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Room temperature alkali treatment of rice straw for enhanced enzymatic hydrolysis.

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ROOM TEMPERATURE ALKALI TREATMENT OF RICE STRAW FOR ENHANCED ENZYMATIC HYDROLYSIS

By

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ABSTRACT

Due to the pollution of fossil fuels due to greenhouse emissions, researchers continue to search for alternative methods that allow for the clean production of energy, fuels, and polymer fibers. A method currently under investigation is the use of lignocellulosic biomass as a solution to current problems with energy and polymer fiber production. Through the process of pretreatment, possibilities for lignocellulosic biomass expand due to improved accessibility of the cellulose and hemicellulose which can be broken down via enzymatic hydrolysis into C5 and C6 sugars such as xylose and glucose. This study investigated the fermentation of the rice straw into fuel and fibers. Previous studies have tested varying pretreatment methods on rice straw, yet those studies require additional energy to obtain higher temperatures. This study demonstrates an alkaline pretreatment method at room temperature to reduce the energy usage of the process. This study found that the following pretreatment conditions: NaOH concentration of 2.5 weight%, treatment time of 6 hours, and biomass loading of 10 weight% yielded the greatest glucose recovery from rice straw. The enzymatic hydrolysis yielded ~75% glucose recovery and ~60% xylose recovery from the rice straw treated at the optimal conditions. The findings from this study are important because the results are a step towards providing low energy alternatives to the high temperature treatments and making a cost-effective method for industrial use.

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I. INTRODUCTION

In recent years there has been a desire to move towards renewable sources of energy with lesser greenhouse gas emissions such as bioenergy, for example ExxonMobil's research of algae[1]. Fossil fuels make up a large aspect of the economy being cheaper than biofuels, but they are also non-renewable. The non-renewable nature of fossil fuels will lead to problems, especially in industries such as fuel and polymers. This shift towards biofuels and biomaterials are on those formed from lignocellulosic biomass, such as crop residue so as not to reduce the food production capabilities[1]. However, the biomass used in research and production varies based on the specific feed source, from corn stover to wood chips to rice straw, all of which can vary in the compositional amounts of contained polymers. With the large variety of biomass, there have been problems developing solutions to access the sugars for all feed sources, due to problems that can arise from attempting to treat on type of biomass[2]. Therefore, bioenergy and biomass research create opportunities for different research groups to develop methods for pretreating the varying specific biomass feed sources.

Lignocellulosic biomass contains hexose and pentose sugars, which are precursors for fuels, chemicals, and plastics[3]. Lignocellulosic biomass consists primarily of three types of natural polymers[4]. Cellulose is the most abundant polymer within lignocellulosic biomass[5]. Proper pretreatment helps to disrupt the crystallinity of the cellulose, which allows for improved conversion of polymers such as glucan to monomers such as glucose. The second most common polymer in lignocellulosic biomass is hemicellulose. Hemicellulose consists of pentoses and hexoses such as xylose and arabinose[4, 6]. The third most prevalent natural polymer within lignocellulosic biomass is lignin. Lignin is an amorphous polymer that acts as the glue for binding together the lignocellulosic biomass[4, 7]. The cellulose, hemicellulose, and lignin contents for biomass generally varies from 40 – 60 weight%, 20 – 40 weight%, and 10 – 25 weight% on a dry basis[8]. These three components glue together tightly with hydrogen bonding to protect plants from insects and diseases, which in turn slows down their upgrading. Therefore, treatment is needed to disrupt the hydrogen bonding and release sugars from cellulose and hemicellulose more easily[9].

There are many forms of pretreatment such as physical pretreatment, chemical pretreatment and biological pretreatment[4, 10]. Physical pretreatment is a method in which the size of biomass is reduced via operations such as shredding, grinding, and milling[7, 11]. By reducing the particle size, this pretreatment focuses on increasing the surface area to improve access to the desired components of the material. However, problems arise from this method because it is highly energy intensive, making the search for another option desirable[4, 7, 12]. Another method of pretreatment is biological, where fungi are used to produce enzymes to break down lignin and other lignocellulosic material[11]. Unfortunately, the process becomes too slow for what is needed for industrial use, for example in a biological treatment 35% of the straw was converted to reducing sugars in five weeks by a specific fungi[11], so biological pretreatment alone is not currently being considered as the solution to improving sugar extraction from biomass. Chemical pretreatment is the last major form of pretreatment in which various chemicals are used to disrupt the lignocellulosic structure, meaning that lignin is removed and since lignin acts like a glue there becomes more access to the cellulose and hemicellulose. The various chemicals used include acids, alkali, organic solvents and ionic liquids[1]. The purpose of these chemicals is primarily to disrupt the lignin structure and allow improved access to the carbohydrates and reduction of cellulose crystallinity[11-12]. The importance of the reduction of cellulose crystallinity comes with the processing during enzymatic hydrolysis because the enzymes are more easily able to convert the amorphous portion of cellulose.

Table 1 provides results from some of the research that has been done and shared within the scientific community. The research from Table 1 focused on the same type of biomass as used in this paper: rice straw. Sodium hydroxide, NaOH, is a common method of pretreatment shared by many papers either alone or in combination with other pretreatment methods. The research previously done on rice straw pretreatment helped plan the process and optimization done in the lab for this study. Along with the benefit of paving the path to the current work, the completed works also provide comparable results, which can show how the current experimentation lines up with previous experiments.

NaOH being an alkaline chemical is important because alkaline chemicals are effective on biomass with lower lignin content, such as rice straw1, 12. The usage of NaOH as a pretreatment method is working towards making a viable industrial pretreatment.

