Report on

Starch Hydrolysis

Submitted to

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ABSTRACT

Enzyme catalysts are important tools of biochemical and biomedical engineering; they provide specific, versatile, and effective catalysis of biological reactions. Enzyme catalyzed reactions are affected by temperature, pH, and substrate concentration, among other things. The Starch Hydrolysis experiment evaluates the effects of initial substrate concentration on the biological enzyme-catalyzed reaction of starch hydrolysis by glucoamylase. It also explores kinetic models to find maximum reaction rate and the reaction rate constant. Using the Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf models for single-substrate-enzyme-catalyzed reactions, experimenters solved for the maximum reaction rate and reaction rate constant for the glucoamylase catalyzed reaction. Experimenters concluded that the initial substrate concentration did not affect the efficiency of the enzyme, and that Michaelis-Menten model gave the most accurate representation of the maximum reaction rate and reaction rate constant.

Executive Summary

The Starch Hydrolysis experiment evaluates the effects of initial substrate concentration on the biological enzyme-catalyzed reaction. The reaction explored in the report is the hydrolysis of starch, using a maltose substrate and glucoamylase enzyme to produce glucose. It is hypothesized that as the initial substrate concentration increases, enzyme performance in starch hydrolysis will also increase.

Additionally, the Starch Hydrolysis experiment explores four kinetic models to find the maximum reaction rate and the reaction rate constant for the enzyme catalyzed reaction: Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf. Each of the models seek to represent the kinetics for single-substrate-enzyme-catalyzed reactions, to varying degrees of accuracy. Therefore, it is hypothesized that the Michaelis-Menten kinematic model will provide the most accurate values for the reaction rate and rate constant of the reaction.

Results of the Starch Hydrolysis experiment gave inconclusive findings for calculating the reaction rate constant, K_m, and maximum rate of reaction, V_m, using the approximation models of Eadie-Hoffstee and Hanes-Woolf. The Michaelis-Menten and Lineweaver-Burk models yielded similar statistical significance seen from R^2 values of 0.8257 and 0.832 respectively, however the values calculated for V_m and K_m varied greatly.

Therefore, it was concluded that the Michaelis-Menten model yielded the best values of K_m and V_m, due to the knowledge that the Lineweaver-Burk model is a derivation from the Michaelis-Menten model. Furthermore, the reactions with higher concentrations of the reaction substrate, maltose, did not affect the overall reaction rate. Therefore, the experimental hypothesis that a higher substrate concentration would increase enzyme efficiency was shown to be invalid.

Contents

Introduction

The Starch Hydrolysis experiment explores the effects of changing substrate concentration on enzyme performance in starch hydrolysis. The experiment also examines the reaction kinetics and reaction rate of the enzyme-substrate formation of glucose. Reaction rate calculations are important in reaction engineering, especially when designing large-scale processes in industry.

Optimizing catalyst usage is crucial for designing efficient and cost-effective chemical reactions. Biochemical and biomedical engineering utilize enzymes for selective catalysis, as well as food processing and pharmaceutical industries.

Calculating the value of the reaction rate constant requires the use of various models such as the Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, or Hanes-Woolf [1]. Each of these models present an equation comparing the rate of product formation, substrate concentration, rate constant, and maximum forward rate of reaction. These methods are used for data analysis, and the results of each model are compared and contrasted in this report.

I hypothesize that as the initial substrate concentration increases, enzyme performance in starch hydrolysis will also increase. I also hypothesize that the Michaelis-Menten will provide the most accurate values for the reaction rate and rate constant of the reaction.

Theory

Enzymes are proteins with high molecular weights that act as catalysts in many chemical and biological processes. Enzyme catalyzed reactions can be manipulated by changing pH, substrate concentration, and temperature, among other things. The glucoamylase enzyme is a digestive enzyme that breaks off a free glucose molecule from the complex sugar-based chains that form starch.

The result of that catalyzed reaction is glucose, which can then be used as a source of energy. Changes in pH can affect the effectiveness of the glucoamylase enzyme, the substrate, or both. Extremely high or low pH values generally result in complete loss of activity for most enzymes, but optimum pH values for an enzyme-catalyzed reaction vary greatly.

