

Article

Pretreatment of Sweet Sorghum Bagasse for Ethanol Production Using Na_2CO_3 Obtained by NaOH Absorption of CO_2 Generated in Sweet Sorghum Juice Ethanol Fermentation

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Abstract: (1) Background: Commercial production of fuel ethanol currently uses sugarcane and corn as feedstocks. Attempts to develop other renewable feedstocks that are more abundant have led to lignocellulosic biomass, which requires pretreatment prior to enzymatic hydrolysis to generate fermentable sugars. One of the largest cost components of pretreatment is chemical cost. Ethanol fermentation also produces large quantities of CO_2 as a co-product contributing to global warming. (2) Methods: Sweet sorghum has emerged as a potential new feedstock for ethanol production. In the present study, the CO_2 produced in sweet sorghum juice (SSJ) fermentation was captured by absorption in 5 M NaOH. The resultant Na_2CO_3 solution was used for pretreatment of sweet sorghum bagasse (SSB), which is the solid residue in SSJ extraction. The pretreated SSB was fermented in SSJ to produce additional ethanol. (3) Results: CO_2 absorption efficiency of 92.0% was observed. Pretreatment of SSB by the obtained Na_2CO_3 solution resulted in no loss of glucan and only 8.1 wt% loss of xylan. Ethanol yield from glucan in the pretreated SSB was 81.7% theoretical. (4) Conclusions: CO_2 from SSJ fermentation captured as Na_2CO_3 could be used for efficient SSB pretreatment. Further study focusing on pretreatment process optimization is needed.

Keywords: ethanol; fermentation; lignocellulosic biomass; pretreatment; enzymatic hydrolysis; sweet sorghum; CO_2 capture; global warming

1. Introduction

Two of the biggest problems that the world is facing are a dwindling supply of fossil fuels and global warming. To alleviate the problems associated with petroleum-based liquid transportation fuels, technology has been developed for commercial production of ethanol via fermentation of sugarcane juice and corn-based starch. Technologies for the production of ethanol from more abundant and renewable feedstock such as lignocellulosic biomass (in short, biomass) have been developed but have not reached the commercial implementation. Whereas the production of ethanol from sugarcane juice and corn-based starch is relatively simple, bioconversion of biomass is more complex. Before biomass can be hydrolyzed with commercial enzymes to release fermentable sugars for use in ethanol fermentation, it must go through a pretreatment step to remove some of the barriers imparted by lignin, thus opening up the fiber structure for enhancement of enzymatic activity [1]. Techno-economic analysis (TEA) results of biomass bioconversion unequivocally indicated that pretreatment chemicals are the largest operating cost and also one of the largest cost components of the overall process [2–4]. Therefore, in future biorefineries, the use of inexpensive and renewable reagents for biomass pretreatment is highly desirable.

Green liquor, which is an intermediate liquid stream containing Na_2CO_3 and Na_2S as the two major components plus other impurities in smaller quantities generated in a kraft pulp mill, can be used for pretreatment of both woody biomass [5,6] and herbaceous biomass [7,8]. Recently, it was demonstrated that near theoretical saccharification of sweet sorghum bagasse (SSB) pretreated with a simulated green liquor could be obtained after enzymatic hydrolysis [9]. In this study, it was shown that the contribution of Na_2S to the efficiency of the pretreatment process was minimal. In other words, it was shown that a highly efficient pretreatment of SSB could possibly be achieved with a Na_2CO_3 solution without Na_2S under certain conditions. It has been demonstrated that a Na_2CO_3 solution could be produced by absorption of CO_2 from an ethanol fermenter in a NaOH solution and subsequently used for dual purposes, i.e., for pH control and to provide the required carbonate in the fermentative production of succinic acid [10]. Carbon dioxide is the major co-product of ethanol fermentation and also is one of the greenhouse gases that contribute to the increase in temperature of the atmosphere. Several countries, which include the United States, have developed energy and climate policies that give incentives to the capture and sequestration of CO_2 from existing biorefineries. This practice could be valued under low-carbon fuels policy, biofuels mandates, supportive carbon capture and sequestration (CCS) policy, and other climate policy instruments [11].

