Report on

Starch Hydrolysis

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The purpose of the Starch Hydrolysis report was to research methods for the optimization of glucose production via the enzyme hydrolysis of starch. This reaction is significant for biofuels research, since starch hydrolysis produces glucose, which is fermented to produce ethanol. Researchers manipulated reactor temperature, enzyme concentration, and substrate concentration, to draw conclusions about the optimization of starch hydrolysis. Spirizyme Fuel and Liquozyme SC DS were both tested as enzymes, with maltose serving as the substrate. It was concluded that Spirizyme produced a higher glucose conversion, with higher initial velocities than Liquozyme. Therefore, it is recommended that Spirizyme be used for a full-plant scale-up in a pre-heated reactor at 75ºC (pretreatment) with a reactor temp of 65ºC, pH of 4.63, and an operating range of 0.0033-0.0066 g/L of Spirizyme per 20 g/L of substrate.

Executive Summary

This report entails the methods used to optimize the production of glucose via enzyme hydrolysis of starch. Glucose production optimization applies to the ethanol sector – maximizing starch conversion to sugars would improve yields, since the sugars are a raw material for ethanol production. To explore this process, researchers tested two different enzymes – Spirizyme Fuel and Liquozyme SC DS – varying enzyme concentration and the concentration of the maltose substrate, as well as the reactor temperature. The optimum reaction would have the highest conversion in the smallest amount of time with large operating ranges for temperature and pH.

With these manipulated variables, researchers hypothesized that as the concentration of the enzyme increased, the maximum velocity of the system would increase. It was hypothesized that Liquozyme SC DS will have a higher yield of glucose because it is an alpha-amylase. Alpha-amylases break the alpha 1-4 bond which is present in the maltose substrate used. Researchers also hypothesized that Spirizyme Fuel would produce higher initial velocities because it was designed to operate in the saccharification step of the hydrolysis reaction.

Spirizyme produced higher glucose conversions than Liquozyme, with higher initial velocities. It was found to perform best when pre-heating the reactor at 75ºC, with a reactor temp of 65ºC at a pH of 4.63. The optimum operating range for Spirizyme was found to be 0.1-0.2 g per 20 g/L of substrate. Therefore, we recommend the use of Spirizyme over Liquozyme, with the implementation of a reactor pre-heating step at a substrate concentration range of 0.0033-0.0066 g/L Spirizyme (0.1-0.2 g Spirizyme in 30mL buffer solution) per 20 g/L substrate.

Limitations of the Starch Hydrolysis report include a limited number of enzymes used, and no variance of solute pH. Since Spirizyme Fuel and Liquozyme SC DS were the only enzymes tested, the only conclusions that can be drawn are between those two materials. The solute pH was also held constant; further study varying the reaction pH will provide more insight into the enzymatic hydrolysis of starch.

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Introduction

The purpose of the Starch Hydrolysis report was to explore research methods for the optimization of glucose production via the enzyme hydrolysis of starch. The breakdown of complex carbohydrates into simple sugars is a practical and important process to research, since those sugars can be metabolized to produce ethanol – a leading industry biofuel. The optimization of starch hydrolysis is particularly important to the USA and Iowa, being the world's largest corn producer, and therefore, ethanol producer [1]. Ethanol production from corn hinges on the saccharification of complex carbohydrates like starch, which is the reaction that this report attempts to optimize.

Ethanol has been used as fuel since 1826, but was not incorporated into mainstream use in the United States until 2005 with the first Renewable Fuels Standard (RFS). The RFS established ethanol production to decrease dependence on imported oil and increase the use of environmentally friendly fuels [2]. Figure 1 shows the trend of ethanol production globally since 1980.

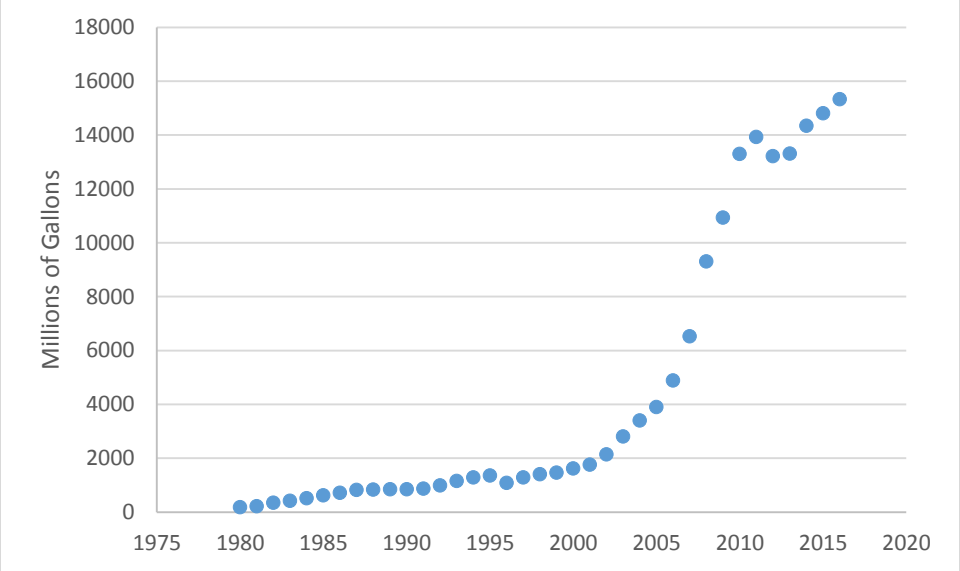

Figure 1: Global ethanol production since 1980 [3]

Ethanol production has greatly increased in the past few decades and is set to maintain a presence as a fuel from The Energy Independence and Security Act of 2007. Ethanol represents significant development in non-petroleum based energy, can be produced domestically, and reduces carbon monoxide emissions [4].

Theory

The processing of starch to make glucose goes through three stages. The first stage is gelatinization. Gelatinization involves the starch granules forming a viscous suspension or a slurry. This stage is achieved by heating starch with water. This process can be thought of as cooking the starch. The next step in starch treatment is the liquefaction step. This involves the partial hydrolysis of the starch. There will be a decrease in viscosity in this step. This step is aided by the use of enzymes. The final step is saccharification which also produces glucose, assisted by enzymes [5].

Liquefaction converts the large starch molecules to dextrins. Dextrin is a step between a complex starch molecule and a simple sugar. The conversion of starch to dextrin is responsible for the loss of viscosity in the starch slurry [6]. Saccharification then breaks the dextrin down further into simple sugars [7].

Maltose is a compound made from the hydrolysis of glycogen and starch. It can be found in germinating grains such as corn. Maltose consists of two molecules of glucose connected by an alpha 1,4 – glycosidic bond. Maltose can be treated with an enzyme to form glucose [8] .

