

Efficient Conversion of Rice Straw to Bioethanol Using Sodium Carbonate Pretreatment

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ABSTRACT: In this study, rice straw was treated with sodium carbonate prior to enzymatic hydrolysis and fermentation. All pretreatments were performed in a high-pressure reactor at 90, 120, 150, and 180 °C by 0.25, 0.5, and 1 M sodium carbonate (Na_2CO_3) solution. The reactor was designed to inject the straw to the reactor at a desired temperature without any preheating effects. The reactor content was continuously mixed, and samples were taken at different time periods. Afterward, enzymatic hydrolysis of the untreated and all treated straws were conducted at 45 °C for 72 h with enzyme loading of 20 FPU cellulase and 30 IU β -glucosidase per gram of substrate. The best pretreatment conditions were obtained to be 0.5 M Na_2CO_3 at 180 °C for 120 min. This pretreatment improved the released glucose from 9.5 g/L for the untreated straw to 43.5 g/L for the treated one. On the other hand, the treatment showed significant improvement on ethanol production from rice straw applying simultaneous saccharification and fermentation. Ethanol production was enhanced from 90.2 to 351.4 g/L by the treatment. The analysis showed that the treatment with sodium carbonate at elevated temperature can significantly reduce the lignin and xylan contents and the cellulose crystallinity and also convert cellulose type I to type II, which is more amenable for enzymatic hydrolysis.

1. INTRODUCTION

According to the Energy Information Administration, world energy consumption would increase from 505 quadrillion Btu in 2008 to 619 and 707 quadrillion Btu in 2020 and 2035, respectively, which shows almost a 53% rise.¹ To meet this demand and decrease various environmental pollutions, lignocellulosic materials are suggested as the most available promising alternative for biofuel production.²

Rice straw is one of the most abundant lignocelluloses available in all over the world which is inexpensive and mainly unused.³ According to a report by FAO (2011), the annual rice production would reach 721 million tons in 2011, which means an increase by 3% from 2010 to 2011. An amount of 1–1.5 kg of rice straw is produced per kilogram of harvested grain. This huge amount of straw has a high potential for bioethanol production.⁴

Cellulose, the main component of lignocellulosic materials, can be enzymatically hydrolyzed to glucose and then fermented to bioethanol. However, lignocellulosic materials have a very complex structure with high crystalline cellulose protected by lignin and hemicelluloses; thus, only up to 20% yield of sugars can be achieved by enzymatic hydrolysis of native lignocelluloses.⁵ For an efficient hydrolysis, a pretreatment process should be considered as a decisive step to modify the structure and remove lignin and hemicellulose.^{6–9} This pretreatment process is nowadays considered as a main key for economically feasible ethanol production from lignocelluloses.¹⁰

Different pretreatment methods such as aqueous-ammonia soaking pretreatment,^{5,11} microwave pretreatment,¹² dilute acid pretreatment,¹³ ultrasonic pretreatment,¹⁴ hot-compressed water pretreatment,¹⁵ and alkaline pretreatment^{16,17} were used to improve saccharification of rice straw. All physical methods suffer from low efficiency and high energy

consumption. In addition, despite having high yields, chemical methods including acid or alkali pretreatments have some operational problems such as corrosive characteristics, need for neutralization, and environmental hazards. To overcome the problems mentioned above, inorganic salts such as sodium carbonate can be used as a pretreatment agent. It is an inexpensive and widely available chemical with no negative environmental effects. This salt has been previously used by some researchers for pretreatment of lignocellulosic materials.¹⁸ However, promising results were obtained only when sodium carbonate has been used in the wet oxidation method. Bjerre et al. (1996) showed that addition of sodium carbonate in wet oxidation can significantly improve the digestibility of wheat straw.¹⁹ In another study, Schmidt and Thomson (1998) applied wet oxidation by addition of sodium carbonate to disintegrate various components of wheat straw. The optimum conditions for 60 g/L straw were 6.5 g/L Na_2CO_3 , at 185 °C and 12 bar O_2 for 15 min reaction time. Under these conditions, 50% of lignin and 80% of hemicellulose were dissolved, while more than 95% of cellulose remained in the solid state.²⁰ Thus, the treatment is potentially a promising method for pretreatment of lignocelluloses. To our knowledge, no previous work was performed on rice straw which contains high amounts of ash (mainly in the form of silica) and shows different behavior in pretreatment compared to other lignocelluloses.

The main purpose of this study was pretreatment of rice straw using sodium carbonate in a high-pressure reactor. Effects of the pretreatment on improvement of enzymatic hydrolysis

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and fermentation were investigated. Compositional, FTIR, and image analyses of untreated and treated straws were provided to obtain more information about the chemical and structural variations as a result of the treatment.

2. EXPERIMENTAL SECTION

2.1. Materials and Microorganisms. Rice straw with a cultivar name of “Sazandegi” was used in all experiments harvested in the Falavarjan field of the Isfahan province in Iran (32°34'N, 51°32'E). It was milled to a size between 0.1 and 0.8 mm lengths. The dry weight of the straw was determined by drying at 105 °C until a constant weight was reached. The composition of native rice straw used in this study is presented in Table 1, and it is compared to some other rice

Table 1. Compositions of Rice Straw Used in the Present and Other Studies

component % (w/w) of dry rice straw	present study	Hsu et al. ¹³	Kim et al. ²¹	Balan et al. ²²	Sindhu et al. ²³
cellulose	40.3	36.6	39.5	37.0	34.1
xylos	23.8	16.1	24.4	20.5	28.4
other polysaccharides	4.1			5.7	
lignin	18.2	14.9	15.9	15.4	18.1
acid insoluble lignin (AIL)	15.5	13.0		14.2	
acid soluble lignin (ASL)	2.7	1.9		1.2	
ash	7.7	14.5		14.2	7.5

straw used by different researchers all around the world.^{13,21–23} Commercial grades of cellulase (Celluclast 1.5L, Novozyme, Denmark) and β -glucosidase (Novozym 188, Novozyme, Denmark) were used for hydrolysis. Activity of the cellulase and β -glucosidase were obtained to be 80 FPU/mL (measured by the Adeny and Baker procedure²⁴) and 240 IU/mL (measured by the method presented by Ximenes et al.²⁵), respectively.

