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Kinetics for Enzymatic Conversion of Biomass to Glucose

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Kinetics for Enzymatic Conversion of Biomass to Glucose

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ABSTRACT

by

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Biofuels are a sought-after alternative for fossil fuels in today's society. More specifically, cellulose-based biofuel is an avenue of research intending to limit waste and provide new renewable energy. Cellulose is a rigid polymer of glucose monomers that is found abundantly across different agriculture crops. However, its stability is a barrier to energy production from this source. Pretreatment followed by hydrolysis of cellulosic materials serves a potential to produce glucose to be used in biofuels in larger quantities compared to other methods. This project studied the effect microwave pretreatment and oxygenation have on hydrolysis of cellulose in Arundo Donax. Arundo Donax ground samples are used in solution with acetic acid buffer (pH= 5.0) along with cellulase and maintained at 50°C. The solution's concentration, in parts per million (ppm), of glucose after hydrolysis was measured over 96 hours using the dinitro salicylic acid method. The Michaelis-Menten constant for cellulase using Arundo Donax and Microcrystalline cellulose before pretreatment were found to be 29.965 g/L and 6.684 g/L, respectively. The concentration of glucose found in Arundo Donax reached a maximum of 310 ppm after 72 hours. In addition, oxygenation, and deoxygenation of buffer and Arundo solution as pretreatment did not yield significantly higher concentrations than Arundo without oxygen manipulation averaging a glucose production of 214.5 ppm with deoxygenation and 209.2 ppm with oxygenation. Microwave pretreatment of Arundo Donax followed by hydrolysis resulted in 29.2 ppm glucose.

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DNS	Dinitro salicylic acid
K_m	Michaelis-Menten Constant
V_{max}	Maximal velocity of the reaction
MCC	Microcrystalline cellulose

CHAPTER 1

INTRODUCTION

Introduction and Purpose of Research

Biomass is organic compounds that are found abundantly across the world. Biomass research has shown a potential for a variety of uses of biomass in more applicable means rather than going to waste. For example, a majority of Biomass has been reused and converted into biofuel such as ethanol and biodiesel[1]. Converting biomass into more applicable means has allowed the United States to benefit from these new energy sources. In 2016, 2% of the energy produced by the United States was from biofuel[2]. However, biofuel begins to formulate the question on whether the crop needs to be raised for food or fuel. One emphasis of this study is using biomass considered, such as celery and corn stalks, for conversion to renewable energy sources, which eliminates waste. Biomass consists of a multitude of different organic compounds such as cellulose and glucose. The question being studied is the effects on enzyme kinetics to convert biomass to glucose for biofuel production.

Cellulose

Cellulose is a polymer of repeating Glucose monomers that are β-linked at the 1 and 4 carbon of glucose monomers. Cellulose is abundant as it makes up 33% of all vegetable matter[3]. Cellulose is mainly found in the stems of plants and skins of fruits. Therefore, foods that are leafy with stalks contain cellulose. The β-linkage of the glucose monomers in cellulose allows for a very rigid structure as the polymer can be linear and stack on each other forming a crystalline like structure. In addition, most biomass samples containing cellulose also contain hemicellulose attached to the cellulose with a shell of lignin forming a rigid, solid structure. This

characteristic of rigidity is why cellulose is common among plants and biomass as the rigidity allows for structural support in the stems of plants. Along with β-linkage, the hydrogen bonding between hydroxyl groups among the sugars adds another layer of structural rigidity to the molecule making this organic compound difficult to breakdown.

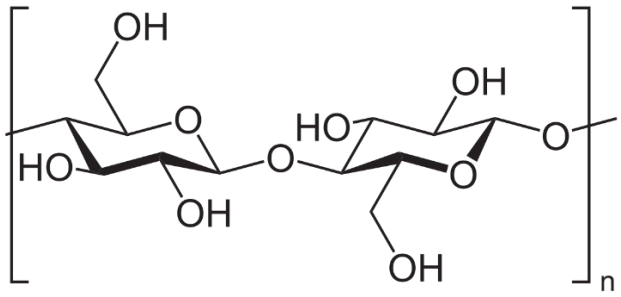

Figure 1. Repeating glucose monomers linked to form cellulose

Also part of the cellulose structure is hemicellulose and lignin fragments that affect the conversion of cellulose to glucose[4]. The presence of these components adds another layer of difficulty to breaking down biomass into high yields of glucose sugars at a low cost. Even though cellulose's abundance provides means for research, recent literature has shown that applying the enzyme cellulase to cellulose shows a slow rate of reaction with low yield of glucose. The addition of biological molecules called "Cellulose-Binding Domains" allows for the rate of the reaction between Cellulose and Cellulase to be enhanced, while also increasing the concentration of glucose that is being produced[5]. The enhancement of cellulose conversion allows more of the biomass be converted to usable product. Since research has already shown that cellulose is an organic compound of interest, the conversion of cellulose to glucose provides a new avenue for a potential energy source.

Biomass Pretreatment

Hydrolysis of cellulose provides a simple, effective method that can produce relatively large quantities of glucose while maintaining a cost-effective strategy. According to literature, enzymatic hydrolysis of cellulose results in a higher percent yield of glucose compared to acid/alkali hydrolysis.