Considerable efforts have been made in the United States and other countries to develop feedstocks other than corn and sugar cane for ethanol production. Among these, sweet sorghum has attracted considerable interest because of its many good characteristics such as rapid growth and high sugar accumulation, high biomass production potential, wide adaptability, drought resistance, lodging tolerance, and salinity resistance. The ability to withstand severe drought conditions and its high water usage efficiency make sweet sorghum a good ethanol feedstock suitable for cultivation in arid regions such as the southern US and many areas in Africa and Asia [12]. Sweet sorghum juice (SSJ), which can be extracted from the stalks with the same equipment used for extraction of sugarcane juice, contains high levels of sugars that can be readily fermented to ethanol. The residual biomass, i.e., sweet sorghum bagasse (SSB), can also be used as a potential feedstock for ethanol production.

In the present investigation, the technical feasibility of capturing the CO_2 produced in SSJ ethanol production by absorption in a NaOH solution and use of the resultant Na_2CO_3 solution for pretreatment of SSB is demonstrated. The potential use of the pretreated SSB together with SSJ for additional ethanol production also is investigated.

2. Materials and Methods

2.1. Materials

SSJ and SSB were obtained from Delta BioRenewables (Memphis, TN, USA). Upon arrival, both SSJ and SSB were kept in a walk-in freezer. Active dry ethanol red yeast culture (*Saccharomyces cerevisiae*) was provided by Lesaffre Yeast Corporation (Milwaukee, WI, USA). The yeast culture in powder form was kept refrigerated. The enzyme products Cellic CTec2 (cellulase) and HTec2 (hemicellulase) were kindly provided by Novozymes (Franklinton, NC, USA). All chemicals were of reagent grade and purchased from various suppliers.

2.2. Methods

2.2.1. SSJ Fermentation and CO_2 Capture

The fermentation of SSJ was performed in a 7-L fermenter (Applikon Biotechnology, Foster City, CA, USA). The frozen SSJ was thawed and 4.74 L was added to the fermenter. Urea (1.9 g) was added to give a concentration of 0.4 g/L. The inoculum was prepared by suspension of the yeast powder in deionized (DI) water at 5 wt%. The mixture was stirred with a magnetic stir bar at ambient temperature for about 30 min then 4.74 mL was added to the fermenter. The initial pH of the fermentation medium was 5.1. At the end of the fermentation, the pH dropped to 4.4. The fermenter was maintained at

32 °C and agitated at 250 rpm. The CO₂ generated in the fermenter was captured by absorption in a solution of 5 M NaOH. The schematic diagram of the CO₂ absorption apparatus is shown in Figure 1. The off-gas from the fermenter was allowed to enter the bottom of a glass column of 26 inches in total height and 3 inches in diameter. The column was randomly packed with 1-inch stainless steel Intalox saddles. The packing height was 21 inches. The volume of the NaOH solution used for CO₂ absorption was 2 L, which occupied a height of 22 inches in the column. The top of the column was closed with a rubber stopper. A stainless steel tube (1/8-inch in diameter and 3-inch in length) was inserted into the rubber stopper to allow venting of the unabsorbed gas. The fermentation was carried out for 72 h. At the end of the fermentation, a sample was removed from the fermenter, centrifuged on a micro-centrifuge, filtered through a 0.2-micron syringe filter and stored in a freezer for high-performance liquid chromatography (HPLC) analysis of ethanol and other metabolites. The Na₂CO₃ solution was drained from the absorption column. A small sample of about 25 mL was removed for analysis of Na₂CO₃ concentration and the rest was placed in a glass bottle, capped and kept at ambient temperature. Several batches of Na₂CO₃ were prepared and combined before the thoroughly mixed solution was used in the SSB pretreatment experiments.