There are various mathematical models of the kinetics of a single-substrate-enzyme-catalyzed reaction, such as catalysis using the glucoamylase enzyme. One of those models is the Michaelis-Menten model, shown in Equation 1, which resembles Langmuir-Hinschelwood kinetics.

$$E + S <-> ES -> E + P \quad (1)$$

where:

E = enzyme
S = substrate
ES = enzyme-substrate
P = product

Using batch reactors allows the use of the quasi-steady-state assumption. After applying this assumption to Equation 1, the resulting equation is shown in Equation 2. Equation 2 is the Michaelis-Menten model which represents the kinetics of a single-substrate-enzyme-catalyzed reaction [1].

$$v = \frac{V_m[S]}{K_m + [S]} \quad (2)$$

where :

v = rate of product formation
$[S]$ = substrate concentration
V_m = maximum forward rate of reaction
K_m = rate constant

Determining values for the parameters of maximum forward reaction rate as well as the rate constant should be determined using initial rate experiments with a known amount of initial substrate and enzyme concentrations. Multiple methods can be used to determine V_m and K_m that are derived from the Michaelis-Menten model, including the Lineweaver-Burk shown in Equation 3, the Eadie-Hofstee shown in Equation 4, or the Hanes-Woolf shown in Equation 5 [1][2].

Equations 2, 3, 4, and 5 all give models to represent the kinetics of single-substrate-enzyme-catalyzed reactions to varying degrees of accuracy. When analyzing data, it is important to understand the accuracy of the models used to calculate the rate constant and rate of reaction. The Hanes-Woolf plot in Equation 5 that more accurately

determines V_m. Similarly, the Lineweaver-Burk plot represented by Equation 3 gives a good estimate of V_m but not necessarily K_m. The Eadie-Hofstee plot in Equation 4 are known to have large errors [1].

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} * \frac{1}{[S]} \tag{3}$$

$$v = V_m - \frac{K_m v}{[S]} \tag{4}$$

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{[S]}{V_m} \tag{5}$$

Equations 3, 4, and 5 are derived from the Michaelis-Menten model to give linear relationships for finding K_m and V_m. The Michaelis-Menten model generates a non-linear saturation curve shown in Figure 1. Instead of using linear methods shown in equations 3, 4, and 5 to find K_m and V_m, the saturation curve in Figure 1 is used.

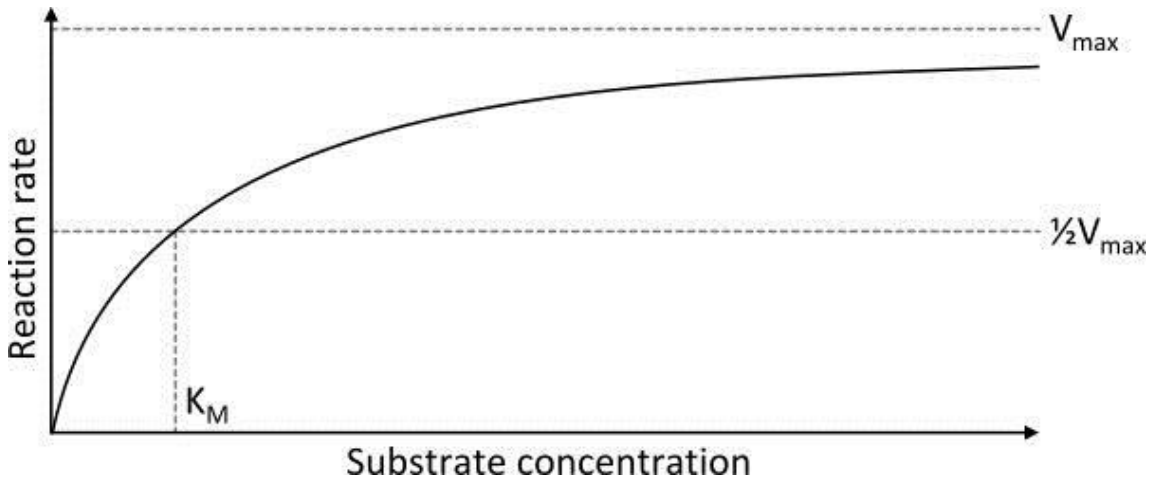

Figure 1: Michaelis-Menten saturation curve used to calculate the maximum rate of reactoin and reaction rate constant for the Michaelis-Menten model for single-substrate-enzyme-catalyzed reaction kinetics.

Materials and Methods

In the Starch Hydrolysis experiment, a water bath will be used to hold the temperature of the solution constant. Erlenmeyer flasks were used as individual reactor vessels, and a glucose analyzer determined the glucose concentration of samples taken throughout the reaction.

Apparatus

The apparatus for the Starch Hydrolysis experiment consists of a scale, six 250ml Erlenmeyer flasks, six 50ml Erlenmeyer flasks, a water bath, sample test tubes, and a Glucose Analyzer machine. A schematic of the apparatus supplies is shown in Figure 1.

