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Compositional Changes in Sugarcane Bagasse on Low Temperature, Long-term Diluted Ammonia Treatment

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Abstract Sugarcane bagasse is the major by-product of the sugar industry. It has a great potential for the production of biofuels and chemicals due to its considerable amount of cellulose and hemicellulose. In this study, we investigated a simple and economic pretreatment process using dilute ammonia for the storage of sugarcane bagasse. Sugarcane bagasse was stored in 0, 0.03, and 0.3% (*w*/*w*) ammonium hydroxide in a closed bottle for 40 days at 30 °C under atmospheric pressure without any agitation or circulation. Samples were taken every 10 days and analyzed for changes on lignin, cellulose, hemicellulose composition, ammonia concentration, and microbial counts. Biomass storage for 40 days at 0.3% ammonium hydroxide removed 46% of lignin and retained 100% cellulose and 73% hemicellulose.

Keywords Sugarcane bagasse · Low temperature pretreatment · Dilute ammonium hydroxide · Lignin · Cellulose · Hemicellulose

Introduction

Over the last few decades, increasing efforts and capital have been invested on research for bio-fuels and chemicals from lignocellulosic plants [1, 2]. The use of lignocellulosics (i.e., bagasse, straw, and grasses) for fuels and chemicals is considered an attractive approach because of their availability and large-scale production [3, 4]. Lignin, cellulose, and hemicellulose are the main components of lignocellulose. Lignin, which binds and encapsulates carbohydrates, is generally resistant to microbial/enzymatic attack in its native state. Pretreatment is a prerequisite process for the production of bio-ethanol and value added sugar-based products. The primary purpose of pretreatment is to disrupt the lignocellulosic matrix, so that enzymes can have access to cellulose and hemicellulose for the release of mono-sugars [5, 6]. Development of an economic pretreatment technology is the main challenge for the biofuel industry [7–9]. Pretreatments using alkaline agents such

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as sodium, calcium, and ammonium hydroxide are more effective than acid catalyzed pretreatments for delignification without having adverse effects on other components [10, 11]. Alkali pretreatments cleave the bonds between lignin and hemicellulose, thus allowing the penetration of water to the inner layers of cellulose increasing its surface area [5]. Ammonia offers certain advantages as a pretreatment such as it is safe to handle, non-polluting and non-corrosive [12] and can be recyclable due to its high volatility [13]. Ammonia pretreatment has been studied on various feedstocks [14–17] using various processes [16, 18–22]. In most processes, ammonia pretreatments have been performed at high-temperatures, resulting in high delignification; however, disadvantages to these processes are high energy input, formation of toxic compounds, and loss of sugars [14, 23]. Low reaction temperature has been reported as an alternative approach to alleviate these challenges [14, 15, 17, 24, 25]. Various reports have indicated that the use of ammonia at room temperature minimized its interaction with hemicellulose [14, 15, 17] and the formation of toxic compounds while increasing bioconversion and fermentation yields. Ammonia is used as a reagent for the removal of lignin [12]. Ammonia cleaves the C–O–C bonds in lignin and the ether and ester bonds between lignin and hemicellulose [12]. Ammonia can also penetrate the crystalline structure in cellulose and cause swelling [12].

The objective of this study was to observe chemical, microbial, and compositional changes of sugarcane bagasse stored in our dilute ammonia technology [16] at low temperature (30 °C) for long periods of time.

Materials and Methods

Pretreatment

Bagasse from sugarcane (*Saccharum officinarum*) was obtained from the Cora Texas Manufacturing Co. in Louisiana. The fractions over 20 mesh were used in all experiments. The composition of sugarcane bagasse was 43.2 wt.% of glucan, 23.4 wt.% xylan, 1.5% arabinan, 23.4% lignin, and 3.8% ash. In each experiment, 30 g bagasse (dry basis) was soaked in 240 ml of water (control), 0.03 or 0.30 wt.% ammonium hydroxide solution in steel bottles at 30 °C for 10–40 days without agitation or shaking during storage. Each steel bottle from different conditions was taken every 10 days, and pH, free ammonia concentration, and total microbial counts were determined. Samples were separated by filtration into solid and liquid portions. Solids were washed with DI water and analyzed for cellulose, hemicellulose, and lignin content following NREL Chemical Analysis and Testing Standard Procedures [26].