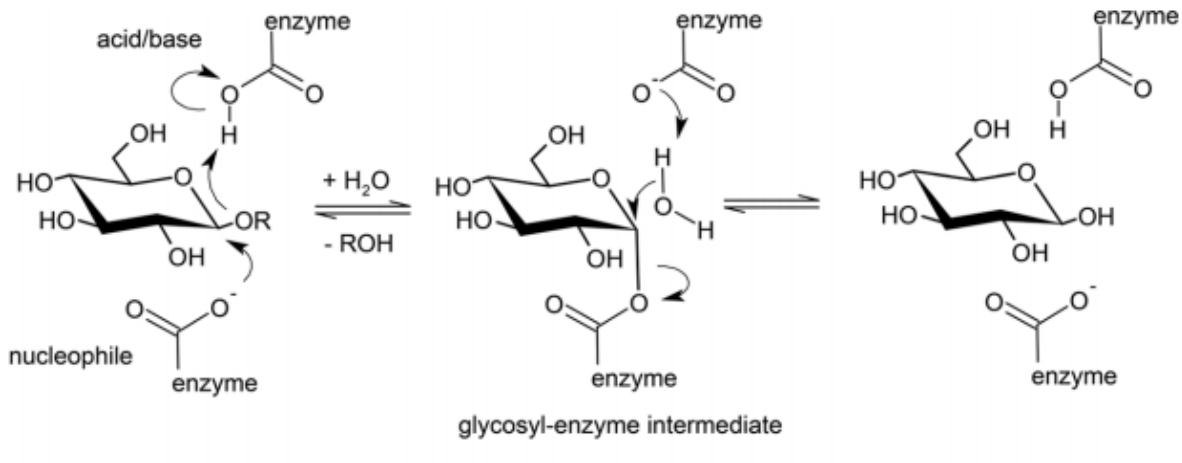

Figure 2: Mechanism of enzymatic hydrolysis of cellulose into glucose[6]

The reported production varies from 75-90% conversion of pure cellulose to glucose[7]. Other forms of hydrolysis include dilute acid, concentrated acid, and hot water compressed hydrolysis. Some favorable conditions for using enzymatic hydrolysis consist of using mild reaction conditions such as pH of 5 and temperature of 45-50°C, while also avoiding corrosion problems that are found in acid/alkaline hydrolysis[8] and preventing inhibition of cellulase. In addition, enzymatic hydrolysis is found to have lower costs when compared to other methods of hydrolysis. However, when enzymatic hydrolysis is applied to raw biomass, the recovery of glucose is not as high as yield as that obtained from pure cellulose. For example, Banu et. al

reported the amount of glucose obtained from direct enzyme hydrolysis of biomass (sourced from sorghum stalk) to be 22.4 mg/g[9]. While pretreatment of the biomass with alkaline contents increased the glucose yield from enzymatic hydrolysis to 34.2 mg/g[11].

In another study, conductive and microwave heating pretreatment were found to enhance glucose production from biomass sourced from rice hulls[10]. Conductive heating produced 5.28 g/L of glucose titer, while microwave heating produced 5.06 g/L at the highest yield. Meanwhile biomass with no pretreatment produced only 0.03 g/L of glucose titer. Other forms of pretreatment include acid hydrolysis, alkaline hydrolysis, CO_2 explosion, and ozonolysis that have shown significant advancements in glucose yield from biomass[11]. Pretreated biomass has been utilized to produce up to 90% higher yield of biomass into fermentable sugars when compared to non-pretreated methods. Pretreatment aids in efficiently extracting more cellulose and then glucose sugars from biomass materials but can also introduce increasing cost. The goal for pretreatment is to find conditions that allow for large production of glucose from cellulose while limiting cost of production.

Cellulase

Cellulase is a broad term that covers specific enzymes such as endo-β-1,4-glucanase and hemicellulases such as β-1,3-glucansase that breaks down hemicellulose[12]. The cellulase enzyme of focus in this research is the endo- β-1,4-glucanase. This enzyme in particular focuses on catalyzing reactions that break down cellulose made out of glucose polymers that have glycosidic bonds at the 1 and 4 carbon of the glucose monomers. The endo- β-1,4-glucanase binds to cellulose molecule and cleaves the substrate at the β 1-4 glycosidic bonds producing smaller oligomers of cellulose and some monomers of glucose (Figure 2).

