The Effect of pH on the Activity of Liver Catalase

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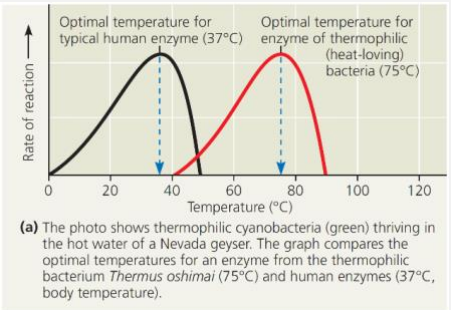
INTRODUCTION

"Humans and other animals produce hydrogen peroxide, a short-lived result of metabolic processes that are damaging to cells." (Wikipedia, 2022) Catalase is present in all living things that breathe oxygen, it is an enzyme that quickly catalyzes the breakdown of hydrogen peroxide into less reactive elements like gaseous oxygen and water molecules. One molecule of catalase can convert 40 million molecules of hydrogen peroxide into water and oxygen each second, making it one of the enzymes with the greatest turnover rates.

The reaction of catalase in the decomposition of living tissue:

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

In this lab, we investigated how pH affects the rate of this reaction by using the liver as the catalase. Human catalase prefers a pH of around 7. As a kind of enzyme, catalase will denature when given to a media that has a pH or temperature that is too high or too low for it to operate properly (Fig. 1). The tertiary structure of the catalase is altered, which changes the shape of the active site of an enzyme (Fig. 2) and decreases the reaction rate.



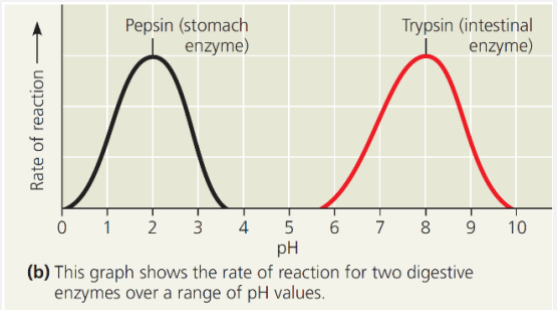


Fig. 1 the effect of temperature (a) and pH (b) on enzyme activity. (*Campbell Biology*, p158, 2021).

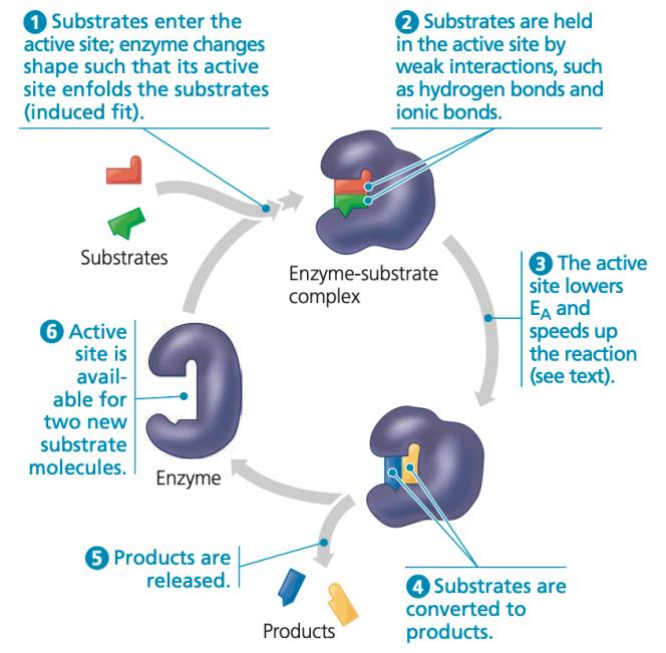


Fig. 2 the active site and catalytic cycle of an enzyme. An enzyme can convert one or more reactant molecules to one or more product molecules. Substrates are bonded with the active site of the enzyme. (*Campbell Biology, p156, 2021*).

So, we can develop the hypothesis that when catalase is added to a higher or lower pH outside its optimal pH range, the catalase activity will decrease, resulting in a decrease in the rate of converting hydrogen peroxide into water and oxygen.