Enzymes are considered to be biological catalysts. These enzymes are responsible for breaking down starch into glucose. Different enzymes act in different ways. Two enzymes that are commonly used for glucose production are Liquozyme SC DS and Spirizyme Fuel Fuel. Liquozyme is active in the liquefaction stage of the process. Where Spirizyme is active in the saccharification step [6]. Spirizyme hydrolyzes alpha 1,4 – and alpha 1,6 – linkages. Liquozyme is an alpha-amylase, which means that this enzyme attacks the alpha 1,4 – linkages of the dextrin.

When an enzyme is added to a substrate a reaction occurs. The enzyme and the substrate form a complex and then this reacts irreversibly to form a product and reproduce a free enzyme. The reaction that takes place between an enzyme and a substrate is represented by Equation 1 shown below.

$$E + S \leftrightarrow ES \rightarrow E + P \quad (1)$$

Where E is the enzyme
S is the substrate
ES is substrate enzyme complex
P is the product

This equation can be further modeled by the Michaelis-Menten equation. The Michaelis-Menten equation describes the reaction velocity as a function of the substrate concentration. Below in Equation 2 is the Michaelis-Menten equation.

$$v = \frac{V_{max}[S]}{K_M + [S]} \quad (2)$$

Where v is reaction velocity
V_{max} is the maximum reaction velocity
[S] is the substrate concentration
K_M is the Michaelis-Menten constant

Using this equation, a Michaelis-Menten plot can be created. The maximum velocity is the value at which the graph levels off. The Michaelis-Menten constant is half of the maximum velocity. The Michaelis-Menten plot shows, in general, how fast the reaction proceeds with different concentrations of substrate [9]. See the sample calculations section for further explanation on analyzing a Michaelis-Menten plot.

Notice that the enzyme concentration is not taken into account directly in this equation. This is because varying the enzyme concentration will change how fast the reaction proceeds. How fast the reaction proceeds is directly proportional to the amount of enzyme that is added to the reaction. So even without directly taking it into account, it will change the Michaelis-Menten plot showing the effect of different enzyme concentrations.

Materials and Methods

The goal of this experiment was to determine which of the two enzymes, Spirizyme Fuel or Liquozyme, worked better to produce glucose from a maltose-acid buffer solution. Table 1 contains a brief summary of the properties of Spirizyme Fuel and Liquozyme.

Table 1: Summary of enzyme properties.

Property	Spirizyme Fuel	Liquozyme
pH Range	3.5 to 5.5 [6]	5 to 6 [6]
Temperature Range	65°C to 75°C [10]	No Data
Density	1.15 g/mL [11]	1.262 g/mL [12]
Bond Broken	α 1,4 and α 1,6 [13]	α 1,4 [7]

These enzymes and maltose were used to carry out the experiment on starch hydrolysis. The results of the experiment were found using the YSI 2700 glucose analyzer.

Apparatus

The starch hydrolysis experiment uses the chemicals Spirizyme Fuel, Liquozyme, maltose, water, a buffer solution controlling the pH of the solution (containing acetic acid and sodium acetate), and a 2.3M Tris solution. A water bath is useful in conducting starch hydrolysis; it keeps the temperature of reaction constant. Erlenmeyer flasks act as individual reaction vessels. The YSI 2700 glucose analyzer determines the amount of glucose produced in the reaction, and a YSI 2710 turntable holds the test tubes for the glucose analyzer, which determined the amount of glucose produced. Figure 2 shows the glucose analyzer and the turntable.

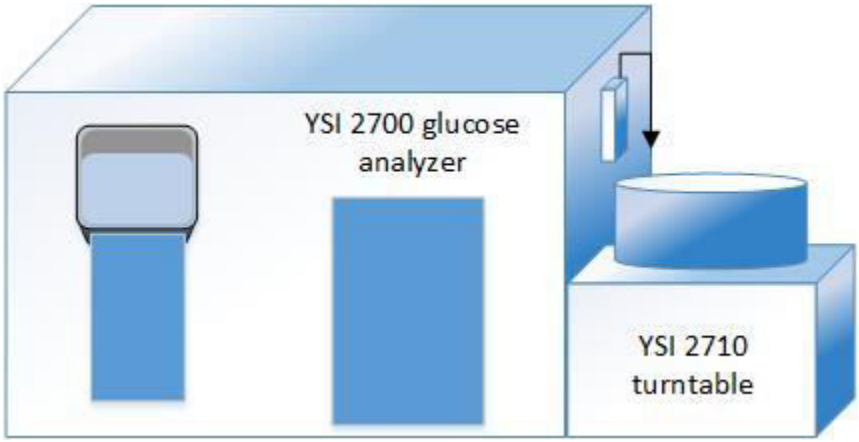

Figure 2: Starch hydrolysis experiment

Methods

Substrate concentration and enzyme concentration were varied using each enzyme. In addition a control trial, which excluded the addition of enzymes, was run.

Varying Substrate Concentration

Four different concentrations of substrate were prepared and used with the enzyme in a reaction vessel in this experiment.

For the first run, 0.15g of maltose was-added to 30mL of buffer solution and heated by placing it in the water bath at 65^0C. Seven test tubes of 1 ml of 2.3M Tris solution were prepared for the first run.

The first run was done with initial substrate (maltose) concentration at 5 g/L. The reaction was started when 0.087 mL, or 0.1 g, of Spirizyme Fuel was pipetted into the flask containing substrate and the stopwatch was started. At 30 seconds, 60 seconds, 90 seconds 120 seconds, 180 seconds, 240 seconds and 300 seconds, 1 ml of solution was taken and put into one of the test

tubes containing Tris. Tris stops the reaction from progressing any further. The test tubes were analyzed for the amount of glucose produced. This was repeated 3 more times with substrate concentrations of 10 g/L, 20 g/L, and 40 g/L. This is shown in Table 2 below.

Table 2 - Concentrations of substrate and times at which reading was taken

Time	30	60	90	120	180	240	300
Concentration of substrate (g/L)							
5 = 0.15g/30mL							
10= 0.3g/30mL							
20= 0.6g/30mL							
40= 1.2g/30mL							

Generally, in larger starch molecules, a pretreatment step is done to break the molecule before it reacts with the enzyme to produce glucose. To test whether heating up the maltose solution helped increase glucose production, trials 5-8 involved a pretreatment step before the reaction took place. The substrate and enzyme concentration were kept the same as in Trials 1-4. After a mixture of the buffer solution and substrate was created, the reaction vessel was kept in a water bath at 75^0C. When the reaction was ready to take place, the flask was moved to another water bath (set at 65^0C) and Spirizyme was added. The samples were taken at the same time as in trials 1-4 and glucose production was analyzed. A similar procedure was followed using Liquozyme.

Varying Enzyme Concentration

Next, the enzyme concentration was varied three times for the substrate concentration of 20 g/L. The first run in this part of the experiment was done with initial enzyme concentration at 0.1 g. The reaction was started when 0.087 mL, or 0.1 g, of Spirizyme Fuel was pipetted into the flask containing substrate and the stopwatch was started. At 30 seconds, 60 seconds, 90 seconds 120 seconds, 180 seconds, 240 seconds and 300 seconds, 1 mL of solution was taken and put into one of the test tubes containing Tris. The samples were analyzed, which reported the amount of glucose produced. This procedure was repeated 2 more times with enzyme concentrations of 0.2 g, and 0.4 g. This is shown in Table 3 below.