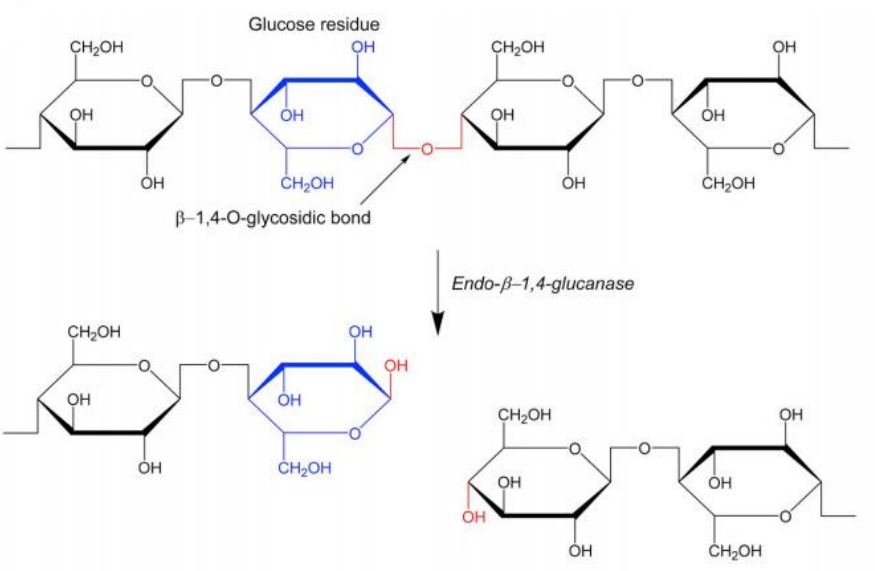

Figure 3: Endo-β-1,4-glucanase breaking down glucose polymers modified from Linton[6]

Cellulase is expressed commonly among animals and bacteria that use this protein to break down the cellulose substrate into useable sugars. However, the enzyme is not found in humans. Humans have enzymes such as amylase to break down glucose polymers of glycogen that have alpha glycosidic bonds, rather than beta bonds. Cellulase can be used to break down biomass containing cellulose into simple glucose monomers that are used in biofuel for reusable energy production. Even though many organisms produce the enzyme, cellulase is still a large cost when used in production settings due to low glucose yield and the demand for the enzyme. Cellulase proves to be an effective enzyme when applied directly to cellulose. However, cellulase efficiency is much lower when used with raw biomass due to lignin contained in the biomass[13]. The lignin of the biomass prevents the enzyme from binding effectively to the cellulose substrate by blocking the accessibility of cellulose and by having non-productive binding of cellulase to the lignin[14]. To aid in enzyme efficiency, pretreatment of the cellulose substrate can aid in allowing more cellulose availability. The emphasis of this research is to find

the effect of microwave pretreatment on cellulase activity. The activity of cellulase was measured using microcrystalline cellulase and Arundo Donax before and after microwave pretreatment. Michaelis-Menten Kinetics was used to determine the effect of oxygen and microwave pretreatment on Arundo Donax.

Michaelis Menten Constant

While converting cellulose to glucose by the enzyme cellulase, the Michaelis Menten constant was determined. The Michaelis-Menten constant depicts how well the enzyme is attaching to the substrate, and how much substrate is needed to reach half of the maximum velocity of the enzyme. The Michaelis-Menten constant is denoted as K_m. For this constant, a small quantity implies a strong affinity of the enzyme for the substrate. The constant is found by using a Lineweaver-Burke plot and determining where half of V_{max} is present. The Line-Weaver Burke plot is derived from the equation used for determining the K_m constant and is given by equation (1.1):

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right)$$

where V is velocity, Vmax is maximum velocity, and [S] is substrate concentration. V_{max} can be described where the enzyme in the reaction is saturated with substrate and maximum velocity of the rate of the reaction is obtained. In this case, cellulose is the natural substrate for cellulase. Carboxymethyl cellulose is another substrate molecule that can be converted into glucose and used to determine the Michaelis-Menten. Previous research has found the enzyme kinetics of cellulase to result in a $_{Km}$ value of 2.5×10^{-5}g/L to carboxymethyl cellulose, which expresses the enzyme has a strong affinity of the substrate[15]. For this research, the K_m value was determined using microcrystalline cellulose and Arundo Donax.

Microwave Pretreatment

In comparison to other pretreatment methods, microwave pretreatment has been found to be simple and effective. The aim of pretreatment is to increase the yield of glucose from cellulose containing biomass by altering the structure of the crystalline cellulose. Microwave pretreatment assists in conversion of cellulose to glucose by removing the lignin found in cellulose and altering the forms of cellulose and hemicellulose to remain in the solid forms and provide accessibility to hydrolytic enzymes[16]. In addition, the cellulose is reported to have more of a crystalline structure after microwave pretreatment by disrupting the hydrogen bonds and removing acetal groups and other amorphous components connected to the cellulose. The crystalline structure of cellulose is thought to allow for hydrolytic enzymes to reach the pure cellulose easier. However, the relationship between crystallinity and cellulase activity is still not understood.

Microwave pretreatment possesses numerous advantages in producing higher yield fermentable sugars. As referred to in the name of the method, microwave pretreatment uses a pressurized microwave to heat the samples at moderate energies to degrade the cellulosic biomass into more pure cellulose. A 300-400 W microwave at about 300 psi is used[16]. The microwave settings compared to other thermal and conductive methods are favorable in large industrial settings due to reducing energy requirements[17]. In addition, microwave pretreatment has been shown to require shorter time periods of operation when compared to simple hydrolysis methods with enzymatic depolymerization due to the enzyme needing less time to reach maximum rate. While microwave pretreatment proves to be simple, the method is also efficient by increasing glucose yield when compared to other methods. Microwave pretreatment has resulted in 50 times higher glucose yield when compared to similar conventional hydrolysis

methods, while also increasing pure cellulose contents in biomass samples and decreasing the percentage of lignin found in the samples[18,16]. Microwave pretreatment has also proved to increase the yield of other reusable wastes such as polystyrene by assisting in pyrolysis[18]. Microwave pretreatment is a desirable method due to its increase in yield of product, its ease of operation, efficiency, and low-cost.