TABLE 1

VARIOUS STUDIES ON RICE STRAW PRETREATMENT AND THEIR RESULT

Pretreatment Type	**Result**
NaOH followed by Hydrothermal [13]	NaOH had 132% biogas yield and NaOH + Hydrothermal had 225.6% biogas yield compared to untreated rice straw biogas yield.
$Ca(OH)_2$/NaOH [14]	Alkaline Loading and reaction time improved delignification, but only loading improved enzymatic hydrolysis for the conditions
Aqueous Ammonia and Dilute Sulfuric Acid [15]	Approximately 97% of cellulose was digested during enzymatic hydrolysis
Foraceline (DES) and Sodium Carbonate [16]	Highest Total Sugar of 42.76 g/L, Butanol Yield of 0.25 g/g(sugar), Productivity of 0.13 g/L h
Sodium cumene sulfonate [17]	Removal of 50% of lignin from rice straw at 5% biomass loading, 121°C for 1 hour
0.5% Sulfuric Acid [18]	Reducing Sugar yield of 0.359 g/g. Enzymatic hydrolysis found to increase after dilute acid pretreatment
Dilute Sulfuric Acid and Aqueous Ammonia [19]	Under optimal conditions ~13.91 g/L (87.24% of theoretical) fermentable glucose was recovered
Dilute Acid [20]	0.35 weight% acid at 162°C and 0.65 weight% acid at 152°C give almost equal sugar recoveries of ~65% from rice straw
Aqueous Ammonia [21]	Enzymatic digestibility of pretreated rice straw was 85% at 60°C and 1 atm.
1% Sulfuric Acid followed by 1.25% NaOH [22]	After both processes a solid fraction contained 72% glucan
Lime ($Ca(OH)_2$) [23]	Optimum pretreatment for rice straw for anaerobic digestion by alkaline disintegration was 225.3 mL/g volatile solids with 9.81% Lime (w/w), 5.89 days PT time and 45.12% Inoculum amount.
NaOH [24]	Maximum glucose yield of 254.4 +- 1.2 g/kg biomass under conditions of 2.96% NaOH concentration, 81.79°C and 56.66 minutes.
NaOH [25]	Highest Glucan Yield achieved with conditions of 12% w/v NaOH for 1 hour at 55°C. Highest Total Carbohydrates achieved with conditions of 2% w/v for 3 hours at 55°C.
NaOH/Urea Solution [26]	Average reducing sugar conversion of 80.22% and H_2 generation of 72.5 mL/g pretreated rice straw
Peroxide Ionic Liquid-water mixture [27]	Maximum lignin removal of 63.8%, enzymolysis efficiency of 92.1%. Recycled IL achieved 251.6 g/L reducing sugar, and 91.9 g/L ethanol at high solid loading.
Sulfuric Acid followed by NaOH [28]	Removal of ~90% hemicelluloses and ~55% lignin. Treated rice straw produced 787 mg/g reducing sugars.
NaOH [29]	Lignin removal reached 82.16% at 80°C and 78.15% at 55°C.

The goal of this study was to determine a low energy, alkali pretreatment method that improved the access to the carbohydrates within rice straw. Operating at room temperature was chosen to reduce the energy input for the pretreatment, which is more favorable in a production environment. This study focused on understanding the effects of the NaOH concentration, treatment time, and the biomass loading during pretreatment. The NaOH concentrations were varied and tested from 1 weight% to 10 weight%, the time was varied and tested from 0.5 hour to 6 hours, and finally the biomass loadings were varied and tested from 1 weight% to 10 weight%. Once pretreatment was concluded the samples went through a solid analysis (or carbohydrate test) and then an enzymatic hydrolysis to identify the amount of glucan that can be extracted from the treated biomass. This work will provide insight into using rice straw in industry using a low energy pretreatment method which could be translated to other types of biomasses.

II. METHODS

A. Process

1. NaOH Treatment of Rice Straw

The pretreatment of rice straw was conducted using the NaOH solution at room temperature. Initially, ~1 gram of rice straw (~5 weight% moisture) was mixed with 20 milliliter of NaOH solution in a 250-milliliter plastic beaker with a magnetic stir bar at 600 rpm for 1 hour. After 1h, the slurry was filtered using Whatman filter paper, Grade 1. The treated solid was washed with ~100 milliliter of DI water on the filter paper. The resulting treated solids were stored in 50 milliliter centrifuge tubes and set to dry in an oven at 60°C overnight. The resulting dry treated solids were used in compositional analysis, enzymatic hydrolysis, and characterization.

To evaluate the effectiveness of this treatment of rice straw, the treatment conditions were varied using NaOH concentration of 1-10 weight%, treatment time between 0.5-6.0h, and solid loading from 1-10 weight%.

2. Enzymatic Hydrolysis of Treated Rice Straw

The treated solid was suspended in solution to obtain 10 grams of glucan per liter. The solution was comprised of a 1 Molar citric acid buffer (pH 4.5) along with 0.1% (weight/volume) NaN_3 (to prevent growth of microbes) and water in a 50-

milliliter centrifuge tube. The enzyme loading for the hydrolysis was 15 mg protein per gram of glucan at 9:1 (volume/volume) Ctec 2: Htec 2. This cocktail (pretreated samples, buffer solution and enzyme) was suspended in an incubator shaker at 50°C and 600 rpm; meanwhile samples were taken at intervals over a 72-hour period. Each time a sample was collected 1 milliliter of Deionized H_2O and 0.1 milliliter of the cocktail were added to a 2 milliliters microcentrifuge tube. The samples were centrifuged at 13,400 rpm for 3 minutes to separate clear solution from the suspended solids. The centrifuged solution was then transferred to 1.5 milliliters HPLC tubes and processed with an 1110 Agilent high-pressure liquid chromatography system (HPLC, Agilent Technologies, Santa Clara, CA, U.S.A)[30].

Enzymatic glucan digestibility was calculated in Equation 1:

$$\text{glucan digestibility}(\%) = \frac{\text{glucan}_{\text{hydrolysate}}}{\text{glucan}_{\text{pretreated solid}}} \times 100 \qquad (1)$$

After 72 hours of the enzymatic hydrolysis, the residual solid was washed with deionized water, dried overnight, and stored for future use.