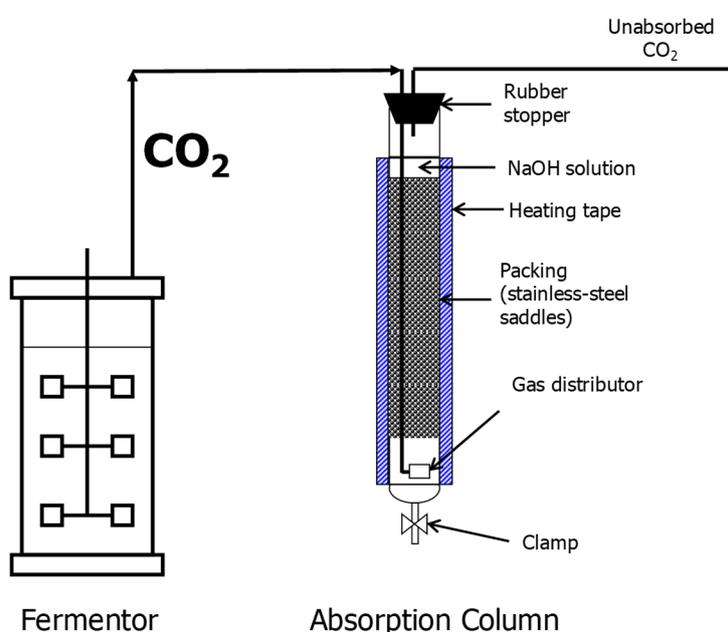


Figure 1. Schematic diagram of the apparatus used for the capture of CO₂ from sweet sorghum juice (SSJ) ethanol fermentation by absorption in 5 M NaOH.

2.2.2. Pretreatment of SSB

The SSB was removed from the freezer, thawed and ground in a Wiley Mill. Ground SSB particles that passed through a 1-mm screen were collected and used for the pretreatment. The pretreatment was performed at two scales. At the small scale, stainless-steel reactors (Figure 2), which were 2.5 cm in diameter and 15 cm in length, were used. In each pretreatment experiment, 4.27 g (dry basis) ground SSB was placed in the reactor then 40 g of the combined Na₂CO₃ solution (described previously) was added. The SSB was allowed to soak in the Na₂CO₃ solution at ambient temperature for 1 h. The reactor then was closed and placed in an oven preheated to 150 °C for 1 h. Upon removal from the oven, the reactor was immediately placed in an ice bath for cooling. The reactor contents were removed, filtered under vacuum on #1 filter paper, and washed with 300 mL DI water. The washed solids were dried in a 55 °C oven for 24 h then were stored in sealed containers at ambient temperature.



Figure 2. Small-scale stainless-steel reactor for sweet sorghum bagasse (SSB) pretreatment study.

At the larger scale, the pretreatment was performed in a 2-L stainless-steel pressure vessel. The solid:liquid ratio was the same as in the case of the small-scale pretreatment experiments. In each experiment, 160.5 g (dry basis) SSB was placed in the reactor and 1500 g combined Na_2CO_3 solution was added. The solids were allowed to soak at ambient temperature for 1 h. The reactor was sealed and placed in the 150 °C oven. Due to the larger quantities of the materials, the pretreatment time was increased to 1.5 h. The reactor was quenched in an ice bath and the reactor contents were removed. The pretreated SSB solids were recovered by centrifugation (600-mL centrifuge bottles filled to about one half, 4000 rpm, 20 min). The supernatant was removed and filtered to recover the lighter solids that floated to the surface during the centrifugation. The recovered solids were added to the pellets in the centrifuge bottles. For washing, the combined light materials and pellets were resuspended in DI water, thoroughly mixed, and centrifuged again. The operation was performed three times. The total amount of DI water used for washing was scaled up proportionally to the amount of solids to give the same ratio of wash water:pretreated solids as used in the small-scale pretreatment. The washed SSB solids were recovered, spread out on aluminum foil, dried in the 55 °C oven for 24 h, and stored in sealed containers at ambient temperature.

In the pretreatment of SSB, several batches were prepared for each scale. The corresponding pretreated SSB batches then were ground in a coffee grinder, combined, and thoroughly mixed before they were used in the fermentation experiments.

