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ORIGINAL ARTICLE

Ethanol production from sorghum by a dilute ammonia pretreatment

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Abstract Sorghum fibers were pretreated with ammonium hydroxide and the effectiveness of the pretreatment evaluated by enzyme hydrolysis and ethanol production. The treatment was carried out by mixing sorghum fibers, ammonia, and water at a ratio of 1:0.14:8 at 160°C for 1 h under 140–160 psi pressure. Approximately 44% lignin and 35% hemicellulose were removed during the process. Untreated and dilute-ammonia-treated fibers at 10% dry solids were hydrolyzed using combinations of commercially available enzymes, Spezyme CP and Novozyme 188. Enzyme combinations were tested at full strength (60 FPU Spezyme CP and 64 CBU Novozyme 188/g glucan) and at half strength (30 FPU Spezyme CP and 32 CBU Novozyme 188/g glucan). Biomass enzyme hydrolysis was conducted for 24 h. *Saccharomyces cerevisiae* D_5A was added post hydrolysis for conversion of glucose to ethanol. Theoretical cellulose yields for treated biomass were 84% and 73%, and hemicellulose yields were 73% and 55% for full strength and half strength, respectively. Average cellulose yield was 38% and hemicellulose yield was 14.5% for untreated biomass. Ethanol yields were 25 g/100 g dry biomass and 21 g/100 g dry biomass for full strength and half strength enzyme concentrations, respectively. Controls averaged 10 g ethanol/100 g dry biomass.

Keywords Ammonia treatment · Sorghum · Ethanol · Lignocellulosic · Biomass · Hydrolysis

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Introduction

A study supported by both the Department of Energy (DOE) and the US Department of Agriculture (USDA) has indicated that the USA has sufficient land resources to sustain production of over 1 billion dry tons of biomass annually, including agricultural (933 million tons/year) and forest resources (368 million tons/year), enough to replace at least 30% of the nation's current consumption of liquid transportation fuels [1, 2]. This supply of biomass would represent a sevenfold increase over the 190 million dry tons of biomass per year used for the production of bioenergy and bioproducts, of which only 18 million are used for the production of biofuels, primarily corn-grain ethanol [1, 3, 4].

Demand for transportation fuels is expected to increase [5] and the use of lignocellulosic biomass as a source for biofuels represents a reasonable approach. Lignocellulosics that show potential for ethanol production include agricultural residues (i.e., corn stover, wheat straw, rice straw), agricultural byproducts (i.e., corn fiber, rice hull, sugarcane bagasse), and energy crops (i.e., switchgrass, sweet sorghum, high-fiber sugarcane, *Miscanthus*) [6]. The major components of lignocellulosics are cellulose (polymers of hexose sugars, 35–50%), hemicellulose (polymers of pentose sugars, 20–35%), and lignin (polyphenols, 10–25%) [4, 6].

The ability to make fuels and/or other value-added products from lignocellulose depends on the ability to separate and/or break down cellulose, hemicellulose, and lignin into their main components. The conversion of lignocellulose to ethanol is more complicated than that of starch or sucrose and this has limited its commercialization. Unlike starch or sucrose, lignocellulose does not compete with the food and cattle feed industries and does not add to the emission of greenhouse gases.

Pretreatment of lignocellulose is a key step for efficient utilization of biomass for ethanol production. Pretreatment softens and ruptures the cell wall and breaks the association of lignin with cellulose in biomass [3, 7]. Pretreatment is also useful in decreasing the crystallinity of cellulose, thus improving enzyme hydrolysis [6]. A number of pretreatment methods have been developed for improving the hydrolysis of lignocellulosics. Most of these technologies suffer from relatively low sugar yields, severe reaction conditions, large capital investment, high processing costs, and great investment risks [2]. Developed technologies include physical disruption, solvent-based approaches, dilute acid, autohydrolysis, wet oxidation, biological, and alkali treatment [3, 6, 8, 9].