3. Product Analysis

Compositional analysis of untreated and pretreated rice straw samples and residual enzymatic hydrolysis solid samples were determined by the National Renewable Energy Laboratory analytical procedures[31]. Initially, 0.1 gram of sample was dissolved in 2 milliliters of 72% H_2SO_4 at 30^{O}C for 1 hour in an incubator shaker prior to dilution of the H_2SO_4 concentration to 4% with deionized water. The sample was autoclaved at 121°C, 15 psi for 1 hour and then filtered by a ceramic filter crucible. The filtrate was analyzed by HPLC for soluble sugars. Residual solids were dried at 80°C overnight. The dried solids mass corresponded to the amount of lignin and ash in the sample. The dried sample mass loss and residual mass after calcination at 575°C for 8 hours corresponded to the amount of lignin and ash respectively.

Soluble sugars were analyzed by HPLC equipped with a refractive index detector (RID) and a diode array detector (DAD). The Aminex HPX-87H column[32] (300 x 7.8 mm, Bio-Rad®, Hercules, CA, USA) separated sugars at 60°C with 0.6 milliliters/minute of 4 millimolar H_2SO_4 used as the mobile phase. The concentrations for the sugars were determined from the RID signals' peak heights. All sugars were calibrated against standards. The rice straw used for the study had a composition of the following: 30.3 weight% glucan, 15.6 weight% xylan, 1.1 weight% arabinan, 21.5 weight% lignin, 13.2 weight% ash, 1.4 weight% acetyl content and 16.9 weight% others (proteins and extractives).

To determine crystallinity, X-ray diffraction was performed on the pretreated rice straw samples using the Bruker D8[33] (Billerica, MA, USA) with CuK_α radiation (λ = 0.15418 Å). The scanning rate was 0.5 seconds/step (0.02 step increment), ranging from 10° to 45°. The change in the degree of crystallinity of biomass was expressed in terms of the crystallinity index (CrI). The CrI value was calculated based on the Segal method[34] using the relationship between the height of the crystalline peak corresponding to (002) lattice plane (I_{002}) and the amorphous region (I_{am}), and were used to solve for the CrI as shown in Equation Two:

$$CrI = \frac{I_{(002)} - I_{am}}{I_{(002)}} \times 100 \quad (2)$$

The changes in the chemical structure of pretreated rice straw relative to untreated samples (bond strength between sugar monomers and lignin-carbohydrates) were characterized by the JASCO 4700 FT-IR Spectrometer[35] (Akron, OH, USA) equipped with Attenuated Total Reflection[36] (ATR, Pike Technologies, Madison, WI, USA). The samples were scanned in the spectral range between 400 and 4000 cm^{-1} for 256 scans at 4 cm^{-1} resolution. The lateral order index (LOI) and total crystallinity index (TCI) were calculated by the intensity ratio of 1423/897 (A_{1423}/A_{897})[37] and 1372/2900 (A_{1372}/A_{2900})[37-38], respectively. The crystalline cellulose bands can be observed at 1423 and 1372 cm^{-1}, and the band at 897 cm^{-1} is associated with amorphous cellulose. The band at 2900 cm^{-1} represents the C-H and CH_2 stretching of the cellulose. These values will be used to represent the proportion of crystalline cellulose (TCI), and the overall degree of crystallinity of cellulose (LOI).

III. RESULTS

To evaluate the changes in composition of the treated rice straw due to NaOH concentrations, a solid analysis was performed on treated solids. As a control, the untreated rice straw sample had a low glucan and xylan compositions, while lignin and other components contributed more to the overall composition. After treatment with NaOH, there was an enhanced glucan and xylan contribution to composition yielding a combined composition of 70%, regardless of the NaOH concentration. There was an observation that the peak for the glucan and xylan composition occurred at 2.5 weight% NaOH concentration. An increase in NaOH concentration higher than 2.5 weight% led to a decrease in the combined glucan and xylan composition for the treated rice straw samples. For these reasons, 2.5 weight% NaOH concentration was applied for the rest of the studies.

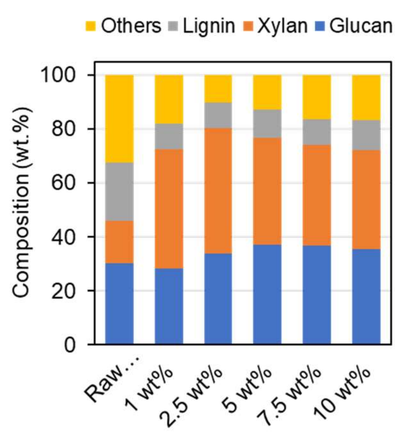

FIGURE 1 - Composition of rice straw after pretreatment by varying the NaOH concentration

To evaluate the effectiveness of the rice straw treatment at various NaOH concentrations, we performed enzymatic hydrolysis of the treated solids. As a control, our untreated rice straw sample had a low glucose yield of 20%. After treatment with NaOH, glucose yield was enhanced with yield improvement of more than 2-fold, regardless of the NaOH concentration. There was an observed NaOH concentration that facilitated the glucose release. An increase in NaOH concentration higher than 2.5 weight% yield nearly 100% glucose yield after 72 hours. Moreover, the NaOH concentration between 5-10 weight% showed similar hydrolysis performance. For these reasons, the 2.5 weight% NaOH concentration was applied for the rest of the studies.

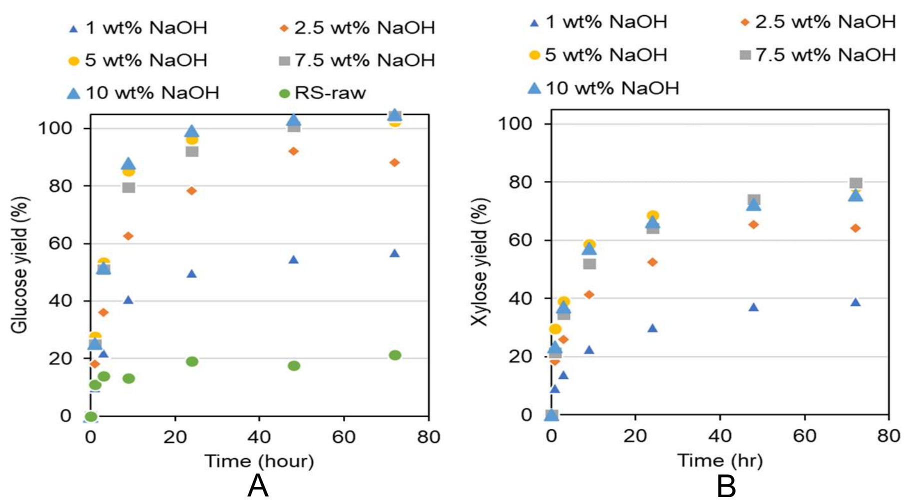

FIGURE 2 - Glucose yield (A) and xylose yield (B) after enzymatic hydrolysis of pretreated rice straw at varied NaOH concentrations

