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Effects of NaOH and Na₂CO₃ pretreatment on the saccharification of sweet sorghum bagasse

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In this work, the chemical composition, chemical structures, and sugar release were evaluated for sweet sorghum bagasse (SSB) biomass in response to alkaline pretreatments and enzymatic hydrolysis. Five different ratios of 2 M sodium hydroxide (NaOH) and sodium carbonate (Na₂CO₃) solutions were used for biomass fractionation along with two heat treatment conditions, 85°C and 150°C for 1 h. Sugar yields from enzymatic hydrolysis were measured at different durations up to 72 h. SSB samples pretreated at high temperature using alkaline solutions containing equal amounts of Na₂CO₃ and NaOH favored retaining cellulose content (up to 90%) while effectively removing hemicellulose (65%) and lignin (92%). The high-temperature pretreatment conditions resulted in improved delignification, higher sugar concentrations, and sugar yields. Pretreatment solutions consisting of 50% or more NaOH produced higher glucose yields (85%–90%) and total sugar concentrations. Moreover, pretreatment solutions containing Na₂CO₃ improved the hydrolysis rates allowing SSB samples reach maximum yields faster than those without it, 48 versus 72 h. Results showed pretreatment methods combining NaOH and Na₂CO₃ were effective at promoting SSB biomass conversion, increasing sugar recovery after hydrolysis, and reducing the hydrolysis duration, all of which are desirable and cost beneficial.

KEYWORDS

sweet sorghum bagasse, biomass pretreatment, NaOH, Na₂CO₃, enzymatic hydrolysis, sugar yields

1 Introduction

Current energy, economic, and climatic factors are driving the search for fossil fuel substitutes that are renewable, locally accessible, and reduce greenhouse gas emissions (Condon et al., 2015; Paris, 2018; Gustavsson et al., 2021; Clauser et al., 2021). Lignocellulosic sources play a key role in these sectors due to their abundance of natural biopolymers found in plant cell walls. Composition varies depending on species, cultivar, and growing environment, but in general lignocellulosic plant cell walls contain cellulose (40%–50%), hemicellulose (20%–35%), and lignin (10%–25%) (de Jong, 2014; Lynd et al., 2017; Chandel et al., 2020; Zhang et al., 2021). All three components can be utilized in conversion processes that aim to produce bio-based fuels, chemicals, and materials (Baral et al., 2019). Regional variables, such as climate and soil conditions, are primary factors driving the decision-making of cultivating particular types of agricultural feedstocks. In the United States (U.S.) corn is cultivated throughout the Midwest due to

these favorable conditions that offer high yields per acre which then allows the subsequent corn grain to be used in fermentation processes to generate ethanol for biofuel applications (Rausch et al., 2019). Corn cultivation also produces corn stover that can be used as a dedicated bioenergy crop. Other bioenergy crops such as switchgrass and wheat straw are grown in high yields in the U.S. due to similar favorable growing conditions (Dai et al., 2016; Jesus et al., 2016). The vast geographic size and variable weather conditions in the U.S. are also suitable for producing less valorized crops such as sweet sorghum.

In 2023, the U.S. produced approximately 5.3 million acres of sweet sorghum valued at over $1.2 billion and projections for 2024-2025 range between 279 and 373 million bushels (Ates et al., 2024). Sweet sorghum bagasse (SSB) is an attractive biomass for second-generation fuel ethanol production given its high fermentable sugar content, high biomass yields, and availability (Zhang et al., 2018; Nunta et al., 2023). Moreover, sweet sorghum is a versatile feedstock capable of enduring different environments and easily convertible into a variety of value-added products such as fuel, fertilizers, and food products (Nghiem and Toht, 2019; Lo et al., 2020; Hu et al., 2022). To efficiently extract lignin-derived aromatics and polysaccharide-derived sugars from the biomass, a variety of biofuel, mechanical, chemical, and thermal pretreatment methods are employed to promote cleavage of molecular bonds. This fractionation increases surface area, thus enabling access to polysaccharides during enzymatic hydrolysis to release sugars for fermentation (Venturin et al., 2018; Bhatia et al., 2020; Li et al., 2022; Hamadou et al., 2023). The general composition of SSB consists of 35%–50% cellulose, 25%–35% hemicellulose, and 20%–30% lignin (Mamma et al., 1995; Piskorz et al., 1998). Previous work presented SSB compositions of 32.3% cellulose, 21.2% hemicellulose, and 8.3% lignin (Dar et al., 2018). Moreover, the composition of sweet sorghum on a dry basis has been measured to be 36% total sugar content, 14%–16% lignin, 3.2%–4% ash, and 46%–48% combined cellulose and hemicellulose. While on a sugar-free basis results were 21.9%–25% lignin, 5%–6.3% ash, and 71.9%–75% combined cellulose and hemicellulose (Piskorz et al., 1998). The composition and sugar yields of sweet sorghum biomass have been previously studied using different parts of the plant and its juice (Kim and Day, 2011; Goshadrou et al., 2011; Yue et al., 2021). For example, sorghum stalks were reported to contain 31%–36.8% cellulose, 22%–29.7% hemicellulose, and 13.9%–22% lignin (Kamireddy et al., 2013; Deshavath et al., 2022). Additional results have shown sorghum stalk juice with a sugar content of 16%–28.1%, able to produce 40–86 L ton^{-1} ethanol yield (Jia et al., 2013; Ekefre et al., 2017).

Current key challenges for biomass valorization include identifying efficient biomass-specific pretreatment methods, developing technologies for processing lignocellulose and lignin-derived products, and reducing fermentative inhibitors from pretreated biomass (Zhao et al., 2022). The choice of pretreatment chemistry plays a significant role in determining process performance. Acidic pretreatments function well by disrupting the chemical bonds between plant cell wall components by hydrolyzing polysaccharide fractions, but degradation by dehydration to sugar monomers can easily occur at too high of processing severity (Junior et al., 2020). Prior research using dilute acid pretreatment on SSB could recover up to 100 g L^{-1}