Ammonia-based pretreatments using anhydrous ammonia (NH_3) or aqueous ammonia (NH_4OH) with or without heat and/or pressure have shown great success in the delignification of lignocellulosic [7, 9–15]. Ammonia, being a selective reagent for lignin, noncorrosive, and a relatively less expensive chemical, is an appropriate choice for pretreatment [11].

The objective of this study was to evaluate the effect of a new pretreatment technology using dilute ammonium hydroxide on the hydrolysis and fermentation of sorghum to ethanol.

Materials and methods

Biomass preparation

Sorghum, *Sorghum bicolor* (L.) Topper, was harvested from the Hill Farm Research Station at Homer, LA. The harvested sorghum consisted of stalks, leaves, grains, and roots. Leaves, roots, and grains were removed by hand. Stalks were crushed in a roller press (Farrel Company, Ansonia, CT) thrice to extract the juice. Approximately, 1.16 kg dry fibers were added into a cylindrical high-pressure reactor (Valley Steel, OH) followed by ammonium hydroxide (28% v/v solution, Fisher Scientific) and water at a ratio of 1:0.5:8, respectively. Fibers were pretreated at 160°C for 1 h under 140–160 psi (0.9–1.1 MPa) pressure. The tumbling reactor (2 rpm) was equipped with a steam jacket for heating and cooling, and pressure and temperature gages for monitoring and controlling operating conditions. Once pretreatment ended, the reactor was cooled down to 50–80°C and a double-walled stainless-steel cylinder was coupled to the automatic valve at the bottom of the reactor prior to discharging the biomass. Approximately 20 kg condensed steam (collected in a stainless-steel container) was pumped over the top of the reactor and through the double-walled cylinder containing the pretreated biomass. The pumped water was then recirculated for 10 min to wash away inhibitors and residual ammonium hydroxide. Pressing of pretreated and washed biomass prior to hydrolysis was done to remove excess water along with dissolved inhibitors of enzyme hydrolysis and fermentation that might have been generated during pretreatment. Biomass (both dilute ammonia treated and untreated) was dried to 20% moisture at 40–45°C overnight. Washed untreated biomass was used as control. Composition analysis for untreated and treated biomass was carried out.

Inoculum preparation

Saccharomyces cerevisiae (D_5A) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were stored at −70°C upon arrival. Yeast cells were grown in YP media [1% yeast extract (Becton–Dickinson and Company, Sparks, MD), 2% peptone (Becton–Dickinson and Company, Sparks, MD), 3% glucose (Sigma–Aldrich, Inc., St. Louis, MO)] at 30°C for 16 h in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) at 200 rpm. Approximately, 10 ml yeast solution was transferred to 2 l YP media. The inoculated media was incubated at 30°C for 24 h in a shaker incubator at 200 rpm. Cells were washed twice with deionized (DI) water and harvested by centrifugation at 8,000 rpm. Rinsed cells were resuspended in 50 ml DI water and stored at 4°C. The final concentration was $\sim 1 \times 10^9$ colony-forming units (CFU)/ml as confirmed on YP media by the pour plate method. The stock solution was analyzed for presence of residual ethanol and sugars.

Enzymatic hydrolysis

Two combinations of enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) containing cellulase and Novozyme 188 (Sigma–Aldrich, Inc., St. Louis, MO) containing β-glucosidase, were evaluated for hydrolysis. The first combination (referred to herein as full strength) consisted of 60 FPU Spezyme CP/g of glucan and 64 CBU Novozyme 188/g of glucan. The half-strength combination was comprised of 30 FPU Spezyme CP/g of glucan and 32 CBU Novozyme 188/g of glucan. One-liter Erlenmeyer flasks were each loaded with 50 g (dry weight) biomass (dilute ammonia treated or untreated), 0.5 g yeast extract, 1 g peptone, 25 g citrate buffer (1 M stock solution, pH 4.8), and water to bring the final weight to 500 g. The pH of each mixture was adjusted to 4.8 with a few drops of concentrated hydrochloric acid. All flasks were autoclaved at 121°C for 30 min. After autoclaving, flasks were cooled to 30°C. Samples (5 ml) were taken prior to the addition of enzymes and labeled time 0. Enzymes (full strength and half strength) were added and all flasks were incubated at

