be of the same size to ensure the same height of the solution for fair

		comparison. The opening of the conical flasks must be of the size to ensure good fitting of the stopper to prevent leaking of the oxygen gas.
PASCO PASPORT Chemistry MultiMeasure Sensor and stopwatch.	The same equipment is used for measurement.	This is to minimise systematic errors and hence improve the accuracy of the data collected.
Room Temperature	All the experiments are conducted at the room temperature of 22 °C.	Different temperature affects the enzyme activity.

Uncontrolled Variables

After the catalase is placed into the conical flask, the stopper of the gas pressure tubing must be connected to the conical flask immediately and the stopwatch must be activated simultaneously. The time lapse between placing the catalase into the conical flask and connecting the stopper of the gas pressure tubing can be an uncontrolled variable. However, it is minimised by shortening the time of the action. The time difference is within 1 second. There are no other significant uncontrolled variables in this experiment.

Equipment and Materials

Equipment and Materials		
Materials	**Equipment**	**Personal Protective Equipment**
▪ 6% Hydrogen peroxide ▪ 1 mole Hydrochloric acid ▪ 1 mole Sodium Hydroxide ▪ 2 mole Sodium Hydroxide ▪ Distilled water ▪ White potato ▪ Chicken livers	▪ 100 ml conical flasks ×10 ▪ 100 ml measuring ×5 cylinders ▪ PASCO PASPORT Chemistry MultiMeasure Sensor with PASCO Scientific pH probe and gas pressure tubing, PASPORT Interface and Data Acquisition Software ▪ Stopwatch ▪ 20 cm plastic ruler ▪ Knife ▪ Pipette ▪ Chopping board ▪ Thermometer ▪ Scientific calculator – Casio FX-CG50 AU ▪ Stirring Rod ▪ Adhesive labelling stickers	▪ Apron ▪ Safety glasses ▪ Safety gloves ▪ Enclosed footwear ▪ Surgical mask

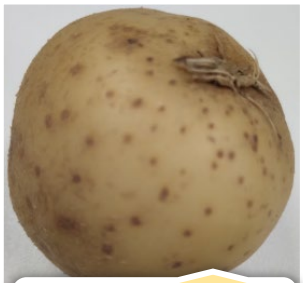
Hydrogen peroxide

1 mole hydrochloric acid

1 mole sodium hydroxide

2 mole sodium hydroxide

Chicken livers



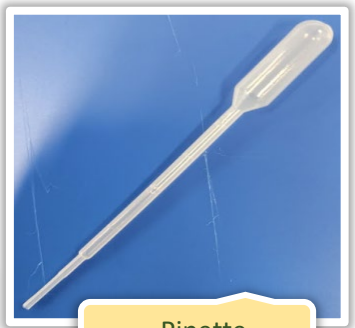
White potato

Measuring cylinder

Stopwatch

Conical flask

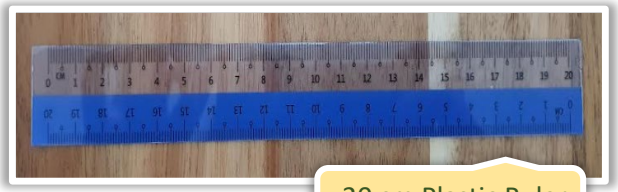
Distilled water



Pipette

PASCO PASPORT Chemistry MultiMeasure Sensor with pH probe and gas pressure tubing

Chopping board



20 cm Plastic Ruler

Thermometer

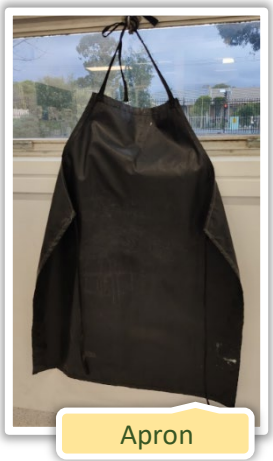
Adhesive labelling stickers

Knife

Stirring rod

Scientific calculator

Safety glasses



Apron

Safety gloves

Enclosed Footwear

Surgical mask

Procedure

STEP 1: Preparation	
1. Put on personal protective equipment: apron, safety glasses, safety gloves, enclosed footwear, surgical mask. 2. Prepare all the equipment and materials. 3. Set up the chemistry sensor with the pH probe and the gas pressure tubing. 4. Cut 5 1cm^3 cubes of potato and chicken liver with a ruler and a sharp knife.	

Step 2: Prepare 100 ml of pH hydrogen peroxide solutions.	
pH 1.6	1. Rinse the 100 ml measuring cylinder with hydrogen peroxide to prevent dilution. 2. Fill the measuring cylinder with 60 ml 6 % hydrogen peroxide. 3. Fill the measuring cylinder with 30 ml of distilled water. 4. Using the pH probe, fill the measuring cylinder with 1 mole HCL until pH is 1.6. 5. Fill the measuring cylinder with distilled water until the total volume of the solution is 100 ml. 6. Use the stirring rod to slowly mix the solution evenly. 7. Label the solution of pH 1.6 with an adhesive labelling sticker.
pH 3.3	1. Rinse the 100 ml measuring cylinder with hydrogen peroxide to prevent dilution. 2. Fill the measuring cylinder with 60 ml 6 % hydrogen peroxide. 3. Fill the measuring cylinder with 38 ml of distilled water. 4. Test the pH using the pH probe which is about pH 5. 5. Fill the measuring cylinder with drops of 1 mole hydrochloric acid until the pH reaches pH 3.3. 6. Fill the measuring cylinder with distilled water until the total volume of the solution is 100 ml. 7. Use the stirring rod to slowly mix the solution evenly. 8. Label the solution of pH 3.3 with an adhesive labelling sticker.
pH 6.5	1. Rinse the 100 ml measuring cylinder with hydrogen peroxide to prevent dilution. 2. Fill the measuring cylinder with 60 ml 6 % hydrogen peroxide. 3. Fill the measuring cylinder with 30 ml of distilled water. 4. Using the pH probe, fill the measuring cylinder with 1 mole sodium hydroxide until pH is 6.5. 5. Fill the measuring cylinder with distilled water until the total volume of the solution is 100 ml. 6. Use the stirring rod to slowly mix the solution evenly. 7. Label the solution of pH 6.5 with an adhesive labelling sticker.
pH 9.9	1. Rinse the 100 ml measuring cylinder with hydrogen peroxide to prevent dilution.

	2. Fill the measuring cylinder with 60 ml 6 % hydrogen peroxide. 3. Fill the measuring cylinder with 30 ml of distilled water. 4. Using the pH probe, fill the measuring cylinder with 1 mole sodium hydroxide until pH is 9.9. 5. Fill the measuring cylinder with distilled water until the total volume of the solution is 100 ml. 6. Use the stirring rod to slowly mix the solution evenly. 7. Label the solution with pH 9.9 with an adhesive labelling sticker.
pH 11.4	1. Rinse the 100 ml measuring cylinder with hydrogen peroxide to prevent dilution. 2. Fill the measuring cylinder with 60 ml 6 % hydrogen peroxide. 3. Fill the measuring cylinder with 35 ml of 1 mole sodium hydroxide. 4. Using the pH probe, test if the pH is 11.4. 5. If the pH is not 11.4, then add drops of 2 mole sodium hydroxide until the pH is 11.4. 6. Fill the measuring cylinder with distilled water until the total volume of the solution is 100 ml. 7. Use the stirring rod to slowly mix the solution evenly. 8. Label the solution of pH 11.4 with an adhesive labelling sticker.

Note: Make sure the pH probe is rinsed with distilled water before testing each solution.

Step 3: Conducting the experiments	
Potato catalase	1. Rinse each conical flask with each pH solution to prevent dilution. 2. Fill each 100 ml conical flask with 50 ml of each pH solution. 3. Label each pH solution of pH 1.6, pH 3.3, pH 6.5, pH 9.9 and pH 11.4 with adhesive labelling stickers. 4. Place a 1cm^3 potato cube in the conical flask for pH 1.6 solution. 5. Immediately place the stopper of the gas pressure tubing in the conical flask to measure the increase of gas pressure at time intervals of 30 sec, 60 sec, 90 sec and 120 sec. 6. Record data between time intervals. 7. Repeat steps 4 to 6 for the other four pH solutions of pH 3.3, pH 6.5, pH 9.9 and pH 11.4.
Chicken liver catalase	1. Rinse each conical flask with each pH solution to prevent dilution. 2. Fill each 100 ml conical flask with 50 ml of each pH solution. 3. Label each pH solution of pH 1.6, pH 3.3, pH 6.5, pH 9.9 and pH 11.4 with adhesive labelling stickers. 4. Place a 1cm^3 chicken liver cube in the conical flask for pH 1.6 solution. 5. Immediately place the stopper of the gas pressure tubing in the conical flask to measure the increase of gas pressure at time intervals of 30 sec, 60 sec, 90 sec and 120 sec. 6. Record data between time intervals. 7. Repeat steps 4 to 6 for the other four pH solutions of pH 3.3, pH 6.5, pH 9.9 and pH 11.4.

