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Corn Stover Pretreatment with Na_2CO_3 Solution from Absorption of Recovered CO_2

Valerie García-Negrón  and Matthew J. Toht

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,
600 East Mermaid Lane, Wyndmoor, PA 19038, USA

* Correspondence: valerie.garcianegron@usda.gov

Abstract: Renewable resources such as lignocellulosic biomass are effective at producing fermentable sugars during enzymatic hydrolysis when pretreated. Optimizing pretreatment methods for delignification while maintaining sustainability and low processing costs requires innovative strategies such as reusing greenhouse gas emissions for materials processing. Corn stover, an agricultural waste residue, was pretreated with 2.2 M Na_2CO_3 produced from CO_2 captured via absorption in a 5 M NaOH solution. Composition analysis of the pretreated corn stover exhibited higher cellulose content (40.96%) and less lignin (16.50%) than the untreated biomass. Changes in the chemical structures are visible in the FTIR-ATR spectra, particularly in the cellulose and lignin-related absorption bands. The sugar release from hydrolysis was evaluated at different time intervals and by varying two enzyme ratios of CTec2-to-HTec2 (2:1 and 3:1). Enzymatic hydrolysis produced higher and more stable glucose yields for the pretreated biomass, surpassing 90% after 24 h using the 3:1 enzyme ratio. Sugar concentrations notably increased after pretreatment and even more when using the cellulase-rich enzyme solution. The maximum glucose, xylose, and arabinose recovered were 44, 19, and 2.3 g L^{-1} . These results demonstrate the viability of capturing CO_2 and converting it into an efficient Na_2CO_3 pretreatment for corn stover biomass. Additional processing optimizations depend on the combination of physicochemical parameters selected.



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1. Introduction

The high dependence on fossil fuels as a primary source for energy generation, primarily for transportation and electric grids, and their environmental impact has led U.S. initiatives to search for efficient renewable solutions in fuel production [1–4]. Moreover, the increased output of greenhouse gas emissions to the atmosphere and air pollution exacerbate current environmental issues. Particularly, carbon dioxide (CO_2) which is the main greenhouse gas and one of the principal waste products released from refineries and large industries [5–7]. Alternative methods and technologies are rapidly adapting and emerging to reduce and mitigate the environmental concerns, for example, capturing CO_2 emissions to transform them into reusable products [8–10].

Current energy demands and societal calls for green energy sources have spurred research on biofuels, which have become attractive due to the worldwide availability of lignocellulosic biomass as an agricultural by-product [11–13]. Ethanol is the most common and techno-economically viable biofuel, mainly produced via fermentation of corn, sugar cane, switchgrass, and sweet sorghum [14–17]. Fermentation efficiency depends on the response of yeast strains to processing conditions and enzymatic digestibility during hydrolysis [18]. Furthermore, to optimize sugar recovery during hydrolysis, pretreatment methods are employed to modify the chemical structures of the biomass by deconstructing lignin structures and cell walls, thus liberating polysaccharides such as cellulose and

hemicellulose. Common pretreatments for lignocellulosic biomass are: extrusion, alkali, organosolv, ammonia fiber explosion, and dilute acid [19–21].

U.S. is one of the largest bioethanol producers from corn sources due to their high-sugar content, abundance, and affordability [22,23]. Corn stover is an abundant agricultural waste residue obtained from harvesting corn grains (1 kg of stover per 1 kg grain) that consists of up to 70% polysaccharides and 15–20% lignin, capable of producing ethanol yields of 370–470 L ton⁻¹ [24–26]. Many studies have evaluated different pretreatment methods and conditions for corn stover and their impact in enzymatic hydrolysis [27–30]. Particularly, alkaline pretreatments are common because they break down ester bonds, remove acetyl groups, and reduce crystallinity due to cellulose swelling, all of which improve enzymatic saccharification and bioethanol conversion from corn stover [31–33]. Sodium hydroxide (NaOH) dissociation has shown to be effective at improving lignocellulose digestibility in different biomasses where corn stover obtained the highest glucose yields when pretreated at a high temperature of 120 °C [34]. Sodium carbonate Na₂CO₃ has also been used in chemical pretreatments as it is accessible, safe to handle, and inexpensive when compared to NaOH and other chemicals. Additionally, Na₂CO₃ serves as a weak base that promotes delignification, increases surface area, and improves cellulose access [35]. A study reports glucose yields for different pretreated biomasses using Na₂CO₃, where corn stover yields of 95.1% were highest. Also, functional groups measured via FTIR have been categorized [36]. Molaverdi et al. pretreated corn stover with Na₂CO₃ which increased the glucan content from 41.9 to 54.6%, while no significant changes were observed for xylan, and obtained 23.4% and 20.5% for pretreated and untreated biomass, respectively [37]. Another study compared glucose yields from corn stover when varying pretreatment conditions such as Na₂CO₃ concentration, temperature, and reaction time. Results showed a glucose yield of 267.5 g kg⁻¹ of biomass using a Na₂CO₃ concentration of 4.1% at 142.6 °C for 18 min of reaction time [38].