of monomeric sugars following enzymatic hydrolysis, although the byproduct formation of acetic acid and furfural are toxic inhibitors to most microbial fermentation processes (Qureshi et al., 2018). Ammonia pretreatments, which fall under alkali chemistry, are also effective on lignocellulose and function by re-orienting the surface structures of the plant cell wall instead of extracting components (Li et al., 2010; Yang and Rosentrater, 2017). Pretreatments that function by extracting and fractionating plant cell wall components are mostly applied using other types of alkaline chemistry or organosolv. Typically, alkaline pretreatments using sodium hydroxide (NaOH) are performed at high temperatures because it helps activate peeling reactions that remove hemicellulose components from the plant cell wall (Hutterer et al., 2016). Organosolv pretreatments can be conducted using several water-to-organic solvent combinations and with or without an acid or alkali catalyst, however, the pretreatment goal remains to produce separate streams of relatively pure polysaccharides remaining in the pretreated lignocellulose, and a dissolved stream of extracted lignin (Guragain et al., 2016). Enzymatic digestibility of SSB has been evaluated with different pretreatment techniques and alkaline mixtures (Zhang et al., 2011; Cao et al., 2012). Alkaline pretreatments on SSB have shown NaOH to be an effective low-energy input reagent compared to others as it alters lignin structures, loosening xylan cross-links, and partially removing hemicellulose (Karp et al., 2015; Kim et al., 2016; Loow et al., 2016; Mafa et al., 2020). Samples pretreated with NaOH have been shown to interact with the ester and ether bonds in hemicellulose and lignin, making these functional groups and linkage structures easier to remove (Wang et al., 2020). The efficiency of chemical pretreatment depends on NaOH concentration, treatment time, temperature, and biomass characteristics (Mirahmadi et al., 2010). Additionally, alkaline pretreatment with NaOH and sodium carbonate (Na_2CO_3) has been demonstrated to improve the digestibility of cellulose by effectively removing the lignin (Yuan et al., 2021). Mild Na_2CO_3 pretreatments have demonstrated to be effective in reducing biomass recalcitrance (Mirmohamadsadeghi et al., 2016). Ideally, all complex lignin structures are removed before hydrolysis as they reduce the accessibility of enzymes to cellulose by blocking binding sites which are essential for the production of bioethanol (Jin et al., 2013; Gao et al., 2014; Huang et al., 2022). The use of enzymes for processing the biomass is advantageous when compared to the other methods as they have shown to be of lower costs of downstream treatment, simpler operation, and lower energy consumption (Meng and Ragauskas, 2014).

This research aims to further understand the impact of combining NaOH and Na_2CO_3 for pretreating lignocellulose. Prior research has demonstrated opportunities for a circular economy strategy by reusing captured carbon dioxide CO_2 outgassed from ethanol fermentation and absorbed in a solution of NaOH at high efficiency to produce an aqueous Na_2CO_3 solution that serves as a suitable medium for chemical pretreatment (Nghiem and Toht, 2019). When using this recovered solution as a pretreatment medium on corn stover, delignification still occurred at a high level and over 90% glucose yields could be achieved after enzymatic hydrolysis (García-Negrón and Toht, 2022). Both the continuous reuse of CO_2 and reducing the fraction of NaOH in alkaline pretreatments with Na_2CO_3 are promising steps towards lowering biomass processing costs with

environmentally safer approaches (Medina-Martos et al., 2022). The objective of this work is to evaluate the effects of NaOH and Na_2CO_3 pretreatments and temperature severity on sweet sorghum bagasse's chemical composition, chemical structures, and hydrolysis yields. Samples of SSB biomass were pretreated with varying concentrations of NaOH and Na_2CO_3 alkaline solutions and exposed to two heat treatments, low and high temperature. The performance of enzyme digestibility of SSB as a function of hydrolysis duration is also evaluated by determining the sugar yields of glucose, xylose, and arabinose via analytical method. This study helps improve the conversion of lignocellulosic biomass into sugars for potential utilization in the agriculture sector and value-added products.

2 Materials and methods

2.1 Pretreatment

Sweet sorghum bagasse was obtained from Delta Biorenewables (Memphis, TN, USA) and milled in a Wiley Mill with a screen size of 1 mm. Pretreatment consisted of mixing the SSB in a solution of Na_2CO_3 (from Sigma Aldrich) and NaOH (from Fisher Scientific) followed by a heating step. Five different 2 M ratios of Na_2CO_3 and NaOH were mixed in a 30 mL alkaline solution with 5% (w/v) solid loading. The ratios of Na_2CO_3 to NaOH were: 100:0, 75:25, 50:50, 25:75, and 0:100. Control experiments included untreated samples washed with water multiple times, then filtered and dried to remove the juices from the raw material. For *Treatment A*, the biomass solution was placed in a 50 mL centrifuge tube and heated to 85°C, then held to 1 h in a water bath. Low-temperature treatments have been shown to be effective and desirable for biomass processing (Wang et al., 2022). For *Treatment B*, the biomass solution was placed in a small-scale stainless-steel reactor and held to 1 h in a convection oven to 150°C [refer to Figure 2 from Nghiem and Toht (2019) for details of the small reactor assembly]. After the heat treatment, the samples were cooled in an ice water bath for 20 min. Samples were removed from the containers and filtered under vacuum with a filter paper grade 1 from *Whatman*, then washed with distilled water to remove the residual alkaline solution. Then, the recovered solids were dried in a convection oven to 55°C for 24 h and stored at room temperature.

2.2 Fourier-transform infrared (FTIR) spectroscopy

FTIR analysis with attenuated total reflectance (ATR) was conducted using a *Spectrum 3* spectrometer (from Perkin Elmer, Inc). In ATR mode, infrared radiation has a high refractive index that allows radiation to reflect within the sample based on the specific vibration modes of chemical bonds corresponding to the energy levels of the molecules. Test samples were dried to 55°C for at least 12 h in a convection oven before FTIR analysis. Spectroscopy experiments were performed at room temperature and configured to a frequency range of 650–4,000 cm^{-1}, a resolution of 32 cm^{-1}, and 32 scans. Triplicate spectra were collected for each pretreated SSB sample.

2.3 Scanning electron microscopy (SEM)

Dried and milled biomass samples were mounted on stubs with carbon tape and gold sputter coated for 1 min (EMS150R ES from Electron Microscopy Sciences, Hatfield, PA, USA). Micrographs were collected with a *FEI Quanta 200 F* scanning electron microscope (Hillsboro, OR, USA), set to an accelerating voltage of 5 kV in high vacuum mode and at 500 times magnification.

2.4 Enzymatic hydrolysis

Hydrolysis experiments were performed for both the initial untreated biomass and the pretreated samples. For each case, 5.1 g of dry biomass was placed in a 250 mL Erlenmeyer flask at 10% (w/v) solid loading in a citric acid buffer with a concentration of 50 mmol L^{-1} and pH of 5. The enzymes used were *Cellic CTec2* (a blend of cellulase, β-glucosidases, and hemicellulase) and *HTec2* (endoxylanase with cellulase background hemicellulase) from Novozymes (Franklinton, NC, USA). The enzyme loadings with respect to dry biomass were 0.075 mL of *CTec2* g^{-1} and 0.025 mL of *HTec2* g^{-1}. Each flask was sealed with a stopper and parafilm to prevent water loss. The hydrolysis took place in a shaker incubator at 50°C and 160 revolutions per minute in triplicates for each sample. Hydrolyzed samples were collected at 0.5, 1, 2, 4, 6, 24, 48, and 72 h and kept frozen until subjected to carbohydrate analysis (glucose, xylose, and arabinose) via high-performance liquid chromatography (HPLC). Saccharification yields for glucose, xylose, and arabinose were calculated using equations described in Zhu et al. (2011); Goshadrou et al. (2011); Tye et al. (2016); Velasco et al. (2017). After 72 h of hydrolysis, the recovered solids were filtered and the hydrolysate content was frozen.