Risk Assessment

Safety Precautions

Prudent laboratory safety practices were followed. Chemical contacts were avoided by putting on personal protective equipment including an apron, safety glasses, safety gloves, enclosed footwear and a surgical mask for preventing inhalation of chemicals. Hair is tied back so that hair does not contact with any chemicals. The experiment was handled with care as the chemicals (H_2O_2, HCL, NaOH) are extremely corrosive. The chemicals can cause serious eye damage, shortness of breath if inhaled and skin irritations as well as burns on skin. When pouring the pH solutions, a slow pouring action was used to minimise the risk of spilling of chemicals. When cutting the potato and chicken liver into cubes, the knife was carefully handled to prevent cuts. Experiments were done in a well-ventilated laboratory. The equipment and apparatus used in this experiment were carefully handled to prevent any accidents.

Environmental Consideration

The experiment was conducted in compliance with the control measures for preparation, usage of laboratory materials and disposal of chemical wastes. There were no significant environmental considerations as the equipment and actions used in this experiment presented no hazard or danger to the environment.

Ethical Consideration

There were no significant ethical considerations as the equipment and actions used in this experiment presented no harm to society or any individual.

Processing and Analysing Data and Information:

Table 3. Experiment 1: The rate of reaction of potato catalase decomposing hydrogen peroxide.

Time Duration	Potato Catalase Rate of Reaction (Change in kPa)														
	pH 1.6			pH 3.3			pH 6.5			pH 9.9			pH 11.4		
	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change
30 sec	100	101.2	**1.2**	100	101.2	**1.2**	100	101.4	**1.4**	100	102.8	**2.8**	100	102.4	**2.4**
60 sec	100	101.3	**1.3**	100	101.5	**1.5**	100	101.9	**1.9**	100	103.4	**3.4**	100	102.8	**2.8**
90 sec	100	101.4	**1.4**	100	101.8	**1.8**	100	102.4	**2.4**	100	104.0	**4.0**	100	103.0	**3.0**
120 sec	100	101.5	**1.5**	100	102.1	**2.1**	100	102.7	**2.7**	100	104.6	**4.6**	100	103.3	**3.3**

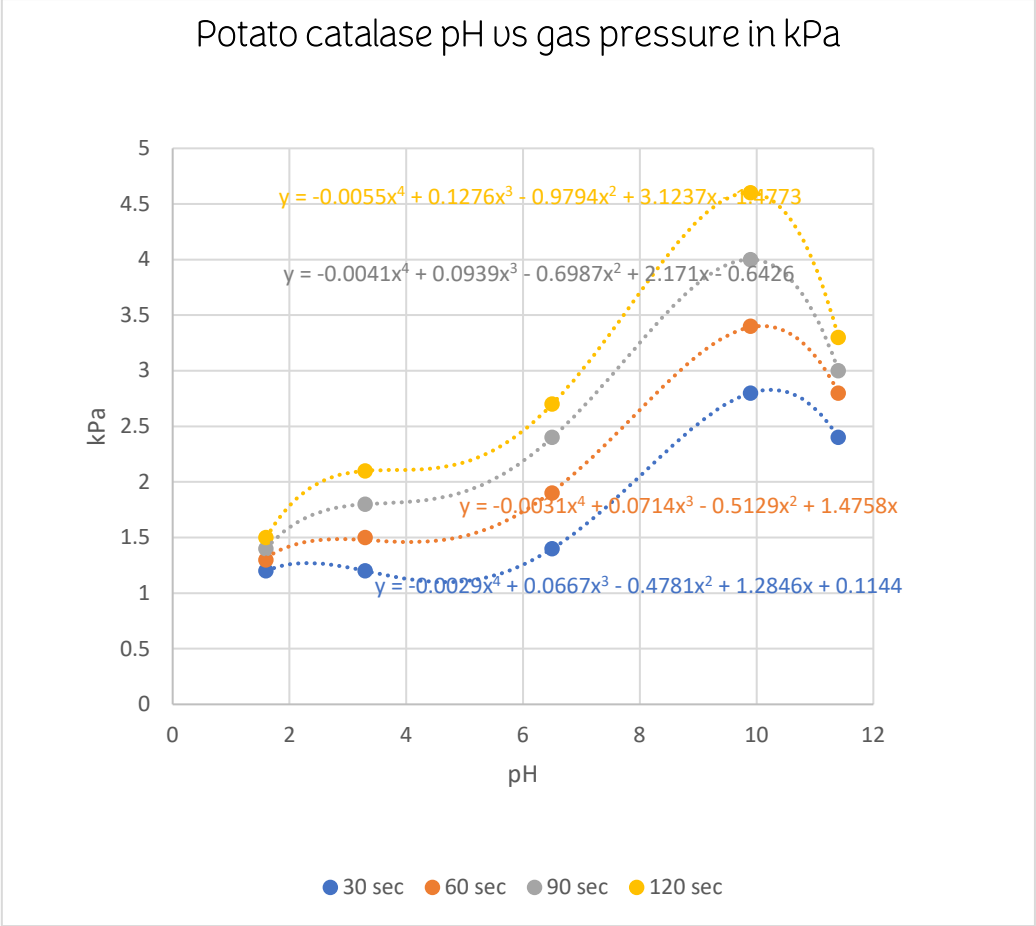


Figure 2a. Graph of potato catalase activity against pH

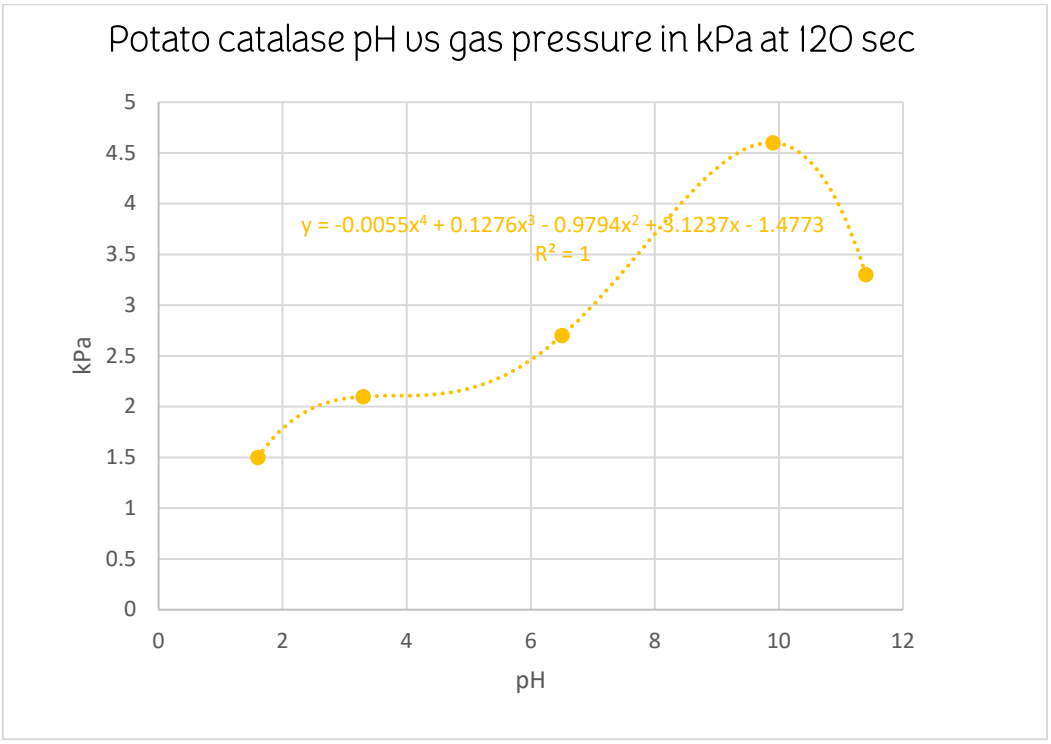


Figure 2b. Graph of potato catalase activity against pH at 120 sec

Table 4. Experiment 2: The rate of reaction of chicken liver catalase decomposing hydrogen peroxide.