To evaluate the changes in the composition of the rice straw due to treatment time, a composition analysis was conducted. As a control, the untreated rice straw had low glucan and xylan compositions with a contribution of just over 40 weight%

of the sample. After treatment with NaOH, enhanced compositions for glucan and xylan were achieved ranging from 70 weight% to 80 weight% composition contributions, regardless of the treatment time. We observed a peak for the combined composition of glucan and xylan at 4 hours. The glucan/xylan composition was similar for treatment times from 2 hours to 6 hours but were all greater than the 0.5-hour treatment. The results from the treatment time compositions in coordination with the enzymatic hydrolysis determined the optimal treatment time.

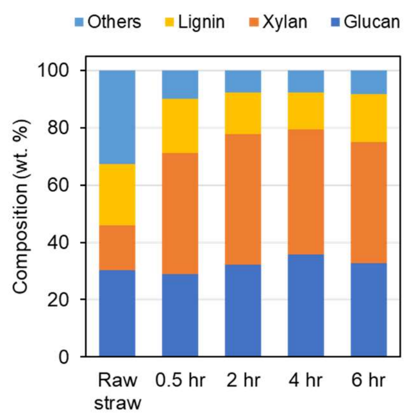

FIGURE 3 - Composition of rice straw after pretreatment varying treatment time

To evaluate the effect of treatment time, an enzymatic hydrolysis was performed on the pretreated solids. As a control, our untreated rice straw had a low glucose yield of 20%. After treatment, all rice straw samples showed glucose yield improvements of greater than two-fold, regardless of treatment time. Yet, the treatment time showed incremental improvement with increased treatment time with the 6 hours pretreatment achieving greater than 90% glucose yield after 72 hours. Xylose yields showed similar improvements in yield based on length of treatment time with the 6 hours pretreatment achieving greater than 80% xylose yield after 72 hours. From the results seen in Figures 3 and 4, the 6-hour treatment time was applied to the rest of the study.

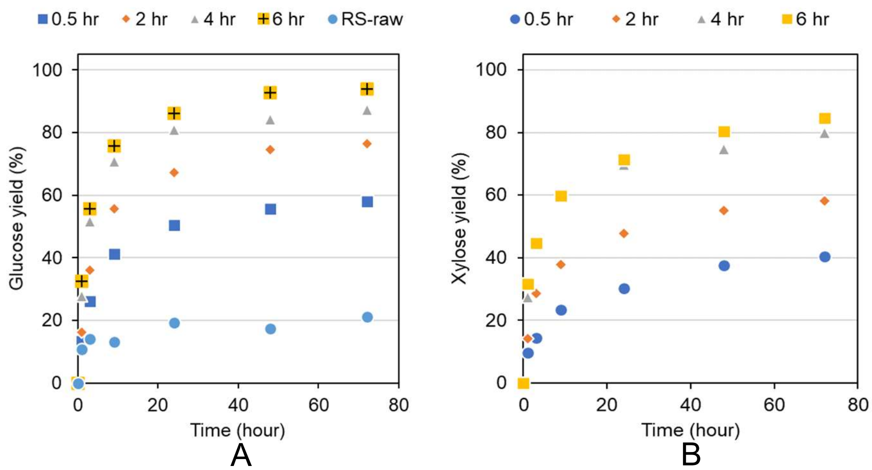

FIGURE 4 - Glucose yield (A) and xylose yield (B) after enzymatic hydrolysis of pretreated rice straw at varied treatment times.

To evaluate the effect of the solid loading on the composition of treated solids, a compositional analysis was performed on the treated solids. As a control, the untreated rice straw had low glucan and xylan compositions with a contribution of just over 40 weight% of the sample. After treatment with NaOH, improvements in the glucan and xylan composition contribution were seen in varying levels of improvement based on the solid loading. The peak of composition for glucan and xylan were found to be at 10 weight% solid loading of rice straw. It was seen that an increase in solid loading greater than 10 weight% yielded lower improvements to the glucan and xylan composition contribution to the sample.

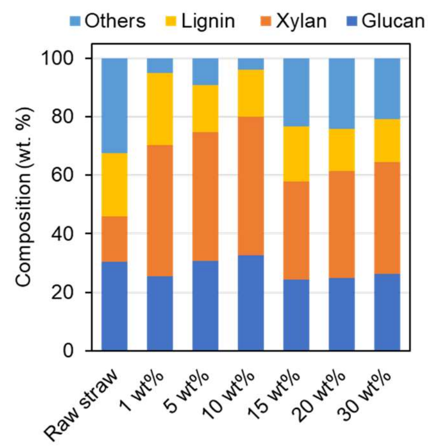

FIGURE 5 - Composition of rice straw after pretreatment varying solid loading

To evaluate the effect of solid loading on the pretreatment efficiency, an enzymatic hydrolysis was performed on the treated solids. As a control, our untreated rice straw had a low glucose yield of 20%. After treatment, all treated rice straw samples were showing improvements in glucose yields. At much higher solid loadings, 20 weight% and 30 weight%, only had minor improvements, whereas the lower and moderate solid loadings had two-fold improvements in glucose yield compared to the control. For the greatest glucose yield the 1 weight% through 10 weight% solid loading performed comparably. Based on the yield data being favorable, along with the composition data from Figure 5 showing that 10 weight% had a greater fraction of glucan and xylan compared to the other treated samples. Finally, 10 weight% loading of rice straw was chosen as the optimal solid loading condition.