Analytical Methods

Cellobiose, glucose, xylose, arabinose, and mannose were separated using an Aminex HPX-87Pb column (Bio-Rad, Hercules, CA) at 85 °C at a flow rate of 1.0 ml/min with water as eluent. HPLC was equipped with a refractive index detector. Free ammonia was determined by neutralization with 1.0066 N H_2SO_4.

Microbiology Methods

A sample was taken from the liquid fraction and serially diluted with 0.85% NaCl. Dilutions were spiral plated on plate count agar (PCA, Difco, MI, USA) and on potato

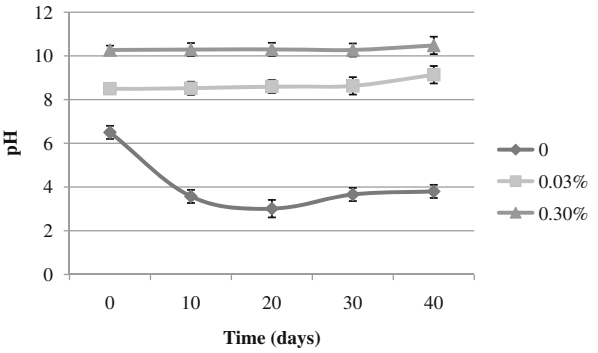

Fig. 1 Changes of pH on ammonium hydroxide concentration and treatment time of sugarcane bagasse. *Note:* The data point in the graph show the mean value (n=4)

dextrose agar (PDA, Difco, MI, USA). Total aerobic bacterial counts were determined on PCA (pH 7.0) while PDA (pH 5.6) was used to enumerate yeast and molds. All plates were incubated at 30 °C for 1–2 days.

Results and Discussion

The change in pH over storage time is shown in Fig. 1. The initial pH for water-treated bagasse was 6.5, 8.5 when treated with 0.03% ammonium hydroxide, and 10.3 in 0.3% ammonium hydroxide. The pH in both ammonium hydroxide treated bagasse samples increased slightly over time; an indication that longer storage times had lower buffering capacity. The pH of bagasse in water decreased to 3.6 after 10 days and remained constant throughout the study. Changes of free ammonia concentration as a function of time are shown in Fig. 2. Free ammonia concentrations in 0.03% ammonium hydroxide-treated bagasse remained constant for 40 days. An 11% decrease was observed after the initial 10-day storage in 0.3% ammonia-treated bagasse. A low ammonia consumption has also been reported in corn stover [14]. Ammonia dissolved in water seems not to interact with bagasse after 10 days.

Microbial growth was observed in all storage tests. Microorganisms in 0.03% ammonium hydroxide-treated bagasse survived in a weak alkaline pH and their concentration (4.2 log CFU/ml of bacteria and 4.1 log CFU/ml of fungi) was stable for 30 days (Fig. 3). The number of bacteria in 0.3% ammonium hydroxide-treated bagasse

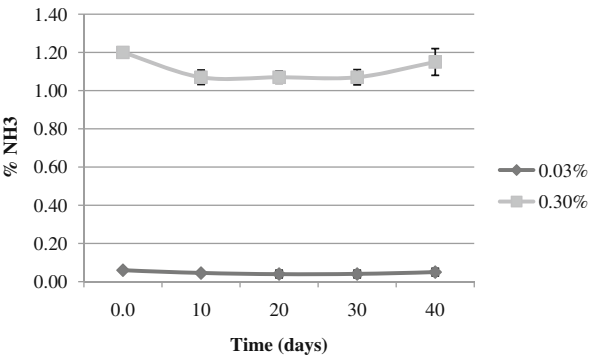

Fig. 2 Concentration of ammonia as a function of time. *Note:* The data point in the graph show the mean value (n=4)