2.2.3. Fermentation of Pretreated SSB in SSJ

Fermentation of the pretreated SSB in SSJ was performed in 125-mL flasks using the SSB pretreated in the small-scale pretreatment reactors. In these experiments, 20 mL SSJ was placed in each flask and 1.2 g pretreated SSB (1.1 g dry) then was added. The SSB solid loading, therefore, was 5.2 wt% (based on the total mass). Urea was added to 0.4 g/L of SSJ. The inoculum was prepared as described previously. The 5 wt% yeast suspension then was added at 0.2 mL/flask. Finally, the enzymes CTec2 and HTec2 were added at 110 μL each per flask. The enzyme loadings, therefore, were 0.1 mL/g dry solids for each enzyme. The flasks were capped with rubber stoppers having a hypodermic needle punctured through for CO_2 venting and incubated in an incubator shaker at 32 °C and 115 rpm for 72 h. Control experiments where only SSJ was used without the pretreated SSB and the enzymes were performed following the same procedure. At the end of the experiments, samples were removed from the flasks for HPLC analysis as described previously. The experiments were performed in triplicate and the average results are reported. Control experiments where only SSJ was used without addition of the pretreated SSB and enzymes also were performed. The experiments were performed in triplicate and the average results are reported.

2.2.4. Fermentation of Untreated SSB in SSJ

To verify the effect of pretreatment, experiments were also performed using untreated SSB in SSJ. The experimental procedure was the same as in the case of treated SSB in SSJ described previously. Control experiments where only SSJ was used without untreated SSB were also performed. Due to the

exhaustion of the SSJ that was used in the experiments performed with the pretreated SSB, a different batch of SSJ was used in the experiments performed with the untreated SSB and the corresponding control experiments.

2.2.5. Analytical Methods

The moisture contents of various solid samples were determined by drying about 3 g of materials in a moisture balance (Model MB45, Ohaus, Parsippany, NJ, USA). The determinations were performed in triplicate and the average results are reported.

The compositions of the raw and pretreated SSB were determined according to the NREL's procedure [13]. To prevent interferences during the compositional analysis by the free sugars that were already present, the samples were thoroughly washed in DI water and dried in an oven maintained at 110 °C prior to use in the analysis.

HPLC was used for analysis of the SSJ and fermentation samples. The system was an Agilent Technologies system equipped with a refractive index (RI) detector. Glucose, fructose, and ethanol were analyzed by HPLC series 1200 equipped with an Aminex[®] HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) using a 0.5 wt% H₂SO₄ solvent. Sucrose and xylose were determined by HPLC series 1100 equipped with an Aminex[®] HPX-87P column using chromatography-grade water as solvent. In both cases the columns were operated at 60 °C and the solvents were pumped at 0.6 mL/min.

The concentrations of Na₂CO₃ in the solutions obtained by absorption of the CO₂ co-product of SSJ ethanol fermentation in solutions of 5 M NaOH were determined by a method used in a previous study [10], which was slightly modified from the method developed by Sutton [14]. Thus, 3 mL sample was added to 30 mL 10% (*w/v*) BaCl₂ (Fisher Scientific, Pittsburg, Pennsylvania) in a 50-mL centrifuge tube. The mixture was thoroughly mixed then centrifuged. The supernatant was discarded and the barium carbonate pellet was washed with 20 mL ice-cold DI water before the tube was centrifuged again. This step was repeated twice. The tube containing the washed barium carbonate pellet then was dried in a 50 °C oven and weighed to determine the weight of the dry pellet, which was used to calculate the amount of the carbonate salt in the sample.

3. Results

3.1. SSJ Fermentation and CO₂ Capture

The results of the SSJ ethanol fermentation experiments and the characteristics of the Na₂CO₃ obtained by absorption of the CO₂ produced in 5 M NaOH solution are summarized in Table 1. The initial sugar concentrations in the fermentation media were determined to be 60.9 g/L glucose, 46.4 g/L fructose, and 52.2 g/L sucrose. The ethanol yield, therefore, was 85.2% of the theoretical value. The total ethanol produced was calculated to be 334.2 g. In ethanol fermentation, the stoichiometric production of CO₂ is 1 mol CO₂ (44 g) per mol ethanol (46 g). Therefore, the total CO₂ production was 319.5 g or 7.3 mol. Since the volume of the 5 M NaOH solution used for CO₂ absorption was 2 L, the total quantity of CO₂ absorbed was 4.6 mol, which gave 63.0% CO₂ removal efficiency. Stoichiometrically, 5 mol CO₂ could be absorbed into 2 L of 5 M NaOH solution. The absorption efficiency, therefore, was 92.0%.