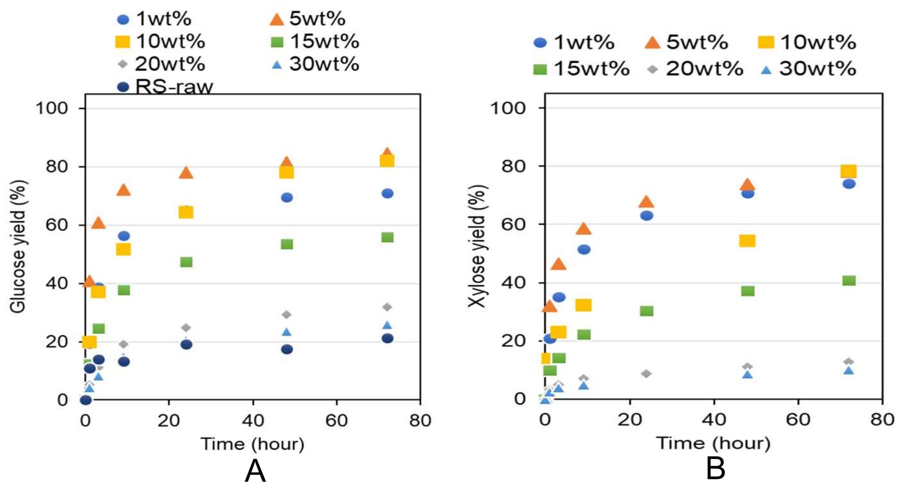

FIGURE 6 - Glucose yield (A) and xylose yield (B) after enzymatic hydrolysis of pretreated rice straw at varied solid loadings.

To evaluate the crystallinity index, an X-ray diffraction was performed on the pretreated solids. As a control the untreated solid was measured as well and for all tests had overall a much lower crystallinity index. In the varied NaOH concentrations (Figure 7A) it is seen that there are two slopes for the variation of the crystallinity index the steeper of which goes from the raw rice straw to 2.5 weight% and the shallower is from 2.5 weight% to 10 weight%. For the time varied crystallinity index (Figure 7B), it is seen that 1-hour sample has the lowest of the pretreated samples and from 2 hours to 6 hours the crystallinity trends down to lower values, yet greater than at 1 hour. For the biomass loading varied crystallinity index (Figure 7C) the trend of the treated solids decreases from 1 weight% to 10 weight% and appears to oscillate from 10 weight% to 30 weight%. The oscillatory nature shows that the data should be rerun as the trend should show a more continual decrease instead of one going up and down again. The optimal conditions as chosen from composition and yield data are seen in the data for crystallinity index to have unique characteristics.

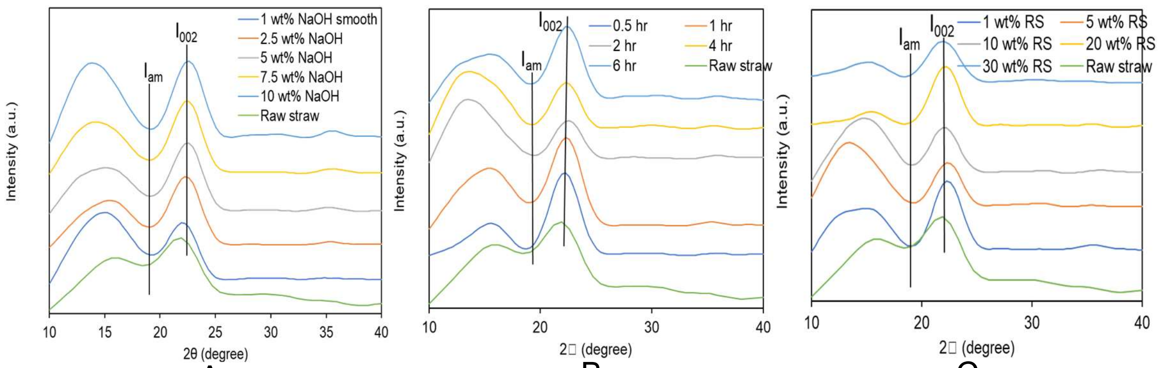

FIGURE 7 - XRD data plotted showing the intensity over the change in angle for the different pretreatment trials of NaOH concentration (A), Time (B), and Biomass Loading (C).

To evaluate the lateral order index (LOI) and the total crystallinity index (TCI), an FT-IR analysis was performed on the pretreated solids. For the NaOH Concentration (Figure 8A), the 2.5 weight% concentration has the greatest LOI, whereas the 5 weight% concentration has the greatest TCI. For the time varied pretreatment (Figure 8B), the LOI is significantly greater than all other values at 0.5 hour, and the next greatest occurs at 6 hours. The TCI of the time varied pretreatment shows a slow, yet steady increase to 6 hours. For the biomass loading (Figure 8C), the LOI and TCI seem to hold a more similar trend in which the 10 weight% loading is the largest and the 1 weight% is greater leaving the 5 weight% value as the minimum value for both LOI and TCI. These were trends gathered from the pretreated solids and observing them allowed for observations in the change in the number of bonds which can reinforce that there was a removal of lignin from the treated samples.