A flocculation strain of *Saccharomyces cerevisiae* (CCUG 53310, Culture Collection, University of Gothenburg, Sweden), isolated from an ethanol plant (Domsjö Fabriker AB, Örnsköldsvik, Sweden), was used as a fermenting organism. The microorganism maintenance and its biomass production were performed according to the procedure presented by Shafiei et al.²⁶

2.2. Pretreatment Procedure. All pretreatments were conducted in a jacketed reactor with the working volume of 3.5 L equipped with a mixer and a temperature controller (Figure 1). The experiments were conducted at different temperatures (90, 120, 150, and 180 °C) and Na_2CO_3 concentrations (0.25, 0.5, and 1 M) for different retention times (30, 60, 120, and 180 min).

The reactor was filled with 2 L of sodium carbonate solution, and the air enclosed in the reactor was purged using pure nitrogen gas to avoid any oxidizing reactions. The reactor was heated by circulation of hot oil at the desired temperature in the reactor jacket, and simultaneously, the reactor pressure was increased to its respective equilibrium value by itself. After reaching the desired temperature, 100 g of rice straw was injected to the reactor (5% solid loading). To eliminate any preheating effects, a side high pressure vessel was connected to the reactor by a valve. The straw was loaded to the vessel and pressurized to about 20 bar using nitrogen gas. Thus, it was possible to inject the straw to the reactor at any time by opening the valve. During reaction, a mixer with the speed of 150 rpm ensured the uniformity of temperature and concentration all over the reactor. An amount of 50 mL samples were periodically taken and after quick cooling to room temperature, filtered, and washed by boiling distilled water to achieve a clear filtrate. The remaining solid fraction was then subjected to hydrolysis, fermentation, and different analyses.

2.3. Enzymatic Hydrolysis. Enzymatic hydrolysis of the untreated and treated straws was conducted at 45 °C for 72 h in a shaker incubator with the speed of 100 rpm. The working volume of the hydrolysis media was 30 mL of solution containing 50 mM sodium citrate buffer with pH of 4.8, 0.5 g/L sodium azide (as an antibacterial agent), and 1.5 g of substrate. The enzyme loadings were 20 FPU cellulase and 30 IU β -glucosidase per gram of dry treated straw. Liquid

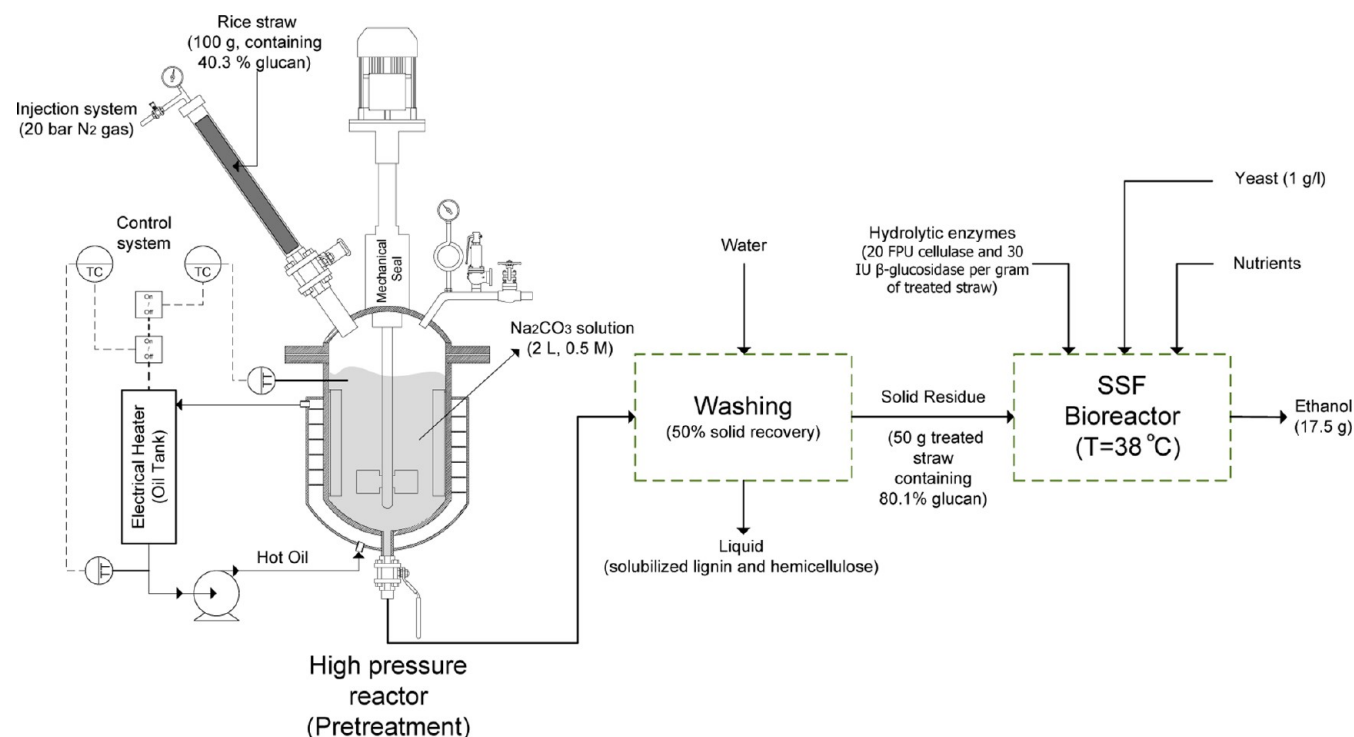


Figure 1. Overall scheme of ethanol production using sodium carbonate pretreatment (the optimum processing conditions and the best obtained results are indicated in parentheses).

samples were taken after 24 and 72 h hydrolysis and stored in a freezer prior to glucose analysis by an assay kit.