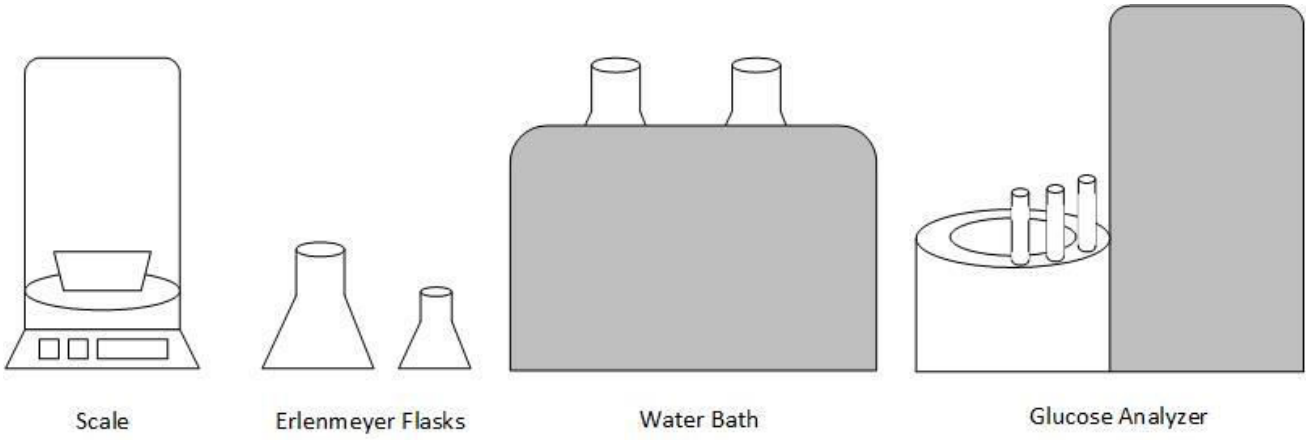

Figure 2: The Starch Hydrolysis apparatus uses multiple parts to conduct the experiment, including a scale, Erlenmeyer flasks, a water bath, and a glucose analyzer.

The scale in the Starch Hydrolysis experiment measures the mass of dry chemicals used in the experiment. The six 250ml Erlenmeyer flasks are used as reaction vessels for the enzyme catalyzed reaction, and the six 50ml Erlenmeyer flasks are used to hold the enzyme solution before the reaction starts.

The water bath holds the reaction's temperature at a constant 50C throughout the reaction. A YSI 2700 glucose analyzer determines the glucose concentration of samples

taken throughout the reactions. The glucose analyzer utilizes a YSI 2710 turntable to hold sample test tubes and submit them to the glucose analyzer for analysis.

Preparation for the Starch Hydrolysis experiment includes cleaning all Erlenmeyer flasks, turning on the water bath and setting it to 50C, and balancing the scale. Additionally, before each reaction is started, 1ml of Tris solution is added six sample test tubes used for the glucose analyzer.

Process

To conduct the Starch Hydrolysis experiment, the performance of the glucoamylase enzyme was monitored during the hydrolysis of a soluble starch. A 250ml Erlenmeyer flask was used to hold 30ml of 2.3M buffer solution in a 50C water bath. The 30ml was measured with a graduated cylinder. Maltose and glucoamylase were carefully weighed using the scale. A maltose solution molarity of 40-15 g/L, 15-8 g/L, or 8-2 g/L was mixed with 0.1g, 0.05g, and 0.25g glucoamylase respectively to start the reaction. The 50mL Erlenmeyer flasks were used to dissolve the glucoamylase in 10ml of buffer.

A timer was started as the glucoamylase-buffer solution in the 50mL flask was added to the 250ml Erlenmeyer flask solution. Every 30, 45, or 60 seconds (depending on the run) an auto-pipet was used to take out 1ml of the reacting solution, and dispense it into a sample test tube containing 1ml of 2.3M Tris solution. When the glucoamylase-maltose-buffer solution mixed with the Tris solution, the reaction was halted.

Six reactions were run at varying initial concentrations of glucoamylase and maltose. Six samples were taken with the auto-pipet every 30 seconds for the first three reactions. The fourth reaction, the six samples were taken every 45 seconds. The fifth and sixth reactions had samples taken every 60 seconds. A total of 36 samples were analyzed by the glucose analyzer. The 1ml samples were mixed with 1ml of Tris solution in a prepared sample tube to be tested in the Glucose Analyzer.