MATERIALS AND METHODS

Table 1. Materials required in the experiment

Item	Quantity
20ml syringe	1
Filter paper	1
Hole puncher	1

Tweezer	1
Grinder	1
Timer	1
Electronic Scale	1
Fresh pig liver/g	1
3% H_2O_2 solution/mL	150
Distilled water/mL	40
1mM HCl (pH = 3)/mL	40
1mM NaOH (pH = 11)/mL	40
pH test paper	sufficient
Parafilm	5

The data of the experiment is quantitative. The independent variable in the experiment is the pH, and the dependent variable is the catalase activity, which could be measured by calculating the rates of the reactions. By keeping track of the amount of gaseous oxygen produced each minute, the rates of the reactions ($ml/\min$)could be calculated. In the experiments, the production volume of gaseous oxygen is measured using a syringe. The liver extract was obtained using the grinder. Our group used an electronic scale to weigh out 1 g of fresh pig liver, then added 20mL of distilled water to the fresh pig liver and ground it until a liver extract solution was obtained. 20 drops of liver extract solution are used in each group.

The experiment consisted of four experimental groups and one control group, each of which contained four different pH values (pH=3,5,9,11) and distilled water (pH=7). Because the optimal pH of human catalase is around 7, the control group is designed to determine whether the influence of pH on catalase activity is positive, negative, or remains constant when compared to the optimal condition.

The experiment requires pH=5 and pH=9 solutions, but the only ones that are readily available are HCl (pH=3) and NaOH (pH=11). In this case, HCl and NaOH must be diluted to achieve the required pH. This was accomplished by adding HCl (pH=3) or NaOH (pH=11) to the liver extract until the pH met the requirements. The pH could be told by the color of the pH test paper.

For each group of the experiment, we used the hole puncher to punch out small filter paper discs. In each of the five groups, three filter paper discs were immersed into the liver extract solution and then placed at the top of the piston. The piston was pushed into the syringe until almost no air was left inside, then we drew 6mL of H_2O_2 solution into the syringe. Because the decomposition reaction began as soon as H_2O_2 entered the syringe, a parafilm was used to quickly cover the syringe's mouth to ensure that no gaseous oxygen escaped during the process.

The production of gaseous oxygen raised pressure, causing the piston to gradually move outward. The total volume of the syringe content was tracked after 1 and 2 minutes to calculate how fast gaseous oxygen was produced, which is the rate of the reaction ($ml/\min$). The rate of the reaction could be calculated using the formula $r = \frac{\Delta V}{\Delta t}$.

During the experiment, the number of filter paper discs, the amount of H_2O_2 solution that is drawn into the syringe, and drops of liver extract solution in each group will affect the experimental results, so these factors need to be controlled.

RESULTS

Table. 2 Rate of volume change in different pH

pH	3	5	7	9	11
rate of volume change during 0~1min/$ml \cdot min^{-1}$	2.2	4	4.4	1.1	0.5
rate of volume change during 1~2min/$ml \cdot min^{-1}$	2.2	3.5	3.6	0	0.5
average rate of volume change/$ml \cdot min^{-1}$	2.2	3.75	4	0.55	0.5

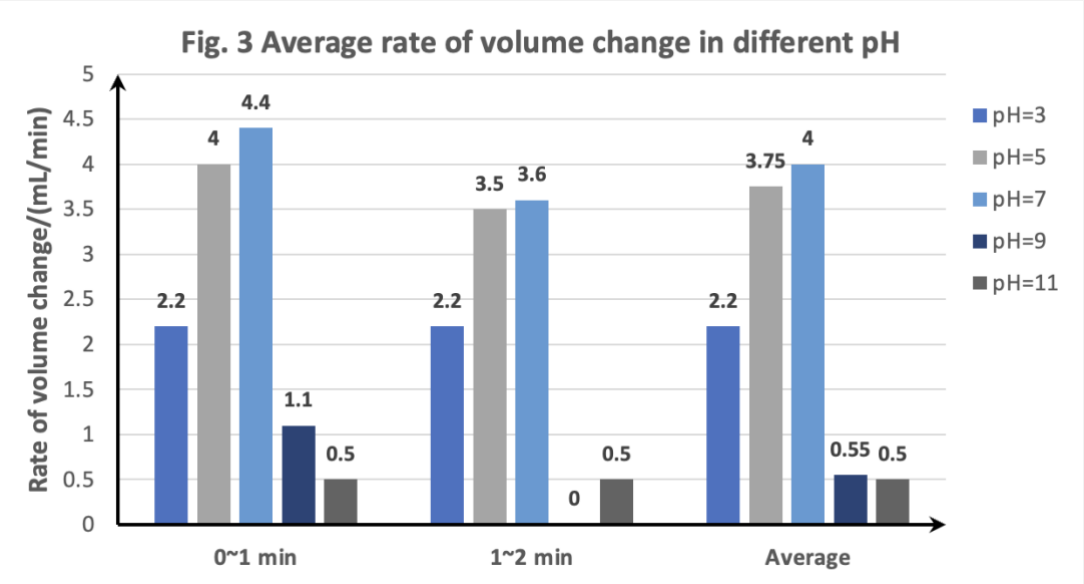


Table. 2 and Fig. 3 show that the rate of volume change decreases when catalase is added to a pH that is higher or lower than its optimal pH range.