2.4. Simultaneous Saccharification and Fermentation (SSF). Simultaneous saccharification and fermentation was performed in a 30 mL medium containing 5 g/L yeast extract, 7.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 3.5 g/L K_2HPO_4 , 0.75 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 50 g/L treated or untreated rice straws in 0.05 M buffer citrate with a pH of 5 ± 0.1 . The medium was autoclaved at 121 °C for 20 min. After cooling, 1 g/L *S. cerevisiae*, 20 FPU cellulase, and 30 IU β -glucosidase enzymes per gram of substrate were added to each medium and mixed at 80 rpm and 38 °C for 72 h. Liquid samples were taken and stored in a freezer prior to metabolite analysis.

2.5. Analytical Methods. Chemical composition of untreated and pretreated straws was determined using the NREL protocol,²⁷ which includes two stages of acid hydrolysis at 30 and 121 °C, respectively. Cellulose and hemicellulose were converted to corresponding monomeric sugars and analyzed by HPLC. Lignin was classified as acid insoluble and determined as solid recovered by filtration, and acid soluble was determined by UV–vis spectroscopy at 240 nm with absorptivity of 25 L/g cm. All the analyses were done in duplicate.

Glucose content of the hydrolysates was analyzed using glucose HK assay kit (Shimenzyme). Ethanol and glycerol contents of SSF metabolites and sugar content of compositional analysis were determined using high-performance liquid chromatography (HPLC) equipped with UV–vis and RI detectors (Jasco International Co., Tokyo, Japan). Ethanol and glycerol were analyzed on an Aminex HPX-87H column (Bio-Rad, Richmond, CA) at 60 °C with 0.6 mL/min eluent of 5 mM sulfuric acid. The sugars were analyzed on an ion-exchange Aminex HPX-87P column (Bio-Rad) at 85 °C with an eluent of deionized water at a flow rate of 0.6 mL/min.

Structural changes of the straws were evaluated by scanning electron microscopy (SEM). The treated straw as well as the untreated one were coated with gold (BAL-TEC SCD 005) and analyzed by SEM (PHILIPS, XL30) at 15 kV.

Crystallinity and chemical bonds of the straw before and after treatments were analyzed using a Fourier transform infrared (FTIR) spectrometer equipped with a universal attenuated total reflection (ATR) accessory and deuterated triglycine sulfate (DTGS) detector (Bruker Tensor 27 FT-IR). Their spectra were obtained with an average of 60 scans from 550 to 4000 cm^{-1} with 4 cm^{-1} resolution.

3. RESULTS

Pretreatment of rice straw in the presence of Na_2CO_3 at high temperatures were applied to enhance enzymatic digestibility of rice straw. The treated and untreated straws were subjected to enzymatic hydrolysis. Then, the treated straws with the highest hydrolysis yields were used for ethanol production by SSF.

3.1. Enzymatic Hydrolysis. The results of enzymatic hydrolysis by commercial cellulase and β -glucosidase enzymes are shown in Figure 2.

As it can be noticed, increasing pretreatment time from 30 to 180 min improved the digestibility at lower temperatures. However, for all Na_2CO_3 concentrations and for pretreatment at 180 °C, the maximum glucose released occurred at 120 min of pretreatment. It means that at higher temperatures, the pretreatment time for an efficient digestibility is less than that at lower temperatures.

The effect of Na_2CO_3 concentration showed minor effects in the pretreatment results, whereas 0.5 M Na_2CO_3 gave the best performance (Figure 2). Glucose released from the straw treated with 0.25, 0.5, and 1 M Na_2CO_3 at 90 °C for 180 min were 27, 29.5, and 29 g/L, while these values at 180 °C were 40.5, 41, and 40.5 g/L, respectively.

In addition, the effect of pretreatment temperature was evaluated at 90, 120, 150, and 180 °C. As indicated in Figure 2, increasing the pretreatment temperature, in all Na_2CO_3 concentrations, resulted in increasing the glucan digestibility