2.5 Analytical methods

The laboratory analytical procedure from the National Renewable Energy Laboratory (NREL-TP-510-42618) was used to perform composition analysis on the untreated and pretreated biomass before and after enzymatic hydrolysis. After pretreatment, the dried solids were measured in triplicates, while after saccharification, the recovered solids were measured once due to the limited amount of solids recovered.

Sugar analysis was performed on the pretreated biomass samples using an *Agilent HPLC Series 1,200*. The collected frozen samples were thawed for the analysis and centrifuged to separate the liquid from the solids and then filtered. Calcium carbonate from Ricca Chemical Company (Arlington, TX, USA) was added to neutralize the aliquot and mixed with a vortex for 10 s. After reaching pH neutrality, liquid samples were filtered into HPLC vials with a 0.2 μm filter. A sample of 10 μL was added to a 1.5 mL HPLC vial with an insert from which two samples were set for injection of 5 μL each. The reference used was NREL-TP-510-42618. The column used was *Aminex® HPX-87H* ion exclusion from Bio-Rad Laboratories (Hercules, CA, USA) operated at 60°C. The solvent used was 0.5 wt% H_2SO_4, pumped at a 0.6 mL min^{-1} flow rate.

TABLE 1 Composition and mass balance analysis for untreated and pretreated SSB samples. For each lignocellulosic component, statistical differences ($p < 0.05$, $df = 9$, $n = 60$) between composition groups, with respect to samples and treatments, are represented with superscript letters. Mass losses are presented in parenthesis below the composition values.

	Sample[1]	Cellulose[5] (%)	Hemi-cellulose[5] (%)	Total lignin (%)	Ash (%)	Solid yield after pretreatment[5] (%)
	SSB-W[2]	35.78	24.38	24.49± 1.13	2.47± 0.99	
Treatment A[3]	100:0	45.91[a](41.36)	29.72[ac](44.29)	16.96± 1.14[a] (68.35 ± 1.18)	0.31±0.30	45.7
	75:25	51.82[ac](41.05)	29.70[ac](50.42)	13.50± 0.34[ab] (77.56 ± 0.38)	0.45±0.13	40.7
	50:50	59.39[ab](16.18)	21.08[ac](56.54)	8.81± 0.25[ab] (81.83 ± 0.29)	0.64±0.04	50.5
	25:75	64.49[ab](44.67)	17.69[a](77.72)	8.07± 0.38[ab] (89.89 ± 0.42)	0.64±0.04	30.7
	0:100	70.27[b](47.76)	12.87[b](85.96)	7.75± 0.92[ab] (91.58 ± 0.96)	0.04±0.04	26.6
Treatment B[4]	100:0	52.71[ac](39.75)	31.88[c](46.52)	8.84± 0.72[ab] (85.24 ± 0.76)	0.05±0.08	40.9
	75:25	53.40[ac](28.81)	26.59[ac](47.98)	7.57± 0.12[ab] (85.26 ± 0.16)	0.65±0.32	47.7
	50:50	66.74[bc](10.84)	17.73[ac](65.24)	4.10± 0.28[ab] (91.98 ± 0.32)	0.76±0.20	47.8
	25:75	69.64[bc](52.31)	15.18[b](84.75)	4.17± 0.77[ab] (95.83 ± 0.81)	0.51±0.12	24.5
	0:100	79.95[b](48.61)	6.98[b](93.42)	2.93± 0.13[b] (97.25 ± 0.17)	0.00±0.00	23.0

[1]Na_2CO_3:NaOH.

[2]SSB-W, represents untreated washed samples.

[3]Pretreatment at 85°C for 1 h.

[4]Pretreatment at 150°C for 1 h.

[5]Composition and mass loss measurements with respective standard deviations no greater than ±0.02 and ±0.06.

2.6 Statistical methods

Statistical analyses were applied to determine significant factors between the different biomass pretreatments, composition measurements, and sugar yields. The independent variables consisted of the pretreatment ratios and heat treatment severity, in single ($df = 4$) and multiple ($df = 9$) variable forms. The evaluations on SSB composition considered three response variables: cellulose, hemicellulose, and lignin compositions. For the analyses on sugar yields, the three response variables were glucose, xylose, and arabinose concentrations, in g L^{-1}. Statistical analyses were carried out using R 4.1.2 via RStudio and with support from library functions found in the Comprehensive R Archive Network, specifically *car (v3.1.2)*, *dunn.test (v1.3.6)*, *corrplot (v0.92)*, and *mvnormtest (v0.1.9.3)* (R Core Team, 2024).

Visual (e.g., histogram, boxplot, Q-Q plot) and statistical (e.g., Shapiro-Wilk) normality tests were applied to biomass composition and sugar concentrations. Although measurements were assumed to belong to normal distributions, inconclusive normality results were attributed to using a limited count of sample measurements ($n = 30$, $n = 60$) with respect to the number of independent variables and groups. Therefore, non-parametric rank-based statistical tests were used to evaluate the data. Spearman rank correlation tests were used to establish linear relationships between the pretreated biomass compositions and the independent variables. Homogeneity of variances was evaluated using the non-parametric Fligner-Killeen test, for which no statistically significant differences were identified between the sample variances for all composition and sugar measurements. Single and multivariate Kruskal–Wallis tests revealed the existence of statistically significant differences between the independent variables with respect to lignocellulosic content and sugar yields. Finally, Dunn's tests, configured with the Bonferroni adjustment for multiple comparisons, were used as *post hoc* tests for pairwise comparisons of biomass compositions between the independent groups.

3 Results and discussion

3.1 Pretreatment

Preteated SSB biomass using varying concentrations of NaOH and Na_2CO_3 exhibited different levels of fractionation. Table 1 presents the composition and mass balance analysis of pretreated SSB biomass samples for two heating methods, *Treatments A* and *B*. Overall, cellulose was the most abundant polymer in the biomass structures, followed by hemicellulose and lignin. The composition of untreated samples consisted of 35% cellulose, 24% hemicellulose, and 24% lignin. For pretreated samples, the respective average content of cellulose, hemicellulose, and lignin were 58, 22, and 11% for *Treatment A*, and 64, 19, and 5% for *Treatment B*. The total lignin measurements took into account insoluble and soluble lignin where the former composed more than 80% of the total lignin. The remaining 5%–10% content was attributed to ash, other carbohydrate structures, extractives, and impurities. Untreated and pretreated samples, respectively, produced less than 1% and 2.5% of ash content.