DISCUSSION

To conclude, the data support the hypothesis. When catalase is added to a higher or lower pH outside its optimal pH range, the catalase activity will decrease, resulting in a decrease in the rate of the decomposition reaction; the bigger difference between the pH in the experiment and the optimal pH, the lower the reaction rate.

This may be due to the fact that: practically all known species that breathe oxygen employ catalase in almost every organ, with particularly high amounts found in the liver, where the pH is roughly 7, making this value also the ideal pH for catalase. Catalase's activity drops as the pH is higher or lower than the optimal pH because the enzyme becomes denatured, this alters its tertiary structure and causes the shape of its active site to shift, resulting in a decrease in the rate of substrate interaction with the enzyme.

The experiments were not repeated because of the time constraint, so the results could be unreliable. This could be improved by repeating the experiment of each group multiple times and computing the average data of all trials.

Because the data of this experiment is quantitative, there are inevitable limits to the methods we used that could affect the data, such as the precision of the volume of H_2O_2, the precision of pH, the temperature in which the experiment is performed, etc.

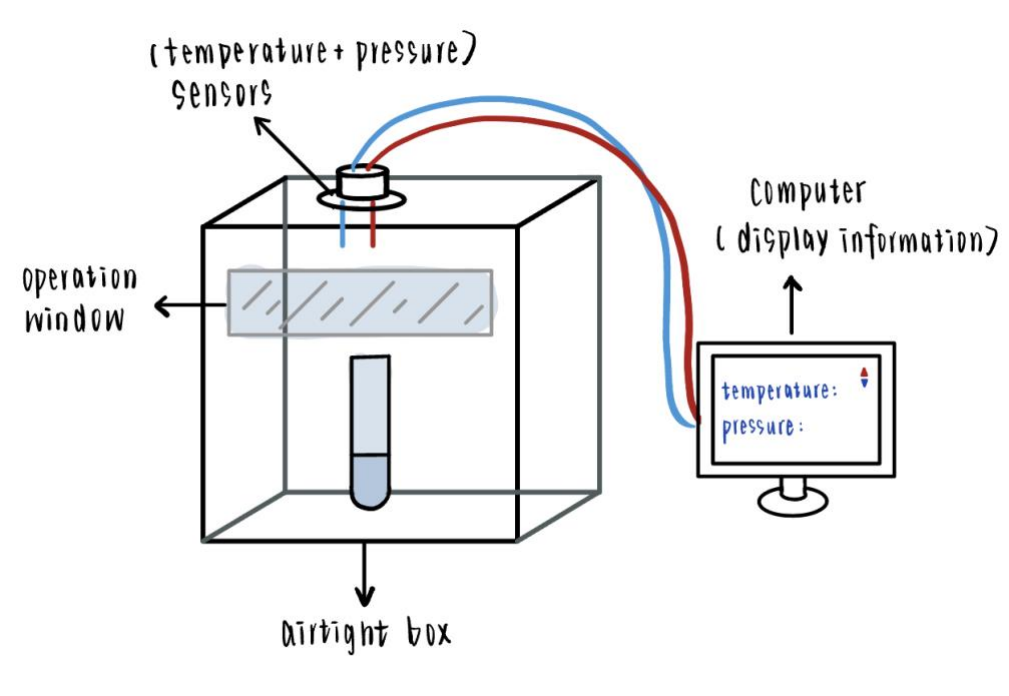


Fig. 4 Device for improvement. Sensors are used to detect the change in temperature and pressure more sensitively. The pressure difference could be obtained by the initial pressure minus the final pressure.

The experiment could be improved by using a more precise device (Fig. 4). The volume of H_2O_2 could not be kept exactly constant due to the limitation of using a syringe, which could affect the result of the experiment. To improve this, we could use a transfer pipette gun to make sure the H_2O_2 is the exact amount required. The optimal temperature of catalase is around 37℃, while the environmental temperature during the experiment is lower than the optimal temperature. This could be improved by using a thermostat to regulate the temperature precisely, thus, increasing the overall rate of reaction. Syringe tightness is also a limitation of the experiment. An airtight box could be used to avoid air from running out. The pressure difference could be calculated by the formula $\Delta P = P_{final} - P_{initial}$.

REFERENCES

https://en.wikipedia.org/wiki/Hydrogen_peroxide (2022)

Campbell, N.A. 2021. The effect of temperature and pH on enzyme activity, the active site and catalytic cycle of an enzyme, Campbell Biology.