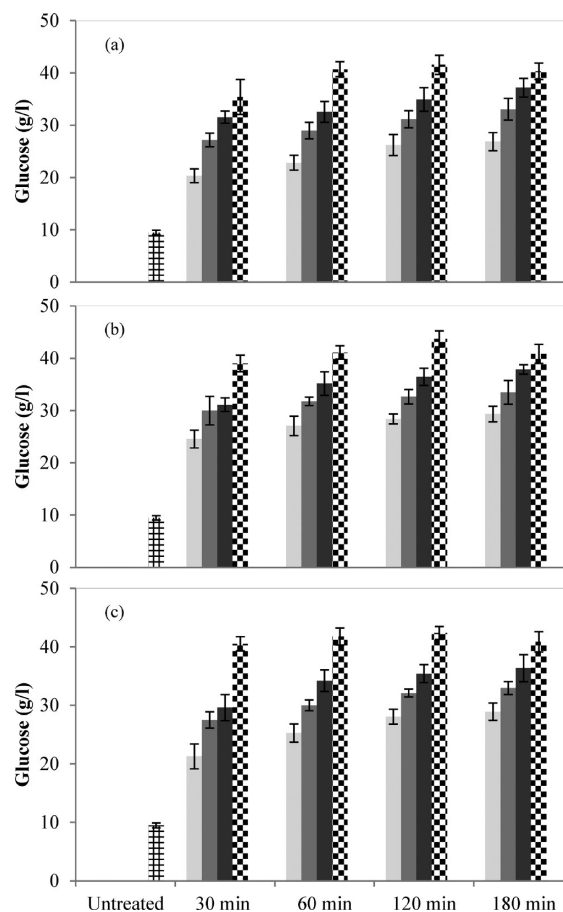


Figure 2. Glucose released after 72 h enzymatic hydrolysis of rice straw treated with (a) 0.25, (b) 0.5, and (c) 1 M Na_2CO_3 at different treatment times of 30, 60, 120, and 180 min. The symbols represent different pretreatment temperatures: (light gray) 90 °C, (dark gray) 120 °C, (black) 150 °C, (checkered) 180 °C, and (grid) untreated.

of the substrate. Increasing the temperature from 90 to 180 °C improved the released glucose from 27 to 40.5 g/L in the case of straws treated with 0.25 M Na_2CO_3 for 180 min. This value was only 9.5 g/L for the untreated straw (Figure 2). Since the best results were obtained in the pretreatment at 180 °C, the straw treated at this temperature was subjected to ethanol production by SSF.

3.2. Lignin Removal by the Pretreatment. The lignin content of untreated and treated straws at various conditions was measured, and data are reported in Figure 3.

As it is indicated by the data presented in Figure 3, increasing the pretreatment temperature resulted in more lignin removal at constant Na_2CO_3 concentration. This reduction in lignin content was greater when the temperature was increased from 90 to 120 °C, while it had a minor effect from 120 to 180 °C. Although lignin removal was increased by increasing temperature, it seems that lignin content was increased at very high temperature.

As shown in Figure 3, increasing pretreatment time resulted in more lignin removal in most cases. That is the case for both acid soluble and insoluble lignin. For instance, acid insoluble lignin content of straw treated with 0.5 M Na_2CO_3 at 150 °C for 30, 60, 120, and 180 min were 9.6, 8.6, 8.4, and 8.2%, respectively. This value was 15.5% for the untreated straw.

Confirmed by data obtained from Figure 3, 0.5 M Na_2CO_3 is the most efficient concentration of Na_2CO_3 which succeeded to