Time Duration	Chicken Liver Catalase Rate of Reaction (Change in kPa)														
	pH 1.6			pH 3.3			pH 6.5			pH 9.9			pH 11.4		
	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change	Initial kPa	Final kPa	kPa Change
30 sec	100	107.2	**7.2**	100	117.3	**17.3**	100	133.3	**33.3**	100	110.3	**10.3**	100	104.8	**4.8**
60 sec	100	110.1	**10.1**	100	125.1	**25.1**	100	141.2	**41.2**	100	122.5	**22.5**	100	105.6	**5.6**
90 sec	100	112.2	**12.2**	100	131.7	**31.7**	100	149.4	**49.4**	100	130.2	**30.2**	100	106.2	**6.2**
120 sec	100	113.8	**13.8**	100	137.5	**37.5**	100	159.3	**59.3**	100	135.9	**35.9**	100	107.0	**7.0**

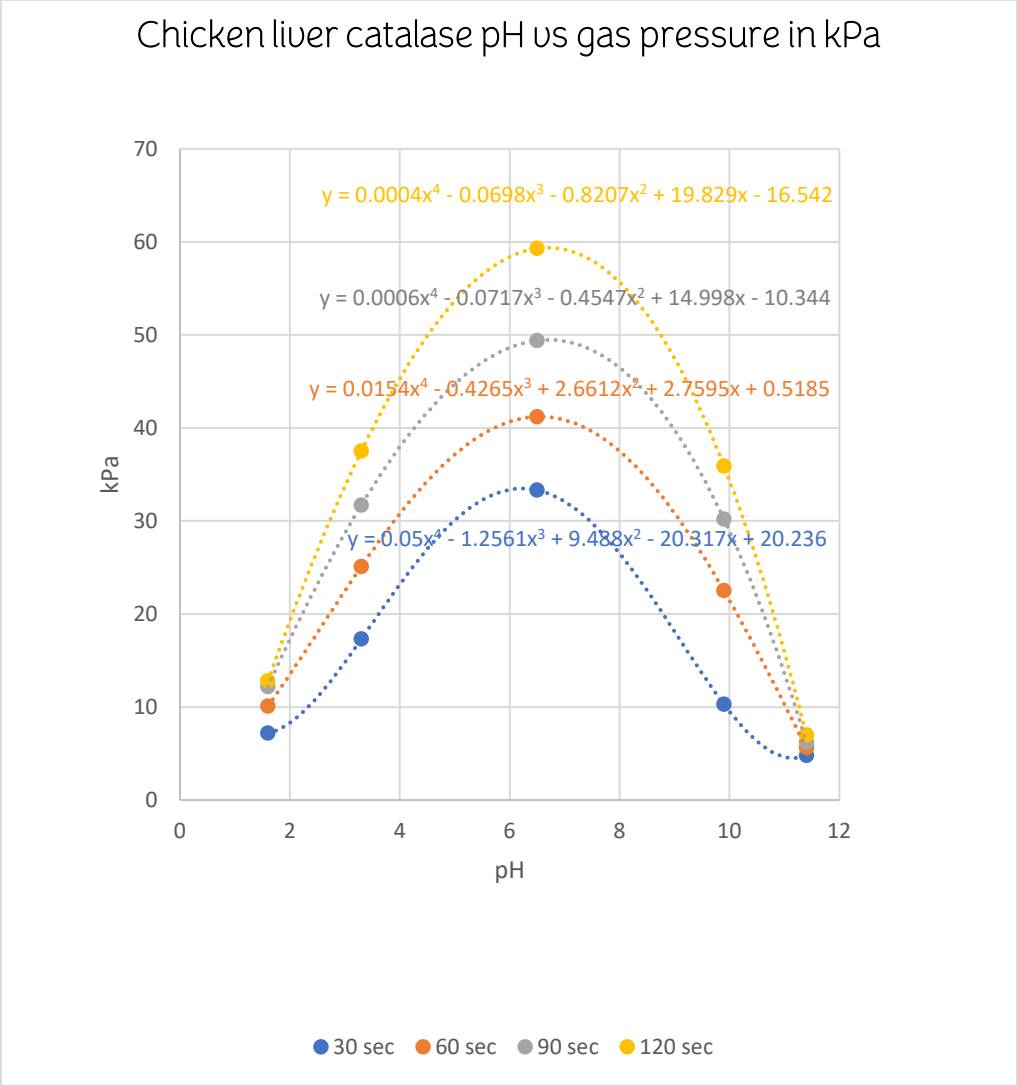


Figure 3a. Graph of chicken liver catalase activity against pH

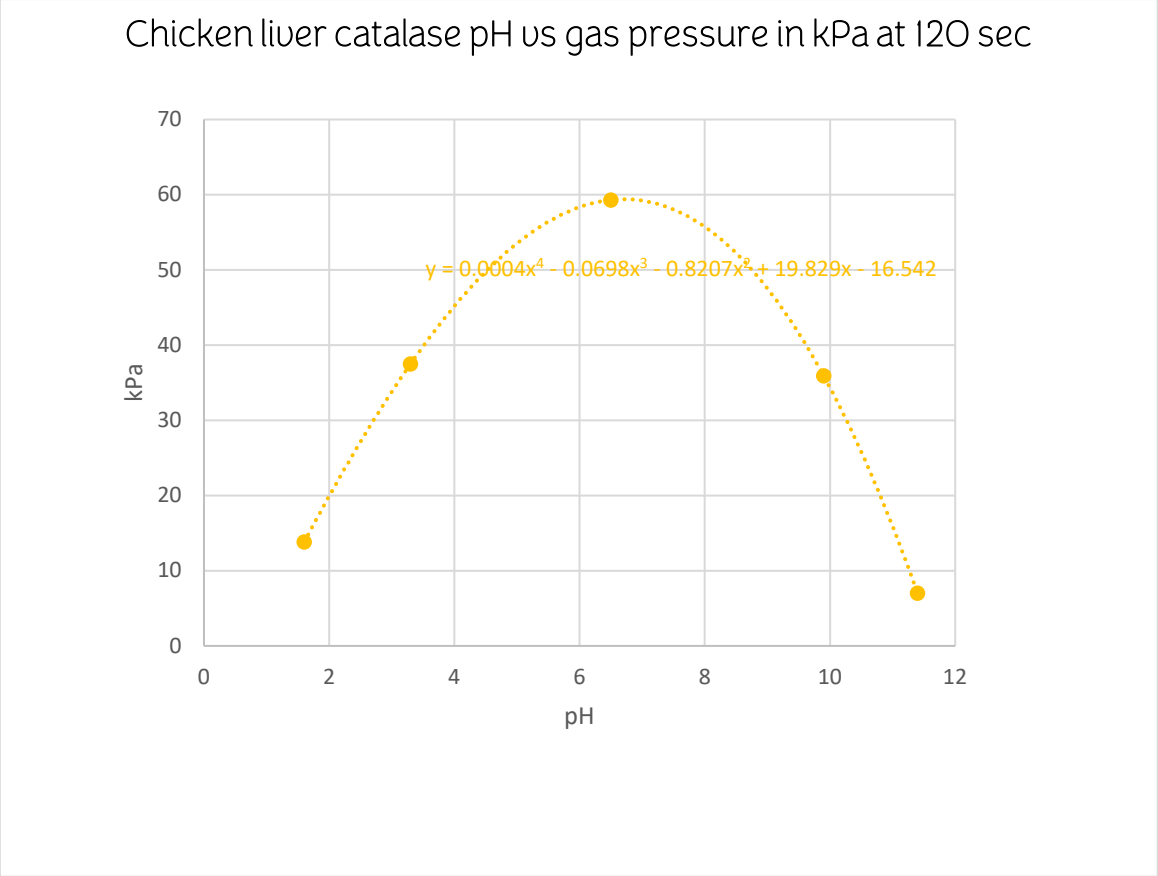


Figure 3b. Graph of chicken liver catalase activity against pH at 120 sec

Table 5. The order of the rate of reaction measured in the change of gas pressure in kPa for potato catalase at 120 seconds.

The order of the rate of reaction	pH	Change in kPa
1st	9.9	4.6
2nd	11.4	3.3
3rd	6.5	2.7
4th	3.3	2.1
5th	1.6	1.5

Table 6. The order of the rate of reaction measured in the change of gas pressure in kPa for chicken liver catalase at 120 seconds.

The order of the rate of reaction	pH	Change in kPa
1st	6.5	59.3
2nd	3.3	37.5
3rd	9.9	35.9
4th	1.6	13.8
5th	11.4	7.0

Table 7. The rate of reaction of potato and chicken liver catalases decomposing hydrogen peroxide at 120 seconds.