Results and Discussion

The hypothesis of the Starch Hydrolysis experiment is that increasing the initial concentration of a catalytic enzyme will increase the generation rate of the desired product. Relating the hypothesis to our experiment, it hypothesizes that increasing the initial amount of glucoamylase enzyme will increase the rate of production of glucose in the reaction.

In order to test our hypothesis, six different initial concentrations of maltose and enzyme glucoamylase were reacted in the Starch Hydrolysis experiment. The initial concentrations of maltose and grams of glucoamylase used in each reaction are shown in Table 1.

Table 1: Concentrations of maltose and masses of glucoamylase used in the Starch Hydrolysis experiment.

	Maltose (g/L) [S]	Glucoamylase Enzyme (g)
Reaction 1	25.5	0.1029
Reaction 2	24.205	0.1022
Reaction 3	11.02	0.0511
Reaction 4	8.00	0.0501
Reaction 5	4.8025	0.0255
Reaction 6	4.5225	0.0247

Data generated from the Glucose Analyzer shows the concentration of glucose generated from each reaction over time. Figure 2 displays the concentration of glucose over time for each of the six reactions conducted. Note the linearity of the trends, with an average R^2 value of 0.987.

The results of Figure 2 allow experimenters to calculate the initial rate of reaction v, using the slope of the equations generated from the linear trends. Calculated values of the initial rate of reaction are shown in Table 3. In order to compare the resulting initial rates from two different enzyme concentrations, the initial rate of Reactions 3, 4, 5, and

6 were multiplied by the ratio of the differing enzyme concentrations. Therefore, Reactions 3 and 4 were multiplied by 2, and Reactions 5 and 6 were multiplied by 4.

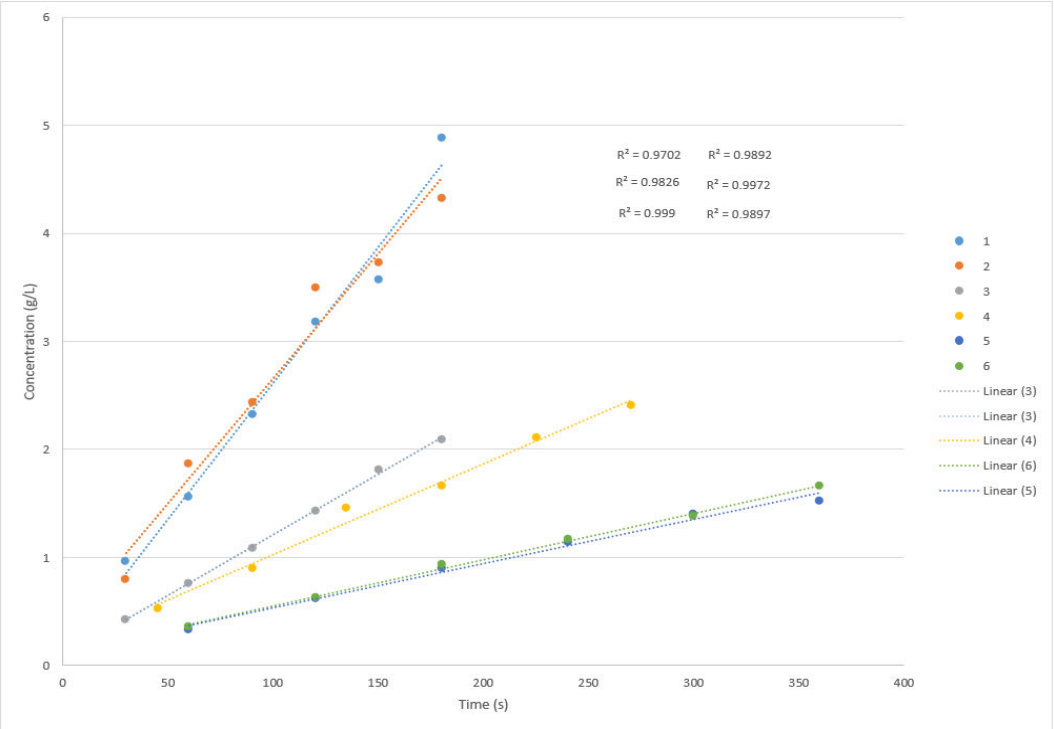

Figure 3: Concentration of glucose vs time for six reactions of the Starch Hydrolysis experiment.

Table 2: Initial rates of reaction, v, calculated from data of glucose concentration vs time.