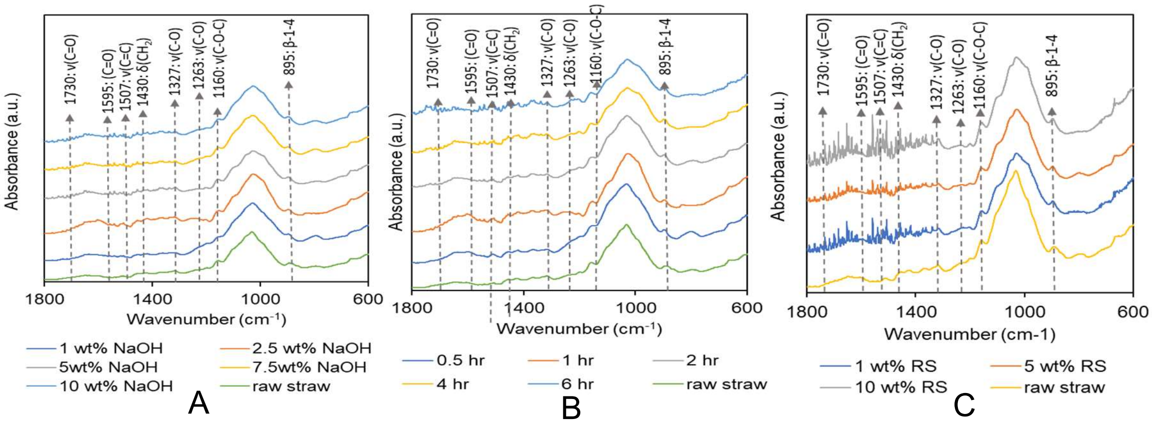

FIGURE 8 - The FT/IR data collected showing the absorbances at varying wavelengths for pretreated samples NaOH Concentration (A), Time (B), and Biomass Loading (C).[39]

IV. DISCUSSION

The lab results from experimentation and optimization of biomass processing can provide benefits to industry by providing a low energy, room temperature, alkaline pretreatment for rice straw. Additionally, the optimal conditions for NaOH, treatment time, and biomass loading were found. The optimal condition for NaOH concentration was found to be 2.5 weight%. At this point it was found that Glucose and Xylose conversions were 90% and 65% conversion. Where NaOH concentrations greater than 2.5 weight% were at the maximum conversion, which only varied from the conversion at 2.5 weight% by about 10%. The optimal treatment time was found to be 6 hours as it yielded the greatest glucose and xylose concentrations when compared to the other treatment times. Biomass loading was shown to be optimal at 10 weight% where most of the glucose and xylose were converted. In addition, more glucose and xylose combined were present in the treated solids at 10 weight% biomass loading than in the other biomass loading treated samples. The optimal process was found through the testing of each variable and compounding the optimal conditions.

The first optimization step that was tested within the experiment was the concentration of NaOH. The testing consisted of using varied concentrations of NaOH for treating the rice straw. This resulted in the new fractional amounts of components within the rice straw as shown in Figure 1. A conclusion drawn from Figure 1 was that the pretreatment was able to remove lignin and other components (like silica) from the rice straw. Part of this study identified that the concentration of the NaOH influenced how much of a decrease in lignin was detected. Overall, the fractional amounts of glucan and xylan were greater for all pretreated rice straw samples than the raw rice straw. Furthermore, the 2.5 weight% NaOH concentration sample had the greatest combined glucan and xylan fraction. It was shown that following the pretreatment with a wash step led to the removal of lignin, but at higher concentrations of NaOH the wash step may have had an unintended effect of washing away some of the desired glucan/xylan.

Figure 2 shows that the conversion of the glucan was ~100% for concentrations of 5 weight% and greater, while 2.5 weight% achieved ~90% compared to only ~20% for the raw rice straw. This similar trend is noted for xylan conversion as well which was ~80% for concentrations of 5 weight% or greater and about 65% for 2.5 weight%. Although the conversions were slightly lower for 2.5 weight% NaOH it was a better choice since the time scale was constant for this optimization step and on the lower side of the range to be tested for the next optimization step. Furthermore, in using a 2.5 weight% solution of NaOH a more dilute chemical will lead to cost savings when preparing industrial scale pretreatments. Further cost savings are obtained beyond the chemical usage as the room temperature operation will also reduce energy costs.

Similar research to this step of the optimization was done in papers by Kim[24] and Harun[25]. In the research done by Kim, an optimization was done with respect to NaOH concentration, temperature, and time. There are some similarities in results as their testing from 1.0% to 4.0% NaOH concentration resulted in an optimal concentration of 2.96%[24]. The best concentration they found from experimentation being 2.96% is very similar to the 2.5 weight% found in lab for this paper. Although, temperatures used in the paper by Kim are higher than those used in this paper as the temperatures varied from 60°C to 100°C, the insight it provides is beneficial in reinforcing the values found in this paper. Another optimization was done by Harun, and they studied NaOH concentration and time, but at a temperature of 55°C. Their experimentation found that 12% weight/volume was the optimum concentration for their highest delignification, while a 2% weight/volume was found to be the optimum condition for total carbohydrate content (glucan, xylan, etc.)[25]. These results from outside research showed similar relationships for NaOH concentrations as found during experimentation.

The second optimization step focused on the treatment time of the rice straw. The treatment time focused on how long the rice straw was placed within the NaOH solution and mixed. The treatment time had an influence on the relative composition of the rice straw as observed in Figure 3; therefore, showing that regardless of how long the rice straw was treated, there was a significant reduction in lignin and other components from the overall composition. The 0.5-hour sample showed the smallest reduction in lignin and other components, whereas the 4-hour treatment appears to show the greatest reduction and yields the greatest relative glucan and xylan. For the optimization, the 6-hour sample was chosen based on the results from the enzymatic hydrolysis.

The treatment time enzymatic hydrolysis results are found in Figure 4. These plots show the conversion of the glucan and xylan over a 72-hour period, and it is seen that even the lowest performing time, 0.5 hour, has greater than twice the conversion of the raw rice straw. The conversion of glucan peaked at ~90% for the 6-hour sample, followed by ~85% at the 4-hour sample. The xylan conversion was ~85% for the 6-hour sample, and ~80% for the 4-hour sample. The conversions for the glucan and xylan were similar at the treatment times of 4 hours and 6 hours. The optimum treatment time was determined to be 6 hours due to the increased conversion achieved from the enzymatic hydrolysis.