55°C in a shaker incubator (Amerex Instruments Inc., Lafayette, CA) for 24 h at 200 rpm. Samples (5 ml) were withdrawn from each flask post enzyme hydrolysis (time 24 h). All samples were analyzed for sugars (glucose, cellobiose, arabinose, xylose), ethanol, glycerol, organic acids (lactic, acetic and formic), hydroxy-methyl-furfural (HMF), and furfurals. Experiments were run in triplicate. Percentage theoretical cellulose and hemicellulose yields were calculated using equations provided by the National Renewable Energy Laboratory (NREL) procedures (LAP#42630).

$$\%\ \text{Theoretical cellulose yield} = \frac{[\text{Glucose}] + 1.053[\text{Cellobiose}]}{1.111f[\text{Biomass}]} \times 100\%, \quad (1)$$

$$\%\ \text{Theoretical hemicellulose yield} = \frac{[\text{Xylose}]0.9 + 0.9[\text{Arabinose}]}{1.111f[\text{Biomass}]} \times 100\%, \quad (2)$$

where [Glucose] is the residual glucose concentration (g/l), [Cellobiose] is the residual cellobiose concentration (g/l), 1.053 is the multiplication factor that converts cellobiose to equivalent glucose, [Biomass] is the dry biomass concentration at the beginning of the fermentation (g/l), f is the cellulose or hemicellulose fraction in dry biomass (g/g), [Xylose] is the residual xylose concentration (g/l), and [Arabinose] is the residual arabinose concentration (g/l).

Simultaneous scarification and fermentation (SSF)

All flasks were cooled down to 30°C post enzyme hydrolysis. Yeast cells (1 ml) from the stock solution were added to each flask and incubated at 30°C in a shaker incubator at 200 rpm for an additional 2 days. Samples (15 ml) were withdrawn at 48 and 72 h and analyzed for sugars (glucose, cellobiose, arabinose, xylose), ethanol, glycerol, organic acids (lactic, acetic, and formic), HMF, and furfurals. Composition analysis and total solids were determined. Percentage theoretical ethanol yield was calculated using the following equation provided by NREL procedure LAP#42630:

$$\%\ \text{Theoretical Ethanol yield} = \frac{[\text{EtOH}]_f - [\text{EtOH}]_0}{0.51\,(f[\text{Biomass}]1.111)} \times 100\%, \quad (3)$$

where $[\text{EtOH}]_f$ is the ethanol concentration at the end of the fermentation (g/l) minus any ethanol produced from the enzyme and medium, $[\text{EtOH}]_0$ is the ethanol concentration at the beginning of the fermentation (g/l), which should be zero, [Biomass] is the dry biomass concentration at the beginning of the fermentation (g/l), f is the cellulose fraction of dry biomass (g/g), 0.51 is the conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, and 1.111 converts cellulose to equivalent glucose.

Analytical procedures

Composition analysis

Composition analysis of treated, untreated, and fermented biomass was carried out following NREL's laboratory analytical procedures (LAPs #42618, 42619, 42620, 42621, 42622). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal sample to ensure accuracy of the procedures.

Sample preparation

Samples taken at time 0, 24, 48, and 72 h were centrifuged at 8,000 rpm and filtered (0.2 μm Syringe Filters, Nagle Company, NY). Dilutions of filtered solutions were made accordingly prior to chemical analysis.

Sugar and glycerol analysis

Cellobiose, glucose, xylose, arabinose, and glycerol were analyzed by high-performance liquid chromatography (HPLC; Agilent 1200 series) with a BioRad Aminex HPX-87P (Pl), lead form, 300 mm × 7.8 mm (ID), 9 μm column, and a differential refractive index (DRI) detector (G1362A Agilent). The eluent solution was filtered water (0.2 μ) at a flow rate of 1 ml/min. Sample volume was 20 μl.