OH^- ions formed from the NaOH and water solutions helped break down the cell walls in the plant's structure, containing hemicellulose and lignin, thus facilitating access to cellulose. The higher temperature of *Treatment B* promoted solubilization, delignification, and additional chemical extraction of hemicellulose. Samples pretreated with 100% NaOH reached maximum compositions of cellulose (70.27%, 79.95%) and

minimum compositions of both hemicellulose (12.87%, 6.98%) and lignin (7.75%, 2.93%) for *Treatments A* and *B*, respectively. However, alkaline pretreatment solutions from *Treatment A* with 100, 75, 50, and 25% of Na_2CO_3 changed chemical structures into approximately 65, 73, 84, and 91% cellulose content relative to using solely NaOH, while for *Treatment B* the relative contents were 65, 66, 83, and 87%. Linear correlations of biomass compositions indicated cellulose (0.95) content increased with respect to pretreatments with more NaOH, and conversely for hemicellulose (−0.97) and lignin (−0.67). Also, lignin content approximately halved (−0.70) with respect to the higher severity treatments.

Table 1 compositions correspond to the recovered solids after biomass pretreatment. The conditions that resulted with the least loss of cellulose content were pretreatments using equal concentrations of NaOH and Na_2CO_3, 10% for *Treatment A* and 16% for *Treatment B*. The mass balance of SSB indicated 50: 50 pretreated samples had the most cellulose content (over 80%), less than 50% hemicellulose, and less than 20% lignin. These effects were more noticeable in *Treatment B*, where 90% of cellulose was recovered and the hemicellulose content was about twice of the NaOH-only case. In general, the solid recovery yields decreased as more NaOH was used, almost halving between the highest yield values and the NaOH-only cases, and yields were lower at the higher heat treatment severity. Linear correlations of mass losses indicated, that as more NaOH was used in the pretreatment, cellulose content decreased (0.57), and even more significantly for hemicellulose (0.98) and lignin (0.79). Also, correlations supported improved delignification (0.59) with *Treatment B*. In other words, the more aggressive NaOH-rich pretreatments retained less amounts of solids, though with more cellulosic components, with the exception of the 50:50 cases. Previous studies have shown pretreatments on SSB with mass losses of cellulosic material in the 10%–40% range (Kim et al., 2012; Sehume et al., 2020).

Statistical tests suggested there were differences between compositions of the sample and treatment groups. In the multivariate case, cellulose ($p \ll 0.001$) and hemicellulose ($p \ll 0.001$) had highly significant differences, and lignin ($p < 0.01$) had significant differences. Considering only temperature, significant differences were measured for cellulose ($p < 0.05$), highly significant differences for lignin ($p < 0.001$), and no significant differences for hemicellulose. Pairwise group comparisons showed most differences in cellulose and hemicellulose composition between the samples with high NaOH and high Na_2CO_3 were highly significant, including for comparisons across temperature treatments. These results support pretreatment ratios had a greater impact on the mass balance of the cellulosic components compared to the temperature effects, while the higher temperature produced more statistically significant effects on delignification.

Previous studies have evaluated different pretreatment strategies on sweet sorghum bagasse. The pretreatment schemes varied in terms of temperature ranges, solvents and concentrations, treatment time, and, in some cases, consisted of multistep approaches (Yu et al., 2011; Cao et al., 2012; Thanapimmetha et al., 2019). Alkaline pretreatments, lime and Na_2CO_3:NaOH, have demonstrated minor loss in cellulose and moderate loss in hemicellulose and lignin (37%) for SSB with similar compositions as obtained in this study (Kim

et al., 2012; Nghiem and Toht, 2019). Also, NaOH pretreatments have outperformed other solvents by reducing biomass recalcitrance and exposing more cellulosic content. Table 1 results exhibit comparable cellulose, hemicellulose, and lignin composition in the 100% NaOH cases. On the other hand, pretreatment mixtures of NaOH and Na_2CO_3 had higher cellulosic content compared to pretreatments using other solvents (Mirmohamadsadeghi et al., 2016; Jafari et al., 2016; Nghiem and Toht, 2019).

3.2 ATR-FTIR spectroscopy analysis

Chemical structures in SSB pretreated biomass were studied using the FTIR spectra shown in Figure 1. The percent of normalized transmittance is presented against wavenumber as a function of different fractions of Na_2CO_3 and NaOH solutions for *Treatments A* and *B*. Bands located between 3,200 and 3,330 cm^{-1} corresponded to the O-H vibrations which are representative of alcohol functional groups. The O-H peaks varied between pretreatments and heat treatment demonstrating there were different effects on the weak intra- and intermolecular hydrogen bonds. Similarly for bands at 2,850 cm^{-1} which indicated C-H stretching from alkene groups and for bands between 1,400 and 1,620 cm^{-1} representing C=C bonds found in aromatic groups (Kotaiah Naik et al., 2017; Mafa et al., 2020). Pretreatment delignification effects were observed with the reduction of C-O vibrations at 1,050–1,150 cm^{-1} and 1,218 cm^{-1} (i.e., the guaiacyl ring), and the low profile of C=O stretching in acid groups found in 1700–1725 cm^{-1}. Additional lignin structures correspond to the bands near 1,440, 1,510, and 1,600, while cellulose is associated with 830 cm^{-1} (Banerji et al., 2013; Perrone et al., 2021). At higher temperature, the NaOH-rich samples presented lower signals of lignin structures, particularly, in the 1,050–1,620 bands. Note that samples pretreated with more NaOH exhibited a smoother profile, even smoother with *Treatment B*, as they were more effective at lignin and hemicellulose removal. Nevertheless, the SSB 50:50 samples had consistent smooth profiles across heat treatments, this can be correlated to the relatively high cellulose content as shown in Table 1.