Table 3 - Concentrations of enzyme and times at which reading was taken

Time	30	60	90	120	180	240	300
Concentration of Enzyme (g)							
0.10							
0.20							
0.40							

A pre-treatment step was also looked at for trials 4-6. After creating the acid buffer and substrate solution, it was heated to a temperature of 75^0C. When the reactants reached this temperature, the reaction vessel was placed in a water bath of 65^0C and the enzyme was added. Substrate and enzyme concentrations were not changed from Trials 1-3. The samples were taken in accordance to Table 2, above. These samples were analyzed for glucose concentration. A similar procedure was followed using Liquozyme.

Control Trials

The control trials were conducted at two temperatures: 65^0C and 75^0C with a substrate concentration of 20 g/L. Samples of 1mL in volume were taken periodically throughout the lab period. The time of each sample was recorded to later be compared with glucose concentration.

Results

In order to compare the two enzymes, the Michaelis-Menten plots and the reaction velocity at different enzyme concentrations were determined. These plots are presented in the following sections. In addition, a control test was conducted with results presented last.

Varying Substrate Concentration

Eight trials (four without and four with pretreatment, respectively) for each enzyme with varying substrate concentrations were conducted to determine the initial velocities for the Michaelis-Menten plots. For Spirizyme Fuel, the initial velocity plots looked similar Figure 3 shown below.

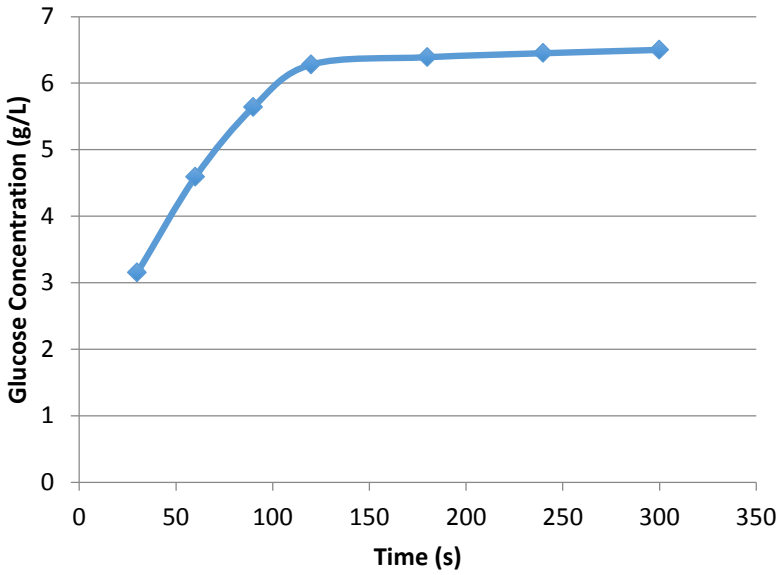

Figure 3: Glucose concentration versus time used to determine the initial velocity (Spirizyme, Trial 7)

Notice the plot levels out after approximately 120 seconds. Each trial using Spirizyme had a similar shape. For Liquozyme, with the same substrate concentration and enzyme weight percent, the initial velocity plot is shown below as Figure 4.

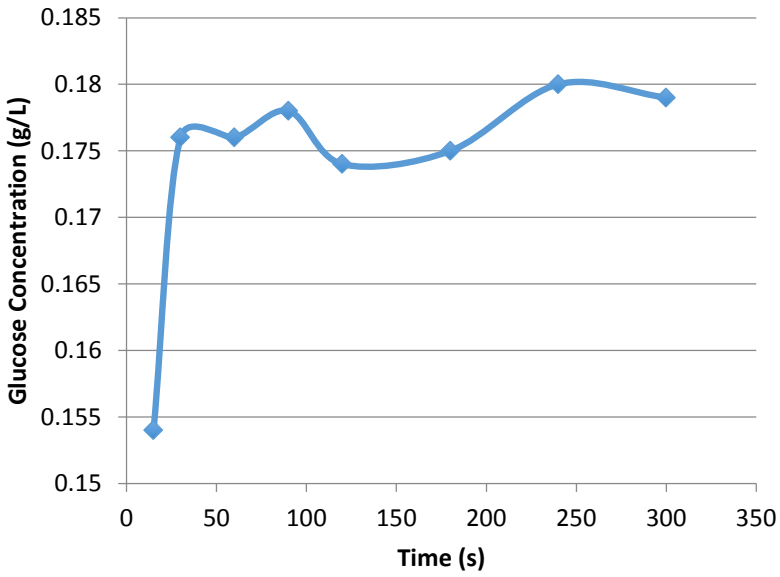

Figure 4: Glucose concentration versus time used to determine the initial velocity (Liquozyme, Trial 7).

Notice the plot oscillates and does not reach a plateau as the trials with Spirizyme did. Many of the Liquozyme trials had more oscillations than this plot. However, the increments on the vertical axis are much smaller in Figure 4 than in Figure 3. If the axis are set to be the same as those for Spirizyme, Figure 4 becomes Figure 5.

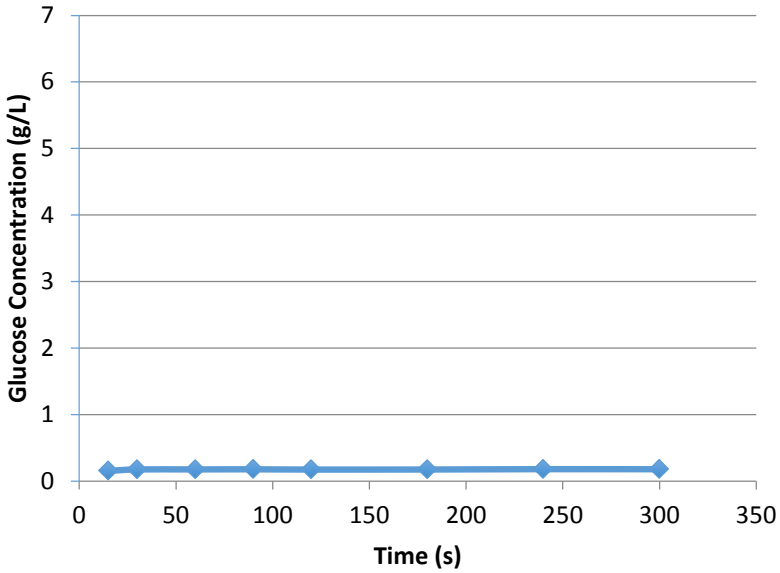

Figure 5: Changed axis for comparison of Liquozyme, Trial 7.

The oscillation becomes undetectable and the trend appears to be level. It is evident that less glucose is produced compared to the Sprizyme trial and there is not a noticeable increase in glucose production. This is true of all of the Liquozyme trials.