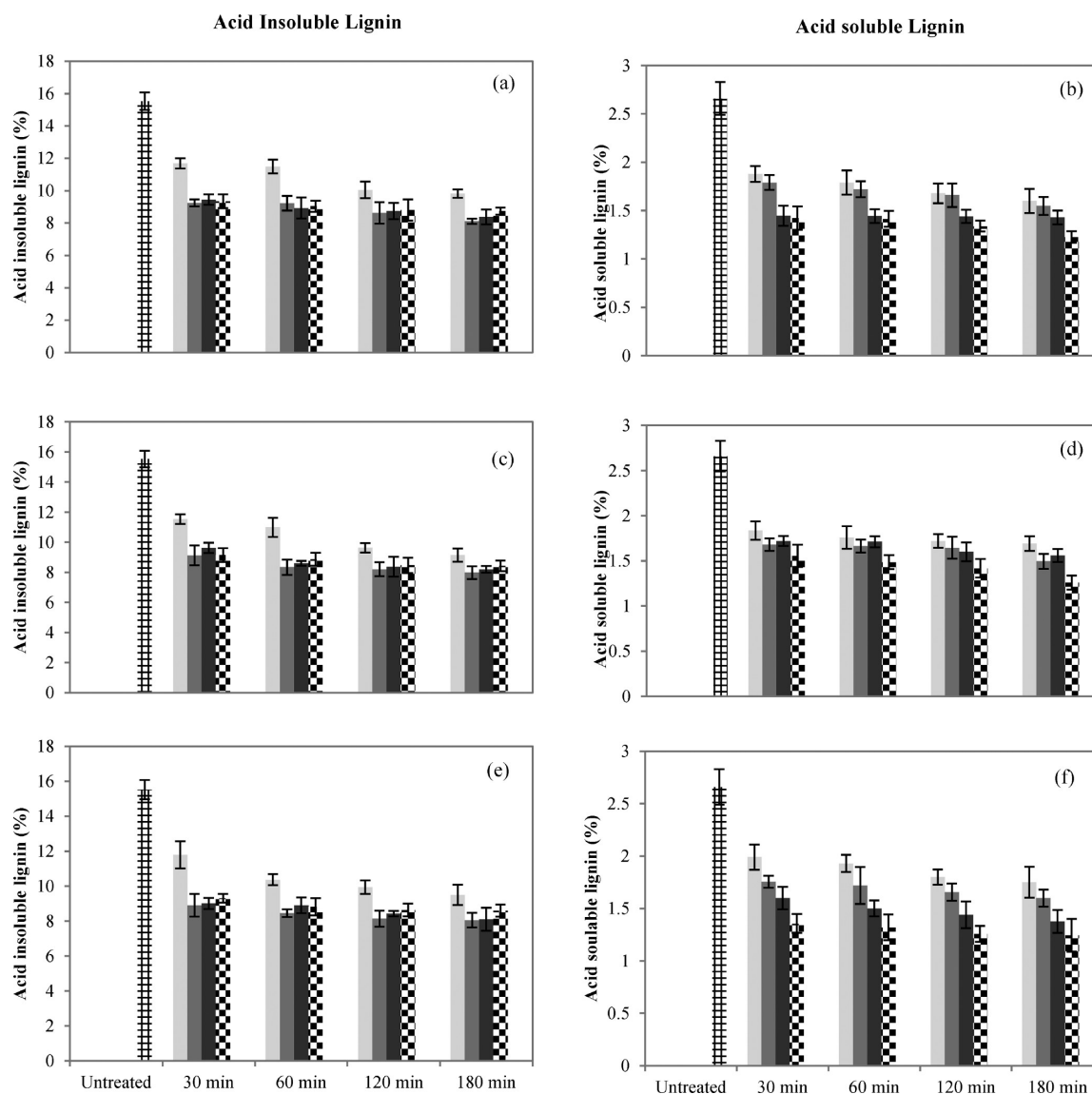


Figure 3. Lignin content of straws treated with (a, b) 0.25, (c, d) 0.5, and (e, f) 1 M Na_2CO_3 at different treatment times of 30, 60, 120, and 180 min. The symbols represent different pretreatment temperatures: (light gray) 90 °C, (dark gray) 120 °C, (black) 150 °C, (checkered) 180 °C, and (grid) untreated.

Table 2. Composition of Untreated and Treated Straws with 0.5 M Na_2CO_3 at Different Pretreatment Temperatures and Times

pretreatment conditions		AIL ^a (%)	ASL ^b (%)	glucan (%)	xylan (%)	other polysaccharides (%)	ash (%)
temperature (°C)	time (min)						
90	180	9.2	1.7	59.1	19.6	4.2	1.7
120	180	8	1.6	65.2	17.3	1.9	1.2
150	180	8.2	1.5	72.5	10	1.8	1
180	120	8.5	1.6	80.1	6.3	1.2	0.8
untreated straw		15.5	2.7	40.3	23.8	4.1	7.7

^aAcid insoluble lignin. ^bAcid soluble lignin.

reduce lignin content of untreated straw from 15.5 to less than 8.4%.

3.3. Effect of Pretreatment on Carbohydrate Contents of Rice Straw. The effect of the pretreatment on carbohydrate contents of rice straw at the best conditions, which was obtained by treatment with 0.5 M Na_2CO_3 (based on the hydrolysis results), was analyzed according to the NREL protocol and the results are shown in Table 2. Both acid soluble

and acid insoluble lignin content of the straw were reduced by increasing the pretreatment temperature. Glucan content was improved from 40.3% for untreated straw to 80.1% for straw treated at 180 °C for 120 min. Other carbohydrates were degraded through the pretreatment, especially at higher temperatures. For example, xylan content of straw treated at 180 °C for 120 min was only 6.3%, while this value for untreated straw was more than 23%. Another effect that should

be considered here is ash removal. Ash content was reduced from 7.7% for untreated straw to 0.8% for the sample treated with 0.5 M Na_2CO_3 at 180 °C for 120 min (Table 2).

3.4. Effect of Pretreatment on Cellulose Crystallinity and Chemical Structures. Effects of pretreatments on the straw composition and cellulose crystallinity were investigated using a Fourier transform infrared (FTIR) spectrometer. The FTIR spectra and bands characteristics are shown in Figure 4 and Table 3, respectively. Lignin contents were reduced as a result of pretreatment, while cellulose was increased through the pretreatments.

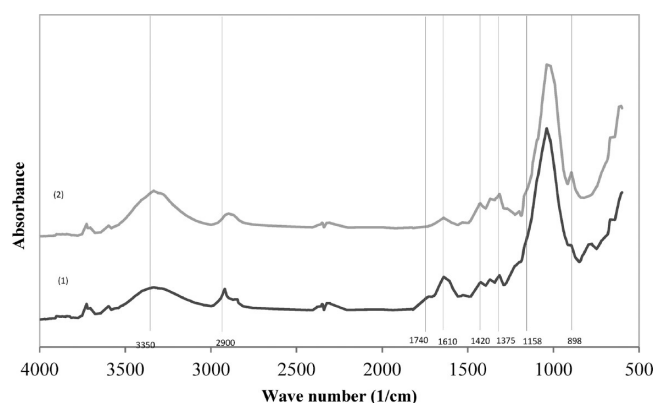


Figure 4. FTIR spectra of (1) untreated straw and (2) straw treated with 0.5 M Na_2CO_3 at 180 °C for 120 min.

The crystalline cellulose type I and II were assigned by the absorption bands at 1430 and 896 cm^{-1} .²⁸ The results indicated conversion of cellulose type I to II.

The absorbance ratio A1430/A896 and A1375/A2900 were considered as the crystallinity index (CI) and total crystallinity index (TCI), respectively.²⁵ These values are 0.43 and 1.45 for untreated straw and 0.39 and 1.36 for straw treated with 0.5 M Na_2CO_3 at 180 °C for 120 min, respectively.

3.5. Effect of the Pretreatment on Morphology. To analyze the morphological destruction effect of pretreatment, SEM images of untreated and pretreated straw were provided (Figure 5). As it can be seen, the images show both macro- and microstructural destruction of rice straw through the pretreat-

ment. By treatment, the packed and inaccessible matrix of straw is converted to an open matrix, which is more accessible for enzymatic hydrolysis.

3.6. Simultaneous Saccharification and Fermentation. The results of simultaneous saccharification and fermentation of the untreated and the straws treated at 180 °C for 120 min with 0.25–1 M sodium carbonate are summarized in Table 4. The treated straws were selected for SSF based on the best results of the enzymatic hydrolysis.

As it can be noticed, ethanol production was improved from 90.2 mg/g for untreated straw to 325.7, 349.2, and 351.4 mg/g for treated straws at 180 °C for 120 min using 0.25, 0.5, and 1 M Na_2CO_3 , respectively.

The yields of glycerol, the dominant byproduct of SSF, also followed the same trend as ethanol production (Table 4). The yields for treated straws were about 4 times more than that of untreated straw.