Sources of catalase	Potato and Chicken Liver Catalase Rate of Reaction (kPa) at 120 sec				
	pH 1.6	**pH 3.3**	**pH 6.5**	**pH 9.9**	**pH 11.4**
	kPa Change	kPa Change	kPa Change	kPa Change	kPa Change
Potato catalase	1.5	2.1	2.7	4.6	3.3
Chicken Liver catalase	13.8	37.5	59.3	35.9	7.0

Table 8. The optimum pH and greatest change in kPa for potato and chicken liver catalases.

Sources	**Potato catalase**	**Chicken liver catalase**
Optimum pH	9.9	6.5
Greatest change in kPa at 120 seconds	4.6	59.3
Chicken liver catalase is 12.9 times faster in the rate of reaction than potato catalase at their optimum pH.		

The rates of reaction for the potato and chicken liver catalases decomposing hydrogen peroxide in five different pH conditions (pH 1.6, 3.3, 6.5, 9.9 and 11.4) are compared. At 120 seconds, the potato catalase shows the greatest increase of gas pressure of 4.6 kPa at pH 9.9, followed by pH 11.4, pH 6.5, pH 3.3. The least increase of gas pressure for potato catalase is 1.5 kPa at pH 1.6. Based on the graph, the optimum pH for potato catalase is pH 9.9 at which it produces the greatest rise of gas pressure of 4.6 kPa at 120 seconds **(Table 3, Table 5, Figure 2b)**.

Comparatively, at 120 seconds, the chicken liver catalase shows the maximum increase of gas pressure of 59.3 kPa at pH 6.5, followed by pH 3.3, pH 9.9, pH 1.6. Chicken liver catalase shows the minimum increase of 7.0 kPa at pH 11.4. Based on the graph, Chicken liver catalase works best at the optimum pH of 6.5 at which it produces the greatest rise of gas pressure of 59.3 kPa at 120 seconds **(Table 4, Table 6, Figure 3b)**.

In the interval of 2 minutes, both catalases show constantly increased rate of reaction. Both catalases exhibit similar trend in change of gas pressure kPa at time intervals of 30, 60, 90 and 120 sec respectively **(Figure 2a, Figure 3a)**.

Interestingly, there is a huge difference in rate of reaction between potato catalase and chicken liver catalase. Chicken liver catalase decomposes hydrogen peroxide more efficiently than potato catalase as more oxygen is produced during the enzyme-catalysed reactions (59.3 vs 4.6 kPa at 120 seconds) **(Table 7, Table 8)**. In other words, chicken liver catalase is much more powerful than potato catalase as chicken liver catalase works 12.9 times faster in the rate of reaction than potato catalase at their optimum pH.

Additionally, the maximum increase of gas pressure in potato catalase at its optimum pH is lower than the minimum increase of gas pressure in chicken liver catalase at its least favourable pH (4.6 vs 7.0

kPa at 120 seconds) **(Table 7, Figure 4)**. The higher the concentration of an enzyme is, the faster the catalytic reaction is. This finding implies that chicken liver contains much higher concentration of catalase per unit volume than potato.

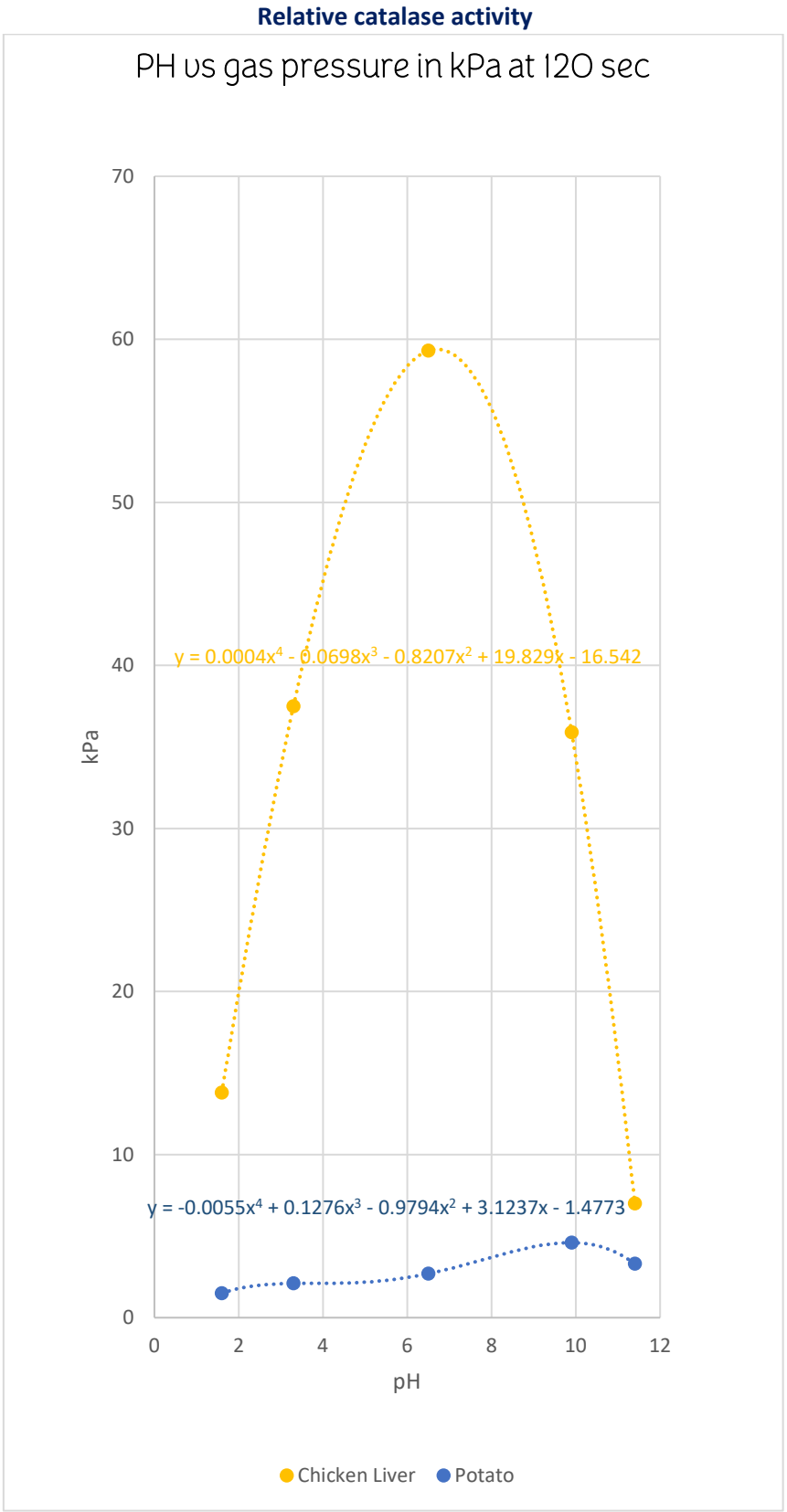


Figure 4. Graph of potato and chicken liver catalase activity against pH at 120 sec

Discussion and Evaluation

Data discussion

The result of the investigation supports the hypothesis that the rate of enzyme activity will reduce when the pH is below or above the optimum pH. All enzymes have an optimum pH at which they function best. Enzymes also have a working range of pH values at which they can still function well. The active site is formed by a specific conformation of the enzyme's amino acid side chains, which has a specific ionization state that forms the resulting specific three-dimensional structure of the enzyme. Changes in pH affects the ionization state of the amino acids which affects the ionic bonds that hold the three-dimensional shape of the enzyme, thereby impacting the effectiveness of the enzyme activity. In living systems, biochemical buffers maintain an optimum pH range for the enzyme to function best. Extreme pH conditions will cause enzyme to denature. Denaturation is a permanent and irreversible change caused by the breaking of the hydrogen and ionic bonds that maintain the three-dimensional shape of the enzyme. Similar to high temperature, acidic and basic pH can disrupt the three-dimensional shape of the enzyme and change the shape of the active site and therefore causing an irreversible change in the function of the enzyme. When the enzyme becomes denatured and loses its structure, the substrate will not bind to the denatured active site **(Figure 5)**. On the other hand, pH also affects the charge and the shape of the substrate, therefore the substrate cannot bind to the active site.