Taking these plots, the linear fit was determined using Excel. For the Spirizyme trials, the linear fit was done after eliminating the plateau regions of the plot (discuss this in discussion). For the Liquozyme, this was done for the entire data set. Using the slope of the trendline and the substrate concentrations, the Michaelis-Menten plots were created. Figure 6 and Figure 7 are Spirizyme and Liquozyme, respectively.

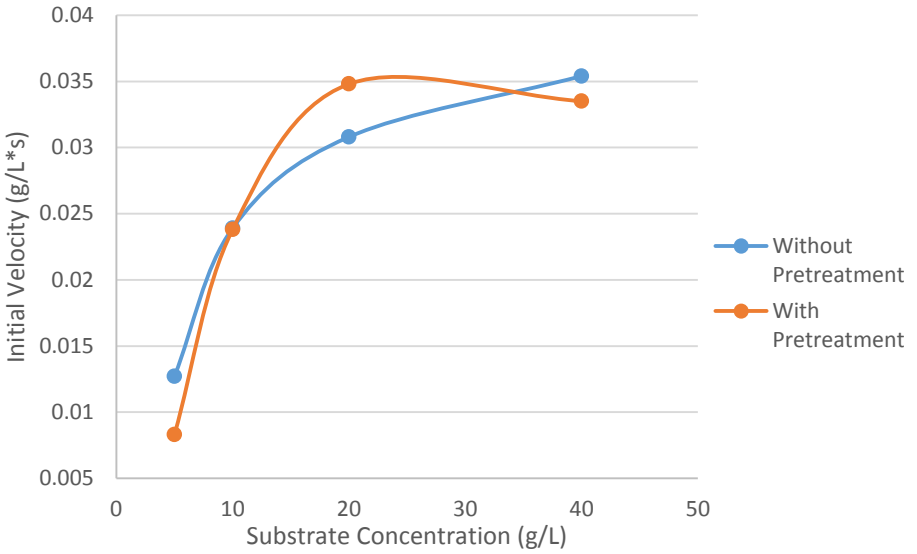

Figure 6: Michaelis-Menten plot for Spirizyme comparing with and without pretreatment.

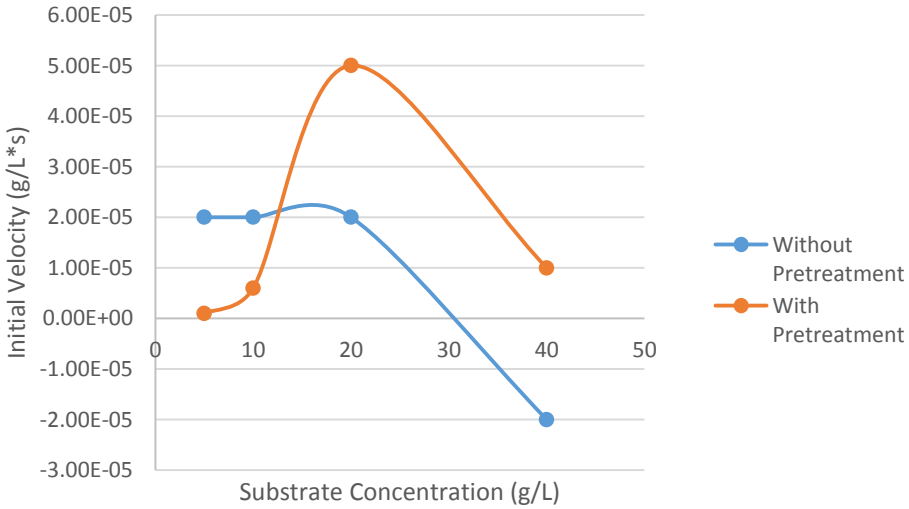

Figure 7: Michaelis-Menten plot for Liquozyme comparing with and without pretreatment.

Figure 8 is the combination of the two plots as shown below.

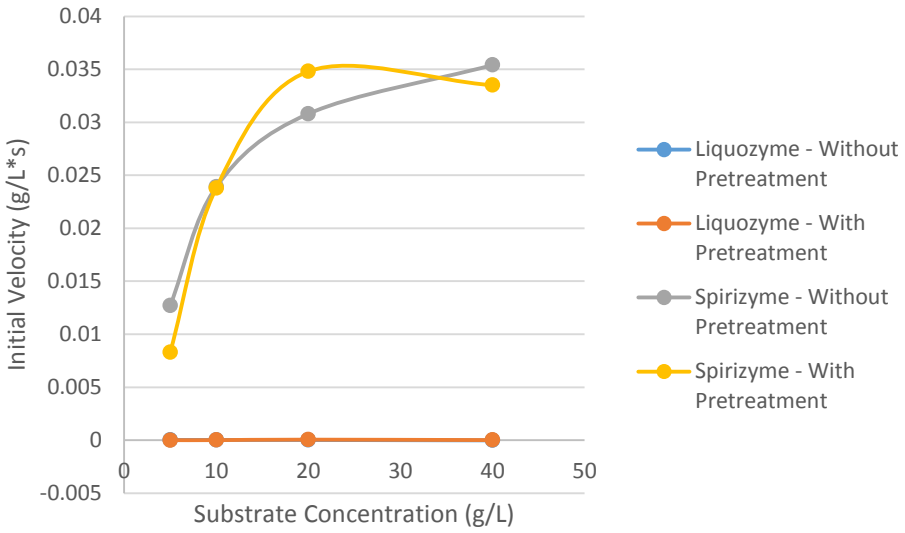

Figure 8: Michaelis-Menten plot comparing the Spirizyme and Liquozyme results.

The results for Spirizyme were much greater in magnitude than those for Liquozyme. In addition, the Spirizyme produced smooth, increasing curves.

Initial Velocity versus Enzyme Concentration

In addition to comparing the initial velocities and the substrate concentrations, the initial velocities and enzyme concentrations were compared. Six trials (three without and three with pretreatment, respectively) for each enzyme with varying concentrations of enzyme were conducted to determine the initial velocities. A similar procedure as shown above was followed. For Spirizyme Fuel, the initial velocity plots looked similar Figure 9 shown below.

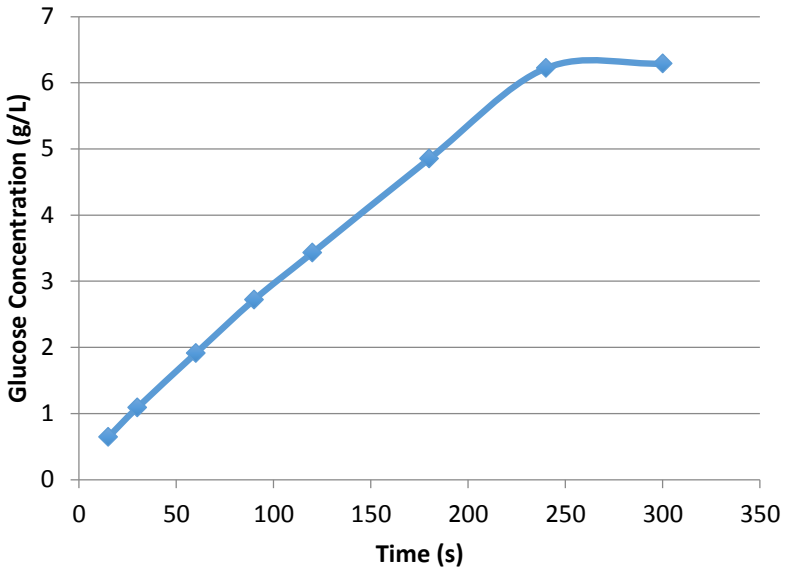

Figure 9: Glucose concentration versus time used to determine the initial velocity (Varying Spirizyme, Trial 1).

