


The Influence of Enzyme Concentration and pH on Turnip Peroxidase Activity

Aarush Jugdar¹ ¹ncssm

Abstract

This study investigates the effects of enzyme concentration and pH on the activity of turnip peroxidase. Enzymes are biological catalysts that accelerate chemical reactions (Schnell (2000)), and their efficiency is influenced by environmental factors (Kontro & Oja (1981)). The experiment analyzed reaction rates by varying enzyme concentration and adjusting pH levels with HCl, using hydrogen peroxide as the substrate. Results showed that increasing enzyme concentration led to a slight increase in reaction rate, though less pronounced than expected, while pH adjustments produced inconsistent data, likely due to experimental errors. These findings emphasize the importance of optimizing enzyme conditions for practical applications in agriculture and industry, and they provide a foundation for further research into enzyme activity under varying conditions.

Keywords Turnip peroxidase, Enzyme kinetics, Enzyme concentration, pH sensitivity, Spectrophotometry

1. INTRODUCTION

Enzymes are biological catalysts that accelerate chemical reactions in living organisms, playing vital roles in processes such as metabolism, digestion, and cellular signaling (Kontro & Oja (1981)). They are typically proteins that lower the activation energy of reactions, thereby increasing reaction rates and enabling essential biological functions. Peroxidases are a diverse group of enzymes that play crucial roles in various biological processes, including oxidative stress response, lignin biosynthesis, and the metabolism of reactive oxygen species (Kontro & Oja (1981)). Among these enzymes, turnip peroxidase stands out due to its unique properties and significant agricultural implications. Extracted from the roots and leaves of turnip (*Brassica rapa*), turnip peroxidase catalyzes the oxidation of various substrates using hydrogen peroxide as an electron acceptor (Belinky et al. (2003)). This enzymatic activity not only aids in plant defense mechanisms against pathogens but also contributes to the plant's growth and development. This experiment aimed to investigate the factors affecting enzyme activity, specifically using peroxidase from turnips and hydrogen peroxide as a substrate. The goal was to understand how enzyme concentration and pH influence reaction rates. Understanding these factors can provide insights into optimizing enzyme activity in agricultural and industrial applications, where enzymes are used for bioremediation, food processing, and biosensors.

The hypotheses for this study were as follows: Increasing enzyme concentration would increase the reaction rate, and peroxidase activity would peak at its optimal pH, with deviations reducing its activity.

2. METHODS

Published Jan 16, 2025

Data Availability

The data that support the findings of this study are openly available in [jterm-2025] at <https://github.com/AarushJ7/jterm-2025>.

The experiment was designed to analyze the effects of enzyme concentration and pH on turnip peroxidase activity. Three groups of reactions were prepared. The control group contained a reaction mixture without any added enzyme to serve as a baseline for comparison. The increased enzyme concentration group used reaction mixtures with higher concentrations of enzyme prepared in molar solutions. The pH variation group used mixtures with varying pH levels adjusted using HCl, measured in molarity. Substrate concentration, temperature, and reaction time were kept constant to ensure the validity of results.

Reaction mixtures were prepared by combining a buffer, substrate (hydrogen peroxide), and varying amounts of enzyme or HCl. Absorbance changes were monitored over time using a spectrophotometer, and values were recorded at consistent intervals. Data were collected across multiple replicates to ensure reliability and reduce variability.

3. RESULTS

The control group showed constant absorbance values, indicating no enzymatic reaction. In the increased enzyme concentration group, absorbance increased slightly over time, suggesting enzyme activity. This effect was observed across multiple molar concentrations, with the reaction rate increasing at higher enzyme concentrations, although less pronounced than anticipated. In the pH variation group, absorbance changes were inconsistent, showing no clear trend. This may have been due to experimental errors or the enzyme's sensitivity to minor pH deviations.

A line graph was generated to depict absorbance changes over time for all experimental groups. Data trends illustrated the influence of enzyme concentration on reaction rates and the variability in results due to pH changes. The results showed that the control group exhibited constant absorbance values, indicating no reaction. When enzyme concentration was increased, there was a slight increase in absorbance over time, suggesting enzyme activity. In contrast, data from the HCl (pH variation) condition displayed fluctuations with no clear trend, likely due to suboptimal pH levels or experimental errors. Observations from [Figure 1](#) indicated that while enzyme concentration positively influenced the reaction, the effect was less pronounced than predicted. Additionally, the fluctuations in pH underscored the enzyme's sensitivity to environmental conditions.