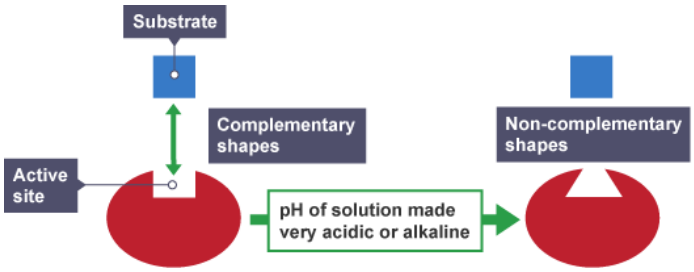


Figure 5. When denaturation occurs at very acidic or alkaline pH values, the altered shape of the active site of the enzyme is no longer complementary to its specific substrate.
Reference:https://www.bbc.co.uk/bitesize/guides/zcr74qt/revision/5#:~:text=Enzyme%20activity%20is%20at%20its,pH%20the%20enzyme%20activity%20decreases

The result also supports the hypothesis that animal cells have more catalase than plant cells. In this experiment, chicken liver has higher concentration of catalase per unit volume than potato. Catalase is found in all aerobic organisms including animal cells, plant cells and human cells. Animal cells have a higher rate of cellular respiration than plant cells, therefore requiring more catalase to decompose hydrogen peroxide which is a toxic metabolic by-product of the cellular respiration.

Extension of knowledge

Enzymes can accelerate the rate of catalytic reaction by 10^6 to 10^{15} folds. Catalase is an antioxidant enzyme which can be found in abundance in liver where toxin is removed. According to Price and Greenfield (1954), rat liver has a range of 160 to 180 units of catalase per gram. In plants, catalase is involved with photorespiration and photosynthesis in peroxisome organelles where hydrogen peroxide is decomposed into water and oxygen gas. There are three types of catalases which are

monofunctional heme-containing catalases, heme-containing catalase-peroxidases, and manganese-containing catalases **(Figure 5)**. One molecule of catalase can decompose millions of hydrogen peroxide molecules per minute.

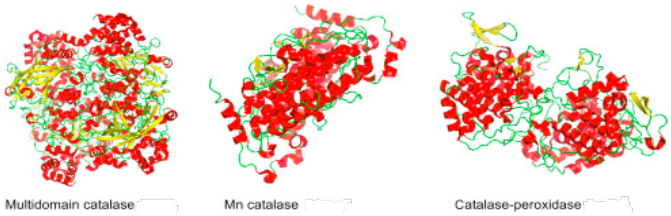


Figure 5. Three types of catalases
Reference: https://www.sciencedirect.com/topics/neuroscience/catalase

Some animals and plants including extremophiles can survive or tolerate in extreme environments, for example, very high or low temperature, very acidic or alkaline surroundings and salinity. Extremophiles have special enzymes called extremozymes that help them to survive. Those enzymes have other applications to our society. For example, thermophiles possess amylases which have many industrial uses such as baking and making paper.

Acatalasemia, a genetic disorder associated with catalase deficiency, is caused by mutations in CAT gene that gives instructions for producing catalase. Study estimates that 1 in 31,250 of individuals in general population have acatalasemia. Low level of catalase activity results in insufficient decomposition and excessive accumulation of hydrogen peroxide in the body. Acatalasemia not only cause body cells to be susceptible to oxidative damage to DNA, proteins and lipids resulting in oxidative stress, but also increases a higher risk of disease and health problems including diabetes mellitus and atherosclerosis. Moreover, a build-up of hydrogen peroxide due to a lack of catalase may contribute to the development of grey hair. Other diseases related to enzyme deficiency include Phenylketonuria (PKU) and Tay Sachs disease, to name a few.

Control Trial

As only catalase is used to assess the effect of pH on decomposition rate of hydrogen peroxide in this study, control trial is not required.

Random error

In Experiment 2c, the stopper popped off the conical flask at about the time interval of 30 seconds. This caused a slight drop in gas pressure, but the stopper was quickly connected to the conical flask. To minimise this error, the stopper should be pressed manually or clipped tightly to prevent leaking of oxygen gas. Additionally, although the chicken liver catalase was measured to a size of 1 cm^3, measurement error may exist due to the texture and irregularity of the chicken liver.

Systematic Error

Systematic error can be minimised with using calibrated equipment that are reliable and functioning accurately including the chemistry sensor and stopwatch.

Limitation and improvement

The limitation of this study is only one trial is conducted. Future experiment can be improved by increasing sample size to repeat more trials to obtain average values, therefore reducing random errors. Furthermore, the experiment design can be improved by investigating more pH conditions to obtain a more accurate data to determine the optimum pH.

Conclusion

The experiment supports the hypotheses that the pH below and above the optimum pH decreases the rate of catalytic reaction of catalase breaking down hydrogen peroxide into oxygen gas and water, and that animal cells contain more catalase than plant cells. Chicken liver that contains higher catalase concentration per unit volume has a faster rate of reaction than potato. In this experiment, chicken liver catalase performs best at pH 6.5 while potato catalase works most effectively at pH 9.9.

Word Count

- 1998 words
- Headings, titles, figure captions, tables, references and logbook/journal are not included in the word count.

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Science Journal

Chloe Yaan Yuit Yew

Notes and Research

Title

An experiment investigating the effect of pH on enzyme activity: How does pH affect the rate of catalytic reaction of catalase breaking down hydrogen peroxide?

Research Question

How does pH affect the rate of catalytic reaction of catalase breaking down hydrogen peroxide?

February - March 2022

- Understanding enzyme through bioluminescence.
- Research broadly on enzymes.
- Website search, library resources.
- Formulate the topic of interest.
- Investigating the factors that determine the enzyme activity.

Reference:

- https://education.nationalgeographic.org/resource/bioluminescence
- https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/25-enzymes/enzyme-experiments.html

April 2022

- Continue investigating the factors that determine the enzyme activity: temperature, concentration, pH, presence of inhibitors or activators.
- Possible enzyme materials of interest: sweet potato, banana peels, broccoli, cabbage, garlic, onion, carrot, white potato, parsnip, yeast, liver, ground meat.
- Common method for measuring catalase activity: UV spectrophotometric method
- Working on research design.
- Discuss with Mr. Starczak to narrow down the topic of interest.
- Refine the research question.

Background information/Introduction

What are enzymes?

- Enzymes are generally named for the reactions of what an enzyme does. One great example is DNA-polymerase which involves in DNA replication, it acts on DNA and synthesizes polymers of DNA. Generally, the suffix "ase" is usually used in most enzymes' names.
- Enzymes are key players in a chemical reaction.
- Enzymes are biological catalysts that speed up the rate of reaction.
- Enzymes either split a substrate apart or combine them together.
- Types of enzymes include catalase, diastase, and pectinase.
- **Induced fit model**: The shape of the active site is very important to determine the function of the enzyme.

Table. Types of enzymes
Reference: https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/25-enzymes/enzyme-experiments.html

Enzyme	Substrate	Products
Catalase	Hydrogen peroxide (H_2O_2)	Oxygen gas + water ($O_2 + H_2O$)
Diastase	Starch	Maltose
Pectinase	Pectin (in plant cell walls)	Simple sugars (releases juices from cells)
Pepsin	Protein	Short polypeptides
Rennin	Soluble casein (milk protein)	Insoluble casein (curdled milk)

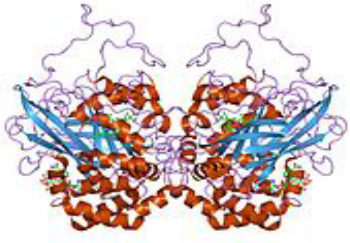
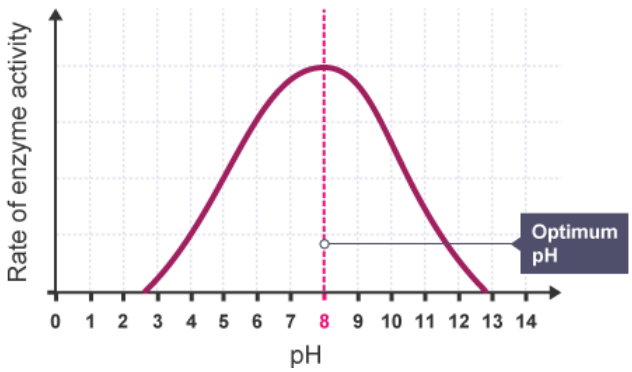