Notice the plot levels out after approximately 240 seconds. Each trial using Spirizyme had a similar shape. For Liquozyme with the same substrate concentration and enzyme weight percent, the initial velocity plot is shown as Figure 10 below.

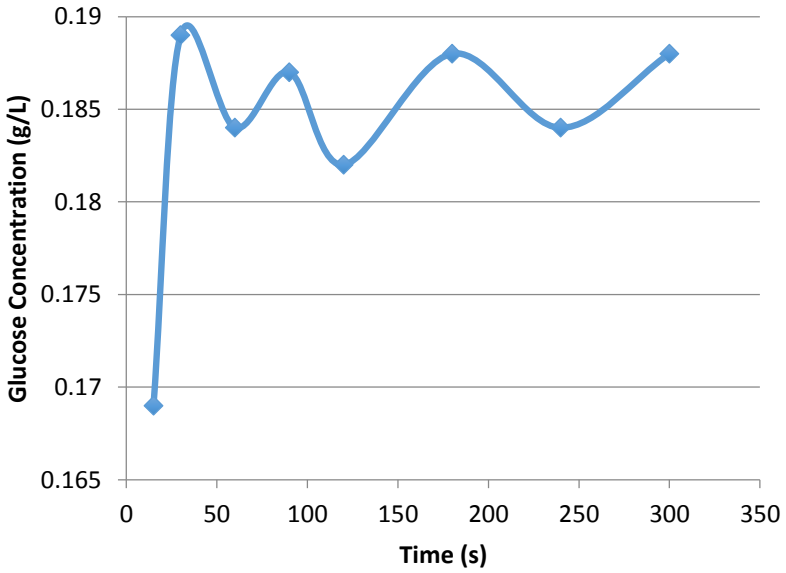

Figure 10: Glucose concentration versus time used to determine the initial velocity (Varying Liquozyme, Trial 1).

Notice the plot oscillates and does not reach a plateau as the trials with Spirizyme did. Many of the Liquozyme trials had more oscillations than this plot. However, the increments on the vertical axis are much smaller in Figure 10 than in Figure 9. If the axis are set to be the same as those for Spirizyme, Figure 10 becomes Figure 11.

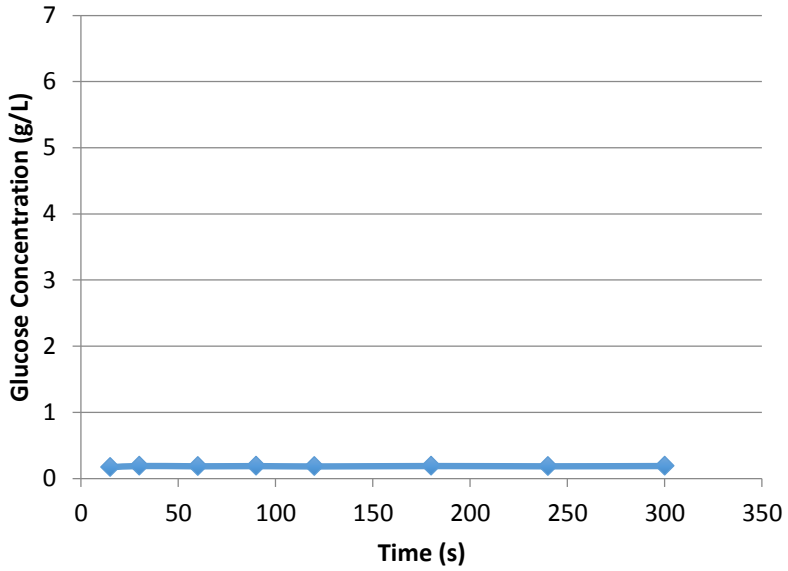

Figure 11: Changed axis for comparison of Varying Liquozyme, Trial 1.

With the change in axis, the oscillation in the data becomes undetectable and the trend appears to be level. It is evident that less glucose is produced compared to the Sprizyme trial and there is not a noticeable increase in glucose production. This is true of all Liquozyme trials.

Taking these plots, the linear fit was determined using Excel. For the Spirizyme trials, the linear fit was done after eliminating the plateau regions of the plot (discuss this in discussion). For the Liquozyme, this was done for the entire data set. Using the slope of the trend line and the substrate concentrations, the following plot was created. Spirizyme and Liquozyme, are shown on the same plot, Figure 12.

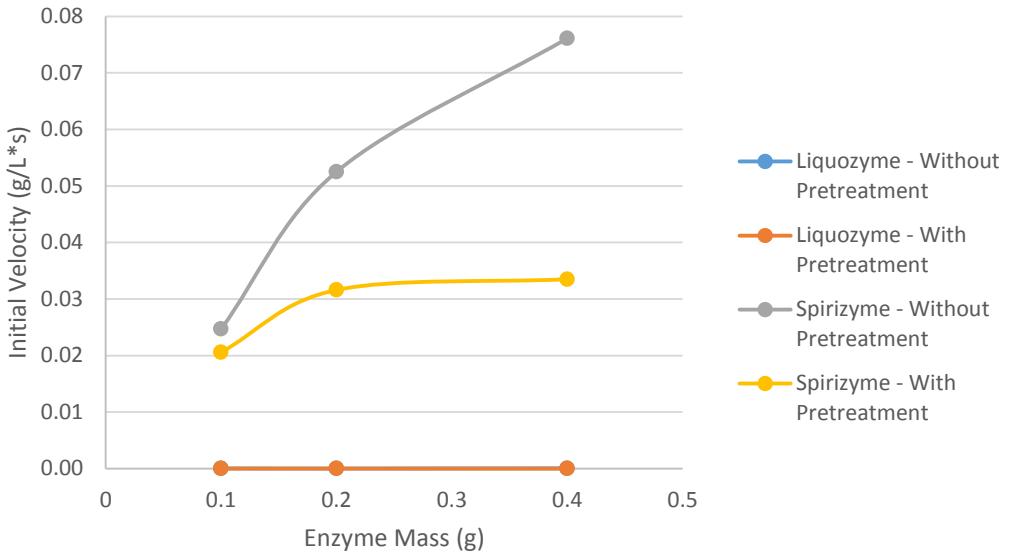

Figure 12: Initial velocity versus enzyme concentration plot comparing the Spirizyme and Liquozyme.