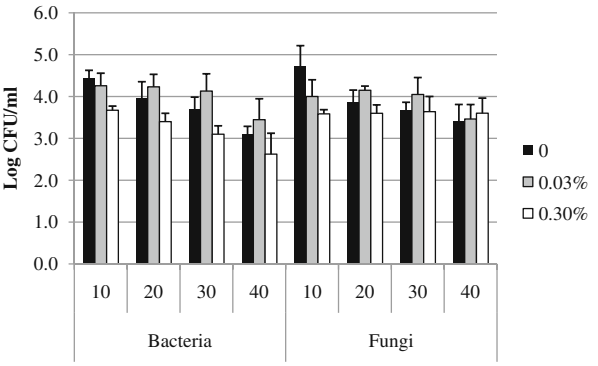

Fig. 3 Microbial changes on ammonium hydroxide concentration and treatment time of sugarcane bagasse. *Note:* The data point in the graph show the mean value (n=4)

decreased from 3.7 log CFU/ml at 10 days to 2.6 log CFU/ml at 40 days; whereas, no change was observed for fungi (Fig. 3). Bacterial numbers decreased linearly with time for water-treated bagasse. Fungi, on the other hand, decreased slowly after 20 days for water-treated biomass (Fig. 3). Microorganisms were unable to reproduce after a 10-day storage because of the unfavorable pH conditions as shown in Fig. 1. Microbial changes in bagasse under alkali storage conditions may have had an impact on the lignin-carhohydrate complex in bagasse; however, more studies are needed to verify their effect on biomass storage.

Compositional changes seen in controls were caused by flora already present in bagasse. Untreated sugarcane bagasse contained 43.2% cellulose, 24.9% hemicellulose, and 23.4% lignin. Compositional changes were observed during a 40-day storage of sugarcane bagasse in water (Fig. 4). Glucan and xylan concentration decreased from 43.19% to 41.56% and from 24.90% to 21.13% for 10 days. After 10 days, they were stable for 40 days. Lignin concentration did not show significant changes for 40 days.

The degrees of delignification were considerably different between 0.03 and 0.3% ammonium hydroxide-treated bagasse samples (Fig. 5). Delignification for 0.03% ammonium hydroxide-treated bagasse began at 20 days and at 10 days for 0.3% ammonium hydroxide treated bagasse. A 10.3% delignification was observed for 40 days and only 23.2% for 40 days in 0.03% ammonium hydroxide-treated bagasse. Delignification increased linearly after 10 days in 0.3% ammonium hydroxide-treated bagasse. Maximum delignification was 46.1% at 40 days in 0.3% ammonium hydroxide-treated bagasse. Reports have sown a 50% deliginification in corn stover soaked in 15% ammonia at 40 °C [14, 15]. A 70%

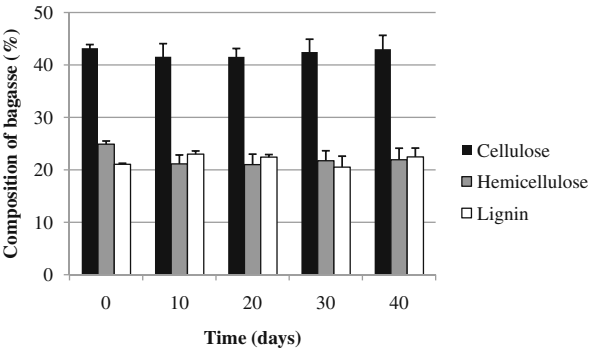

Fig. 4 Changes of carbohydrates and lignin in sugarcane bagasse in water over time. *Note:* (1) The data in the graph show the mean value (n=4). (2) The data in the graph were based on the dry untreated sugarcane bagasse

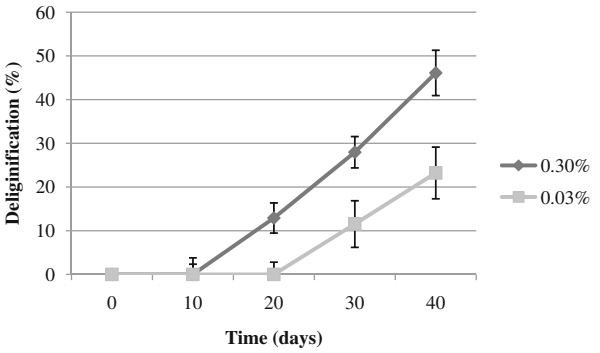

Fig. 5 Delignification of ammonium hydroxide treated sugarcane bagasse at 30 °C. *Note:* (1) The data points in the graph show the mean value (n=4). (2) The data point in the graph based on the dry untreated sugarcane bagasse

delignification was observed when corn stover was soaked in 30% ammonia for 30 days at room temperature [14, 15].