Figure. Catalase
Reference: https://en.wikipedia.org/wiki/Catalase

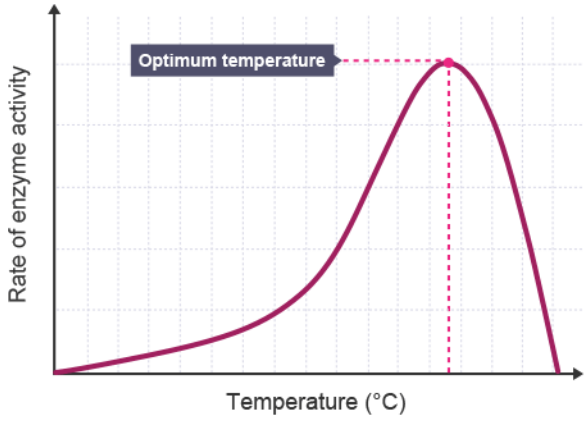
25/05/2022

Factors affecting enzyme activity

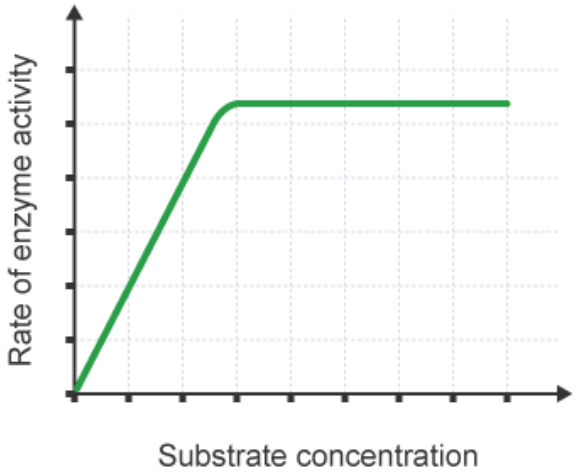
- Factors: Temperature, concentration, pH, presence of inhibitors or activators.



Reference:https://www.bbc.co.uk/bitesize/guides/z9jrng8/revision/3#:~:text=Extremes%20of%20pH%20also%20denature,best%20within%20this%20pH%20range



Reference:https://www.bbc.co.uk/bitesize/guides/z9jrng8/revision/3#:~:text=Extremes%20of%20pH%20also%20denature,best%20within%20this%20pH%20range



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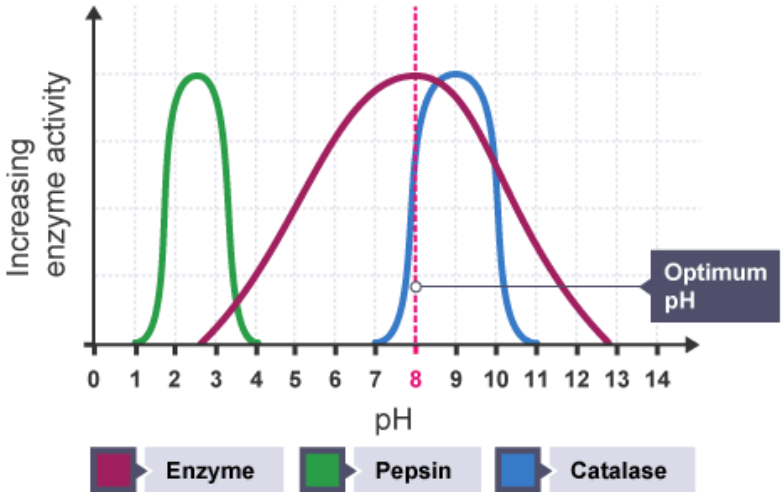


Reference:https://www.bbc.co.uk/bitesize/guides/zcr74qt/revision/5#:~:text=Enzyme%20activity%20is%20at%20its,pH%20the%20enzyme%20activity%20decreases

Effect of pH on enzyme activity

- The active site is formed by a specific conformation of the enzyme's amino acid side chains, which has a specific ionization state that forms the resulting specific three-dimensional structure of the enzyme.
- Biochemical buffers in living systems maintain an optimum pH range for the enzyme to function best.
- In humans, catalase works optimally within the pH range of 7 and 11.
- Within the enzyme molecule, positively and negatively charge amino acids attract with each other, resulting in the folding/conformation of the enzyme molecule, its shape and the shape of the active site.
- Changes in pH cause the atoms and molecules of the amino acids to ionize, change the shape of the active site of the enzyme and impair its function, therefore impacting the rate of enzyme activities.
- If the pH level falls outside its optimum pH range, the enzyme becomes denatured and loses its structure. Denaturation takes place when an enzyme loses its shape.

Enzyme	Optimum pH
Salivary amylase	6.8
Stomach protease (pepsin)	1.5–2.0
Pancreatic protease (trypsin)	7.5–8.0

Reference:

- https://sciencing.com/ph-levels-catalase-6826245.html
- https://www.bbc.co.uk/bitesize/guides/z9jrng8/revision/3#:~:text=Extremes%20of%20pH%20also%20denature,best%20within%20this%20pH%20range
- https://www.bbc.co.uk/bitesize/guides/zcr74qt/revision/5#:~:text=Enzyme%20activity%20is%20at%20its,pH%20the%20enzyme%20activity%20decreases
- https://www.creative-enzymes.com/resource/effect-of-ph-on-enzymatic-reaction_51.html

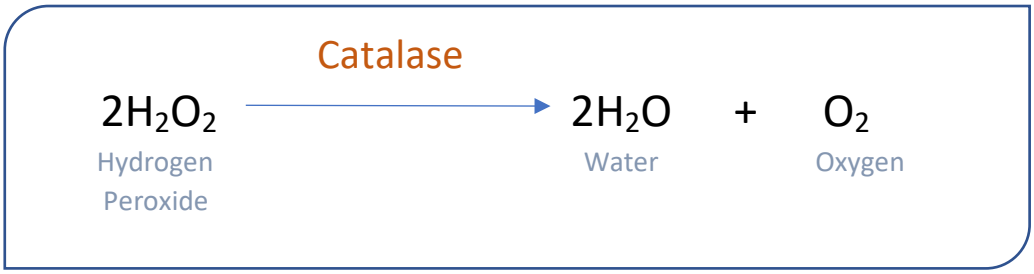
What is pH?

- PH is a scale measure whether a chemical is acidic or basic and describes how much ionic hydrogen (H+) or hydroxide (OH) it contains.
- Formula: pH = -log (H^+)

	solutions	pH scale
Strong acid	Hydrochloric acid (HCL)	1.5 to 3.5
Weak acid	Sulfurous acid (H_2SO_3)	5.1
Neutral	Distilled water (H_2O)	7
Weak basic	Ammonium hydroxide (NH_4OH)	7 to 10
Strong basic	Sodium Hydroxide (NaOH)	13 to 14

What is hydrogen peroxide?

- When catalase is placed in hydrogen peroxide, the hydrogen peroxide will split apart to produce oxygen and water.
- It is also a poisonous by-product of cellular metabolism produced by living cells.
- Antiseptic properties.
- Oxidising and bleaching agent.



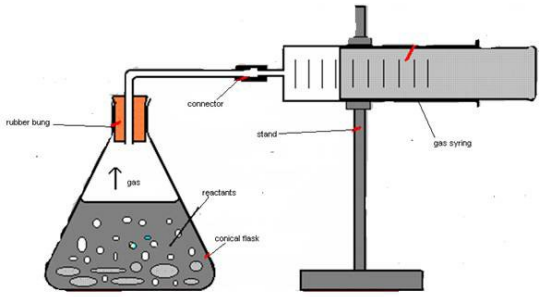


Figure. Basic experiment set up to collect oxygen gas

27/05/2022

- Discussing the experiment with Mr. Starczak and Mr. Wren.
- Visiting the lab: identify the materials and equipment.

29/05/2022

- Study the induced fit model
- Draw the diagram of induced fit model using Paint 3D.
- Construct the aim and hypotheses of the investigation, outline pilot study.

Reference:

- https://www.khanacademy.org/test-prep/mcat/biomolecules/enzyme-structure-and-function/v/the-induced-fit-model-of-enzyme-catalysis

The aim

- To investigate the effect of pH on catalase activity (5 pH conditions).
- To observe the catalytic action of two sources of catalase on hydrogen peroxide (to investigate the difference between animal and plant catalases).

Hypotheses

Hypothesis 1: If the pH is below or above the optimum pH, the rate of the catalytic action of catalase will decrease.

Hypothesis 2: Animal cells contain more catalase than plant cells. Chicken liver that contains higher catalase concentration per unit volume has a faster rate of catalytical reaction than potato.