The results for Spirizyme were much greater in magnitude than those for Liquozyme. In addition, the Spirizyme produced smooth, increasing curves.

Control Test versus Data

A control test was done at the reaction temperature (without pretreatment) and the pretreatment temperature (with pretreatment) at a substrate concentration of 20 g/L. However, no enzyme was added to the reactor. The control test was carried out to track glucose production without the presence of an enzyme. Samples were taken from the control trials periodically throughout a lab period and were analyzed. Figure 13 below compares the two trials.

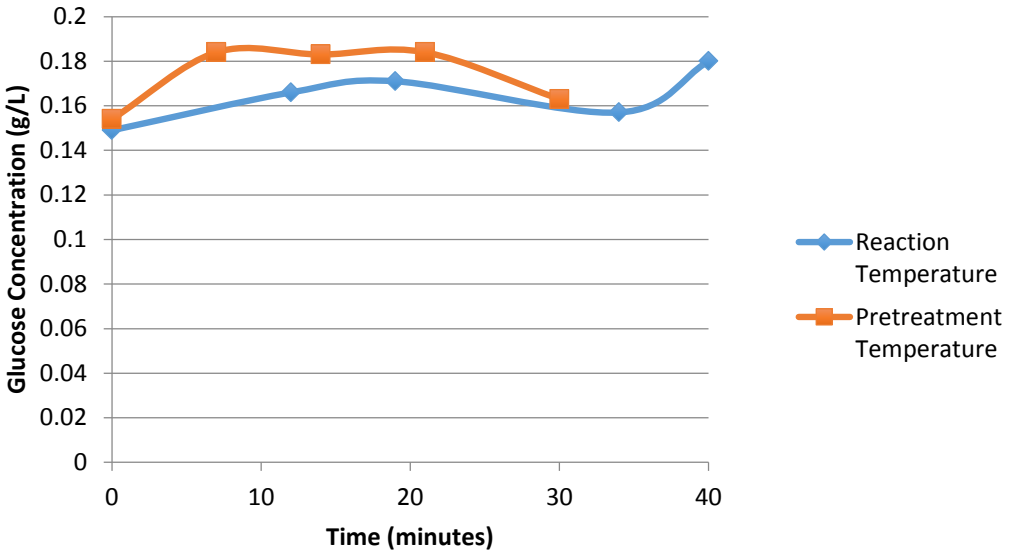

Figure 13: Glucose concentration versus time without enzyme present.

Figure 13 shows that the concentration of glucose is not zero initially and increases overtime.

The control data presented in the above figure was fitted with polynomial trend lines. These equations were used to predict the concentrations of glucose at a certain time if no enzyme was present. These predictions were then compared to the actual data. Figure 14 compares the control predictions and the actual results for substrate concentrations of 20 g/L (with an enzyme mass of 0.1 g) for Spirizyme.

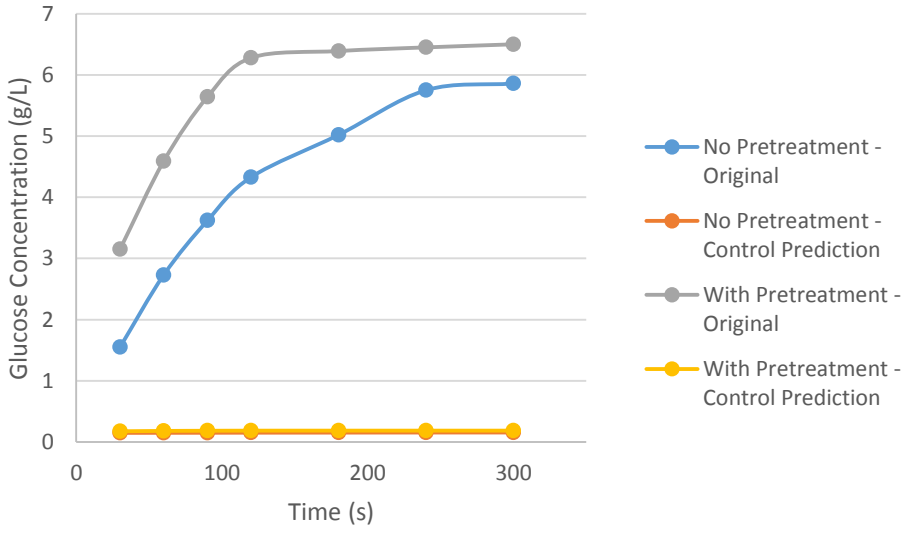

Figure 14: Control predictions compared to actual results for Spirizyme.

Note that there is a large difference between the actual results and the predicted results with no enzyme.

Figure 15 shown below summarizes Liquozyme results compared to the predicted results from the control with no enzyme.

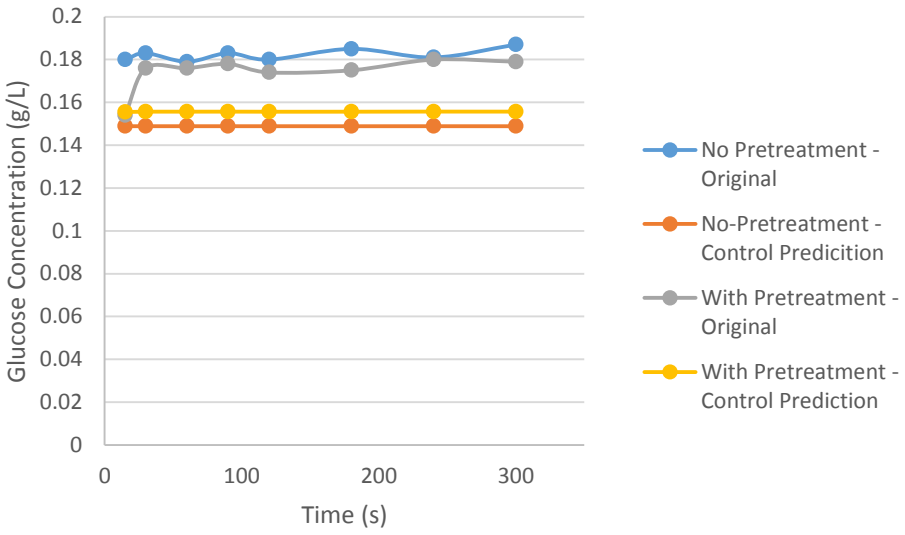

Figure 15: Control predictions compared to actual results for Liquozyme.

Note there is not a large difference between the predicted results and the actual results without enzyme. The average difference between the actual data and the predicted data is summarized in Table 4 shown below.

Table 4 - Average difference between control predictions and actual results.

Enzyme	Treatment Status	Average Difference
Spirizyme	No pretreatment	3.971
	With pretreatment	5.388
Liquozyme	No pretreatment	0.033
	With pretreatment	0.018

As shown, the highest difference between the control (no enzyme) prediction and the actual results (with enzyme) is for Spirizyme with pretreatment temperatures. Compared to the actual values and the predicted values, there is a negligible difference.