3.7. Mass Balance. Mass balance was evaluated for pretreatment and simultaneous saccharification and fermentation steps, and the results are summarized in Figure 6 for the straw treated with 0.5 M Na_2CO_3 at 180 °C for 120 min. Because of partial dissolution of hemicellulose, lignin, and ash in addition to possible inefficient filtration, solid recovered after pretreatment was less than 50 wt % of the initial straw. However, determination of glucan content before and after pretreatment showed no degradation effect on this component. Because of the pretreatment, the glucose and ethanol released, respectively, in the hydrolysis and SSF were significantly improved.

4. DISCUSSION

Rice straw, one of the most available lignocellulosic biomass, has a high potential for ethanol production. Similar to other lignocelluloses, packed and complex structure of the straw increases the significance of a pretreatment process to achieve an efficient conversion. In order to maximize the glucose and subsequently ethanol yield from the straw, sodium carbonate solution was chosen as a pretreatment medium. In contrary to previous studies in which sodium carbonate was used as an alkaline agent in an oxidative pretreatment,²⁰ no oxidizing component was used. It can be proposed that due to the high lignin content of untreated rice straw and its complex structure,

Table 3. Characteristics and Variations of Bands in FTIR Spectra and Crystallinity Changes of Treated and Untreated Straw

wavenumber (cm^{-1})	functional group	band assignment	untreated straw	treated straw with 0.5 M Na_2CO_3 at 180 °C for 120 min
3175	–OH stretching intramolecular hydrogen bonds	cellulose II	0.06	0.13
2918	C–H stretching	cellulose	0.11	0.15
1730	C=O stretching of acetyl or carboxylic acid	hemicellulose and lignin	0.07	0.06
1627	C=C stretching of the aromatic ring	lignin	0.17	0.09
1598	C=C	lignin	0.14	0.08
1510	C=C stretching of the aromatic ring	lignin	0.1	0.08
1465	Asymmetric bending in C–H3	lignin	0.12	0.12
1423	C–H2 symmetric bending	cellulose	0.15	0.18
1430	C–H2 bending	cellulose	0.15	0.18
1375	C–H bending	cellulose	0.16	0.20
1335	–OH (in plane bending)	cellulose	0.16	0.20
1315	C–H2 wagging	cellulose	0.19	0.22
1158	C–O–C asymmetric stretching	cellulose	0.35	0.33
896	asym., out of phase ring stretching (cellulose)	cellulose	0.35	0.45

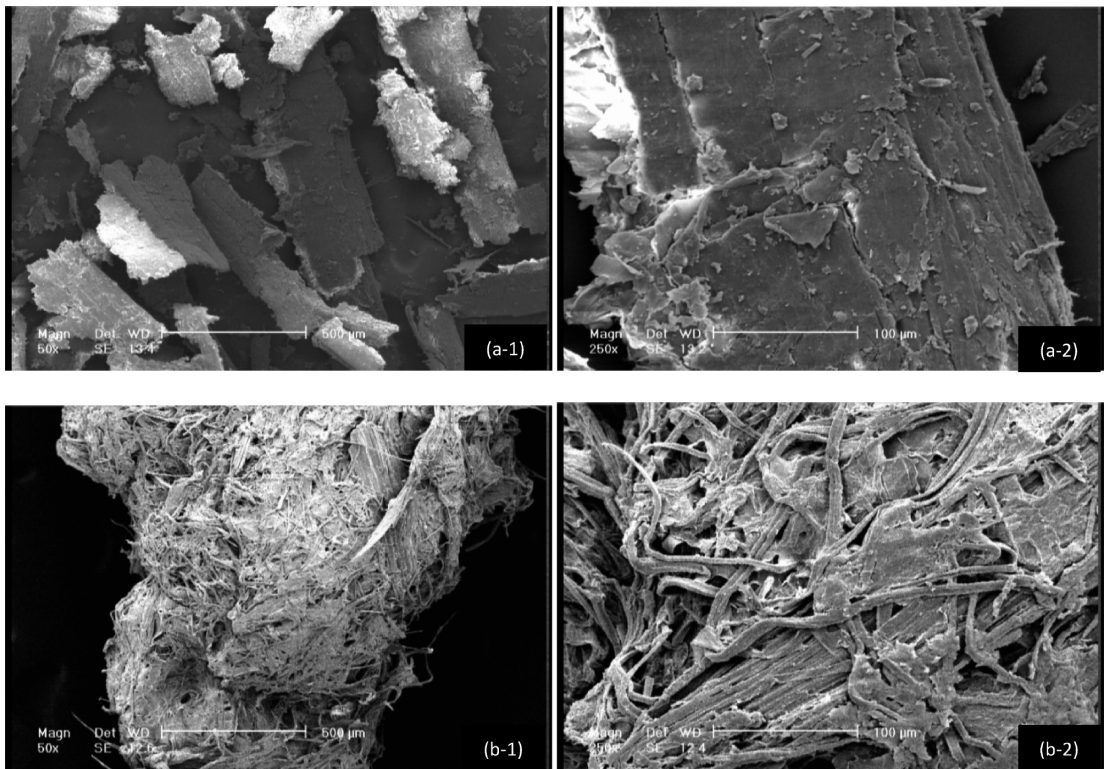


Figure 5. SEM images of (a) untreated straw and (b) pretreated straw with 0.5 M Na₂CO₃ at 180 °C for 120 min.