	Initial Rate of Reaction v	Scaled v
Reaction 1	0.025	0.025
Reaction 2	0.023	0.023
Reaction 3	0.011	0.022
Reaction 4	0.008419	0.016838
Reaction 5	0.0004086	0.0016344
Reaction 6	0.000429	0.001716

The data of concentration of glucose over time from Figure 3 give the initial rates of reactions calculated in Table 3. These initial reaction rates are used in the Lineweaver-Burk, Eadie-Hofstee, Hanes-Woolf, and Michaelis-Menten models of enzyme-catalyzed reaction kinetics.

The Lineweaver-Burk model examined in Equation 3 is known to give a good estimate of V_m [1]. Using data from Table 3, the initial concentration of maltose [S], and Equation 3, values of the maximum rate of reaction V_m and the rate constant K_m can be calculated from the equation generated in Figure 4. With an R^2 value of 0.832, there is somewhat significant statistical evidence that our data fits the Lineweaver-Burk model.

The Eadie-Hofstee model examined in Equation 4 is known to subject large error when calculating K_m and V_m [1]. Using data from Table3, the initial concentration of maltose [S], and Equation 4, values of K_m and V_m can be calculated using the equation generated in Figure 5. The Eadie-Hofstee model did not fit to the data obtained, yielding an R^2 value of 0.3706. This evidence, as well as the known inaccuracies of the Eadie-Hofstee, show that the K_m and V_m values obtained from the Eadie-Hofstee model are inaccurate.

The Hanes-Woolf model examined in Equation 5 is known to give an accurate value of V_m [1]. Using data from Table 3, the initial concentration of maltose [S], and Equation 5, values of V_m and K_m can be calculate from the equation generated in Figure 6. The equation formed from Figure 6 gives an R^2 value of 0.2361, signifying minimal statistical significance that our data fits the Hanes-Woolf model for finding K_m and V_m, concluding that the values found are likely to be inaccurate.

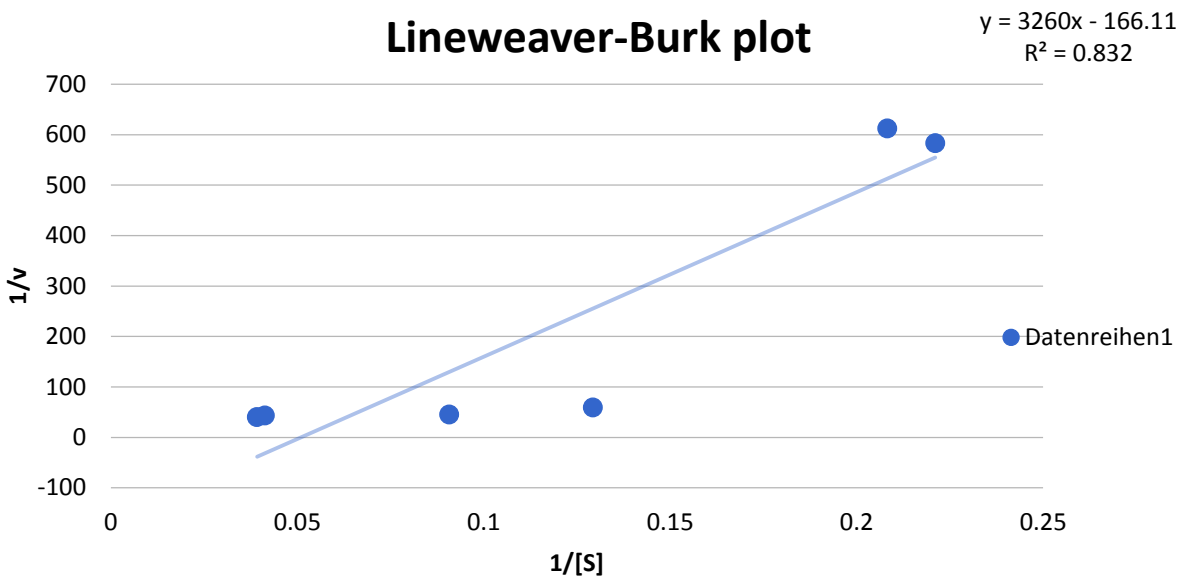

Figure 4: Lineweaver-Burk plot to calculate the rate constant, Km, and max reaction rate, Vm, of the glucoamylase catalyzed reaction using Equation 3