Effect of Oxygen on Cellulose Hydrolysis

Conversion of cellulose to glucose at high yields can be achieved through multiple scenarios, however, utilizing the least amount of a costly enzyme with highest glucose yield is preferred in industrial biofuel production. Oxygenation/deoxygenation of a catalytic reaction shows potential for increasing product yield. Catalytic reactions involving enzymes such as oxidases show high selectivity towards dissolved molecular oxygen in order to be completely effective[19]. The discovery of how dissolved oxygen affects enzyme rate can aid in producing a more effective catalytic reaction in order to produce more product at lower cost. Studies have shown that as the air-liquid interfacial area increases, the amount of cellulose conversion from cellulase decreases[7]. Therefore, the increasing exposure of air may cause for deactivation of cellulase at low enzyme concentrations. However, when dissolved oxygen is continuously provided in solution, the glucose product concentration is 3-fold higher than that obtained using ten times the concentration of enzyme[19]. Other studies have shown the dependence of enzymes on molecular oxygen by aiding in oxidizing glycosidic bonds to break polysaccharides into monomer sugars and using less enzyme concentration with lytic polysaccharide mono-oxygenase[6]. Results from these previous studies show a potential effect that oxygen and air can have on the rate of enzyme in cellulosic conversion. The effect of oxygenation of solution paired

with microwave pretreatment of Arundo Donax was studied in this work and compared to previous results.

CHAPTER 2

EXPERIMENTAL METHODS

Materials

All trials were performed with commercial microcrystalline cellulose, carboxymethyl cellulose, and Arundo Donax was provided by Dr. Ramey (North Carolina State Agricultural Science) grown in Asheville, NC. The Arundo Donax was allowed to dry and then blended into a fine powder for use in trials. Other reagents used in these trials were also A.C.S. reagent grade from commercial buyers and were used without further purification. These other reagents included sodium hydroxide, potassium tartrate, 3,5 Dinitro salicylic acid, deionized water, pure molecular oxygen from Airgas, cellulase, glacial acetic acid, and D-glucose. Enzymatic hydrolysis of cellulose was carried out in acetic acid buffer (0.05M, pH=5.0).

DNS Assay

The DNS assay was used to analyze the solution by measuring absorbance with a Vernier SpectroVis Plus® at 540.6 nm along with Logger Pro 3 software. The DNS assay was used to quantify glucose. A stock solution of 3,5-dinitrosalicylic acid was prepared by taking 1.0 g DNS and adding to 50.012 g of deionized water. The solution was then heated. A solution of NaOH/ Potassium tartrate was prepared by adding 1.600 g of NaOH and 3.010 g of potassium tartrate to deionized water with stirring and dilution to 50.016 g. A 2,010 ppm Glucose stock solution was prepared by dissolving 0.402 g d-glucose in 200 mL deionized water. The solution was heated and stirred until dissolved. Table 1 shows the standard samples that were prepared with the stock solution of glucose and boiled for five minutes. The solutions were then placed in an ice bath to cool. Each sample was measured in a spectrophotometer at wavelength 540.6 and the absorbance

was recorded. A calibration curve was developed plotting absorbance and concentration. Figure 4 is a color picture of each prepared solution.

Table 1. DNS dilution and Glucose analysis

DNS (mL)	NaOH-K Tartrate (mL)	Glucose Stock Solution (mL)	DI Water (mL)	Absorbance	Concentration (PPM)
0.500	0.500	0.2	4.8	0.101	67
0.500	0.500	0.4	4.6	0.172	134
0.500	0.500	0.6	4.4	0.265	201
0.500	0.500	0.8	4.2	0.415	268
0.500	0.500	1.0	4.0	0.498	335
0.500	0.500	1.2	3.8	0.586	402
0.500	0.500	1.4	3.6	0.708	469
0.500	0.500	1.6	3.4	0.807	536
0.500	0.500	1.8	3.2	0.971	603
0.500	0.500	2.0	3.0	0.981	670

Figure 4. Results of DNS assay with Glucose serial dilution

Due to COVID regulations, lab closure resulted in the DNS calibration curve being retested when returned to lab. A new solution of Sodium hydroxide and Potassium Tartrate was made from the following reagents (Table 2).

Table 2. Retested Solution of Sodium Hydroxide and Potassium Tartrate Quantities

NaOH	K-Tartrate	DI Water
1.611g	3.002g	45.417g

The 2,010 ppm stock solution of glucose was able to be reused and the DNS calibration was similar to before the lab closure.