Ethanol analysis

Ethanol was analyzed by gas chromatography (GC; Hewlett Packard 5890 series II) with a wax column (Zebron ZB Wax Plus, 60 m × 0.32 nm × 0.50 μm) and GC-flame ionization detector (GC-FID, 280°C). Operating conditions were: injector at 250°C, split flow rate at 10.4 ml/min, and column flow at 1.0 ml/min. Sample volume was 1 μl. Initially, the sample was held at 75°C for 5 min and then the temperature was increased (at the rate of 10°C/min) to 200°C and held for 1 min throughout the total run time of 18.5 min.

Organic acids analysis

Lactic acid, acetic acid, and formic acid were analyzed by HPLC (Metrohm Peak Ion Chromatography). The column used was a Dionex Ion Pac AS-11 HC anion exchange column with Anion Trap Ion Pac ATC-1. A 50 μl sample volume was used with a gradient method. The eluents used were 50 mM NaOH and DI water. Total run time was 54 min at flow rates ranging from 0.8 to 1.4 ml/min.

HMF and furfural analysis

HMF and 2-furfural were analyzed using reverse-phase HPLC (Agilent 1100) with a C18 column, 150 mm × 4 mm × 5μm (Agilent Eclipse). A diode array detector was configured to collect absorbance at 280 and 330 nm. The gradient method had a flow rate of 1 ml/min, and a total run time of 15 min. Methanol and water were used as eluents at concentrations of 5% and 95% for 2 min, 30% and 70% for 3 min, 50% and 50% for 5 min, and 5% and 95% again for the last 5 min.

Results and discussion

Effect of pretreatment on biomass composition (delignification)

Composition of untreated and dilute-ammonia-pretreated sorghum is summarized in Table 1. A total of 881 g (76%) carbohydrates (cellulose and hemicellulose) were present in the untreated material. Approximately, 518 g (45%) cellulose was available for conversion to ethanol by *S. cerevisiae*. Twenty-eight percent hemicellulose was present in the untreated material. This composition (45% cellulose, 28% hemicellulose, and 22% lignin) was found to be within the range of published results. Mamma et al. [16] and Gnansounou et al. [17] reported values of 48–49%, 22–26%, and 19–20%, respectively. It was observed that 276 g (24%) of the total mass was lost during pretreatment, mostly attributed to lignin removal. Approximately, 44% of the initial lignin was removed during pretreatment. Inhibitors such as 2-furfural and HMF were not formed during pretreatment and were not present during enzyme hydrolysis or fermentation as indicated by HPLC analysis. These compounds are regarded as toxic to both enzymes and microorganisms during hydrolysis and fermentation steps [18, 19]. Pretreatment technologies involving the use of hot water, sulfuric acid, or organosolv enhance the formation of these compounds [5, 20].

Table 1 Composition analysis of untreated and dilute-ammonia-treated sorghum

	Untreated biomass (g)[a]	Treated biomass (g)[a]
Ash	3.624001	4.419739
Ethanol extractives	22.00287	34.81672
Total lignin	256.3981	143.7768
Arabinan	32.48658	20.83591
Xylan	322.4067	209.5317
Mannan	8.41286	6.043317
Glucan	517.8439	467.3198
Total	1,163.175	886.744