The results presented in this section are discussed in the following section.

Discussion

In order to compare the two enzymes, the Michaelis-Menten plots and the reaction velocity at different enzyme concentrations were created. In addition, a control test was conducted. The following is a discussion of those results.

Criteria for the Optimum Enzyme and Operating Range

The problem to be addressed was the optimized process of producing glucose from maltose given two different enzymes. The overarching assumption of this report was that an existing production facility is in place. This means that certain variables were held constant, such as pH, because in order to adjust various parameters additional process would need to be added. For example, in the case of pH adjustment, an acid pump would need to be added along with additional piping. For this manufacturing process, the focus was on saccharification, the hydrolysis of complex sugars. Given that maltose is a disaccharide formed from two glucose units, a high yielding hydrolysis reaction would have a large concentration of glucose.

An optimized process would do this quickly with the smallest amount of enzyme used to the largest amount of raw material, in this case maltose. Therefore, Spirizyme and Liquozyme were evaluated based on the yield of glucose and how well the mechanism behaves. In addition, factors such as large operating ranges for temperature and pH that were provided from the manufacturer literature were considered. Operating range was considered because if the process had an upset, an enzyme with a large range would have a reduced risk of deactivation.

Once the optimum enzyme was chosen, the operating range was determined. This includes reaction duration, temperature, pH and the concentrations of enzyme and substrate. Temperature and concentrations were varied in this experiment. However, evaluating all possibilities was out of scope for this report. Therefore, the optimum operating range was provided based on the conditions tested. It was assumed that the pH of the operating process is fixed at a pH of 4.63. Note that this pH is within the optimum operating range of Spirizyme but is just under the operating range of Liquozyme [6].

Implications of Varying Substrate Concentration

Initial data collected for each enzyme showed very different trends. For Spirizyme, there would be a linear trend until at some time the glucose concentration would reach a maximum and level off. This behavior was expected. As time went on, the enzyme would react with more substrate until the substrate was depleted. Each Spirizyme trial was consistent with the results shown in Figure 3 of the results section. When analyzing these results to find the initial velocity, the linear trend line was fit to the linear piece of the results. This was done so an accurate slope could be

attained. The plateau, level piece indicated that there was no further conversion to glucose and the reaction was over.

However, this same method could not be used with the results for Liquozyme. The initial results shown in Figure 4 and Figure 5 do not follow the same trend as Spirizyme did. Instead, the results appear to oscillate. However, when the axis are adjusted to match those of Spirizyme, it appears to be linear. Compared to the magnitude of Spirizyme, Liquozyme did not produce nearly as much glucose as Spirizyme did. This was true of all Liquozyme trials. When analyzing results to find the initial velocity, a linear trend line was fit to the entire data set. This yielded slopes that were very small including a negative slope. Which is much different than the increasing slopes found with Spirizyme. The results of varying substrate concentration are best summarized in Figure 8. This figure compares both enzymes with the different initial treatments.

The results for Spirizyme were much greater in magnitude than those for Liquozyme. In addition, the Spirizyme produced smooth, increasing curves. Smooth, increasing curves were expected based on the theory surrounding enzymes. The rate of reaction should increase as substrate concentration increases due to the higher likelihood of enzyme encountering a substrate molecule. At some point, the initial velocity will plateau when the enzyme is saturated with substrate meaning each enzyme molecule has formed a enzyme-substrate complex and the reaction cannot speed up without adding more enzyme.

Spirizyme produced the expected plots. Comparing the two conditions (with and without pretreatment), similar results are found. Based on this data alone, a conclusive decision on operating point cannot be determined. Therefore, the initial velocity versus enzyme concentration was considered.

Implications of Varying Enzyme Concentration

In addition to comparing the initial velocities and the substrate concentrations, the initial velocities and enzyme concentrations were compared. As expected, the initial results for both enzymes were similar to those for varying substrate concentrations. The general curve for glucose concentration with Spirizyme was expected as it was previously. Again the general shape of Liquozyme, as seen in Figure 10, was oscillatory until the vertical axis was adjusted as seen in Figure 11.

As done before, the initial results were used to determine the initial velocities. The results for each enzyme with each initial treatment were plotted as shown in Figure 12. Again, as seen in the varying substrate concentration part of the experiment, the Spirizyme produced plots of the expected shape. This shape was expected because as your enzyme concentration increases, the likelihood of an enzyme molecule encountering the substrate molecule is increased and the reaction can be carried out quickly. The Liquozyme did not produce graphs with the expected shape.

Comparing the two Spirizyme trials, the Spirizyme without pretreatment performed much better at higher enzyme concentration than the Spirizyme with pretreatment. The pretreated Spirizyme appears to reach its plateau at approximately 0.2 g of enzyme added. This indicates that the additional Spirizyme was no longer being effective because it was out of substrate to react with. The Spirizyme without pretreatment has not reached the plateau for the enzyme concentrations chosen. Therefore, the Spirizyme with pretreatment produced more glucose faster than the Spirizyme without pretreatment. Based on the results in Figure 12, the maximum initial velocity reached with a substrate concentration of 20 g/L is achieved at 0.2 g of Spirizyme or larger than 0.4 g of Liquozyme.

In industry, saving money is important. Assuming both enzymes cost the same amount, using the minimum amount of enzyme to convert the same amount of substrate would be preferred. Therefore, using Spirizyme at a mass of 0.2 g with pretreatment for a substrate concentration of 20 g/L is optimal.

Implications from the Control Test

Figure 13 showed that there is glucose present in the solution without the presence of enzyme. This also showed that the pretreatment temperature had a higher concentration of glucose. However, there is not a large difference between the two values. The control group proved that there is glucose present in the samples without the use of any enzyme.

Looking at the values of glucose concentration, these can be considered minimal when compared to the magnitude of the reactions with the use of enzymes. This is shown in Figure 14 with Spirizyme. The predicted values, using the fitted lines, for the untreated control were minimal compared to the glucose concentrations obtained in the experiment. Therefore, it can be concluded that the heat did not contribute much to the production of glucose in the Spirizyme trials. The same cannot be said for Liquozyme.

Figure 15 showed the control predictions compared to the results of the Liquozyme trials. The largest difference is less than 0.04 g/L between the glucose concentrations of the actual data and of the control predictions. Unlike Spirizyme, there is not a large difference between these two values. The average difference between the control predictions and the actual results is summarized in Table 3.

From the table, it is seen that the largest difference is for Spirizyme with pretreatment. This means that the enzyme, Spirizyme, produced the most glucose without the assistance of additional heat that was predicted by the control trial. From these results, it was concluded that the best performing enzyme was Spirizyme with pretreatment.

Optimum Enzyme and Operating Range

As discussed previously, the optimum enzyme would have the highest conversion in the smallest amount of time with large operating ranges for temperature and pH.