Hydrolysis Experiments

To determine the amount of glucose generated, hydrolysis was performed without cellulase to determine if glucose was present. These amounts were subtracted from trials using cellulase to determine the amount of glucose and reducing sugars generated due to only enzymatic hydrolysis. MCC and Arundo Donax were used for hydrolysis experiments using water and buffer in separate experiments. A 0.05M acetic acid buffer was prepared by adding 25.555 g of sodium hydroxide to 2 L of deionized water, followed by the addition of 58 mL of glacial acetic acid. pH was tested with litmus till a pH of 5 was show. Approximately 2.5 g of MCC or Arundo was put into 250 mL of water or buffer solution for hydrolysis carried out in a stopper sealed Erlenmeyer flask in a water bath controlled at 50°C and magnetically stirred. During hydrolysis, liquid samples (6 mL) was pipetted out every 24 hours over 96 hours, then centrifuged with Sorvall Legend XT (Thermo Scientific) at 4500 rpm for 5 minutes. The centrifuged solution (1 mL) was analyzed using the DNS assay.

Background samples were also collected after putting 2.5 g of MCC and Arundo Donax in buffer solution after boiling to remove oxygen, as well as after bubbling pure oxygen for 30

minutes into the buffer solution to determine the effect of oxygen on hydrolysis. Hydrolysis of the MCC and Arundo Donax was carried out and the liquid sampled as previously described. Liquid samples, 6 mL, were collected every 24 hours for 96 hours, centrifuged and DNS assay performed. Background glucose amount were subtracted to calculate the amount of glucose produced.

During the study, cellulase was purchased one year apart. To determine the effectiveness of both sources of cellulase, microcrystalline cellulose and Arundo Donax was used to determine the concentration of glucose produced from each enzyme after 96 hours of hydrolysis. The DNS assay was performed for both old and new enzyme with 2.501 grams of microcrystalline cellulose in 250 mL of buffer solution with 0.125 grams of each enzyme in separate experiments. DNS assay was performed after 0, 2, 4, 8, 16, 24, 48, 72, and 96 hours. From the spectroscopic results, the old enzyme was used for the remaining trials because of higher absorbance values recorded indicating production of more reducing sugars.

Once background samples for buffer, water, cellulase, oxygenation/deoxygenation, and microwave were performed, the DNS assay was conducted for Arundo Donax, MCC, Oxygenation/Deoxygenation of Arundo Donax, and microwave pretreatment of Arundo Donax with cellulase. Separate experiments were performed using Arundo Donax and MCC. For each reaction condition, 2.500 g of Arundo Donax, 2.519 g of MCC, and 0.125 g of cellulase was used with 250 mL of acetic acid buffer (0.05 M, pH=5.0) kept at 50° C and magnetically stirred. Three liquid samples, 6 mL, were pipetted out for each condition, centrifuged, and the DNS assay was performed. The Michaelis-Menten constant was found for MCC and Arundo Donax by using different amounts of substrate. The enzyme concentration was kept the same at 0.013 grams of cellulase enzyme followed by 250 mL of acetic acid buffer for each substrate

concentration. The DNS assay was performed using 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, and 2.50 g of Arundo. All samples were magnetically stirred in a 50°C water bath for 24 hours. Six liquid samples, 6 mL, were pipetted, centrifuged, and ran in the DNS assay for each substrate mass.

Microwave Pretreatment

Microwave pretreatment was conducted by adding 10.021 g of Arundo Donax into 60.0 mL of distilled water and microwaving at low heat settings (30%) for 30 minutes. Once microwaved, the solution was decanted and the remaining Arundo substrate was retrieved. 1.008 g of Arundo was added to 0.013 g of cellulase and 100.0 mL of acetic acid buffer and was kept in a 50°C water bath for 24 hours. After 24 hours, the DNS assay was repeated..

CHAPTER 3

RESULTS

DNS Calibration Curve

From the DNS assay, the absorbance at 540.6 nm was graphed against the concentration of the glucose to determine a linear relationship. A linear relationship proves to be a vital result as the slope of this line can be used to calculate the concentration of glucose by reading its absorbance. With a correlation coefficient of 0.9928, the plot is linear for the purpose of glucose concentration determination.