CO₂ is a co-product to ethanol generated during fermentation. Additionally, CO₂ is not only present in fermentation process, it is also readily generated from industrial processes that can serve as an alternative source in addition to the recycled stream. CO₂ is available in processes where organic material is decomposed, in petroleum and fossil fuels, and in food industries such as beer, wine, and sugars. The goal of this work is to evaluate the potential to reuse CO₂ in a circular process where it is captured in the form of Na₂CO₃ and that Na₂CO₃ is used as a reagent of pretreatment of biomass. This mild alkaline pretreatment process allows for cleavage of ester bonds and glycosidic linkages present in the cell wall increasing the effectiveness of enzymatic hydrolysis. Previous work captured CO₂ in NaOH solution from fermentation of sweet sorghum juices to produce Na₂CO₃, and subsequently used it to pretreat sweet sorghum bagasse for bioethanol conversion [39]. In this work we investigate the use of corn stover as a potential agricultural residue pretreated with a solution that contains Na₂CO₃ and NaOH from CO₂ generated during fermentation process and absorbed in a NaOH solution. The study presents the pretreatment of corn stover and its effect on composition and hydrolysis as a function of enzymes' ratio and reaction time.

2. Materials and Methods

2.1. Self-Made Na₂CO₃ Solution

Na₂CO₃ was made in-house by absorption of CO₂ formed as by-product from the fermentation process. The absorption column followed a similar setup as in previous related work [39]. It consisted of a glass column from Ace Glass, Inc. (Vineland, NJ, USA) with 3 inches of inside diameter and 26 inches in height. Stainless steel Intalox saddles of 1 inch were randomly packed in the column. A 5 M NaOH 2 L solution was added to the packed column and recirculated with a peristaltic pump at a flow rate of 100 rpm to maintain a homogeneous solution and prevent precipitation during CO₂ absorption. A heating tape was wrapped around the column and connected to a temperature controller set to 30 °C to maintain a stable operating temperature and also help prevent precipitation.

A 7 L fermenter from Applikon Biotechnology was used as the CO₂ source. A synthetic media solution was prepared by mixing 4.75 L of deionized (DI) water with a concentration of 200 g L⁻¹ of *Cerelose*[®] dextrose from Corn Products CPC International Inc. and 0.4 g L⁻¹ of urea from Mallinckrodt Specialty Chemicals Co., England, UK *Ethanol Red*[®] yeast provided by Lesaffre Yeast Corp. was prepared with DI water at 5 wt% and stirred for 30 min, then it was added to the fermenter. For the CO₂ capture, fermentation took place for 72 h at 32 °C and 250 rpm. The pH in the fermenter was initially set to 5.1 and maintained by adding 2 N NaOH buffer solution via injection when needed.

2.2. Biomass Pretreatment

Corn stover (*Zea mays*) (CS) biomass was obtained from USDA-ARS's Wheat, Sorghum, and Forage Research Unit in Lincoln, NE and milled using a Wiley mill with a 1 mm screen size. Pretreatment was prepared with a solid loading of 5% (*w/v*) dry biomass in 1 L of the self-made Na₂CO₃ solution, then added to a 2.5 L stainless steel reactor. The reactor was placed in a convection oven for 1.5 h at 150 °C. Solids were recovered via vacuum filtration using a *Whatman* paper filter grade 1, water washed to remove the residual alkaline solution, and dried to 55 °C.