Figure 6 shows the compositional changes of carbohydrates and lignin in ammonium hydroxide-treated sugarcane bagasse. Cellulose in 0.03% ammonium hydroxide-treated bagasse was stable over the entire storage period (Fig. 6a). Hemicellulose content was decreased from 24.90 to 19.93% for a 10-day storage in 0.03% ammonium hydroxide-treated bagasse. A decreased of lignin from 21.98 to 18.64% was observed between a 20- and 30-day storage.

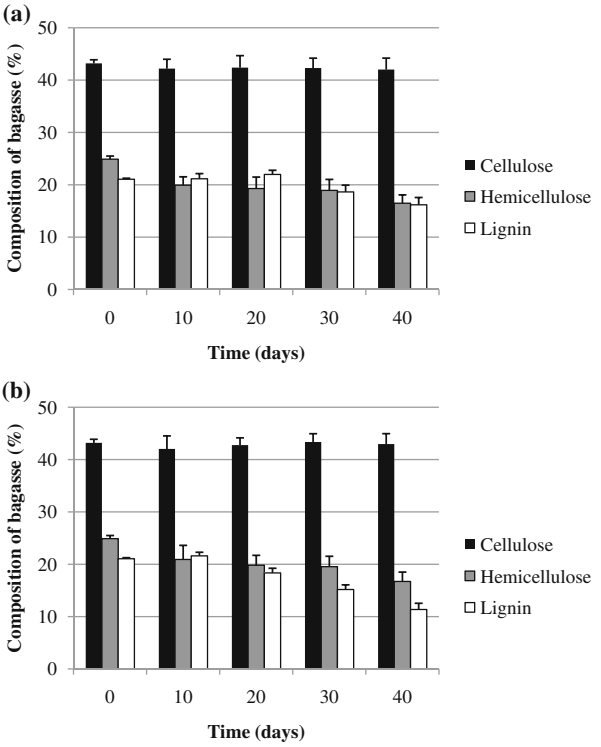

Fig. 6 Changes of carbohydrates and lignin in ammonia treated-sugarcane bagasse over time: **a** sugarcane bagasse stored in 0.03% ammonia solution, **b** sugarcane bagasse stored in 0.3% ammonia solution. *Note:* (1) The data in the graph show the mean value (n=4). (2) The data in the graph based on the dry untreated sugarcane bagasse

Compositional changes of sugarcane bagasse in 0.3% ammonium hydroxide were remarkable in lignin contents (Fig. 6b). Lignin was removed with increasing storage time. Cellulose fraction remained unchanged for a 40-day storage. Similar patterns for cellulose and hemicellulose in 0.3% ammonium hydroxide-treated bagasse were observed in 0.03% ammonium hydroxide-treated bagasse. After a 40-day storage of bagasse in 0.3% ammonium hydroxide solution, composition of bagasse was 42.96% of cellulose, 16.71% hemicelluloses, and 11.36% lignin. The effectiveness of alkali pretreatment at low temperature reported here are comparable to those reported in other studies [12, 17, 27]. Ammonia-treated corn stover (30%) at room temperature delignified 67% and retained 100% of cellulose and 83% of hemicellulose [12]. Kim et al. [17] previously reported that 37.1% of lignin and 4.9% of xylan were removed and glucan content was not changed for 14 days in barley hull soaked in 30% of aqueous ammonia. Lime-treated sugarcane bagasse at 30 °C removed 15% of lignin and retained 80% of cellulose for 30 days [27].