3.3 SEM analysis

SEM was used to study the morphological and textural features present in the untreated and pretreated SSB samples. Figure 2A shows the untreated washed sample as a representative lignocellulosic structure composed of rigid and ordered fibrils along with smooth cell walls containing few longitudinal grooves and having low specific surface areas. Figures 2B–F correspond to SSB samples pretreated with different alkaline mixtures using *Treatment B*. Pretreated samples showed that lignin was not completely removed but rather exhibited distorted fibers containing rougher surfaces with cracks and pores (Mafa et al., 2020). Hydroxyl groups found in cellulose, hemicellulose, and lignin break down as a consequence of the alkaline pretreatment, thus improving the accessibility to the biomass structure (Beukes and Pletschke, 2010; Jetti and Kishore, 2024). Particularly, samples
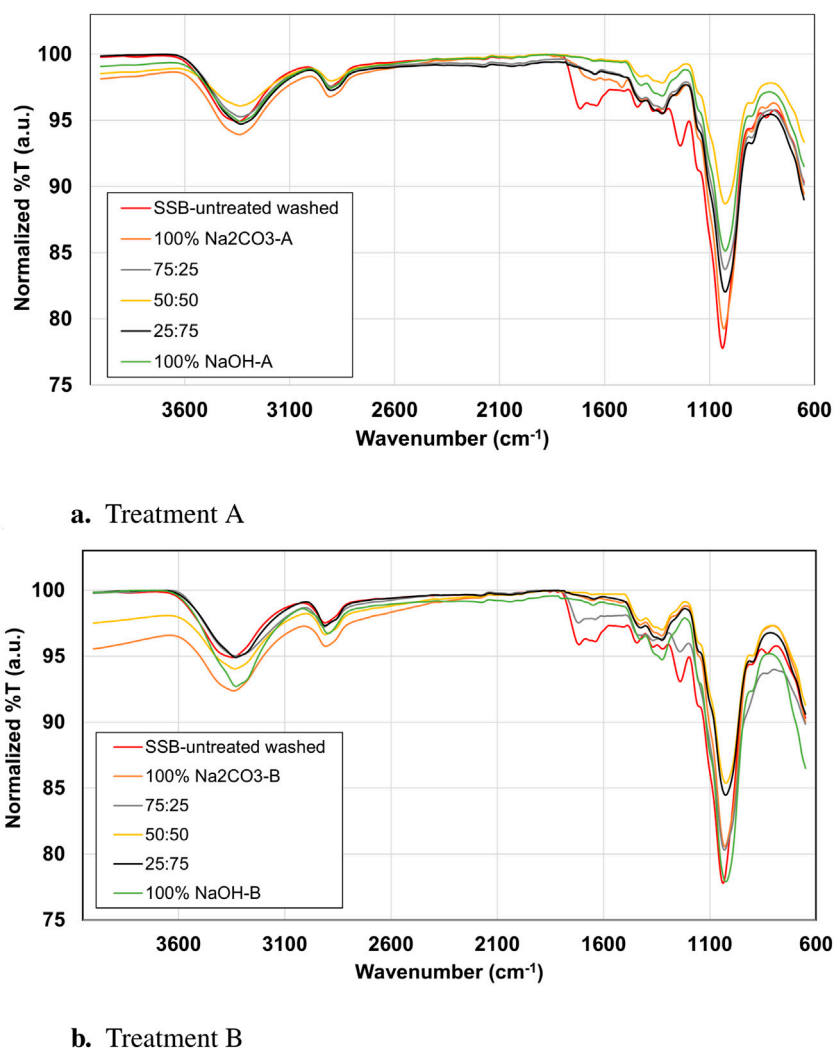
pretreated with high concentrations of NaOH ($\geq$ 50%) displayed additional changes in the microstructure, such as cell wall degradation and rougher surfaces, indicating higher specific surface areas (Saini et al., 2013). In contrast, samples pretreated with more Na_2CO_3 showed less cell wall breakage. These SEM observations correlate with the composition analysis from Table 1 where the most delignified samples correspond to pretreatment solutions consisting mainly of NaOH.

3.4 Enzymatic hydrolysis

Enzymatic hydrolysis was used to assess the saccharification of pretreated SSB biomass. Sugar yields were calculated to evaluate the efficiency of SSB characteristics, pretreatment chemical selection, heat treatment severity, and hydrolysis duration. Figures 3–5 present glucose, xylose, and arabinose yields measured at various times during hydrolysis. A higher yield was achieved for all sugars by the pretreated samples compared to the untreated samples which did not reached a 20% yield. The largest increments in yields occurred

during the first 24 h of hydrolysis where samples reached approximately 85% of their maximum yield, indicative of a high concentration of amorphous cellulosic biomass.

Glucose yields (Figure 3) were highest when the pretreatment solution included both NaOH and Na_2CO_3, achieving at least 90% for *Treatment A* and 85% for *Treatment B*. Particularly, SSB 25:75 reached the highest yield, followed by SSB 50:50 and 75:25 which had similar hydrolysis results. The 100% NaOH cases also reached over 90% for *Treatment A* after 72 h, although for *Treatment B* they underperformed. Conversely, the 100% Na_2CO_3 samples underperformed for *Treatment A* and had similar glucose profiles to the mixed cases for *Treatment B*. Figure 4 shows xylose yields had relatively similar behavior between heat treatments, although yield rates varied among corresponding samples. Samples pretreated with more NaOH resulted in higher xylose yields ($p < 0.001$) achieving maximum yields of 85%–90% for *Treatment A* and over 90% for *Treatment B*. In *Treatment B* xylose yields of at least 70% were measured after 24 h hydrolysis time while *Treatment A* required 72 h to achieve 70% yields for samples pretreated with high Na_2CO_3 content, except for SSB 100% Na_2CO_3 yield which capped at 60%.
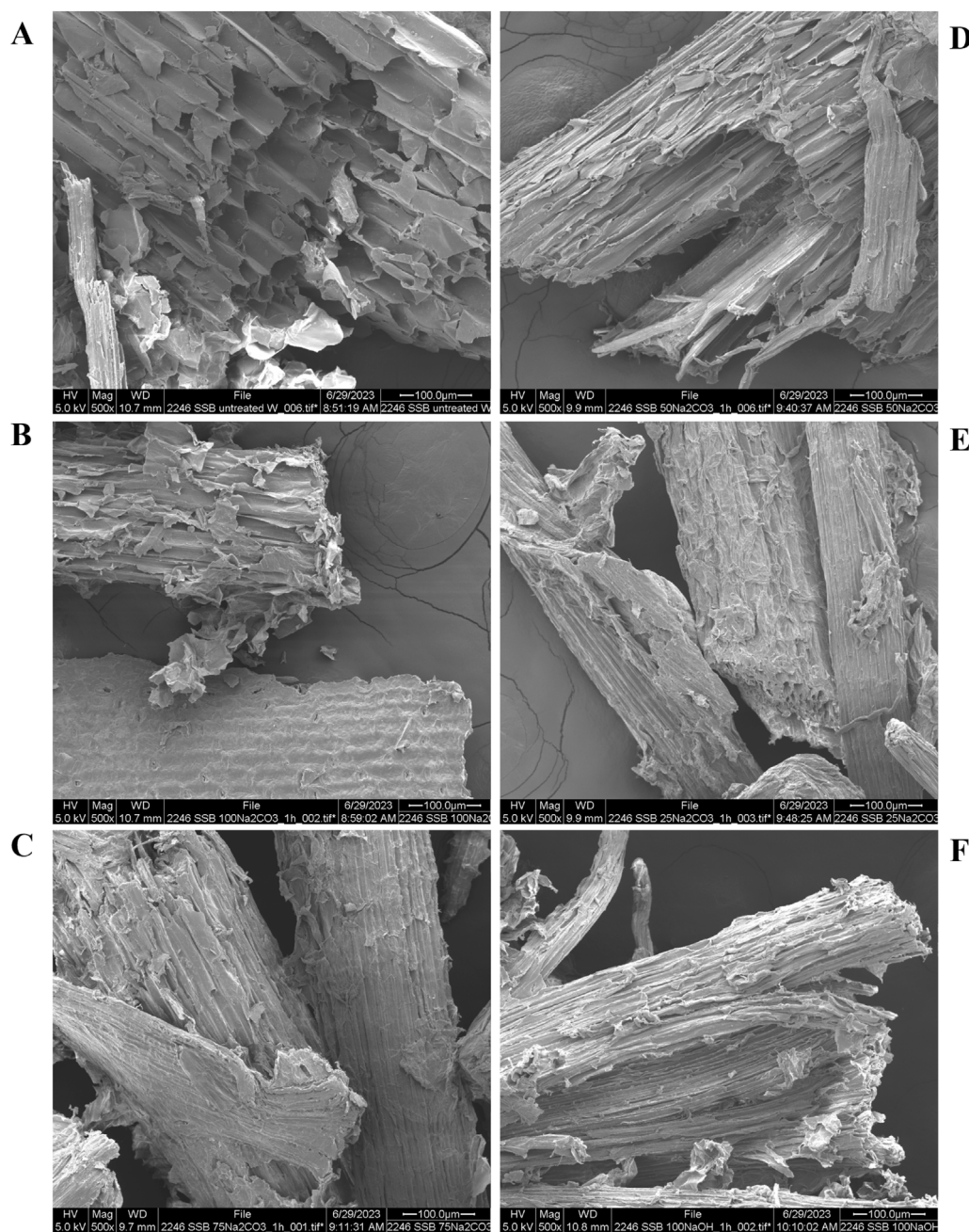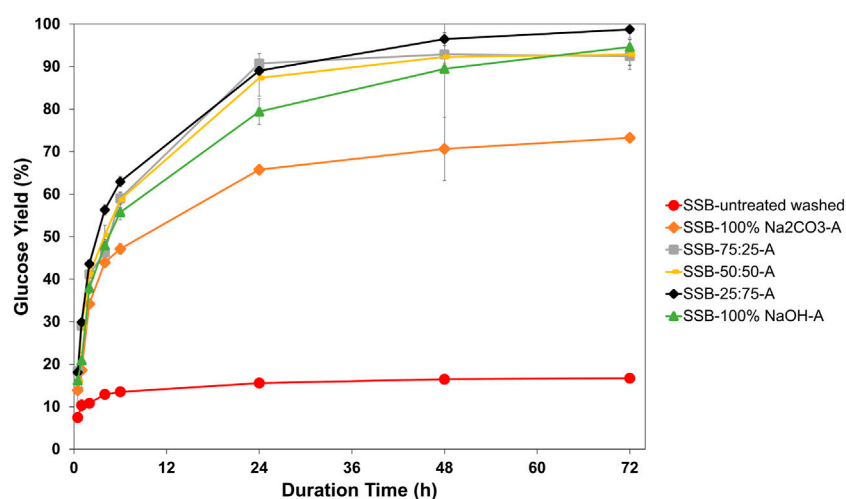
FIGURE 2
SEM micrographs of SSB samples pretreated with different ratios of Na_2CO_3 and NaOH for Treatment B. **(A)** untreated sample and **(B–F)** pretreated samples using solutions ordered from 100% Na_2CO_3 to 100% NaOH.