[a] Dry weight

Considerable amounts of hemicellulose (35%) and lignin (44%) were removed during the process. However, more than 90% (467 g) of the cellulose was retained in the treated biomass. Loss of hemicellulose was expected, as ammonia has been reported to remove hemicellulose along with lignin [7]. Kim and Lee [13] reported 53–79% delignification with 75–97% removal of hemicelluloses and 4–11% removal of celluloses from corn stover by a two-stage hot water and ammonia recycle percolation (ARP) process at high temperature (170–210°C), whereas in another study, 70–85% delignification was achieved by ARP process (170–210°C, 2.3 MPa pressure) from corn stover presoaked overnight in ammonia (15% wt) [11]. Soaking in aqueous ammonia (SAA) treatment of corn stover for an extended time (1–60 days) at moderate temperature (room temperature to 90°C) resulted in 55–74% delignification [12, 14]. Most of the data available on ammonia pretreatments is on low-lignin-containing corn stover, which has shown high delignification values due to lower recalcitrance [21] as compared with high-lignin-containing sorghum. Exposure time (ranging from 1 to 60 days) and temperature (room temperature to 210°C) played an important role in delignification in the above-mentioned studies as compared with our study (1 h, 160°C). Another important feature of the dilute ammonia pretreatment evaluated in this study is the significant delignification (44%) obtained using lower ammonia concentrations (0.14 parts of ammonia per part of dry biomass, or 1.5% in the total slurry) as compared with the above-mentioned studies (1–15 parts ammonia per part of dry biomass). Although significant amounts of hemicellulose were detected in the filtrate post pretreatment, the main focus of this study was the conversion of cellulose to ethanol. We believe that lignin removal increases the area and porosity of the biomass, thus enhancing enzyme hydrolysis and fermentation. Studies on recovery and isolation of hemicellulose and cellulose from filtrate and its utilization for production of value-added materials are being investigated in our laboratory. We are also investigating recovery and recycling of the ammonium hydroxide from filtrate due to its volatile nature.

Enzymatic hydrolysis and fermentation of sorghum at different enzyme concentrations

Untreated and dilute-ammonia-treated sorghum fibers were hydrolyzed for 24 h using combinations of two enzymes. The concentrations were 60 FPU Spezyme CP/g of glucan and 64 CBU Novozyme 188/g of glucan (full strength) and 30 FPU Spezyme CP/g of glucan and 32 CBU Novozyme 188/g of glucan (half strength). Changes in sugars and

ethanol concentrations during saccharification and fermentation are depicted in Fig. 1. At the end of saccharification (24 h), glucose concentrations were 47 and 40 g/l for dilute-ammonia-treated sorghum at full-strength and half-strength enzyme concentrations, respectively. For controls, these values were 17 g/l at both full-strength and half-strength enzyme concentrations. Xylose concentrations were 17 and 13 g/l for dilute-ammonia-treated sorghum and 3 g/l for controls at full-strength and half-strength enzyme concentrations, respectively. Glucose (400–470 g/kg dry sorghum) and xylose (130–170 g/kg dry sorghum) yields achieved in this study were higher than those previously observed by Kurakake et al. [22]. Sugar yields of hydrolyzed ammonia water-treated sugarcane bagasse were 255 g glucose/kg dry biomass and 62 g xylose/kg biomass [22]. The comparison was possible as sugarcane bagasse, like sorghum, is a grass and has similar composition.

Percent theoretical cellulose and hemicellulose yields are given in Table 2. Cellulose digestibility for ammonia-treated sorghum was 84% and 73% at full-strength and half-strength enzyme concentrations. Xylose digestibility for treated biomass was 73% and 55%, respectively. Approximately, 38% cellulose and 14.5% hemicellulose digestibility was observed for controls regardless of enzyme concentration. The high percentage digestibility in the treated material can be attributed to lignin removal. During ammonia pretreatment C–O–C bonds in lignin and other ether and ester bonds in the lignin-carbohydrate complex are cleaved [11]. The cleavage of the lignin-carbohydrate complex can result in pore formation and swelling of biomass, thus increasing surface area and subsequently improving enzyme accessibility [5, 11].