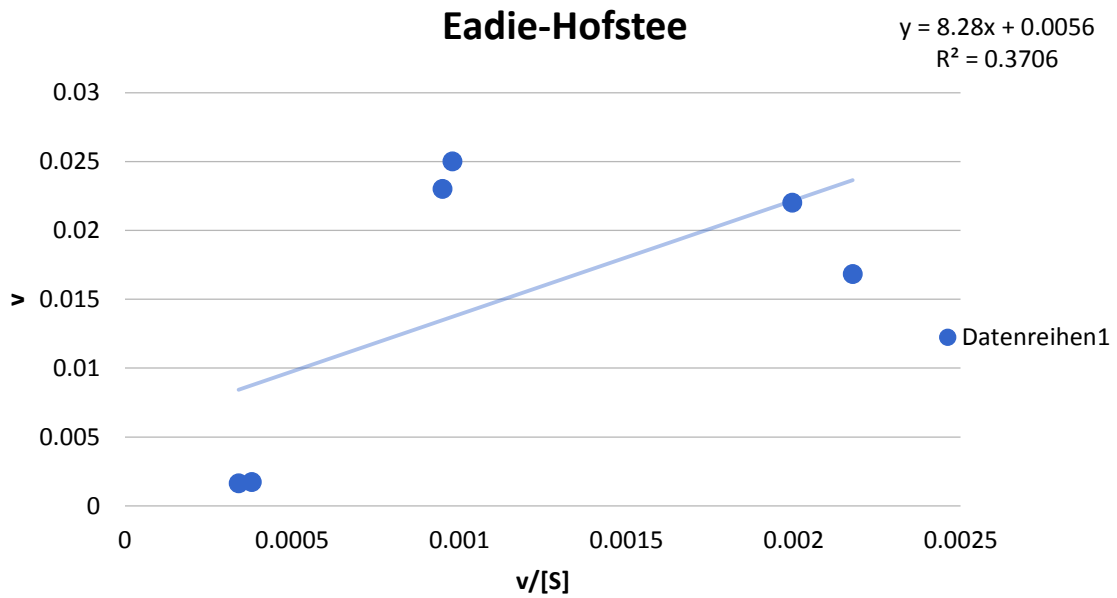

Figure 5: The Eadie-Hofstee uses the initial rate of reaction, the initial concentration of maltose, and Equation 4 to solve for Km and Vm

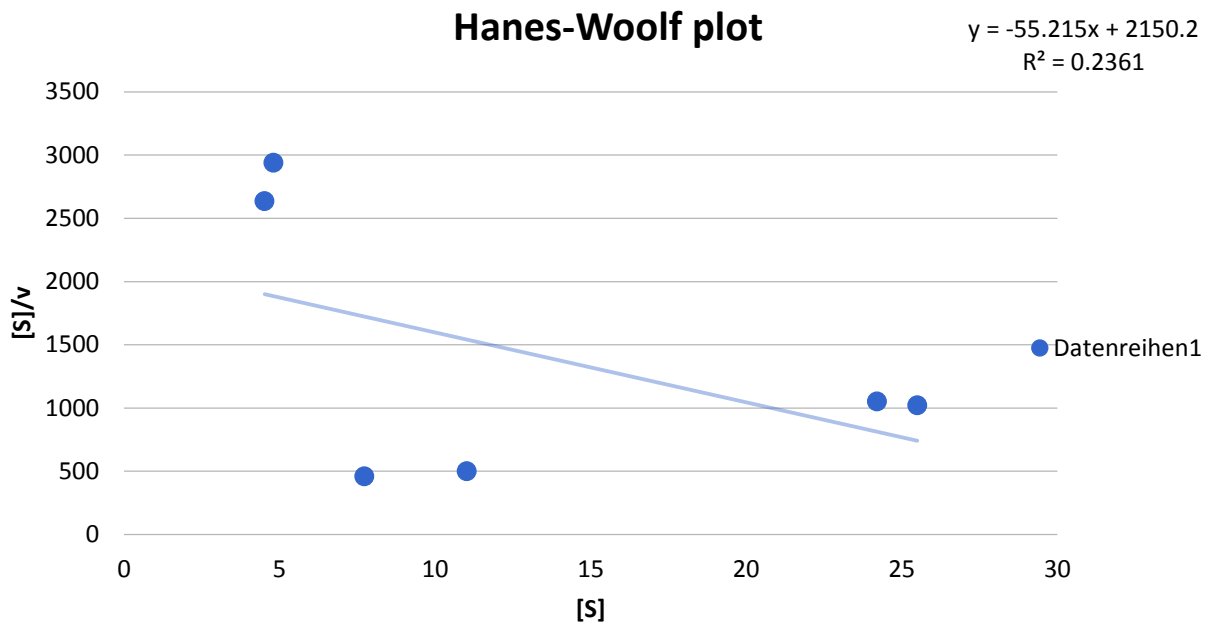

Figure 6: The Hanes-Woolf model uses the initial rate of reaction, the initial concentration of substrate, maltose, and Equation 5 to give estimates for Vm and Km