On the other hand, arabinose yields differed based on the treatment used, although yield increments remained small after hydrolyzed for 5 h (refer to Figure 5). *Treatment A* samples had similar maximum arabinose yields when pretreatment solution included Na_2CO_3, with SSB 25:75 reaching almost 50%. *Treatment B* samples pretreated with more Na_2CO_3 content resulted in higher yields, followed by the other mixed samples. In both temperature treatments, the 100% NaOH samples notably underperformed. Also, *Treatment A* yield rates varied less among samples and reached maximum values between 40% and 50% while *Treatment B* maximum yield rates ranged from 20%–50%.

Statistical correlations indicated significant associations between sugar concentrations (glucose, xylose, arabinose) and pretreatment ratios (0.88, −0.87, −0.82), and low associations with respect to heat treatment (0.38, 0.07, −0.36). Single and multivariate Kruskal–Wallis tests showed significant differences ($p \ll 0.05$) between the independent variables with respect to sugar yields. Glucose and arabinose yields had significant differences ($p < 0.005$) with respect to treatment temperature while xylose did not.

Sugar yields obtained for the SSB samples are comparable to related works that make use of a variety of pretreatments on sorghum biomass before hydrolysis. SSB enzymatic hydrolysis
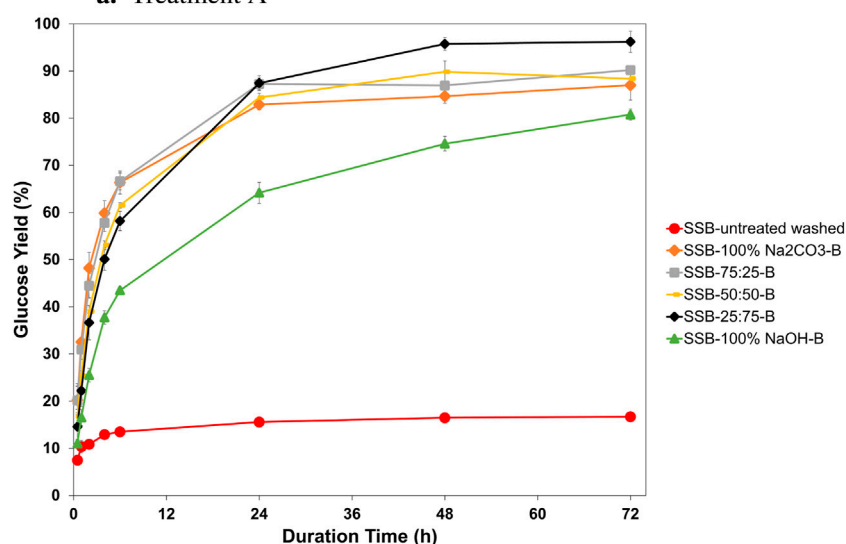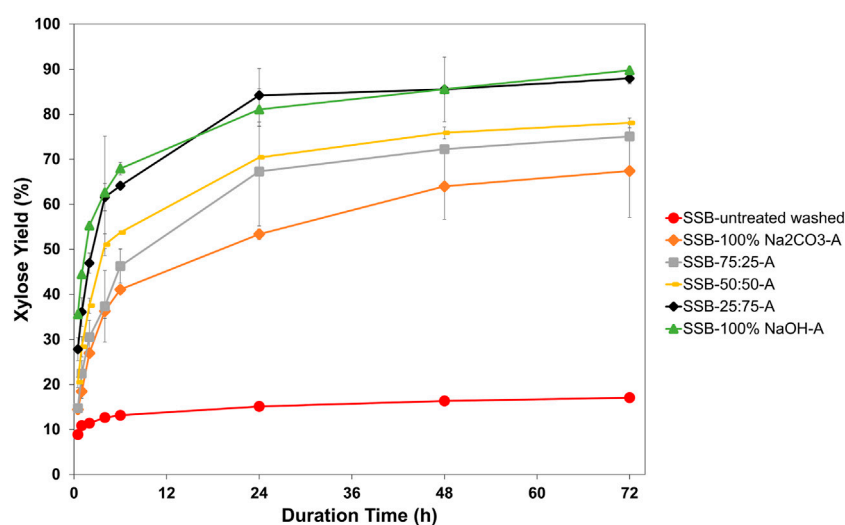
FIGURE 3
Glucose yields measured at various intervals during enzymatic hydrolysis for SSB samples pretreated with different ratios of Na_2CO_3 and NaOH and heat Treatments A and B. **(A)**: Treatment A, **(B)**: Treatment B.