Table 4. Ethanol Production by Simultaneous Saccharification and Fermentation

pretreatment conditions			ethanol (mg/g of substrate)	glycerol (mg/g of substrate)
Na ₂ CO ₃ concentration	temperature	time		
0.25 M	180 °C	120 min	325.7 ± 21	41.4 ± 0.5
0.5 M	180 °C	120 min	349.2 ± 27.9	57.1 ± 4.7
1 M	180 °C	120 min	351.4 ± 34.6	56.6 ± 6.7
untreated straw			90.2 ± 4.2	13.5 ± 2

a long pretreatment time is needed in the oxidizing pretreatments. However, despite its positive effects on lignin removal and xylan degradation, the used oxidant in long pretreatment can destruct the cellulose that is not favorable.²⁶ Hence, the

sodium carbonate at high concentration can be used to compensate the lack of oxidizing agent and improve the pretreatment without losing cellulose.

There are several studies in which improvement of ethanol production of rice straw was pursued. Hsu et al.¹³ succeeded to remove xylan using a dilute sulfuric acid solution in pretreatment and therefore improved the glucose yield up to 83%. In another study, alkaline pretreatment of rice straw by 20 wt % ammonia solution was performed. Lignin was removed from the straw to less than 5%, and the maximum glucose yield of 80.6% was attained.¹¹ However, the results obtained in the present study indicate a hydrolysis yield of 97%. According to the results of this study (production of 175 g of ethanol per 1 kg of rice straw), considering the average ratio of rice straw to harvested grain to be 1.25 and based on annual rice production,

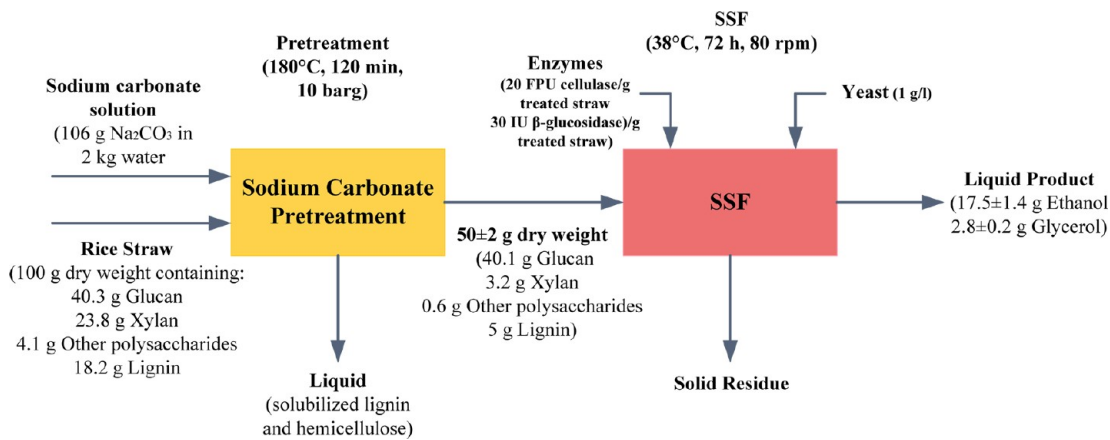


Figure 6. Material balance flow diagram for pretreatment and simultaneous saccharification and fermentation (SSF) for the straw treated with 0.5 M Na₂CO₃ at 180 °C for 120 min.

it can be claimed that more than 200 billion liters of bioethanol can be produced annually from rice straw using an inexpensive chemical without the necessity of neutralization and pollutant production.

Xylan or lignin removal are frequently shown to improve the enzymatic hydrolysis of lignocelluloses.^{6,9,29,30} Some researchers have shown that removal of both xylan and lignin can further improve the hydrolysis, and thus two step pretreatment processes such as lignin removal from straw by ammonia pretreatment and xylan removal by dilute sulfuric acid pretreatment were among the most efficient pretreatments for improvement of rice straw hydrolysis.²¹ The results of the present study indicated the ability of Na₂CO₃ pretreatment in removing both xylan and lignin in one stage. Alkaline characteristic of this salt is probably more responsible for lignin removal, and high pretreatment temperature leads to xylan dissolution, although it cannot be purely divided. Regarding the data presented in Table 2, the xylan content of the treated straw was significantly decreased with increasing the temperature from 90 to 180 °C at the same Na₂CO₃ concentration, and this result confirms other studies indicated that by increasing the temperature, specifically more than 150 °C, degradation of hemicellulose compounds starts.²⁶ Although the lignin is removed as well, this significant decrease in xylan content leads to an increase in lignin concentration in the remaining solid. Thus, considering the results presented in Table 2 and Figure 3, it can be concluded that the dominant phenomenon is lignin removal at low temperature, while at high temperature, in addition to the lignin removal, xylan degradation is another major phenomenon which leads to higher lignin percentage in the residual solid.

Moreover, FTIR analysis of the treated and untreated straws signifies the cellulose crystallinity reduction as another reason for the observed improvements, and it shows that by converting cellulose type I that is less digestible to cellulose type II, higher digestibility of the cellulose can be achieved. In addition, SEM images also show that the pretreatment opens up the compact structure of the straw and its morphology is significantly modified.

Since all of the changes, i.e., xylan and lignin removal, cellulose crystallinity reduction, and more accessible structures affect the hydrolysis and subsequently the ethanol yields, it is difficult to find which one of the changes has the dominant effect on the observed improvements by Na₂CO₃ pretreatment. A combination of these changes may be the reason for the observed improvements.

One of the main requirements for economically feasible application of highly concentrated chemical pretreatments is regeneration of the treating chemical after the pretreatment and its reuse. Moreover, water should also be reused in the process in order to minimize the water consumption and wastewater pollutions. Thus, in continuation of this work, further study is required to efficiently reuse the sodium carbonate and recycle the water used for the pretreatment.