Table 1. The results of SSJ ethanol fermentation and capture of the produced CO₂ by absorption in 5 M NaOH solution.

Results of Ethanol Fermentation			
Final Ethanol Concentration (g/L)	Ethanol Yield (% theoretical)	Total Ethanol Production (g)	Total CO ₂ Production (g)
64.7 ± 1.4	85.2	334.2	319.5 (7.3 mol)
Characteristics of Na ₂ CO ₃ Solution			
pH	Na ₂ CO ₃ concentration (M)	Absorption efficiency (%)	CO ₂ removal efficiency (%)
11.2	2.30 ± 0.01	92.0	63.0

3.2. Pretreatment of SSB

The compositions of the raw and pretreated SSB are summarized in Table 2. The total amounts of solids recovered after the pretreatment using the small-scale reactor were determined and used for mass balance calculation, which indicated no loss of glucan, 8.1% loss of xylan, 24.7% loss of arabinan, and 37.7% loss of lignin. The removal of lignin during the pretreatment caused the increases in glucan and xylan contents in the pretreated SSB.

Table 2. Compositions and mass balance of SSB pretreated with Na₂CO₃ solutions obtained by absorption of the CO₂ produced in SSJ fermentation in 5 M NaOH.

	Glucan (%)	Xylan (%)	Arabinan (%)	AI lignin (%)	AS lignin (%)	Total Lignin (%)
Raw SSB	38.3 ± 3.9	20.6 ± 1.9	2.3 ± 0.3	17.7 ± 1.7	1.2 ± 0.2	18.9 ± 1.8
Pretreated SSB in small reactor	47.0 ± 0.9	23.1 ± 0.4	2.1 ± 0.1	13.5 ± 0.3	1.0 ± 0.0	14.4 ± 0.3
Pretreated SSB in large reactor	44.6 ± 1.5	24.4 ± 1.0	2.8 ± 0.1	14.7 ± 0.8	1.5 ± 0.0	16.2 ± 0.8
Loss during pretreatment in small reactor (%)	0	8.1	24.7	37.8	35.0	37.7

3.3. Fermentation of Pretreated SSB in SSJ

The results of fermentation of the pretreated SSB in SSJ at 5.2 wt% solid loading are summarized in Table 3. With addition of the pretreated SSB, the final ethanol concentration was increased by 10 g/L. The theoretical ethanol yield from glucan in the pretreated SSB was calculated to be 81.7%. The final xylose concentration was 7.5 g/L, which indicated 56.9% conversion of xylan in the pretreated SSB. The theoretical yield calculations are shown in Appendix A.

Table 3. Results of ethanol fermentation using Na₂CO₃-pretreated SSB in SSJ.

	Ethanol (g/L)	Xylose (g/L)	Ethanol Produced from SSB (g/g Pretreated SSB)	Xylose Yield from Xylan in SSB (% Theoretical)	Ethanol Yield from Glucan in SSB (% Theoretical)
SSJ only (control)	65.4 ± 2.1				
SSJ + Pretreated SSB	75.3 ± 1.3	7.5 ± 0.7	0.15	56.9	81.7

The results of fermentation of the untreated SSB in SSJ are summarized in Table 4. Calculations performed using the procedure that is described in Appendix A indicated that there was no increase in the quantity of ethanol produced when the untreated SSB was added to the SSJ. In other words, there was insignificant enzymatic hydrolysis of glucan in the untreated SSB. The final xylose concentration was 1.3 g/L, which indicated only 11.0% conversion of xylan in the untreated SSB.

Table 4. Results of ethanol fermentation using untreated SSB in SSJ.