Similar research to this step of the optimization was done in papers by Kim[24] and Harun[25]. In the research by Kim, an optimization based on NaOH concentration, temperature, and time was done. The time that they found to be optimal for their experimentation was 56.66 minutes while testing from 30 minutes to 90 minutes, and they were also testing at a higher temperature (between 60°C and 100°C)[24]. The study conducted by Harun had a focus on time as well in their optimization experimentation with times of 1 hour and 3 hours. The study found that for improved delignification a treatment time of 1 hour along with the high NaOH concentration was optimal, whereas for a treatment time of 3 hours with a low NaOH concentration achieved higher levels of glucan and xylan[25]. The research by Harun also was at 55°C which is closer to the room temperature (25°C) used in this current paper, therefore it helps reinforce that a longer treatment time at lower temperatures will yield similar results to those done at higher temperatures for shorter time periods.

The third optimization step focused on the biomass loading of the treatment, how much rice straw was placed in the NaOH solution. The amount used for the first two optimization steps was 5 weight%. The biomass loading was tested at varied amounts in this step to see the limit of what could be loaded in the same amount of fluid before resulting in diminished returns. The diminished returns is referring to the amount of rice straw fed in where there is roughly no improvement or even a decrease in the results achieved. Figure 5 shows the results of the pretreatment and what relative composition was present. The figure shows that there is an immediate increase in glucan/xylan combined relative contribution to the composition after pretreatment compared to the raw rice straw. After 10 weight% it is seen that there are diminishing returns for the increase in biomass loading immediately. Therefore, 10 weight% which had the greatest combined glucan/xylan contribution to its composition was the desirable option for the optimization. For after 10 weight% the composition seems to have a lesser effect from the treatment as the lignin and other categories for composition are much greater than the 10 weight%.

The results of the biomass loading enzymatic hydrolysis are found in Figure 6, where the glucan and xylan conversions are plotted. These allow for the comparisons between the varied biomass loadings to be compared such as the conversion of 20 weight% and 30 weight% are comparable to the raw rice straw, 15 weight% being a moderate improvement in conversion, and the greater conversion values are found in the 1 weight% to 10 weight%. A point of note is that the 10 weight% sample lost some excess fluid between 24 hours and 48 hours leading to the large jump in conversion. If the 10 weight% sample were to be rerun, the values of glucan conversion would likely have been at ~80% for 1 weight%, ~75% for 10 weight% and ~65% for 5 weight%. Furthermore, the xylan conversions would be ~75% for 5 weight%, ~70% at 1 weight% and ~55% at 10 weight%. Due to the greater presence of glucan and xylan within the 10 weight% biomass loading sample, combined with the conversion (even though slightly less than max) led to choosing 10 weight% biomass loading as the optimum loading.

In a research paper by Dong[26], a study was done on an NaOH/Urea pretreatment with a focus on using higher solid loadings of rice straw to improve hydrogen production. In the study, a focus was on the amount of rice straw that was pretreated in the NaOH/Urea solution, which varied from 10% weight/volume to 200% weight/volume. The study shows that increasing the loading of rice straw provides yields that increase to a maximum before the yields begins to decrease. This study seems to show similar results, albeit on a different scale, to what was observed within the study done in this paper. The trend from the research Dong did was seen in the experimentation completed for this paper as well. Once the rice straw loading exceeded 10 weight% the yield and compositional changes were less effective than 10 weight% and less.

Data collected from the XRD shown in Figure 7 allowed for the calculation of the crystallinity. This information is important because the crystallinity of the sample helps indicate that lignin is removed. The disruption of the lignin which is acting like a bonding agent to keep the glucan and other carbohydrates contained within the biomass was observed. Therefore, due to lignin's amorphous nature and the major carbohydrates such as glucan and xylan are crystalline in nature, the crystallinity of the sample becomes an indicator of how well lignin was disrupted and allows access to the carbohydrates. From Figure 7, the raw rice straw is shown to have the lowest crystallinity indicating that there is more amorphous material including the lignin which is desired to be removed. But with pretreatment there is an increase in crystallinity, such as treatment with 2.5 weight% NaOH the crystallinity doubles from the raw rice straw crystallinity.

From the NaOH concentration in Figure 7A, there are two observed slopes: the first is the major change in which most of the amorphous material is removed/dissociated, then the second slope is a slight increase with increasing NaOH concentration. The concentration that was chosen happens to be at the change of slope point showing that enough of the lignin and amorphous material is removed to allow access to the carbohydrates. Figure 7B shows that the difference between the 002-plane height and the amorphous region height increases from untreated to treated sample. Also the trend seems to show that the difference in the heights decreases as treatment time increases, but that changes with an increase at the 6 hour sample indicating that the XRD scans should be run again in case there was an erroneous measurement. Figure 7C shows the biomass loading versus intensity and the trend shows an immediate increase from the raw condition to the 1 weight%, but a slow decline to 10 weight%. The values after 10 weight% are not useful due to the poor conversions achieved by the higher biomass loading and the appear that they need to be run again like some of the samples in Figure 7B.

Figure 8 shows peaks that allow for the calculation of the Total Crystallinity Index (TCI) and Lateral Order Index (LOI) for the different samples across the optimization steps. Total Crystallinity Index is used for identifying how crystalline the cellulose is within the sample. Cellulose crystallinity becomes useful when samples are used for enzymatic hydrolysis because the enzymes used like to latch on and convert amorphous cellulose more than crystalline cellulose. From the values found, which show the ratio of crystalline to amorphous, there is more amorphous cellulose which is easily converted.

The LOI is an empirical system that also is used for noting the crystallinity of samples that were measured with the FTIR for the experimentation. The LOI is a ratio just like the TCI which shows that the relationship between the LOI and TCI that were plotted should be slightly similar as noted within the appendix. The first optimization step had the greatest LOI value at the optimal NaOH concentration and like with the TCI the greatest LOI values were at the optimal conditions. The second optimization step had a similar trend between TCI and LOI with a general increase as the time increased. The trend holds true when the 0.5-hour point is treated as an outlier. For the biomass loading optimization, a trend is seen like that with the treatment time in which there is a general increase for LOI and TCI with increased loading of rice straw. Figure 8 shows that for both TCI and LOI a greater crystallinity ratio correlates to optimal results for rice straw pretreatment conditions.