studies of up to 72 h showed maximum glucose yields of 92% for NaOH pretreatment and 79% for phosphoric acid pretreatment (Goshadrou et al., 2011). A case study evaluated four SSB pretreatment methods (ionic liquid, steam explosion, lime, and dilute acid) where the steam explosion produced a maximum cellulose conversion of 70% (Zhang et al., 2011). Previous work demonstrated SSB pretreatments combining ensiling and NaOH to attain reducing sugar yields ranging in 87%–94% (Zhao et al., 2024). Hydrolysis experiments of washed SSB and forage sorghum using ammonia fiber expansion pretreatment showed high glucan and xylan conversions at 140°C, and up to a 7% increase with xylanase loading. Glucan and xylan conversions were respectively about 80% and 90% for SSB and 80% and 83% for forage sorghum (Li et al., 2010). Dilute sulphuric acid hydrolysis of SSB after different pretreatment severity resulted in up to 82% C5 and 26%

C6 sugar yields (Banerji et al., 2013). Note that some of these observations support processing SSB at high-temperature and with NaOH-based pretreatments to obtain high sugar yields during hydrolysis.

Table 2 presents the post-hydrolysis composition analysis of the recovered solids which includes the relative content of polysaccharides and lignin. The remaining contents of the mass balance correspond to other carbohydrates, extractives, hydrocarbons, polyphenolic compounds, sugar acids, and other components. Moderate-to-high temperature alkaline pretreatments helped extract additional phenol derivatives compared to low temperature pretreatments, in addition to acid hydrolysis promoting the release of lignans, low-molecular-weight compounds, and phenolic acids (Yu et al., 2011; Li et al., 2015; Crowe et al., 2019). These effects manifested in the mass balance of

a. Treatment A
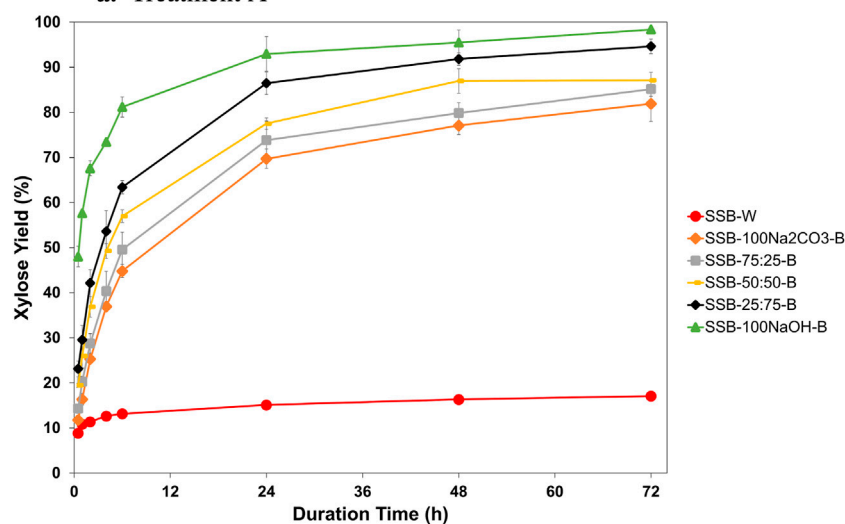
b. Treatment B

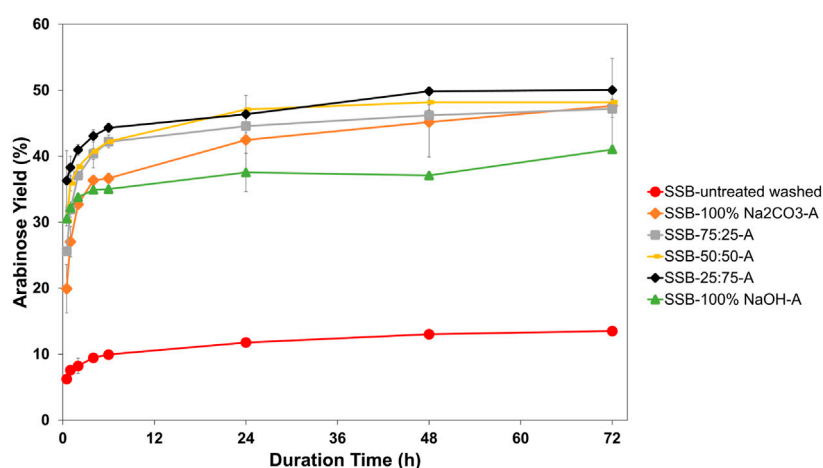**FIGURE 4**
Xylose yields measured at various intervals during enzymatic hydrolysis for SSB samples pretreated with different ratios of Na_2CO_3 and NaOH and heat Treatments A and B. **(A)**: Treatment A, **(B)**: Treatment B.

the recovered solids where *Treatment B* samples had more content attributed to cellulose, hemicellulose, and lignin (85% total average) compared to *Treatment A* (75% total average), given that more components were released during pretreatment. The untreated hydrolyzed samples still consisted of 25% cellulose indicating that enzymes were not able to easily access cellulosic structures. All pretreated hydrolyzed samples consisted mainly of lignin, in most cases over 60%, and low polysaccharide content, less than 20% for cellulose and 4% for hemicellulose. The amount of cellulose and hemicellulose decreased as more NaOH was present in the pretreatment solutions, although hemicellulose's changes were relatively small. Biomass samples from *Treatment B* had cellulose content that was approximately half of those from *Treatment A*. Similarly, samples from *Treatment B* had less hemicellulose. The total lignin content was greater for mixtures and less variable

between them, with the SSB 50:50 samples having the most lignin content which can be associated with the high cellulose-to-lignin content observed in the composition analysis after pretreatment. Moreover, increasing NaOH in the pretreatment made cellulose content in the hydrolyzed solids exhibit a larger difference compared to the content in the pretreated samples, indicating that NaOH was effective at cellulose fractionation and produced improved hydrolyzable structures.