	Ethanol (g/L)	Xylose (g/L)	Ethanol Produced from SSB (g/g Pretreated SSB)	Xylose Yield from Xylan in SSB (% Theoretical)	Ethanol Yield from Glucan in SSB (% Theoretical)
SSJ only (control)	68.2 ± 0.1				
SSJ + Pretreated SSB	69.5 ± 0.6	1.3 ± 0.1	0	11.0	0

4. Discussion

In the present study, a process has been developed for the production of ethanol from SSJ and SSB together in a single fermenter. The CO₂ produced in SSJ fermentation was captured in a NaOH solution and the resultant Na₂CO₃ solution was used for pretreatment of the SSB to facilitate high-efficiency enzymatic hydrolysis of the pretreated material. In theory, the whole sorghum plant can be cut into small pieces and used for ethanol fermentation. However, from a process engineering point of view, the use of the whole plant suffers from several disadvantages. These disadvantages include: (1) Difficulty in maintaining uniform temperature profiles in a heterogeneous system to achieve the optimum metabolic activity of the yeast; (2) restricted access of the sugars in the unextracted juice to the yeast; and (3) significant quantities of sugars in the juice may be destroyed during the pretreatment process, which is required for high-efficiency enzymatic hydrolysis of the fibers. In the proposed process, instead of escaping to the atmosphere, the CO₂ produced in the SSJ fermentation was captured and fixed as Na₂CO₃. The use of the resultant Na₂CO₃ for pretreatment of the SSB undoubtedly will generate a waste stream containing residual NaOH, Na₂CO₃, and lignin. This waste stream is characteristically similar to the waste waters generated in a Kraft paper mill but will have much lower strengths because the reagent used is Na₂CO₃, which will result in much lower lignin solubilization compared to strong NaOH solutions typically used in Kraft mills. Treatment of the waste stream generated in the proposed process, therefore, is expected to be much less expensive than paper mill wastewater treatment. Techno-economic analysis (TEA) is needed to verify this point. A TEA, however, is not within the scope of the present study.

The results shown in Table 1 indicated that 5 M NaOH solution was quite efficient for CO₂ absorption. The absorption efficiency was determined to be 92.0%. With the apparatus used in the study, 63.0% of the CO₂ produced from the ethanol fermenter was removed as Na₂CO₃. Since the absorption efficiency was quite high at 92.0%, it is anticipated that most of the rest of the CO₂ produced probably could be removed in a second absorption column using 5 M NaOH solution. The feasibility of using the resultant Na₂CO₃ solution for SSB pretreatment also was demonstrated. Under the conditions used for the pretreatment, 37.7% of lignin was removed. On the other hand, the pretreatment resulted in no loss of glucan and only 8.1% loss of xylan. The loss of arabinan was higher at 24.7%. However, arabinan normally is only a minor source of fermentable sugar due to its low content in most biomass feedstocks. The arabinan content in the SSB was only 2.3 wt% (Table 2). The loss of arabinan, therefore, will not be a strong negative factor on the subsequent bioconversion of the pretreated biomass for production of fuels and value-added chemicals. In a previous study using simulated green liquor for pretreatment of SSB, under the conditions optimized for production of fermentable sugars by enzymatic hydrolysis, the retentions of glucan and the combined xylan + arabinan + galactan were

91.8 wt% and 76.7 wt%, respectively [9]. The results obtained in the present study, therefore, compared quite favorably to those obtained with synthetic $\text{Na}_2\text{CO}_3/\text{Na}_2\text{S}$ solutions.

The Na_2CO_3 solution used in the present study actually was not pure Na_2CO_3 but rather a mixture of 2.3 M Na_2CO_3 and 0.4 M NaOH (1.6% *w/v* NaOH). In the study by Cao et al. [15], SSB was pretreated with 2% *w/v* NaOH for 1 h at 121 °C, which was significantly lower than the temperature used in the present study. These investigators observed only 6.6% loss of glucan but 67.8% loss of hemicellulose. In their enzymatic hydrolysis using 2 wt% solid loading, which was lower than the solid loading used in the present study, 72.1% theoretical yield of glucose was observed. All these results clearly demonstrate the advantage of using the 2.3 M Na_2CO_3 solution obtained by absorption of ethanol fermentation-derived CO_2 in a 5 M NaOH solution compared to the use of pure NaOH for pretreatment of SSB.