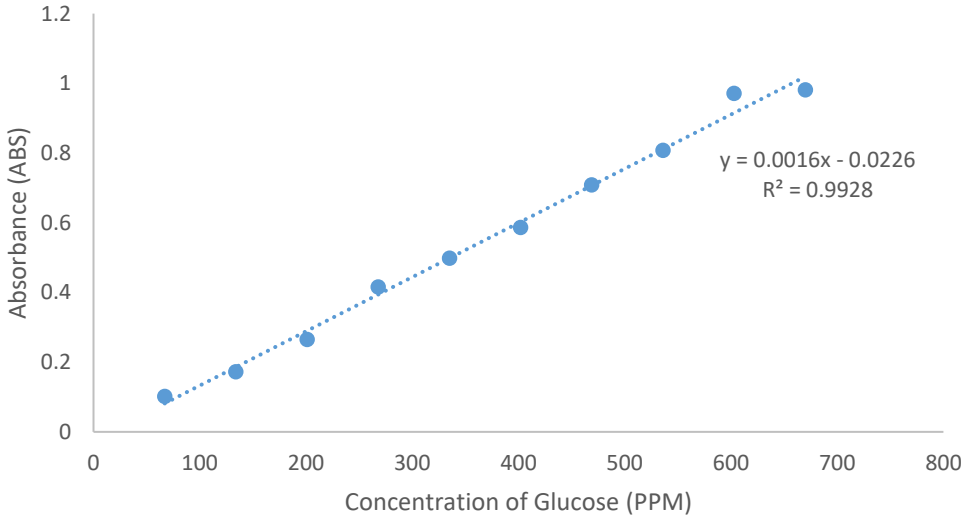

Figure 5. DNS Calibration Curve with Linear equation

Hydrolysis of Microcrystalline cellulose and Arundo Donax

Multiple trials for each substrate were used for enzyme hydrolysis to determine glucose yield after subtracting the background. The average concentration of glucose produced for MCC and Arundo Donax with the introduction of cellulase and no pretreatment is shown in Figure 6.

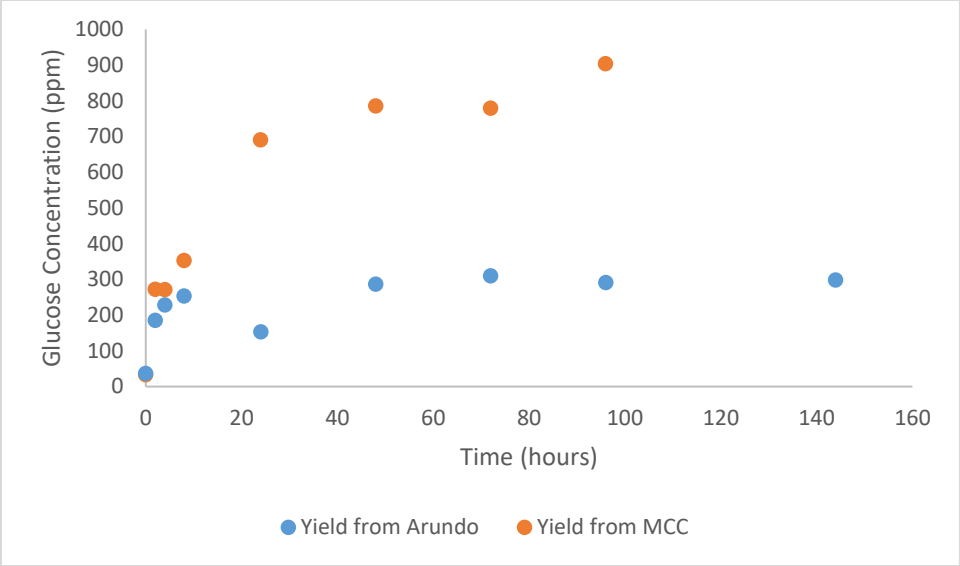

Figure 6: Average Concentration of glucose over time for Microcrystalline cellulose and Arundo Donax

The MCC was found to produce more glucose over time than the Arundo Donax. To determine enzyme affinity for each substrate, a Line-Weaver Burke plot was utilized, and the Michaelis Menten equation was used for calculation of the K_m constants (Figure 7).

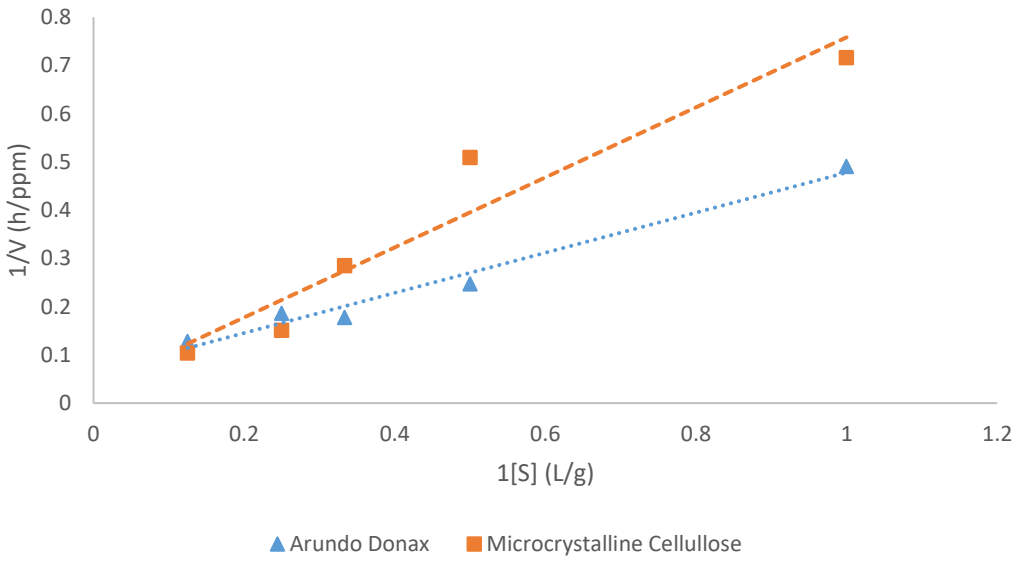

Figure 7: Line-weaver Burke plots comparison of Arundo Donax and MCC

In Figure 7, Equation 1.1 can be applied to determine the K_m constant to quantify enzyme activity. Arundo Donax produced a K_m of 29.97 g/L and MCC produced 6.685 g/L. The K_m constant for Microcrystalline cellulose proved to be smaller indicating a greater affinity for MCC than Arundo Donax. The affinity of cellulase to MCC can be shown by comparison of both Line-weaver burke plots in Figure 7. The slope for MCC is larger than Arundo resulting in a smaller K_m value.