The Michaelis-Menten model for enzyme-catalyzed reactions seen in Equation 2 does not give a linear relationship between the initial reaction rate v and initial concentration of maltose [S]. Figure 7 shows an inverse logarithmic relationship between v and [S]. Using the initial reaction rate, initial concentration of maltose [S], and saturation curve shown in Figure 1, V_m and K_m can be calculated with the Michaelis-Menten model. The Michaelis-Menten model gives an equation with an R^2 value of 0.8257, which is similar to the R^2 value found in Figure 4 using the Lineweaver-Burk model. Therefore, the Lineweaver-Burk and Michaelis-Menten kinetic models fit best for the single-substrate-enzyme-catalyzed reaction of starch hydrolysis with glucoamylase.

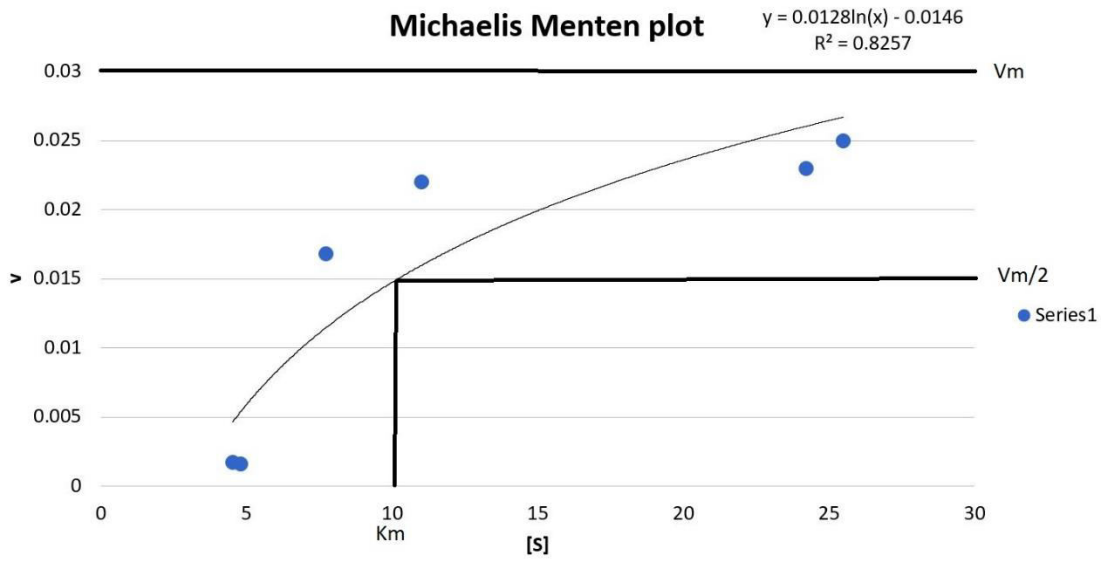

Figure 7: Michaelis-Menten model of glucoamylase catalyzed reaction finds Km and Vm using the initial rate of reaction, concentration of maltose [S], and saturation curve seen in Figure 1.

Values of the maximum reaction rate and reaction rate constant are calculated from Figures 4, 5, 6, and 7. These values are shown in Table 3. Note the disparities and wide range of values calculated for K_m and V_m. The models explored in the Starch Hydrolysis experiment give varied accuracies for calculating K_m and V_m. Lineweaver-Burk gives a good estimate of V_m but not necessarily K_m, the Eadie-Hofstee model is subject to large errors, and the Hanes-Woolf gives a more accurate value of V_m but not of K_m. These discrepancies give explanation for the range of K_m and V_m values calculated.

Table 3: Calculated values of Vm and Km using the Lineweaver-Burk, Eadie-Hofstee, Hanes-Woolf and Michaelis-Menten model.