V. CONCLUSION

The research and experimentation done for this study was able to provide a step in forming a low energy alkaline treatment process for rice straw. The optimization steps completed for the experiment helped examine how different factors of the process affected the sugar output for the enzymatic hydrolysis. This study helped to address the potential for a low temperature pretreatment opportunity. The optimal conditions for the pretreatment were found to be a NaOH concentration of 2.5 weight%, a treatment time of 6 hours, a biomass loading of 10 weight%, and a temperature of 25°C.

A question to pose for future work is whether it will be possible to apply this sort of pretreatment to more forms of biomass than just rice straw. Another question to pose for future work would be is there a way to maintain the conversion of sugars achieved, but with a shorter treatment time. Furthering the research would involve finding a way to use the pretreatment conditions but develop a method that doesn't rely on a wash step after pretreatment. A potential solution to remove a washing step would be to attempt correction of the pH using a buffer.

This research is beneficial to the field of biomass science and pretreatment because it is providing the ability to pretreat biomass without using large quantities of harsh chemicals and with lesser energy usage, due to the process occurring at room temperature. The pretreatment method used in the study could help move toward the cost-effective industrial applications of biomass to allow entrance of bio-based fuels and fibers into consumer markets.

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APPENDIX I. CRYSTALLINITY INDEX

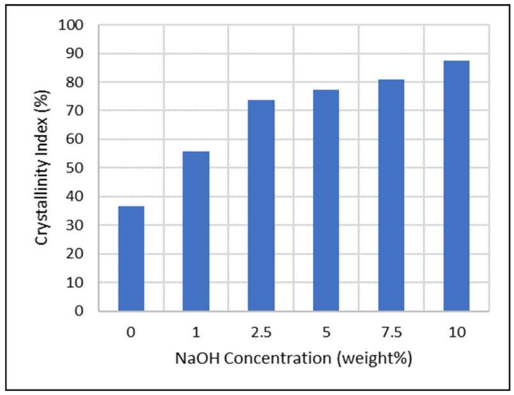

FIGURE 9 – Calculated crystallinity index for treated rice straw at different NaOH concentrations

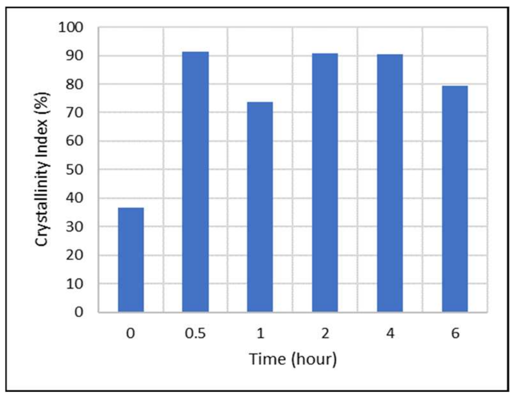

FIGURE 10 – Calculated crystallinity index for treated rice straw at different treatment times.

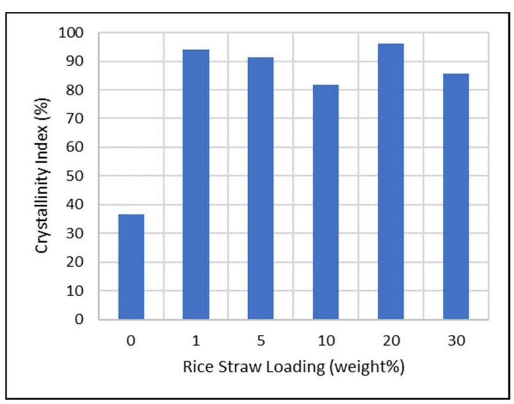

FIGURE 11 – Calculated crystallinity index for treated rice straw at different rice straw loadings (biomass loading)

APPENDIX II. LOI & TCI

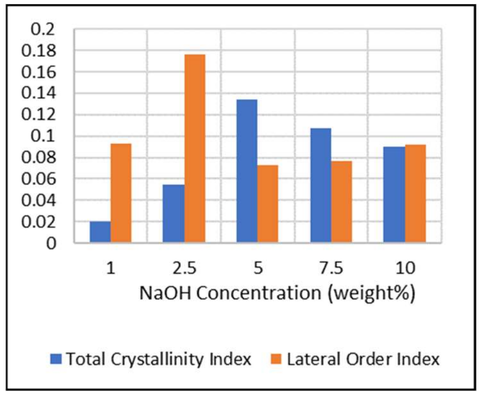

FIGURE 12 – Calculated LOI and TCI for treated rice straw at varied NaOH concentrations

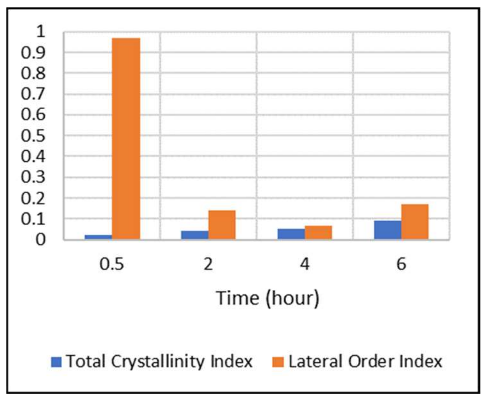

FIGURE 13 – Calculated LOI and TCI for treated rice straw at varied treatment times

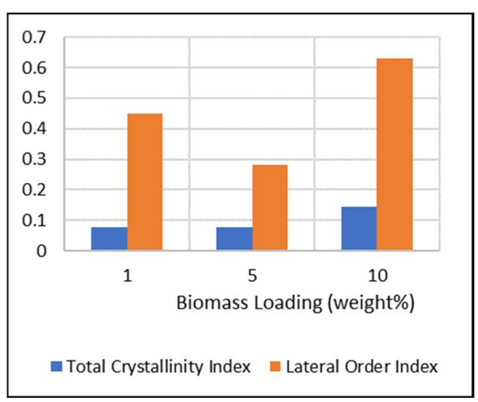

FIGURE 14 – Calculated LOI and TCI for treated rice straw at varied biomass loadings