Effect of Oxygen on Hydrolysis

The amount of glucose generated by enzymatic hydrolysis of Arundo Donax was found to be similar under deoxygenated and oxygenated conditions. Figure 8 shows the ppm glucose amounts obtained under these conditions compared to normal enzymatic hydrolysis conditions without oxygen manipulation.

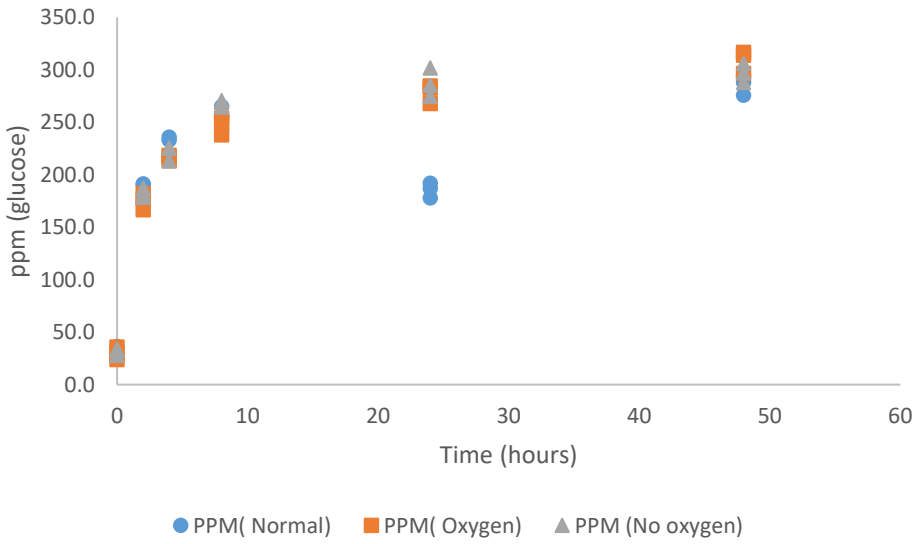

Figure 8: Comparison of Oxygenation, deoxygenation, and normal conditions of enzymatic hydrolysis over time.

With 30 minutes of oxygenation and deoxygenation of buffer solution, the production of glucose does not change significantly over time. There is a small deviation from the trend found at 24 hours that is a potential outlier, however, the other points indicate no significant difference in glucose production between each of the reaction conditions.

Microwave Pretreatment

To determine the effect microwave pretreatment has on the production of glucose from enzymatic hydrolysis, 10.021 g of Arundo was microwaved and used for enzymatic hydrolysis. The solution was analyzed using the DNS assay method. Six trials of the same microwaved Arundo were tested and the results are shown in Table 4.

Table 3: Microwave Pretreatment glucose production of Arundo Donax after 24 hours

Sample	Glucose Production (ppm)
1	34.125
2	29.125
3	27.875
4	24.125
5	30.375
6	29.75

The microwave treated Arundo Donax resulted in an average yield of 29.2 ppm of glucose, which was similar to the results of the blank trials indicating little/no glucose yield.

CHAPTER 4

DISCUSSION

DNS Assay

The DNS assay performed was a desirable method for the purpose of determining glucose yield because an absorbance measurement may be used to determine glucose concentration. DNS solution is naturally yellow. When heated with glucose under basic conditions the solution turns more orange with an increase in glucose concentration. A UV-Vis spectrometry measurement may be used to determine the absorbance at 540.6 nm. A linear plot of absorption against concentration for glucose is easily used to indicate the amount of glucose produced. When DNS and glucose are combined, the glucose undergoes oxidation while the DNS undergoes reduction to create an orange color in solution. During the reaction, the nitro group of DNS is reduced to an amino group, while the aldehyde group of glucose is oxidized to a carbonyl group. Since glucose is a reducing sugar, the redox reaction will have glucose consume hydroxide ions in solution; therefore, adding a basic media will increase the reaction rate and stabilize the reaction. In addition, potassium tartrate is used for DNS assays as well to help the DNS reagent keep from dissolving oxygen so that the color change is maximized and allows for accurate spectroscopic results. For the DNS assay performed, the detection limit was calculated to correlate to an absorbance of 0.073.