Composition analysis was performed to untreated and pretreated corn stover samples following the protocol from the National Renewable Energy Laboratory (NREL) NREL-TP-510-42618. In this procedure, carbohydrate structures are determined from the liquid portion recovered from the acid hydrolysis. The aliquot was centrifuged and filtered to remove any residual solids. Calcium carbonate from Ricca Chemical Company (Arlington, TX, USA) was added to neutralize the aliquot and mixed in a vortex shaker for 10 s. After reaching pH neutrality, 10 µL liquid samples were filtered into 1.5 mL high-performance liquid chromatography (HPLC) vials using a 0.2 µm filter. HPLC runs consisted of duplicate measurements each using a 5 µL injection. Sugar analysis was performed using an *Agilent HPLC Series 1200* instrument with an *Aminex*[®] *HPX-87H* ion exclusion column from Bio-Rad Laboratories (Hercules, CA, USA). The column was operated at 60 °C and 0.5 wt% H₂SO₄ solvent was pumped at a 0.6 mL min⁻¹ flow rate.

2.3. FTIR-ATR

Fourier-transform infrared spectroscopy - attenuated total reflectance (FTIR-ATR) was conducted with the *FT-IR Spectrum 3* spectrometer from PerkinElmer, Inc. sampling in the frequency range of 650 to 4000 cm⁻¹, using 32 scans, and a resolution of 32 cm⁻¹. Prior to conducting the experiment, the untreated and pretreated CS biomass samples were dried overnight at 55 °C. Triplicate spectra scans were collected at room temperature.

2.4. Enzymatic Hydrolysis

Enzymatic hydrolysis was performed to untreated and pretreated CS dried samples. Solid loadings of 5.1 g of dry biomass at a 10% (*w/v*) in a citric acid buffer solution of 50 mM and pH of 5 were added to 250 mL Erlenmeyer flasks. The enzymes used were *Cellic*[®] *CTec2* (cellulase) and *HTec2* (hemicellulase) from Novozymes (Franklinton, NC, USA). Two different ratios of enzymes based on 0.025 mL g⁻¹ dry biomass were studied: 3:1 and 2:1 (*CTec2:HTec2*). The hydrolysis took place in a shaker incubator at 50 °C at 160 rpm. Each flask was sealed with a stopper and parafilm to prevent water loss. Hydrolyzed samples were collected at multiple duration (0.5, 1, 2, 4, 6, 24, 48 and 72 h) and evaluated using HPLC for carbohydrate analysis of glucose, xylose, and arabinose.

3. Results and Discussion

3.1. Self-Made Na₂CO₃ Solution

CO₂ by-product from the synthetic fermentation was captured in the absorption column of 5 M NaOH solution producing 2.2 M of Na₂CO₃ and 0.6 M of nonreactive NaOH. The initial glucose concentration in the fermenter was 17.10 g L⁻¹ and the final glucose

concentration was 0.5893 g L^{-1} after 72 h. The ethanol produced reached a concentration of 81.17 g L^{-1} .

3.2. Biomass Pretreatment

Pretreatment effects were evident in the composition analysis made to the untreated and pretreated corn stover samples. Table 1 shows cellulose increased from 34.50 to 40.96% after pretreatment while hemicellulose, lignin, and ash content decreased. The decrease was more notable for lignin content (25.50 down to 16.50%) than for hemicellulose content (21.95 down to 18.35%). These changes in composition correspond to a 18% increase in cellulose content, 16% less hemicellulose, and a 35% decrease in lignin mainly due to lignin's higher susceptibility to solubilization in an alkaline solution than its polysaccharides counterparts. The lignocellulose underwent chemical structural degradation during pretreatment from improved hydrolysis of ester bonds attacked by the hydroxide ions dissociated from NaOH. Na_2CO_3 (at high temperature and pH above 11) also aids in deterioration of lignin [35]. Easier accessibility to cellulose boosts enzyme digestibility during enzymatic hydrolysis. The remaining constituents of the total composition are attributed to impurities present in the biomass samples. Moreover, during pretreatment some biomass material is released in the soluble phase, these were not recovered.

Table 1. Composition analysis of untreated and pretreated corn stover.