5. CONCLUSIONS

Treatment with Na₂CO₃ at high temperature and relatively short time is a promising pretreatment method to improve enzymatic hydrolysis and ethanol production from rice straw. Complete conversion of rice straw cellulose to glucose is possible after pretreatment with 0.5 M Na₂CO₃ at 180 °C for 120 min. The pretreatment can significantly remove the lignin and xylan from the straw and efficiently modify its structure.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Energy Information Administration (EIA). International Energy Outlook 2011, September 2011; available from www.eia.doe.gov/oiaf/ieo/index.html.
- (2) Claassen, P. A. M.; van Lier, J. B.; Lopez Contreras, A. M.; van Niel, E. W. J.; Sijtsma, L.; Stams, A. J. M.; de Vries, S. S.; Weusthuis, R. A. *Appl. Microbiol. Biotechnol.* **1999**, *52* (6), 741–755.
- (3) Kim, S.; Dale, B. E. *Biomass Bioenergy* **2004**, *26* (4), 361–375.
- (4) Binod, P.; Sindhu, R.; Singhania, R. R.; Vikram, S.; Devi, L.; Nagalakshmi, S.; Kurien, N.; Sukumaran, R. K.; Pandey, A. *Bioresour. Technol.* **2010**, *101* (13), 4767–4774.
- (5) Ko, J. K.; Bak, J. S.; Jung, M. W.; Lee, H. J.; Choi, I. G.; Kim, T. H.; Kim, K. H. *Bioresour. Technol.* **2009**, *100* (19), 4374–4380.
- (6) Taherzadeh, M. J.; Karimi, K. *Int. J. Mol. Sci.* **2008**, *9* (9), 1621–1651.
- (7) Sun, Y.; Cheng, J. *Bioresour. Technol.* **2002**, *83* (1), 1–11.
- (8) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. *Bioresour. Technol.* **2005**, *96* (6), 673–686.
- (9) Alvira, P.; Tomas-Pejo, E.; Ballesteros, M.; Negro, M. J. *Bioresour. Technol.* **2010**, *101* (13), 4851–4861.
- (10) Yang, B.; Wyman, C. E. *Biofuels, Bioprod. Biorefin.* **2008**, *2* (1), 26–40.
- (11) Zhong, C.; Lau, M.; Balan, V.; Dale, B.; Yuan, Y.-J. *Appl. Microbiol. Biotechnol.* **2009**, *84* (4), 667–676.
- (12) Ma, H.; Liu, W.-W.; Chen, X.; Wu, Y.-J.; Yu, Z.-L. *Bioresour. Technol.* **2009**, *100* (3), 1279–1284.
- (13) Hsu, T.-C.; Guo, G.-L.; Chen, W.-H.; Hwang, W.-S. *Bioresour. Technol.* **2010**, *101* (13), 4907–4913.
- (14) Wongsorn, C.; Kangsadan, T.; Kongruang, S.; Burapatana, V.; Pripanapong, P. Ultrasonic pretreatment enhanced the enzymatic hydrolysis of rice straw. In *Chemistry and Chemical Engineering (ICCCE), 2010 International Conference*, 2010.
- (15) Yu, G.; Yano, S.; Inoue, H.; Inoue, S.; Endo, T.; Sawayama, S. *Appl. Biochem. Biotechnol.* **2010**, *160* (2), 539–551.
- (16) Cheng, Y.-S.; Zheng, Y.; Yu, C.; Dooley, T.; Jenkins, B.; VanderGheynst, J. *Appl. Biochem. Biotechnol.* **2010**, *162* (6), 1768–1784.
- (17) Xu, F.; Sun, J. X.; Liu, C. F.; Sun, R. C. *Carbohydr. Res.* **2006**, *341* (2), 253–261.
- (18) Vaccarino, C.; Lo Curto, R. B.; Tripodo, M. M.; Bellocchio, E.; Laganfi, G.; Patan, R. *Biol. Waste* **1987**, *20*, 79–88.
- (19) Bjerre, A. B.; Olesen, A. B.; Fernqvist, T. *Biotechnol. Bioeng.* **1996**, *49*, 568–577.
- (20) Schmidt, A.; Thomsen, A. *Bioresour. Technol.* **1998**, *64*, 139–151.
- (21) Kim, J.-W.; Kim, K. S.; Lee, J.-S.; Park, S. M.; Cho, H.-Y.; Park, J. C.; Kim, J. S. *Bioresour. Technol.* **2011**, *102* (19), 8992–8999.
- (22) Balan, V.; da Costa Sousa, L.; Chundawat, S.; Vismeh, R.; Jones, A.; Dale, B. J. *Ind. Microbiol. Biotechnol.* **2008**, *35* (5), 293–301.
- (23) Sindhu, R.; Binod, P.; Janu, K.; Sukumaran, R.; Pandey, A. *World J. Microbiol. Biotechnol.* **2012**, *28* (2), 473–483.
- (24) Adney, B.; Baker, J. *Laboratory Analytical Procedure, NREL LAP-006*; 1996.

- (25) Ximenes, E. A.; Felix, C. R.; Ulhoa, C. J. *Curr. Microbiol.* **1996**, *32* (3), 119–123.
- (26) Shafiei, M.; Karimi, K.; Taherzadeh, M. J. *Bioresour. Technol.* **2010**, *101* (13), 4914–4918.
- (27) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Laboratory Analytical Procedure, NREL/TP-510-42618*; 2008.
- (28) Colom, X.; Carrillo, F.; Nogués, F.; Garriga, P. *Polym. Degrad. Stab.* **2003**, *80* (3), 543–549.
- (29) Hendriks, A. T. W. M.; Zeeman, G. *Bioresour. Technol.* **2009**, *100* (1), 10–18.
- (30) Girio, F. M.; Fonseca, C.; Carvalheiro, F.; Duarte, L. C.; Marques, S.; Bogel-Lukasik, R. *Bioresour. Technol.* **2010**, *101* (13), 4775–4800.