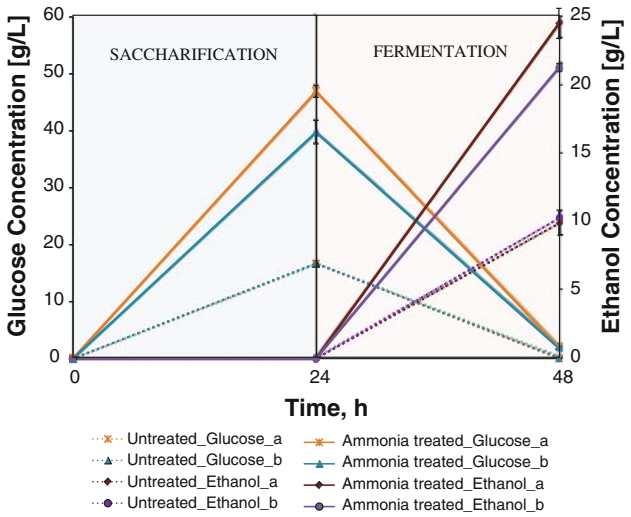

Fig. 1 Saccharification and fermentation of sorghum at two enzyme concentrations. *a* Full strength (30 FPU Spezyme CP/g of glucan and 32 CBU Novozyme 188/g of glucan); *b*, half strength (15 FPU Spezyme CP/g of glucan and 16 CBU Novozyme 188/g of glucan)

Ethanol concentration reached its highest peak at 48 h (Fig. 1). No difference in ethanol concentrations was observed at 72 h (data not shown). At the end of the fermentation process, ethanol concentrations reached 25 g/l for dilute-ammonia-treated sorghum as compared with 10 g/l for untreated sorghum at full-strength enzyme concentration (Table 3). Half-strength values were 21 g ethanol/l and 10 g ethanol/l for dilute-ammonia-treated and untreated sorghum. Theoretical ethanol yields were 84% and 73% for dilute-ammonia-treated biomass and 44% and 45% for untreated biomass at full- and half-strength enzyme concentrations; respectively. Ethanol yields from dilute-ammonia-treated sorghum were 250 g/kg dry sorghum and 210 g/kg dry sorghum. These yields are higher than those reported using sulfuric-acid-pretreated sorghum in solid-phase fermentation (141 g/kg dry sorghum) [23]. Ethanol yields in our study were also higher than phosphoric-acid-treated sorghum, where cellulose and hemicellulose fermentation resulted in 145 g ethanol/kg dry sorghum [24]. Mamma et al. [16] reported 115 g/kg dry sorghum ethanol yields from sorghum fibers using a mixed culture of *Fusarium oxysporum* and *Saccharomyces cerevisiae*. In another study Mamma et al. [25] reported ethanol yields of 160–258 g/kg dry sorghum by fermentation of soluble (glucose and sucrose) as well as insoluble (cellulose) sugars from sorghum juice and fiber. Yu et al. [26] were able to achieve higher ethanol yields (316 g ethanol/kg dry sorghum) by fermenting both acid-treated sorghum (30% sulfuric acid) and sorghum juice. Unlike in our study, both sorghum juice and fibers were converted to ethanol using a mutant strain of baker yeast.

Analytical studies

Maiorella et al. [27] reported that concentrations of acetic acid >0.5–9 g/l, lactic acid >10–40 g/l, and formic acid >0.5–2.7 g/l inhibited *Saccharomyces cerevisiae* by interfering with functions involved in cell maintenance. Glycerol at high concentrations (450 g/l) alters the cell's osmotic pressure [27], and furfurals at concentrations of 3 g/l are considered antagonistic to cell growth [19]. In our study, organic acids (acetic acid <1 g/l, lactic acid <0.05 g/l, and formic acid <0.12 g/l), glycerol (<2.1 g/l), and HMF and furfurals (undetected) concentrations were insufficient to produce any inhibitory effect.