Model	Maximum reaction rate V_m	Reaction rate constant K_m
Lineweaver-Burk	-0.0060	-19.6
Eadie-Hofstee	-0.0056	-8.3
Hanes-Woolf	-0.018	-38.9
Michaelis-Menten	0.03	10.0

The values of K_m and V_m calculated from the approximation methods of Eadie-Hofstee, and Hanes-Woolf are inaccurate due to the lack of statistical significance when using data from the Starch Hydrolysis experiment. The Michaelis-Menten and Lineweaver-Burk models fit experimental data to about the same degree, with R^2 values of 0.8257 and 0.832 respectively. However, when comparing calculated values of K_m and V_m for the two models in Table 3, the Lineweaver-Burk model gives a reaction rate constant of -19.6 and a maximum reaction rate of -0.006. These values contrast greatly with the values of K_m and V_m calculated from the Michaelis-Menten model.

With this discrepancy in data and knowledge that the Lineweaver-Burk model is derived from the Michaelis-Menten model, it can be concluded that the Michaelis-Menten model does give the most accurate values for the reaction rate constant and maximum reaction rate. Consequently, the experimental hypothesis that the Michaelis-Menten model would give the most accurate results of the reaction rate constant and maximum reaction rate is shown to be sound.

Shown in Figure 4, reactions with higher concentrations of the substrate maltose showed higher initial reaction rates, however the high concentration of maltose was inconsequential when calculating the overall maximum reaction rate. The inconclusive data produced from the kinetic models are not affected by the initial concentration of substrate in the Starch Hydrolysis experiment. Therefore, experimenters conclude that the initial concentration of substrate has no effect on the efficiency of the catalytic reaction, proving the first half of the hypothesis invalid.

The Starch Hydrolysis experiment explores how changing substrate concentration changes the reaction rate, and examines how kinetic models fit to experimental data. Some follow up questions to the study would be: How does changing pH affect the enzyme performance? How does changing the temperature affect enzyme performance? Since temperature and pH are known to affect enzyme catalyzed reactions, it would be interesting to find an optimal enzyme catalyzed reaction environment while changing temperature and pH.

Conclusion

The Starch Hydrolysis experiment examined the effect of substrate concentration on the rate of reaction, as well as exploring kinematic models of enzyme catalyzed reactions. It was hypothesized that if the initial substrate concentration was higher, it would increase the overall rate of reaction. It was also hypothesized that the Michaelis-Menten model would give the best results when calculating the maximum reaction rate and reaction constant.

Results of the Starch Hydrolysis experiment gave inconclusive findings for calculating the reaction rate constant, K_m, and maximum rate of reaction, V_m, using the approximation models of Eadie-Hoffstee and Hanes-Woolf. The Michaelis-Menten and Lineweaver-Burk models yielded similar statistical significance seen from R^2 values of

0.8257 and 0.832 respectively, however the values calculated for V_m and K_m varied greatly.

Therefore, it was concluded that the Michaelis-Menten model yielded the best values of K_m and V_m, due to the knowledge that the Lineweaver-Burk model is a derivation from the Michaelis-Menten model. Furthermore, the reactions with higher concentrations of the reaction substrate, maltose, did not affect the overall reaction rate. Therefore, the experimental hypothesis that a higher substrate concentration would increase enzyme efficiency was shown to be invalid.

Errors in the Starch Hydrolysis experiment could be due to the lack of constant stirring of the reactions. Proper mixing of the reaction solution is important in when conducting reaction rate experiments, so not having a constantly stirred reaction would cause errors in data.

Understanding how substrate concentration affects enzyme catalyzed reactions is important in biochemical and biomedical engineering. To design the most efficient catalytic reaction, understanding that initial substrate concentration has little effect on the efficiency of the enzyme allows experimenters to look into changing pH or temperature to manipulate the desired rate of reaction.

Sample Calculation:

Finding K_m and V_m for the Lineweaver-Burk model – Reaction 1

Equation 3

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} * \frac{1}{[S]}$$

From Table 1 and Figure 3

[S] = 25.5 g/L

v = 0.025

From Figure 4

$\frac{1}{v}$ = 3260$\frac{1}{[S]}$ - 166.11

R^2 = 0.832

$$\frac{K_m}{V_m} = 3260$$

$$\frac{1}{V_m} = -166.11$$

$$\boldsymbol{V_m = -0.006}$$

$$\boldsymbol{K_m = -19.62}$$

References

[1] S. Loveland, *Starch Hydrolysis Lab Manual*. Ames, IA: Iowa State University, 2013.

[2] H. Lineweaver, D. Burk, “The determination of enzyme dissociation constants,” *Journal of the American Chemical Society.* Vol 56, no. 3, pp. 658-666. 1934.

[3] T. Shafee, *Michaelis-Menten Kinetics.*Available at https://commons.wikimedia.org/w/index.php?curid=38914698. Accessed Nov 20, 2016.