Conclusion

Pretreatment using dilute ammonia at low reaction temperature for sugarcane bagasse is an economic and efficient technology due to low energy input and simple processing. Longer storage of bagasse in ammonia solution resulted in more delignification after delignification was started at 20 days in 0.03% ammonia solution and 10 days in 0.3% ammonia solution. Sugarcane bagasse stored in 0.3% ammonia accomplished the most delignification (46.11% of lignin). Most hemicellulose was decreased during the first 10 days of storage. Cellulose contents in ammonia-treated bagasse were stable for the entire storage time. High delignification and cellulose concentration may be useful for the production of lignocellulosic ethanol. Future work requires the investigation of the effect of this process on enzyme digestibility and ethanol production.

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References

1. Kristensen, J. B., Thygesen, L. G., Felby, C., Jørgensen, H., & Elder, T. (2008). *Biotechnology and Biofuels, 1*, 1–9.
2. Pandey, A., Soccol, C. R., Nigam, P., & Soccol, V. T. (2000). *Bioresearch Technology, 74*, 69–80.
3. Duff, S. J. B. & Murray, W. D. (1996). *Bioresource Technology, 55*, 1–33.
4. Wyman, C. E. (1999). *Annual Review of Engineering Environment, 24*, 189–226.
5. Balat, M., Balat, H., & Öz, C. (2008). *Progress of Energy Combined Science, 34*, 551–573.
6. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., et al. (2005). *Bioresource Technology, 96*, 673–686.
7. Hamelinck, C. N., van Hooijdonk, G., & Faaij, A. P. C. (2005). *Biomass and Bioenergy, 28*, 384–410.
8. Eggeman, T. & Elander, R. T. (2005). *Bioresource Technology, 96*, 2019–2025.
9. Chen, Y., Sharma-Shivappa, R. R., & Chen, C. (2007). *Applied Biochemistry and Biotechnology, 143*, 80–92.
10. Kaar, W. E. & Holtzapple, M. T. (2000). *Biomass Bioenergy, 18*, 189–1999.
11. McMillan, J. D. (1997). *Renewable Energy, 10*, 295–302.

12. Kim, T. H. (2004). PhD. Dissertation, Auburn University.
13. Dale, B. E. & Moreira, M. J. (1982). *Biotechnology and Bioengineering Symposium, 12*, 31–44.
14. Kim, T. H. & Lee, Y. Y. (2005). *Applied Biochemistry and Biotechnology, 121/124*, 1119–1131.
15. Kim, T. H. & Lee, Y. Y. (2007). *Applied Biochemistry and Biotechnology, 137/140*, 81–92.
16. Aita, G. M. & Stradi, B. (2007). PCT/US2009/033173.
17. Kim, T. H., Taylor, F., & Hicks, K. B. (2008). *Bioresource Technology, 99*, 5694–5702.
18. Holtzapple, M. T., Jun, J., Ashok, G., Patibandla, S. L., & Dale, B. E. (1991). *Applied Biochemistry and Biotechnology, 28/29*, 59–74.
19. Reshamwala, S., Shawky, B. T., & Dale, B. E. (1995). *Applied Biochemistry and Biotechnology, 51/52*, 43–55.
20. Dale, B. E., Leong, C. K., Pham, T. K., Esquivel, V. M., Rios, I., & Latimer, V. M. (1996). *Bioresearch Technology, 56*, 111–116.
21. Iyer, P. V., Wu, Z. W., Kim, S. B., & Lee, Y. Y. (1996). *Applied Biochemistry and Biotechnology, 57/58*, 121–132.
22. Kim, T. H., Kim, J. S., Sunwoo, C., & Lee, Y. Y. (2003). *Bioresource Technology, 90*, 39–47.
23. Yang, B. & Wyman, C. E. (2008). *Bioproducts and Biorefining, 2*, 26–40.
24. Morris, P. J. & Mowat, D. N. (1980). *Canadian Journal of Animal Science, 60*, 327–336.
25. Streeter, C. L. & Horn, G. W. (1982). *Animal Feed Science and Technology, 7*, 325–329.
26. NREL (2008). *Chemical analysis and testing standard procedures*. Golden, CO: National Renewable Energy Laboratory.
27. Cotlear, C. B. G. (2004). PhD. Dissertation. Texas A&M University.