Considering the highest conversion rate, Spirizyme had the largest initial velocity and glucose conversions both with and without pretreatment. Unfortunately, temperature data was not found on either enzyme. Therefore, it is not clear from these trials what the ideal temperature range is and temperature range was not included in this decision. However, based on the literature, Spirizyme had the largest pH range of 3.5 to 5.5 compared to Liquozyme's range of 5 to 6. Therefore, Spirizyme is considered to be the preferred enzyme.

The optimum operating point for Spirizyme includes set points for pH, temperature, reaction duration, and concentrations of enzyme and substrate. The pH was held constant during this experiment therefore, the optimum operating set points are based on a pH of 4.63. The optimum operating temperature for Spirizyme was with pretreatment. This is supported by the implications of varying enzyme concentration and the implications of the control test with the implications of varying substrate concentration being inconclusive. These were all discussed in the previous sections. Therefore, a temperature of 75°C was reached with the maltose before the enzyme was added and reaction was carried out at a temperature of 65°C.

To determine the optimum operating set point for the concentrations of substrate (maltose) to Spirizyme, the initial velocity plots were considered. For a constant enzyme mass of 0.1 g added, the highest initial velocity occurred after a substrate concentration of 20 g/L. For a constant substrate concentration of 20 g/L, the highest initial velocity occurred after an enzyme mass of 0.2 g was added to the substrate. Therefore, the optimum operating range for Spirizyme is 0.1 to 0.2 g per 20 g/L of substrate.

Sources of Error

Sources of error for this experiment include the assumptions made about the pH of the starch solution. From industry experience, it was known that the pH of the starch milk entering without pH adjustment is approximately 4.6. After a pH adjustment with caustic, for example, the pH is increased to 5.8. Therefore in industry, the pH can be adjusted to meet the needs of the enzyme used. However, the caustic pump could fail. The same pH was used for both enzymes with it only being in the optimum range for Spirizyme. With the pH outside of the Liquozyme operating range, it was likely deactivated. Therefore, it makes sense that the Spirizyme outperformed the Liquozyme.

Another source of error is the measurement of the glucose concentration of the glucose analyzer. The machine is approximately 20 years old had multiple technical difficulties during experimentation. Finally, the method at which samples were taken could be subjected to human error. Therefore, not all samples could have been taken as time accurately as possible. In addition, hand-mixing the reactor was not as consistent as a reactor, such as a CSTR, would have been. This could explain discrepancies in the results of the glucose analyzer.

Conclusions

This experiment explored the effect of substrate concentration, enzyme concentration and temperature on production of glucose from maltose. One goal of the experiment was to determine which of two enzymes—Spirizyme Fuel and Liquozyme-- would break down the substrate better. It was predicted that an increasing enzyme concentration would increase glucose production and increasing substrate concentration would lead to an initial increase in glucose production then glucose production would level off. Secondly, it was hypothesized that Liquozyme would work better than Spirizyme to create glucose as an earlier piece of literature stated that it will be able to break the 1-6 bonds. Thirdly, it was believed that Spirizyme Fuel may work better as an enzyme to break down maltose as literature was found stating that Spirizyme acted in the saccharification step of breaking down glucose [7].

The Michaelis-Menten model was used to analyze which enzyme worked better at the different conditions conditions. Various substrate concnetrations, enzyme concentrations, and temperatures were changed and the effects were analyzed.

In conclusion, it was found that Spirozyme Fuel was a better enzyme to use to break down glucose than Liquozyme. Pretreatment was also found to be useful in increasing the amount of glucose formed in each reaction. Pretreatment was also beneficial as a smaller amount of enzyme was used to get more glucose.

Recommendations

The overall purpose of this experiment was to determine the best enzyme for converting starch into glucose in an industrial process. In industry, the process would start with starch from a source such as corn. It is recommended that a glucose polymer is used (either starch or a maltodextrin) to determine the effectiveness of the enzymes on a larger molecule. In addition, starch has a tendency to gel depending on its amylose content which makes it viscous. A higher viscosity is harder to pump, therefore, before implementing any enzyme into the process, a series of viscosity tests should be conducted.

Further testing to should challenge the assumption of a constant pH and verify the operating ranges provided by literature. As well as provide a larger operating range of temperatures for the enzymes.

Furthermore, this process lacked proper agitation that would be provided in industry. All future experiments should consider using consistent agitation to promote the reaction.

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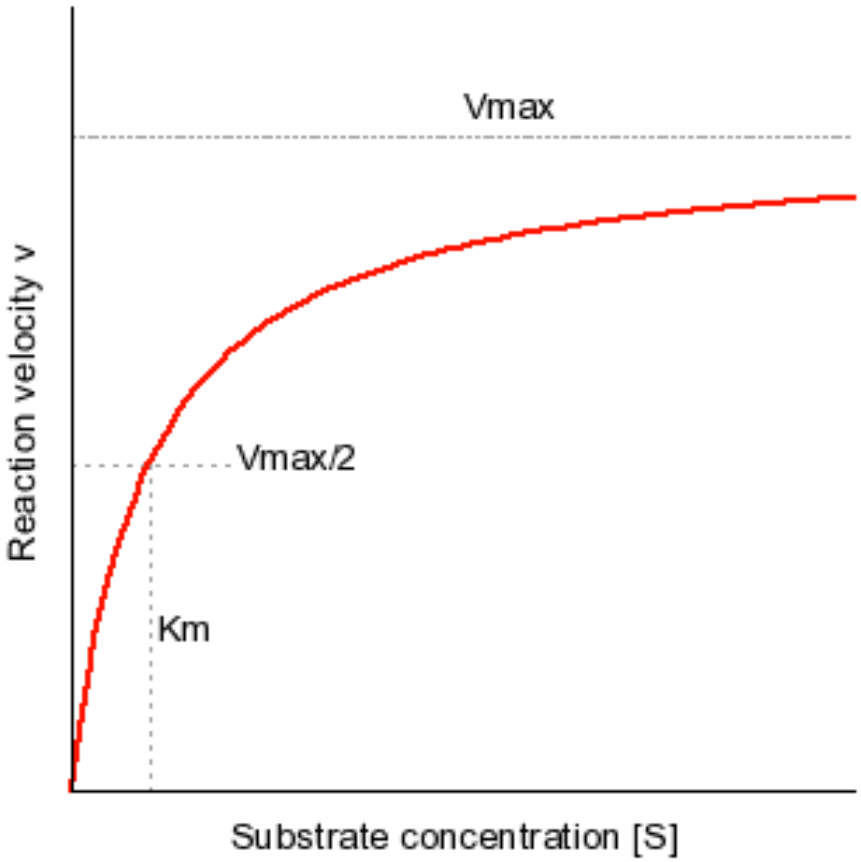
Appendix

Sample Calculations



The Michealis Menten plot is shown above. As the reaction levels out the substrate and enzyme has reached its maximum rate of reaction. The value where the graph levels out is the maximum reaction velocity. The Michealis Menten constant is the maximum reaction velocity divided by two. This process was used to form the Michealis Menten plots.