02/06/2022

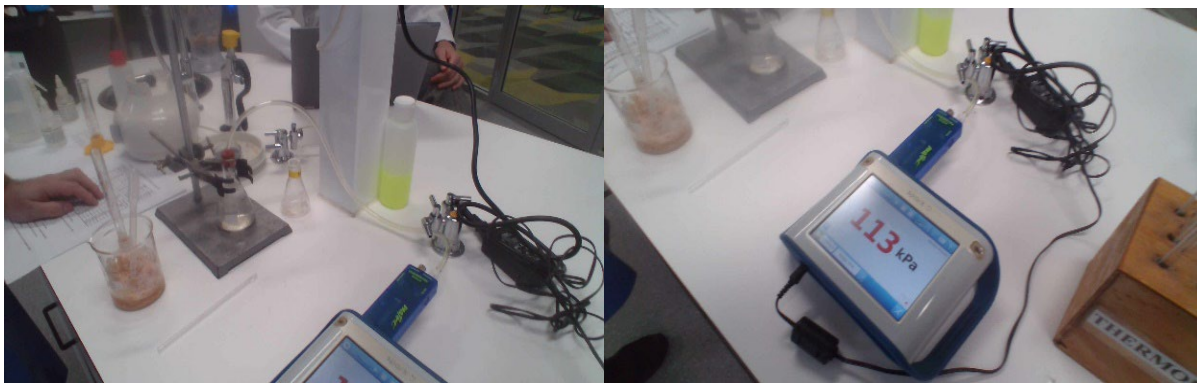
- Design experiment: procedure, materials, and equipment.

03/06/2022

PILOT STUDY 1 (LAB DAY)

- Conduct pilot study to determine:
  - time duration for measuring oxygen pressure
  - volume of H_2O_2
  - volume of enzyme
- Both enzyme and substrate concentration can be a limiting factor.
- Measure the pH of each substrate sample by using PASCO PASPORT Chemistry MultiMeasure Sensor with PASCO scientific pH probe.
  - The pH electrode produces a voltage proportional to the pH of the solution. This voltage is measured by the chemistry sensor which computes pH.
- Gas pressure in the conical flask is measured by connecting the conical flask to the chemistry sensor using a piece of tubing.
- Readings are obtained using Spark Learning System PS-2008A.

- Substrate samples of a total volume of 100 ml:
  1. pH 1: 50 ml 6% H_2O_2 + 10 ml distilled water + 40 ml 2 mole HCL
  2. pH 6.2: 50 ml 6% H_2O_2 + 50 ml distilled water
  3. pH 12: 50 ml 6% H_2O_2 + 44 ml distilled water + 6 ml 2 mole NaOH
- Catalase: 3 ml of blended potato



Result

Table. Potato catalase rate of reaction in kPa

Potato Catalase Rate of Reaction (kPa)			
Duration	pH 1	pH 6.2	pH 12
60 sec	2 kPa	10 kPa	7 kPa
120 sec	2 kPa	13 kPa	10 kPa

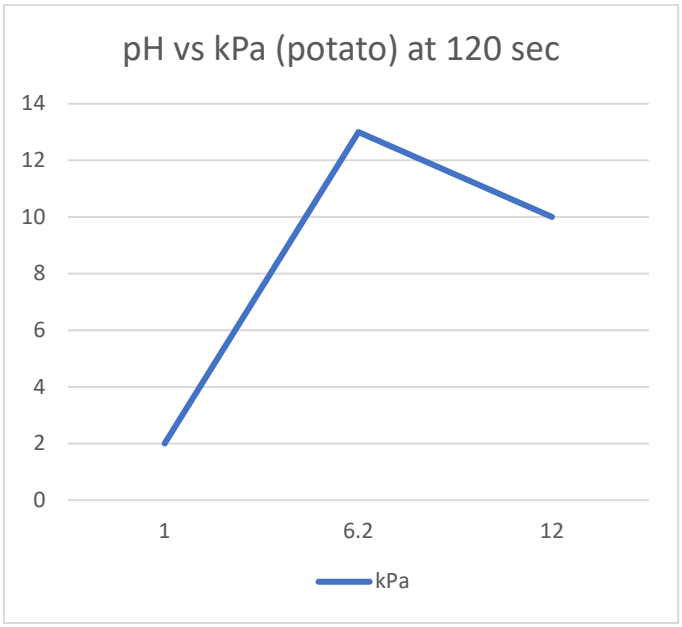


Figure. Graph of potato catalase activity against pH

Reference:
https://cdn.pasco.com/product_document/PASPORT-Chemistry-Sensor-Manual-PS-2170.pdf

04/06/2022

Calculation

Q: In a laboratory, oxygen gas can be obtained by the decomposition of H_2O_2. What volume of oxygen is produced from the decomposition of 10 ml of 6% of hydrogen peroxide?

Balanced equation:
$2H_2O_2$ (l) → 2 H_2O (l) + O_2 (g)

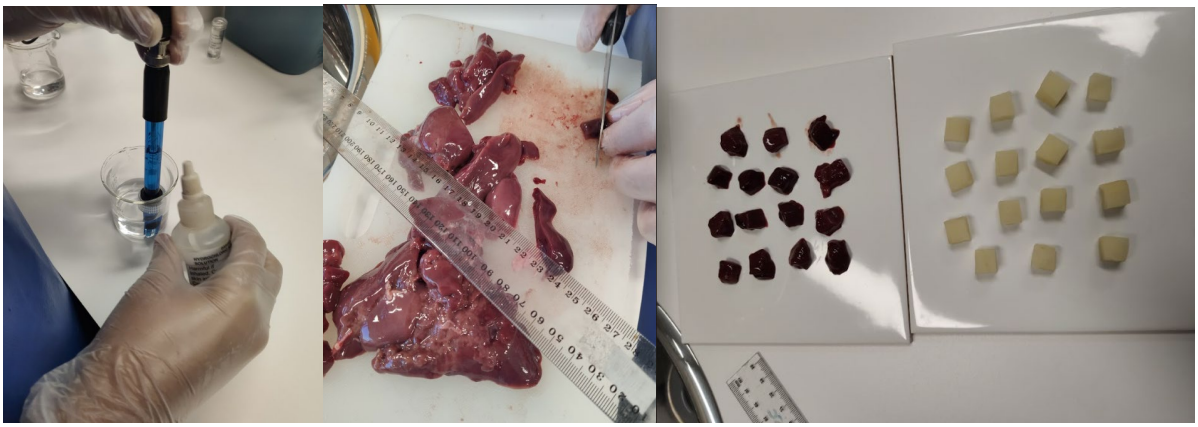
The molar ratio between H_2O_2 and O_2 = 2 : 1
The molar mass of H_2O_2 = 34 gram per mole
At STP condition, 1 mole of any ideal gas occupies volume of 22.4 L
10 ml of 6% of hydrogen peroxide solution:
10 ml x 6% = 0.6 ml of H_2O_2
0.6 g/34 g/mole=0.0176 mole
0.0176 mole of H_2O_2 will produce 0.0088 mole of O_2
Thus, the volume of 0.0088 mole of O_2 = 0.0088 mole X 22.4 L/mole = 0.1976L O_2 gas = 197.6 ml of O_2 gas at STP condition.

Reference:
- https://www.quora.com/If-the-theoretical-volume-of-oxygen-in-6ml-of-6-hydrogen-peroxide-is-132ml-why-were-my-results-20-6-ml

06/06/2022

Pilot study 2 (LAB DAY)

- Design pilot study:
  - Determine time duration for measuring oxygen pressure, volume of H_2O_2 and volume of enzyme
  - Measure the pH of each substrate sample by using the PASCO PASPORT Chemistry MultiMeasure Sensor with PASCO scientific pH probe.
  - Measure the gas pressure in the conical flask by connecting the conical flask to the chemistry sensor using a piece of tubing.
- Substrate samples of 100 ml:
  a. pH 1.9: 60 ml 6% H_2O_2 + 30 ml distilled water + 2 mole HCL
  b. pH 3: 60 ml 6% H_2O_2 + 38 ml distilled water + 2 mole HCL
- Catalase: 3x 1 cm^3 liver cube
- Readings are obtained using PASPORT Interface and Data Acquisition Software.



Result

	pH 1.9	pH 3
60 sec	12 kPa	Experiment stopped due to overflowing of oxygen gas.
120 sec	24 kPa	-

What I have learnt:

1. The stopper of the gas pressure tubing must be placed immediately to the conical flask so that the oxygen gas produced does not leak out.
2. When 3 pieces of 1 cm^3 chicken liver cube were added to 100 ml of pH 3 hydrogen peroxide solution, the reaction was too powerful with effervescence overflowing out of the conical flask (Figure). Overflowing happens when a lot of oxygen is produced. Solution: use a lower volume hydrogen peroxide solution, i.e., 50 ml, lesser catalase, i.e., 1 piece.