Sample	Cellulose%	Hemicellulose%	Lignin%	Ash%
CS-untreated	34.50	21.95	25.50	3.47
CS-pretreated	40.96	18.35	16.50	1.15

3.3. Fourier-Transform Infrared Spectroscopy Analysis

FTIR spectra (see Figures 1 and 2) were collected for untreated and pretreated CS biomass. Results are comparable to previous investigations which were used to assign functional groups and chemical components [36,40–42]. The stronger the transmittance signal is detected (the lower the inverted peak), the less likely absorption from bonds vibrating at the expected mode/frequency are found. Note that all spectra exhibited two regions with significant transmittance peaks approximately corresponding to absorption bands $830\text{--}1750 \text{ cm}^{-1}$ and $2900\text{--}3500 \text{ cm}^{-1}$. These wavenumbers were associated with single and double bonds mostly attributed to the complex lignin structure. The broad peak near 3330 cm^{-1} was attributed to hydroxyl groups in polysaccharides, mainly cellulose, which the CS-pretreated samples presented more of. The band near 2900 cm^{-1} in CS has been associated with the stretching vibration of C-H bonds [40]. Absorption bands $1680\text{--}1750 \text{ cm}^{-1}$ were attributed to C-O double bonds found in the ester bonds between hemicellulose and acetate. CS-untreated spectra contains these features while they are less pronounced in the CS-pretreated spectra as a result of lignocellulose degradation. CH_2 and C-H vibrations ($1319\text{--}1430 \text{ cm}^{-1}$) also decreased after pretreatment. The sharpest peak in region $1029\text{--}1156 \text{ cm}^{-1}$ resulted from C-O and C-C stretching in cellulose. Overall, pretreated CS biomass presented changes in the chemical structures, particularly in the cellulose and lignin-related absorption bands.

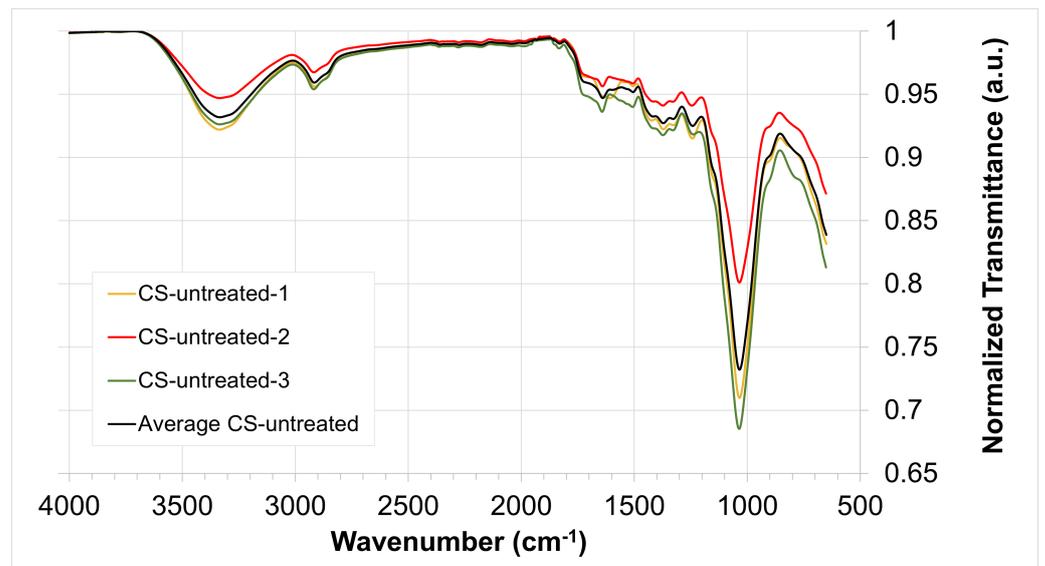


Figure 1. FTIR profiles of corn stover without pretreatment. Samples presented were ran in triplicates and averaged.

