# Effects of enzyme concentration or addition of hydroxylamine on the rate of a peroxidase reaction

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## Abstract

*Purpose:* Many different factors affect the performance of enzymes. Groups studied various factors and their impact on the rate at which the peroxidase reaction occurred. These experiments tested the effects of enzyme concentration and of the presence of hydroxylamine on the rate of the peroxidase reaction.

*Methods:* Turnip peroxidase, guaiacol and hydrogen peroxide were used as reactants in this experiment. Guaiacol was used to visibly see the reaction occuring. A spectrophotometer measured the absorbance of a specific wavelength at set intervals during the reaction which was used to determine the rate.

*Results:* The first experiment revealed that an increase in enzyme concentration leads to an increase in the rate of the reaction. The second experiment verified that hydroxylamine is in fact an inhibitor, and due to the knowledge of its chemical structure, that it is a competitive inhibitor. In higher doses, the reaction will almost come to a complete halt.

## Introduction

Enzyme speeds up the rate of a specific reactions by acting as biological catalysts. They lower the activation energy needed for a reaction to begin by bringing together two or more substrates in the proper orientation for their reaction, or by stressing bonds within a single substrate. Enzymes can also create microenvironments which are different in some environmental factor than the rest of the cell (Urry, et al. 2017). As these experiments will reveal, many factors play into how well an enzyme effectively performs its duties. An increase in enzyme concentration will increase the rate of reaction, up to a specific saturation plateau. After that point is reached, higher enzyme concentrations will not have an effect on the rate of reaction (Jaime-Fonseca 2016). Allosteric activation involves a molecule binding to a site on the enzyme. It makes the enzyme more likely to accept its substrates and thus increases the rate at which the reaction occurs. Inhibition decreases the rate of, or completely stops, the reaction. Noncompetitive inhibition occurs when a molecule binds to an allosteric site on the enzyme which changes the shape of the active site, while competitive inhibition involves a molecule that is similar to that of the substrate. That molecule enters the active site and prevents the reaction from occuring on that particular enzyme for a period of time (Urry, et al. 2017).

This particular reaction involves peroxidase enzymes which are common in plant and animal cells and are often involved in removing hydrogen peroxide from the cells. Hydrogen peroxide is a product of many oxidation-reduction (REDOX) reactions, but it is toxic to the cells, so it must be removed. This particular reaction is composed of the reactants guaiacol and hydrogen peroxide and the products tetraguaiacol and water. Guaiacol is used as a donor to determine the rate because it changes colors when it is reduced to tetraguaiacol. The specific enzyme being used is derived from a turnip (Lab Handout 2018).

$$\text{2 guaiacol} + 2\ H_2O_2 \xrightarrow{\text{turnip peroxidase}} \text{tetraguaiacol} + 4\ H_2O$$

This experiment aims to find the effects of various factors on the rate of this reaction. The two

experiments performed involved changing the concentration of the enzyme in the solution or the addition of hydroxylamine which is chemically similar to hydrogen peroxide, meaning that it could be hypothesized as being an inhibitor. The results supported the hypotheses that enzyme concentration would positively correlate with the rate of reaction and that the addition of hydroxylamine would negatively correlate with the rate of reaction.

## Methods

### Baseline Measurements

The baseline reactions were the same for both experiments. 0.1mL guaiacol was mixed with 1.0mL turnip extract and 8.9mL distilled water (dH2O). This was placed into a cuvette and used as a blank in the spectrophotometer. Once calibrated, 0.1mL guaiacol, 0.2mL hydrogen peroxide (H2O2) and 4.7mL dH2O were mixed with 1.0mL turnip extract and 4.0mL dH2O. For all reactions, absorbance vs time was collected with measurements every 10 seconds for 2 minutes. If performed with different batches of turnip extract, the baseline reaction had to be repeated for the second experiment. The spectrophotometer needed to be calibrated before each reaction took place.

### Enzyme Concentration

For the enzyme concentration reaction, solutions were made with double and half the base enzyme amount. The 2x enzyme blank included 0.1mL guaiacol, 2.0mL turnip extract and 7.9mL dH2O. For the reaction, 0.1mL guaiacol, 0.2mL H2O2 and 4.7mL dH2O were mixed with 2.0mL turnip extract and 3.0mL dH2O. The ½x reaction blank consisted of 0.1mL guaiacol, 0.5mL turnip extract and 9.4mL dH2O. The reaction called for 0.1mL guaiacol, 0.2mL H2O2 and 4.7mL dH2O to be mixed with 0.5mL turnip extract and 4.5mL dH2O.

### Addition of Hydroxylamine

In the hydroxylamine reaction, the only values that differed from the baseline were the addition of 1 drop of 1% hydroxylamine for the first reaction and 1 drop of 10% hydroxylamine for the second reaction.