Postfermentation composition of treated and untreated biomass

Composition analysis of dilute-ammonia-treated and untreated sorghum was carried out post fermentation, following NREL procedures. Initial mass for both dilute-ammonia-treated and untreated biomass was 50 g dry

Table 2 Glucose and xylose yields for untreated and dilute-ammonia-treated sorghum

	Glucose		Xylose	
	g/l	Digestibility (%)	g/l	Digestibility (%)
Untreated[a]	16.68 ± 0.20	38	3.44 ± 0.00	15
Ammonia treated[a]	46.86 ± 1.02	84	17.3 ± 0.79	73
Untreated[b]	16.70 ± 0.42	38	3.02 ± 0.07	14
Ammonia treated[b]	39.75 ± 2.06	73	13.03 ± 0.67	55

[a] Full strength (30 FPU Spezyme CP/g of glucan and 32 CBU Novozyme 188/g of glucan)

[b] Half strength (15 FPU Spezyme CP/g of glucan and 16 CBU Novozyme 188/g of glucan)

Table 3 Ethanol yields for untreated and dilute-ammonia-treated sorghum

	g/l	Theoretical yield (%)
Untreated[a]	9.89 ± 0.90	44
Ammonia treated[a]	24.53 ± 1.10	84
Untreated[b]	10.27 ± 0.02	45
Ammonia treated[b]	21.28 ± 0.29	73

[a] Full strength (30 FPU Spezyme CP/g of glucan and 32 CBU Novozyme 188/g of glucan)

[b] Half strength (15 FPU Spezyme CP/g of glucan and 16 CBU Novozyme 188/g of glucan)

weight. Final mass post fermentation was approximately 21 and 25 g for dilute-ammonia-treated biomass and approximately 33 and 31 g for untreated biomass at full-strength and half-strength enzyme concentrations, respectively (Fig. 2). For dilute-ammonia-treated biomass the total solids remaining were less as compared with untreated biomass as most of the glucan was converted to ethanol (Fig. 2). Similarly, more xylan was hydrolyzed from dilute-ammonia-treated biomass as compared with untreated biomass (Fig. 2). Other components such as ash, lignin, arabinan, and mannan either decreased in mass or remained the same after fermentation. Ethanol extractables showed a slight increase, mostly in the treated biomass.

Scanning electron microscope (SEM) images

SEM images of untreated and dilute-ammonia-treated sorghum pre and post fermentation are shown in Fig. 3. Untreated biomass showed compact and rigid fibril structures (Fig. 3a) which swelled after pretreatment due to lignin and xylan degradation (Fig. 3b). Ban et al. [24] reported destruction of crystal structures in sorghum treated with phosphoric acid. Ammonia treatment showed swelling and scaling prominently for sorghum, as compared with acid treatment where only hemicelluloses are hydrolyzed [24]. Untreated biomass retained most of its rigid structure post fermentation (Fig. 3c), though some

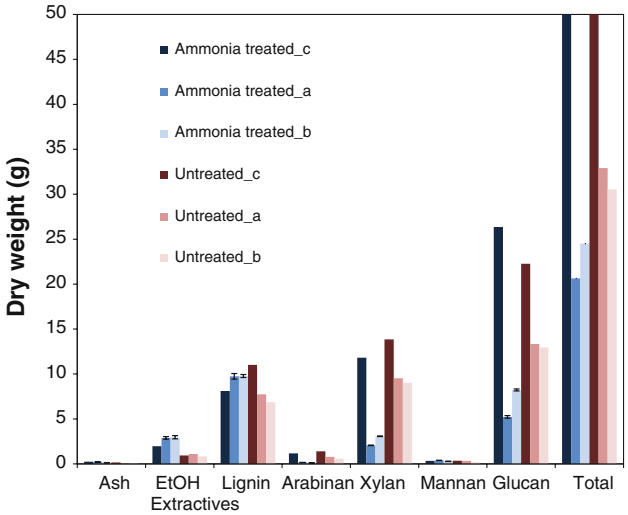

Fig. 2 Composition analysis pre and post hydrolysis and fermentation. *a* Post fermentation at full-strength enzyme concentration; *b* post fermentation at half-strength enzyme concentration; *c*, prefermentation. *Note*: Data was available in duplicate for ammonia-treated samples post fermentation at full- and half-strength enzyme concentrations

swelling and scaling was observed. Small fiber skeletons (most probably lignin) remained from the dilute-ammonia-treated biomass, suggesting that most of the cellulose was hydrolyzed and converted to ethanol, as confirmed by glucose and xylose digestibility (73–84% and 55–73%). Results from composition analysis post hydrolysis also confirmed that the composition of lignin was higher in treated biomass (40–47%) as compared with untreated biomass (16–23%). Cellulose composition post fermentation on the other hand was higher in untreated biomass (13–14 g) than in treated biomass (5–8 g).