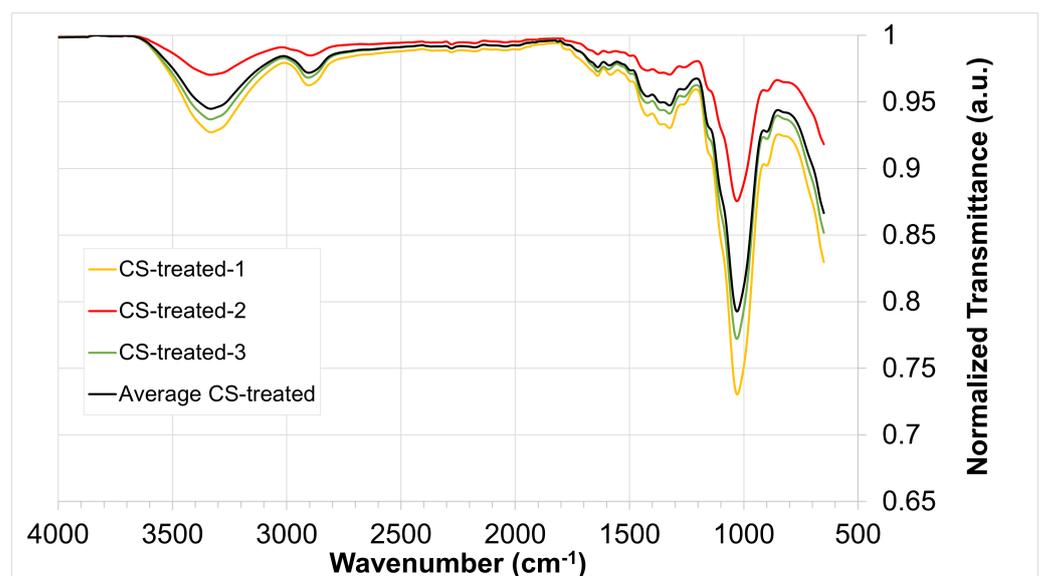


Figure 2. FTIR profiles of corn stover pretreated with recovered solution from CO₂ absorption column. Samples presented were ran in triplicates and averaged.

3.4. Enzymatic Hydrolysis

The effects of the corn stover biomass, Na₂CO₃ pretreatment, and enzyme ratios on the sugar released from enzymatic hydrolysis were evaluated. Sugar yields and concentrations of glucose, xylose, and arabinose were measured via HPLC at different times during hydrolysis for two enzyme ratios (refer to Section 2.4 for a detailed description of the hydrolysis solutions). Figure 3 presents glucose yields where the pretreated CS resulted in a significant yield increase over the untreated samples and after 24 h of hydrolysis reached near-to-maximum yields of 83% and 92% for the 2:1 and 3:1 enzyme solutions, respectively. On the other hand, the untreated biomass had a maximum glucose yield of 42% after 48 h of hydrolysis. Since the 3:1 ratios contained more cellulase to catalyze cellulolysis, the corresponding CS samples yielded more glucose than the 2:1 ratios. In addition, the β -glucosidases present in the enzymes hydrolyze the strong glycosidic linkages found in the backbone of the polysaccharides.

Sugar concentrations for glucose, xylose, and arabinose are included in Figures 4–6. For all cases, the pretreated CS outperformed the untreated samples by more than double, and the enzyme ratio of 3:1 produced higher concentrations than the 2:1 cases while maintaining a similar profile. That is, enzyme ratios had negligible effect in the sugar release rate. Moreover, sugar concentrations of pretreated samples exhibited their largest increase during the first 24 h, followed by a steady increase in concentration. Note that glucose’s concentration saturated after 72 h hydrolysis, contrast to xylose and arabinose, indicating their limited accessibility. After 72 h of enzymatic hydrolysis, maximum concentrations were attained as follows: 44 g L⁻¹ of glucose, 18 g L⁻¹ of xylose, and 2.3 g L⁻¹ of arabinose.