Glucose conversion efficiency during SSB hydrolysis was evaluated by relating the initial conversion rate as a function of lignin concentration, see Figure 6. Samples exposed to the higher heat treatment severity exhibited increased delignification, with lignin concentrations reduced by 5%–10%, and those with 50% or more NaOH fraction had the lowest lignin concentration (less than 5%). Whereas changes in glucose rates varied differently based
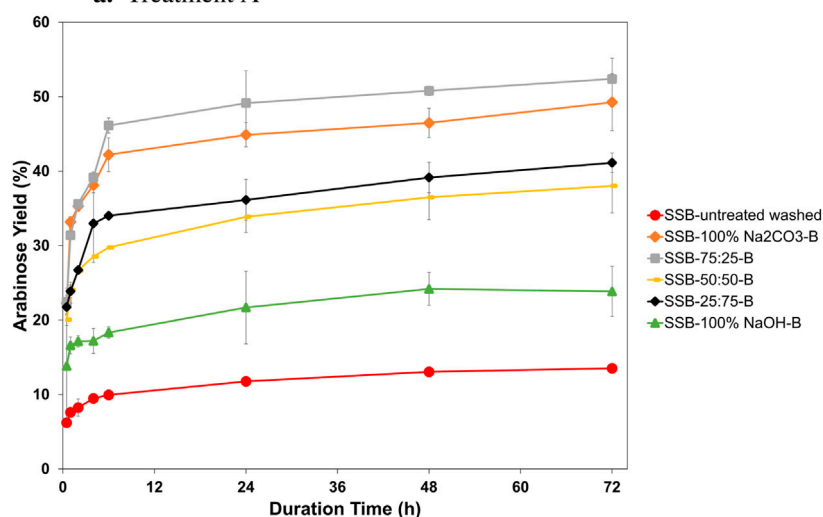
FIGURE 5
Arabinose yields measured at various intervals during enzymatic hydrolysis for SSB samples pretreated with different ratios of Na_2CO_3 and NaOH and heat Treatments A and B. **(A)**: Treatment A, **(B)**: Treatment B.

on both the heat treatment and pretreatment solution. For example, SSB samples pretreated with equal or more Na_2CO_3 had increasing glucose rates with *Treatment B*, while as the NaOH fraction dominated, the rates decreased. The average variation of glucose rates due to differences in heat treatment severity was 0.02 mol L^{-1} h^{-1}, except for the 100% Na_2CO_3 samples which varied five times as much. These observations coupled with Figure 3 indicate that high-temperature treatment along with mixtures of pretreatment alkaline solutions is suitable for delignification and enzymatic saccharification.

4 Conclusion

Sweet sorghum bagasse biomass was used to understand the effects from varying ratios of Na_2CO_3 and NaOH in pretreatment

and using different heat treatments. Composition analysis showed that NaOH was more effective than Na_2CO_3 in changing chemical structures to allow enzymes to access cellulose and degrade hemicellulose and lignin. SSB samples pretreated with 100% NaOH were favorable to cellulose content while samples pretreated with high Na_2CO_3 concentrations favored hemicellulose. Pretreatment solutions with 50% Na_2CO_3 recovered approximately twice the solid yields compared to the NaOH-dominant treatments. Moreover, the pretreatments with equal amounts of Na_2CO_3 and NaOH resulted in the least mass loss of cellulose content, demonstrating that the balanced interaction between these chemicals helped retain cellulose while still being effective and reducing hemicellulose and lignin components. This suggested alkaline solutions containing Na_2CO_3, a chemical that can be produced from captured CO_2, can be effective in SSB sugar conversion.

TABLE 2 Composition analysis for recovered solids from untreated and pretreated SSB samples after hydrolysis.

	Sample[1]	Cellulose (%)	Hemi-cellulose (%)	Total lignin (%)	Ash (%)
	SSB-W[2]	24.97	7.13	28.42	3.22
Treatment A[3]	100:0	18.63	4.02	37.33	0.80
	75:25	11.44	3.57	66.13	1.54
	50:50	8.79	3.34	71.87	1.88
	25:75	7.82	3.27	63.14	1.59
	0:100	6.40	3.01	15.58	0.00
Treatment B[4]	100:0	11.99	3.33	58.85	2.09
	75:25	5.10	2.77	77.48	5.54
	50:50	4.49	2.78	81.32	5.06
	25:75	3.87	2.65	70.71	4.57
	0:100	3.08	2.37	70.61	6.51

[1]Na_2CO_3:NaOH.
[2]SSB-W, represents untreated washed samples.
[3]Pretreatment at 85°C for 1 h.
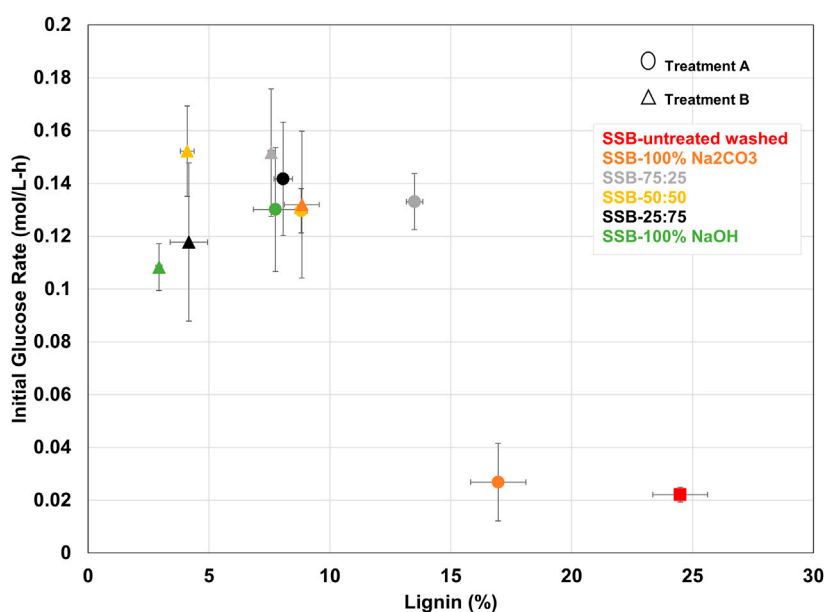[4]Pretreatment at 150°C for 1 h.

FIGURE 6
Glucose rates of the first 8 h of hydrolysis as a function of lignin concentration for SSB samples pretreated with different ratios of Na_2CO_3 and NaOH for Treatments A and B.

The performance of enzyme digestibility of SSB as a function of hydrolysis duration was evaluated by determining the sugar recovery of glucose, xylose, and arabinose via HPLC. Results confirmed pretreatment methods were effective at promoting SSB biomass conversion and increasing sugar recovery after enzymatic hydrolysis. The higher temperature pretreatment, *Treatment B*, produced additional changes in the chemical structures, thus providing access to more cellulose content and disintegrating more hemicellulose and lignin. Overall, samples pretreated with the conditions of *Treatment B* showed better stability and sugar release. Pretreatment solutions with Na_2CO_3 reached higher yields at less enzymatic hydrolysis duration than those with NaOH (48 vs. 72 h). Consequently, reducing hydrolysis time by 33% which is desirable as it is cost-effective. These results indicate the viability of using Na_2CO_3 in biomass to obtain high sugar concentrations after pretreatment and high sugar yields after hydrolysis. Further studies will evaluate pretreatment effects and hydrolysis

performance for agricultural residues and energy crops such as corn stover and switchgrass.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

VG-N: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing–original draft, Writing–review and editing, Formal Analysis, Project administration, Validation. RS: Conceptualization, Investigation, Writing–review and editing. MT: Investigation, Methodology, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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