Figure. Overflowing of oxygen gas for pH 3 solution.

3. According to Nature, there is an initial rapid evolution of oxygen which lasts for about two minutes, depending on the peroxide concentration. After this, oxygen is given off at a steady rate which slowly decreases in the course of an hour.

Reference:
https://www.nature.com/articles/160041a0

08/06/2022

Experiment flowchart

- Designing the experiment flowchart using Canva.

14/06/2022

Experiment Day

Conducting the experiment

Variables

Independent Variables:

- 5 pH conditions
- 2 sources of catalase

Dependant Variable:

- Gas pressure exerted by oxygen in kPa (rate of reaction).
- At constant temperature and volume, the gas pressure exerted by oxygen gas depends on the total number of moles of gas present.

Reference:

- https://chem.libretexts.org/Bookshelves/General_Chemistry/Map%3A_Chemistry_-_The_Central_Science_(Brown_et_al.)/10%3A_Gases/10.06%3A_Gas_Mixtures_and_Partial_Pressures#:~:text=6%20in%20a%20more%20general,even%20hundreds%20of%20gaseous%20species.

Controlled Variables:

- Number and volume of potato and chicken liver
- Concentration and Volume of hydrogen peroxide in each pH solution
- The same potato is used
- Measuring time for gas pressure
- PH conditions for the substrate
- Size of conical flask
- PASCO PASPORT Chemistry MultiMeasure Sensor
- Room Temperature

Uncontrolled Variables:

- The time lapse between placing the catalase into the conical flask and connecting the stopper of the gas pressure tubing.

Equipment and Materials

- Working on list for equipment and materials.
- Personal protective equipment.

Procedure

- Working on procedures.
  - Step 1: Preparation.
  - Step 2: Prepare 100 ml of pH hydrogen peroxide solutions.
  - Step 3: Conducting the experiments.

Risk Assessment

Safety Precautions

- Putting on personal protective equipment: an apron, safety goggles, safety gloves, enclosed footwear, surgical mask.
- Hair is tied back.
- The chemicals (H_2O_2, HCL, NaOH) were handled with care.
- When pouring the pH solutions, a slow pouring was done to minimise the risk of spilling of chemicals.
- When cutting the potato and chicken liver into cubes, the knives was carefully handled to prevent cuts.
- Experiments were done in a well-ventilated laboratory.
- The equipment and apparatus used in this experiment were carefully handled to prevent any accidents or hazards.

Environmental Consideration

- The experiment was conducted in compliance with the control measures for preparation, usage of laboratory materials and disposal of chemical wastes.
- The equipment and actions used in this experiment presented no hazard or danger to the environment.

Ethical Consideration

- The equipment and actions used in this experiment presented no harm to society or any individual.

17/06/2022

Processing and Analysing Data and Information:

Data analysis

- Experiment 1. The rate of reaction of potato catalase decomposing hydrogen peroxide
- Experiment 2: The rate of reaction of chicken liver catalase decomposing hydrogen peroxide.
- The order of the rate of reaction measured in the change of gas pressure in kPa for potato catalase at 120 seconds.
- The order of the rate of reaction measured in the change of gas pressure in kPa for chicken liver catalase at 120 seconds.
- Comparing the greatest change in kPa at 30, 60, 90, 120 seconds for potato and chicken liver catalases.
  - Both catalases show consistent trend in rates of reaction at all time intervals.
- Comparing the optimum pH and greatest change in kPa at 120 seconds for potato and chicken liver catalases (59.3 vs 4.6 kPa).
  - The greatest increase for potato catalase is lower than the smallest increase of chicken liver catalase in kPa at 120 seconds (4.6 vs 7.0 kPa).
  - Chicken liver catalase is 12.9 times faster in the rate of reaction than potato catalase at their optimum pH.
  - This finding supports that chicken liver contains much higher concentration of catalase per unit volume than potato.
- Creating tables and graphs of line of best fit.

19/06/2022

Discussion and Evaluation:

Data Discussion

- The result of the experiment supports the hypothesis that the rate of enzyme activity will reduce when the pH is below or above the optimum pH.
  - Changes in pH cause the atoms and molecules of the amino acids to ionize, alter the shape of the active site of the enzyme and impair its function, therefore affecting the effectiveness of the catalase activity.
  - Changes of pH conditions can disrupt the three-dimensional shape of the enzyme.
  - Denaturation of an enzyme is irreversible.
- Animal cells have higher concentration of catalase than plant cells because animals have a higher rate of cellular respiration than plants.

Extension of knowledge

1. According to Stubbe's lecture (MIT, 2013), enzymes can accelerate the rate of catalytic reaction by 10^6 to 10^{15} folds.
2. Catalase is involved in photosynthesis and photorespiration in plants.

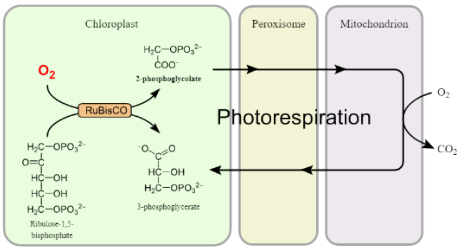


Figure. Photorespiration

Reference: https://en.wikipedia.org/wiki/Photorespiration

3. Types of catalases
4. Acatalasemia, Phenylketonuria (PKU) and Tay Sachs disease.
5. Animals and plants can survive in extreme environments with special enzymes.
6. Hydrogen peroxide contributes to the development of grey hair.

Reference:

- https://medlineplus.gov/genetics/condition/acatalasemia/
- https://www.news-medical.net/health/What-is-Acatalasemia.aspx
- https://www.sciencedirect.com/science/article/abs/pii/S1383574213000689
- https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/catalase
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- https://pdb101.rcsb.org/motm/57
- https://www.sciencedirect.com/topics/neuroscience/catalase
- https://www.intechopen.com/chapters/70428

Control Trial

As only catalase is used to assess the effect of pH on decomposition rate of hydrogen peroxide in this study, control trial is not required.

Random error

1. In Experiment 2c, the stopper popping off the conical flask caused a slight drop in gas pressure. The immediate action was quickly connecting the stopper to the conical flask to minimise error.
2. Measurement error may exist when cutting due to the texture and irregularity of the chicken liver (accuracy to 1 mm).

Systematic Error

1. Systematic error can be minimised with using calibrated equipment which can provide reliable and accurate readings including PASCO PASPORT Chemistry MultiMeasure Sensor.

Limitations

1. Only one trial is conducted. Time constraint.

Improvements

1. Repeating more trials to increase sample size, thereby reducing random errors.
2. More pH conditions are to be investigated to provide more accurate data.

Conclusion

1. The pH outside the optimum pH decreases the rate of catalytic reaction of catalase breaking down hydrogen peroxide into oxygen gas and water.
2. Animal cells contain more catalase than plant cells.
3. Chicken liver that contains has higher catalase concentration per unit volume has a faster rate of reaction than potato.
4. In this experiment, chicken liver catalase performs best at pH 6.5 while potato catalase works most effectively at pH 9.9.

Assistance and Acknowledgement:

I acknowledged that Mr. Starczak and Mr. Wren assisted me in devising the experiment.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

NAME: Chloe Yaan Yuit Yew ID: 0445-001

SCHOOL: Norwood International High School

Activity: Give a brief outline of what you are planning to do.

This experiment investigates the effect of 5 different pH conditions on enzyme activity on breaking down hydrogen peroxide and observes the catalytic action of 2 sources of catalases on hydrogen peroxide.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?

Risks	How I will control/manage the risk
Chemical risks	Prudent laboratory safety practices were followed. Chemical contacts were avoided by putting on personal protective equipment including an apron, safety glasses, safety gloves, enclosed footwear and a surgical mask for preventing inhalation of chemicals. Hair is tied back so that hair does not contact with any chemicals. The experiment was handled with care as the chemicals (H_2O_2, HCL, NaOH) are extremely corrosive. The chemicals can cause serious eye damage, shortness of breath if inhaled and skin irritations as well as burns on skin. When pouring the pH solutions, a slow pouring action was used to minimise the risk of spilling of chemicals. Experiments were done in a well-ventilated laboratory. The equipment and apparatus used in this experiment were carefully handled to prevent any accidents.
Sharp Risks	When cutting the potato and chicken liver into cubes, the knives was carefully handled to prevent cuts.

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Chloe Yaan Yuit Yew

SIGNATURE(S): Chloe Yew

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Luke Starczak

SIGNATURE:  DATE: 27/5/2022