Conclusions

Dilute ammonia treatment removed 44% of the original lignin and 35% of the original xylan, and retained 90% of the glucan in the treated material. High glucan digestibility

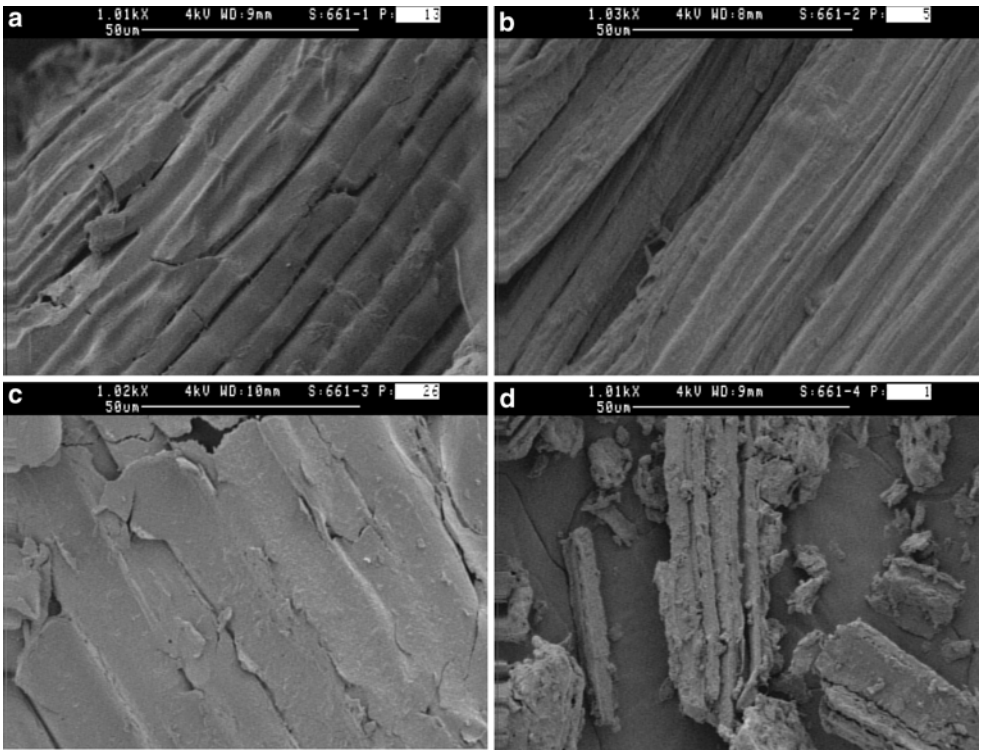

Fig. 3 Sorghum SEM images: **a** untreated, **b** dilute ammonia treated, **c** untreated (post fermentation), and **d** dilute ammonia treated (post fermentation). All images at ×1,000 magnification

was observed in treated biomass due to increased surface area and porosity (~84% with full-strength enzyme concentrations and 73% with half-strength enzyme concentrations) as compared with untreated sorghum fibers (~38% with both enzyme concentrations). The highest ethanol concentration was observed at 48 h. Ethanol yields were 84% for full strength and 73% for half strength as compared with 44% and 45% for untreated sorghum. Enzyme hydrolysis and ethanol yields of dilute-ammonia-treated sorghum were comparable to or better than those obtained from favored technologies. Glycerol, organic acids, and furfurals concentrations were below the toxicity level to enzymes and yeast cells.

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