Hydrolysis Experiments

For both Arundo Donax and MCC, Line Weaver-Burke plots were developed to determine the K_m constant. The calculated K_m constants for both Arundo Donax and MCC displayed expected results with MCC having a lower value than Arundo Donax at 6.68 g/L, while Arundo Donax was 29.97 g/L. The reason for a lower value for MCC is microcrystalline

cellulose is pure cellulose that is partially depolymerized into smaller subunits of cellulose and glucose making the cellulase enzyme have easier access to the substrates. Arundo Donax, however, is a biomass that contains hemicellulose and lignin, which both aid in the encapsulating the cellulose and disallowing cellulase to bind to the preferred substrate. Therefore, cellulase is expected to have a greater activity for MCC than Arundo Donax since more cellulose is available for the enzyme to access and perform the catalyzed reaction, and MCC is cellulase's natural substrate. The average yield of glucose for Arundo Donax was found to be 234.7 ppm over 96 hours with the highest yield coming at 72 hours with a concentration of 315.1 ppm. On the other hand, MCC had an average production of 554.2 over 96 hours and had its highest yield of 971.8 ppm at 96 hours. The average yield for each substrate demonstrates that glucose can be readily produced from pure cellulose, MCC, while lignocellulosic biomass proves to be difficult to produce high yields of glucose.

Effect of Oxygenation on Hydrolysis

For the oxygenation and deoxygenation of buffer solution, the resulting glucose concentration produced showed to have no effect from the oxygen manipulation. Both with 30 minutes of oxygenation and deoxygenation, the average glucose concentration was no different from normal enzymatic hydrolysis condition results, which indicates that dissolved oxygen may have no effect on cellulase enzyme, or that consistent oxygenation of the buffer solution during enzymatic hydrolysis should be tested. However, the expense of consistent oxygenation of buffer solution may drastically increase costs and should be considered before testing. The results from this experiment suggest that dissolved oxygen is not a needed cofactor for cellulase to improve enzymatic conditions unlike other select enzymes like oxidases. To be considered, the addition of potassium tartrate to the DNS method may have an effect on the effect of dissolve oxygen as

this reagent helps to control the amount of dissolved oxygen so that the DNS reagent can be reduced from glucose. A potential future experiment is to determine the effect that oxygenation pretreatment may have on microwave pretreatment and enzymatic hydrolysis. Overall, the oxygen manipulation did not prove to increase glucose yield.

Microwave Pretreatment

The K_m constants were calculated before microwave pretreatment. The expected result for the K_m constants for both Arundo Donax and MCC was lower values for both, however, the performed microwave pretreatment did not yield high concentrations of glucose. The pretreatment method was not successful in increasing glucose concentration. Potential cause for low values of glucose can include not having Arundo Donax sample temperature during microwave pretreatment reach sufficient enough levels (180°C). The high temperatures from the microwave was expected to break down the lignocellulosic biomass into more pure cellulose substrates so that increased catalytic activity could be displayed. The technique of microwaving the Arundo samples was difficult to perform due to the sample boiling over and losing biomass material. Lower levels of microwave power were performed to maintain biomass levels and consistently microwave the sample. Alternative techniques for the microwave pretreatment would include refluxing the biomass samples during pretreatment to keep from boiling over and increase temperature of Arundo samples.

Conclusions

As fossil fuels continue to be depleted, an alternative energy resource will always be in need to produce sufficient, cleaner energy. The research for conversion of cellulosic biomass into fermentable sugars used in biofuels has been a long subject of study, yet small yields of glucose and high enzyme loads increase the cost of the previously studied methods. The pretreatment of cellulosic biomass has been proven to increase the production of glucose; however, more research is needed to continue the enhancement for enzymatic hydrolysis in order to produce sufficient amounts of fermentable sugars. In this study, the effect of oxygenation and microwave pretreatment on glucose production from Arundo Donax was performed. The Michaelis-Menten constant for Arundo Donax and Microcrystalline cellulose before pretreatment were found using Line Weaver-Burke plots and resulted in values of 29.97 g/L and 6.68 g/L, respectively. The intended goal was to compare K_m values before and after microwave pretreatment, however, the microwave pretreatment was not successful. This is due to the amount of reducing sugars generated before pretreatment using MCC and Arundo Donax (209.2 ppm and 214.5 ppm) being approximately 10 times greater compared to microwave pretreatment (29.2 g). In addition, oxygen manipulation of the buffer solution before the DNS method was carried out included oxygenating and deoxygenating the solution. There were no significant differences in the amount of reducing sugars produced using deoxygenation compared to oxygenation suggesting that oxygen manipulation does not affect enzymatic hydrolysis.

Future Work

Future alternatives for this study include microwaving Arundo Donax biomass material with the inclusion of oxygen, alternative microwave pretreatment with corn samples, and use of ionic liquid chemical hydrolysis. The hinderance of the microwave pretreatment was shown with the inability to sufficiently heat the biomass solution to high enough temperatures. Refluxing the sample with microwaving can potentially allow for increased temperature of the sample and higher yields of glucose. Comparably, the effect of oxygenation of microwaved pretreated samples has not been published and shows an area of interest to determine how continuous oxygenation with a paired pretreated biomass sample effects glucose production. Finally, ionic liquid chemical hydrolysis may be an alternative method to enzymatic hydrolysis conversion of biomass by adding water drop wise to a chloride ionic liquid containing catalytic acid. This method excludes the need for cellulase and ultimately decrease the cost of procedure with potential of high sugar yield.

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