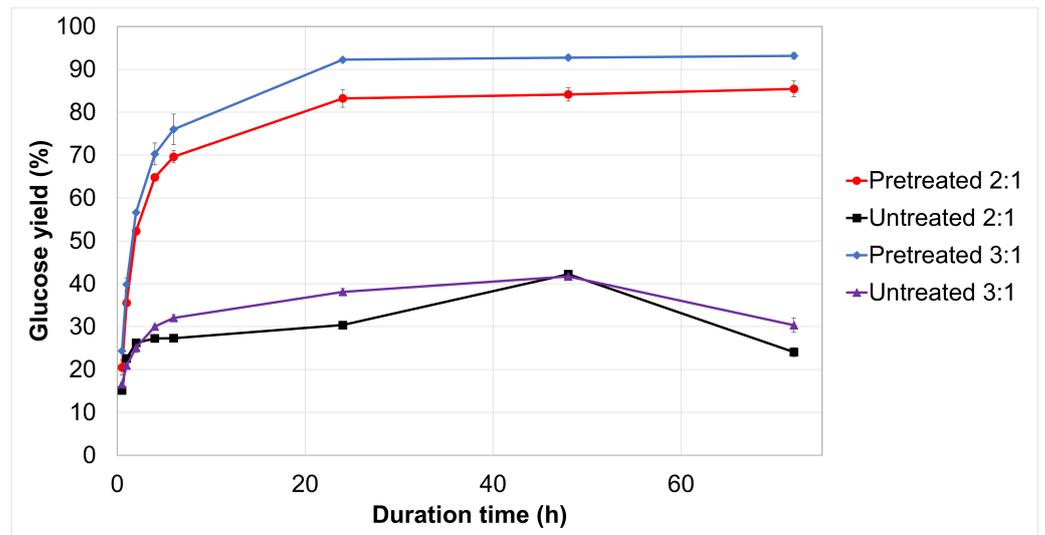


Figure 3. Glucose yield for untreated and pretreated corn stover. Experiments were performed for two different ratios of CTec2:HTec2 enzymes.

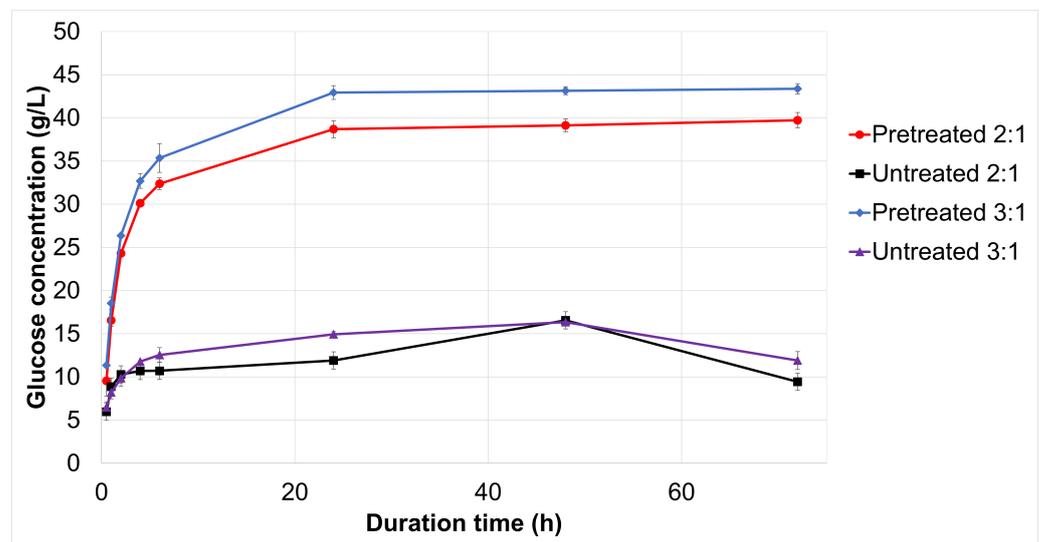


Figure 4. Glucose concentration for untreated and pretreated corn stover. Experiments were performed for two different ratios of CTec2:HTec2 enzymes.

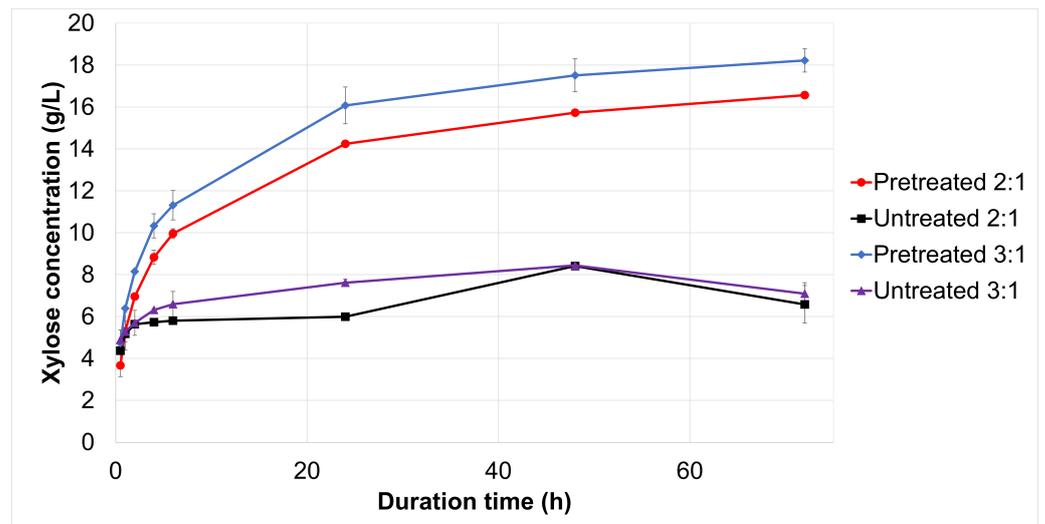


Figure 5. Xylose concentration for untreated and pretreated corn stover. Experiments were performed for two different ratios of CTec2:HTec2 enzymes.