The results of the ethanol fermentation using the pretreated SSB in SSJ (Table 3) clearly demonstrated the high efficiency of the pretreatment using the Na_2CO_3 solution obtained by absorption of the ethanol fermentation co-product CO_2 in 5 M NaOH solution. As shown in Table 3, the ethanol yield from glucan in the pretreated SSB was 81.7% of the theoretical value. Since there was no loss of glucan during the pretreatment process, this value also is true for glucan in the original SSB. The ethanol yield was calculated based on glucan because the yeast strain used in this study was only capable of metabolizing glucose but not the C5 sugars (xylose and arabinose). The yield of xylose was relatively low at 56.9% of the theoretical value based on the xylan content of the pretreated SSB. A xylose yield value of about 70% or higher probably will be more desirable. However, it should be noted that the fermentation was a simultaneous saccharification and fermentation (SSF) process. In other words, the hydrolysis of the pretreated SSB proceeded in the presence of ethanol, which has been shown to be a potential inhibitor of cellulases and hemicellulases [16]. Higher xylose yield can possibly be achieved in a modified process that includes a biomass hydrolysis stage at the optimum temperatures of the cellulolytic enzymes (50–55 °C) for 24 h followed by lowering the temperature to 30–32 °C and inoculation with a yeast culture to start the SSF process. It is likely that higher ethanol yield from glucan can also be achieved in this modified process.

5. Conclusions

The feasibility of capturing the CO_2 produced in SSJ ethanol fermentation by absorption in 5 M NaOH and use of the resultant Na_2CO_3 solution in pretreatment of SSB for subsequent ethanol production at high efficiency was demonstrated. The process developed, however, needs to be further investigated. In the future study, optimization of the pretreatment needs to be performed and the results used to establish detailed mass balance of the integrated process. A TEA of the integrated process also is highly recommended.

Declaring: Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA). USDA is an equal employment provider and employer.

Author Contributions: N.P.N. conceived the concept idea, designed the experiments, interpreted the experimental results, performed the calculations, and prepared the manuscript. M.J.T. performed the experiments and sample analyses, and assisted in interpretation of the experimental results.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

1. SSJ ethanol fermentation:

Basis: 1 L of SSJ.

Mass balance equation:

$$V_{T1} = 1 + V_{E1}, \quad (A1)$$

where, V_{T1} = total final volume and V_{E1} = volume of ethanol produced.

Since the final ethanol concentration was 65.4 g/L (0.0654 kg/L) and the density of ethanol is 0.79 kg/L, $V_{E1} = (0.0654 \times V_{T1}) \div 0.79 = 0.0828 V_{T1}$. Substitution of this value into equation A1 and solving for V_{T1} gives $V_{T1} = 1.0903$ L. The amount of ethanol produced, therefore, was 0.0903 L or 71.3 g.

2. SSB-in-SSJ ethanol fermentation:

Basis: 1 kg total mass (0.052 kg SSB and 0.948 kg water).

Mass balance equation:

$$V_{T2} = 0.948 + V_{E2}, \quad (A2)$$

where, V_{T2} = total final volume and V_{E2} = volume of ethanol produced.

Since the final ethanol concentration was 75.3 g/L (0.0753 kg/L), $V_{E2} = (0.0753 \times V_{T2}) \div 0.79 = 0.0953 V_{T2}$. Substitution of this value into equation A2 and solving for V_{T2} gives $V_{T2} = 1.0479$ L. The amount of ethanol produced, therefore, was 0.0999 L or 78.9 g. The amount of ethanol produced from SSJ only was 71.3 g/L \times 0.948 L = 67.6 g. The amount of ethanol produced from the pretreated SSB, therefore, was 78.9 g – 67.6 g = 11.3 g.

Since the glucan content of the pretreated SSB was 47.0%, the theoretical yield of ethanol from glucan was 52 g SSB \times 0.47 (g glucan/g SSB) \times 1.11 (g glucose/g glucan) \times 0.51 (g ethanol/g glucose) = 13.84 g ethanol. The ethanol yield, therefore, was (11.3 g \div 13.84 g) \times 100% = 81.7% of theoretical value.

Since the xylan content of the pretreated SSB was 23.1%, the theoretical yield of xylose from xylan was 52 g SSB \times 0.231 (g xylan/g SSB) \times 1.15 (g xylose/g xylan) = 13.81 g xylose. The amount of xylose produced was 7.5 (g/L) \times 1.0479 = 7.86 g. The xylose yield, therefore, was (7.86 g \div 13.81 g) \times 100% = 56.9% of the theoretical value.

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