### Measuring Absorbance

A SpectroVis Plus Spectrophotometer was used to determine the rate of reaction. The program Logger Pro was used in collaboration with the spectrophotometer to test the absorbance of the solution. A blank had to be used each time the components of the reaction were changed. The absorbance was tested at a particular wavelength every 10 seconds for 2 minutes, creating an absorbance vs time graph. The data was then transferred to Microsoft Excel for further analysis, including determining the rates with trendlines and the fit by using $R^2$ coefficients.

## Results

The figures show all of the measurements taken by the spectrophotometer, along with the trendline for each set of data, the equation for that trendline and the $R^2$ coefficient.

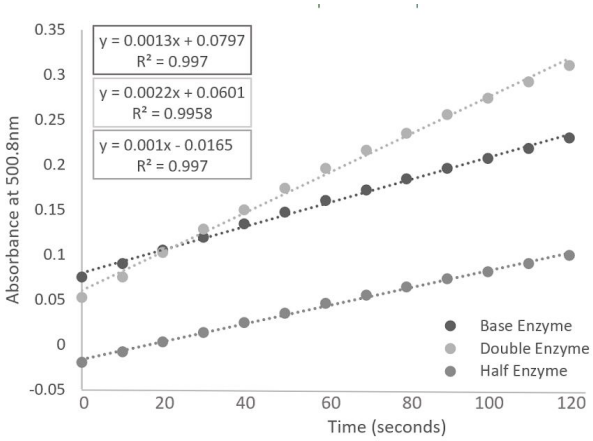


**Fig. 1** Effects of enzyme concentration on the rate of the peroxidase reaction. The rate of reaction increased as the concentration of the enzyme increased.

**Effects of Enzyme Concentration**

The rate of the peroxidase reaction positively correlates with the concentration of the enzyme in the solution. The 2x enzyme's rate is 0.022, which is more than those of the base and ½x enzyme, which are similar to each other, at 0.0013 and 0.001 respectively. The difference of the base and ½x enzyme absorbances exist at the starting point and remains the same throughout the duration of the experiment because of the similar slopes. The data points closely fit to the trendlines, meaning that enzyme concentration is related to the rate of reaction.

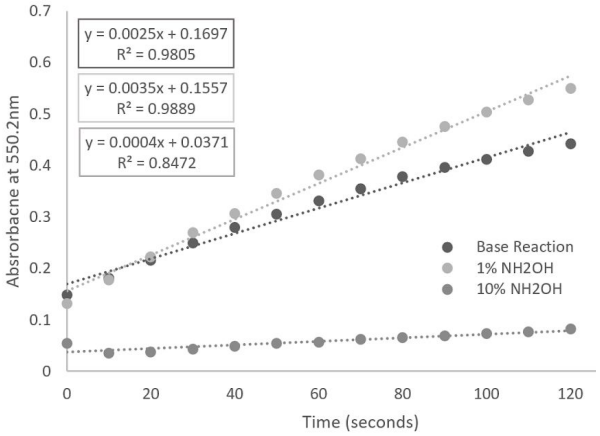


**Fig. 2** Effects of the addition of hydroxylamine on the peroxidase reaction. 1% increased the rate of reaction and 10% almost stopped the reaction from occuring.

**Effects of Hydroxylamine**

In higher concentrations (10%), the addition of hydroxylamine has a large effect on the rate of the peroxidase reaction, bringing it down to just 0.0004. At lower concentration (1%), the rate of the reaction increased from 0.0025 to 0.0035. The rate of reaction seems to negatively correlate with the concentration of one drop of hydroxylamine being present in the solution, but due to the 1% increasing the rate, it can not be determined. The base reaction and 1% reaction trendlines had slight curves, but were still fit with the data points. The 10% reaction may have had some error because the absorbance at 10 seconds was less than that at the start time.

## Discussion

The hypotheses are generally supported for both experiments. If the rate of a reaction needs to be increased, the concentration of the enzyme can be increased. If a reaction needs to be decreased, the concentration of enzyme can be decreased, but this does not affect the rate, but rather that the starting absorbance is less and it stays the same distance from the base reaction for the duration of the experiment because of the similar slopes. A more effective way to decrease the rate of the reaction is to add an inhibitor such as hydroxylamine. In high concentrations this can stop the reaction from occurring, but at a low concentration it increased the rate of reaction which conflicts with the definition of an inhibitor. Enzyme concentration and the presence of an inhibitor are just two of the factors that can affect the rate of an enzyme reaction.

I would recommend that the 10% hydroxylamine be repeated if the results are similar to this because the absorbance should not have decreased after the reaction began. It is also odd that the 1% hydroxylamine increased the rate of reaction because it too should have been an inhibitor. The accuracy of these results could easily be confirmed by repeating the experiment as there could have been a measurement error with either of the tests. Additional tests could be performed with other concentrations of hydroxylamine to see exactly how the rate is impacted, but it is not necessary. Using concentrations at intervals between 1% and 10%, or even to higher concentrations, would allow us to see if the effects are constant at different percentages. The same could be done with the enzyme concentration by increasing or decreasing the concentrations accordingly.

## References

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