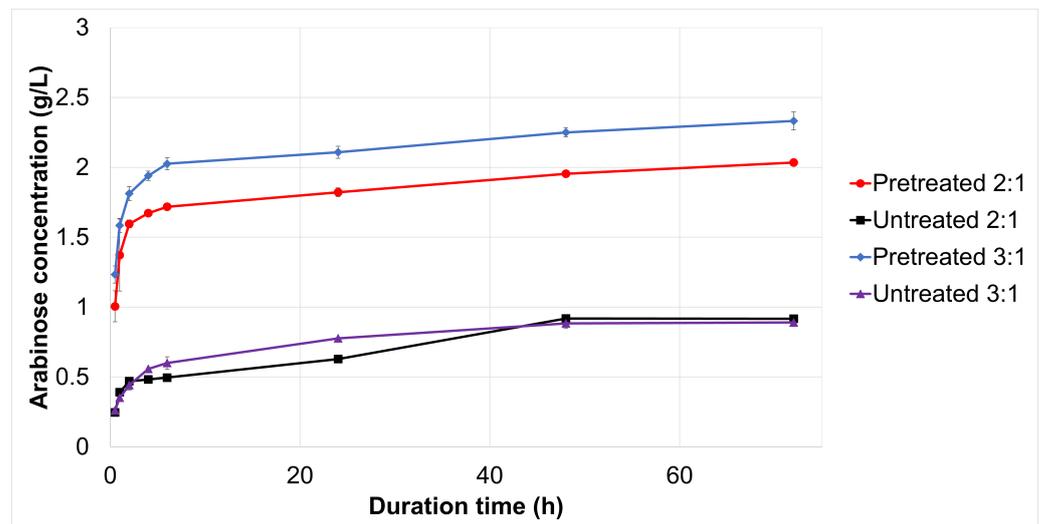


Figure 6. Arabinose concentration for untreated and pretreated corn stover. Experiments were performed for two different ratios of CTec2:HTec2 enzymes.

4. Conclusions

Renewable sources such as lignocellulosic biomass are effective for producing fermentable sugars via enzymatic hydrolysis. The effect of Na_2CO_3 pretreatment on corn stover biomass and enzymatic hydrolysis were investigated. Na_2CO_3 has the attractive potential of being formed by CO_2 absorption, the primary greenhouse gas and by-product of carboxylation processes. Conceptually, bioethanol fermentation could provide the CO_2 for pretreatment preparation, which in turn enhances hydrolysis efficiency and subsequent fermentation of biofuels and chemicals. This study demonstrated the scenario where CO_2 output from synthetic fermentation was captured to form Na_2CO_3 in order to pretreat corn stover biomass. Considering the amount of synthetic glucose used and the amount of biomass pretreated, the CO_2 to be produced from the ethanol fermentation may not be sufficient to recreate the same conditions of the initial pretreatment, therefore, an external CO_2 source would be needed.

Pretreated samples contained higher cellulose and less lignin content than when no treatment was employed, indicative that the Na_2CO_3 and NaOH solution was effective. The pretreatment step clearly promoted lignin degradation, reducing its content by 35% while increasing cellulose content by 18%. The easier accessibility to cellulose improved the

solubilization of polysaccharides, thus higher sugar concentrations were measured during hydrolysis. Also, the enzyme mixture with significantly more cellulase than hemicellulase was more effective at producing glucose, xylose, and arabinose in less hydrolysis time. Additional experiments can be made to evaluate the effect other physicochemical parameters have in pretreatment and hydrolysis. Optimizing pretreatments to increase biomass yields, recycle CO₂, and reduce processing cost helps design fermentation for fuel production. Further studies will be integrated in the process for understanding the performance during biofuel as well as studying other pretreated biomass feedstock.

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Abbreviations

The following abbreviations are used in this manuscript:

CS	Corn stover
HPLC	High-performance liquid chromatography
NREL	National Renewable Energy Laboratory
FTIR	Fourier-transform infrared spectroscopy

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