Time (s)	Control	Increased Enzyme Concentration	HCl Added
0	0.138	0.142	-0.017
30	0.697	0.605	0.011
60	0.698	0.665	0.01
90	0.695	0.668	0.009
120	0.697	0.67	0.01
150	0.694	0.666	0.009
180	0.695	0.667	0.009
210	0.696	0.669	0.01
240	0.694	0.67	0.008
270	0.639	0.668	0.01
300	0.644	0.667	0.01

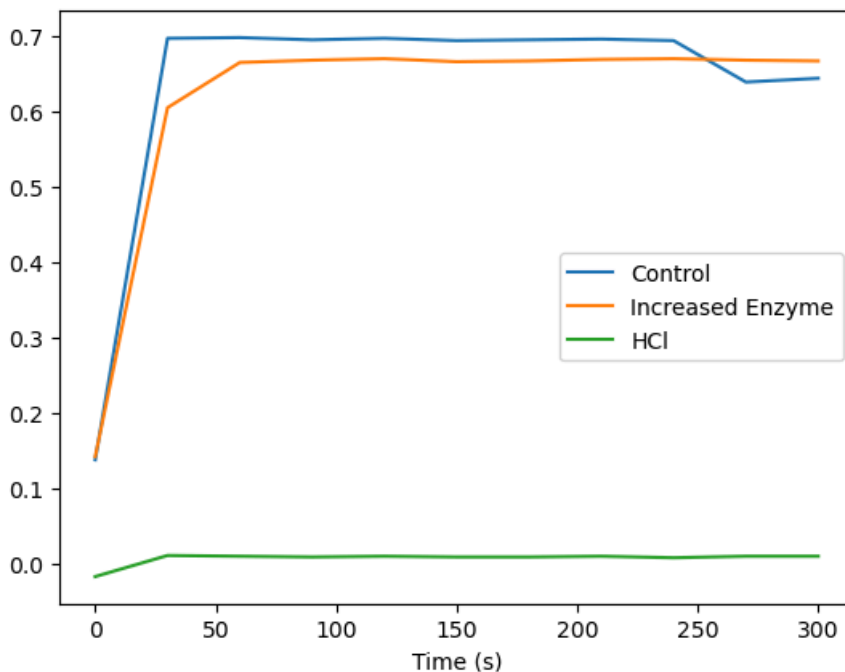


Figure 1: This reflects the effects of different variables on enzymatic activity. The activity fluctuates per each variable as time goes on after one of the variables are added.

4. DISCUSSION

The results partially supported the hypothesis that increasing enzyme concentration enhances reaction rates. The slight increase observed aligns with the principles of Michaelis-Menten kinetics (Schnell (2000)), where reaction rates rise with enzyme concentration until substrate availability becomes limiting. The effect of pH on enzyme activity was inconclusive, with inconsistent absorbance readings likely caused by errors in pH adjustment or insufficient replication of experiments. Turnip peroxidase's sensitivity to pH highlights the importance of precise experimental controls and the need for further research to clarify its activity profile.

These findings demonstrate the potential applications of turnip peroxidase in industrial processes, where enzyme optimization is critical. Variability in pH data underscores the necessity for improved methodologies and accurate calibration of experimental conditions.

5. CONCLUSION

This study confirmed that turnip peroxidase catalyzes reactions with detectable increases in absorbance, validating its enzymatic activity. While increasing enzyme concentration showed some effect on reaction rates, pH effects were inconclusive due to experimental limitations. These results emphasize the need for refined techniques and broader testing ranges to optimize enzyme performance for agricultural and industrial applications.

REFERENCES

- Belinky, P. A., Flikshtein, N., Lechenko, S., Gepstein, S., & Dosoretz, C. G. (2003). Reactive Oxygen Species and Induction of Lignin Peroxidase in *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*, 69(11), 6500–6506. <https://doi.org/10.1128/aem.69.11.6500-6506.2003>

- Kontro, P., & Oja, S. S. (1981). Enzyme Kinetics. In *Methods in Neurobiology* (pp. 265–337). Springer US. https://doi.org/10.1007/978-1-4684-3806-2_5
- Schnell, S. (2000). Enzyme Kinetics at High Enzyme Concentration. *Bulletin of Mathematical Biology*, 62(3), 483–499. <https://doi.org/10.1006/bulm.1999.0163>