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Pretreatment of corn stover using low-moisture anhydrous ammonia (LMAA) process

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ABSTRACT

A simple pretreatment method using anhydrous ammonia was developed to minimize water and ammonia inputs for cellulosic ethanol production, termed the low moisture anhydrous ammonia (LMAA) pretreatment. In this method, corn stover with 30–70% moisture was contacted with anhydrous ammonia in a reactor under nearly ambient conditions. After the ammoniation step, biomass was subjected to a simple pretreatment step at moderate temperatures (40–120 °C) for 48–144 h. Pretreated biomass was saccharified and fermented without an additional washing step. With 3% glucan loading of LMAA-treated corn stover under best treatment conditions (0.1 g-ammonia + 1.0 g-water per g biomass, 80 °C, and 84 h), simultaneous saccharification and cofermentation test resulted in 24.9 g/l (89% of theoretical ethanol yield based on glucan + xylan in corn stover).

1. Introduction

Lignocellulosic biomass is most available potential feedstock for production of bio-ethanol, which is currently the most widely used liquid biofuel alternative to fossil fuels (Demirbas, 2004). Lignocellulosic biomass consists primarily of three different types of polymers, cellulose, hemicellulose and lignin, which are tightly associated with each other (Fengel and Wegener, 1984; Hendriks and Zeeman, 2009). The lignin-hemicellulose association shields the cell wall polysaccharides from enzyme hydrolysis, and thus a pretreatment process is required to permit saccharification. In the past several decades, various pretreatment methods have been suggested to enhance the enzymatic digestibility and fermentability of lignocellulosic biomass (Chang et al., 1998; Holtzapple, et al., 1992; Laser et al., 2002; Yang and Wyman, 2004; Kim and Lee, 2005; Mosier et al., 2005; Teixeira et al., 1999; Zhu et al., 2004). Although a few of them may be effective, several cost barriers which prohibit scale-up exist including high chemical input and excessive water use (Yang and Wyman, 2008; Zheng et al., 2009).

Ammonia is one of the most effective pretreatment reagents because of its many useful properties, which include delignification effect (Kim and Lee, 2005; Streeter and Horn, 1982), swelling effect (Bariska, 1975; Dale, 1986; Foster et al., 2001; Holtzapple et al., 1992; Mosier et al., 2005), and high preservation of cellulose and hemicellulose (Kim and Lee, 2007). From the previous studies, it was reported that ammonia caused swelling of cellulose structure and ammoniation prevents methoxyl groups on lignin from adsorbing cellulases, thus it enhanced the enzyme hydrolysis rates of lignocellulosic biomass and yields of fermentable sugars (Kawamoto et al., 1992; Sewalt et al., 1997). In addition, the antimicrobial effect of ammoniating allowed long-term storage of biomass with minimal biodegradation of carbohydrates (Tajkarimi et al., 2008). In our group, various types of ammonia pretreatments have been investigated, which could achieve high enzymatic digestibility (Kim et al., 2003; Kim and Lee, 2005, 2007; Li and Kim, 2011). Although ammonia pretreatments are effective in improving the applications of biomass, there are still some economical issues with high water and chemical consumption. Table 1 summarizes the chemical and water consumptions in various ammonia pretreatment methods and the maximum ethanol yield at optimal pretreatment conditions. Ammonia recycle percolation (ARP) needed relatively low amounts of liquids (0.5 g ammonia/g biomass and 2.8 g water/g biomass) (Table 1), and gave effective delignification (70–90%). However, significant amounts of xylan (~50%) were removed during the pretreatment. Moreover, the reaction temperature was relatively high (170–210 °C) and additional energy was consumed to recover and reuse the

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Table 1
Emical and water inputs in various ammonia pretreatment methods and their maximum ethanol yields.

Methods	Reaction conditions	Ammonia input [g/g-biomass]	Water input[a] [g/g-biomass]	Maximum ethanol yield & concentration[b] [%] ([g/l])
ARP[c]	170 °C, 10 min, 3.3 g-liquid/g-solid, 15 wt.% NH_3	0.5	2.8	71 (19.4)
SAA[d] at room temp.	Ambient temp., 10 days, 8.0 g-liquid/g-solid, 29.5 wt.% NH_3	2.4	5.6	72 (19.8)
SAA[e] at moderate temp.	60 °C, 12 h, 6.0 g-liquid/g-solid, 15 wt.% NH_3	0.9	5.1	70 (19.2)
LLA[f]	30 °C, 4 week, 2.0 g-liquid/g-solid, 30 wt.% NH_3	0.5	1.5	70 (19.2)
LMAA[g]	80 °C, 84–96 h, 50–70% MC	0.1	1.0–2.3	89–91 (24.9–25.1)

[a] Water input does not include washing water.
[b] Maximum ethanol yield and concentration are based on the optimal reaction conditions; ethanol yields are calculated based on total glucan and xylan in untreated corn stover.
[c] ARP: Ammonia recycle percolation (Kim et al., 2006).
[d] SAA: Soaking in aqueous ammonia (Kim and Lee 2005).
[e] SAA: Soaking in aqueous ammonia (Kim and Lee 2007).
[f] LLA: Low liquid ammonia (Li and Kim 2011).
[g] LMAA: Low moisture anhydrous ammonia.

ammonia and water in the process (Kim et al., 2006). Soaking in aqueous ammonia (SAA) was developed to alleviate these problems (Kim and Lee, 2005, 2007). It was tested at both ambient and moderate temperatures. The SAA was a low-severity process, so high ethanol yields (70–72%) were achieved with lower energy consumption. However, ammonia and water consumptions were much larger than ARP (Table 1). Recently, Li and Kim (2011) developed low liquid ammonia (LLA) process to significantly reduce the liquid throughput. This process gave a similar ethanol yield (70%) as that of SAA but using much less amounts of liquids (0.5 g ammonia/g biomass and 1.5 g water/g biomass). However, all of the aforementioned methods require an additional washing step to remove and recover the ammonia still associated with the biomass after pretreatment. From the previous study, the amounts of washing water required were 17.2 ml/g biomass for SAA-treated biomass and 20.9 ml/g biomass for LLA-treated biomass. For this reason, the actual water consumptions of these processes are much higher than the numbers presented in Table 1.

In order to eliminate the additional water washing step and to improve the cost-effectiveness of ammonia pretreatment processes, the low moisture anhydrous ammonia pretreatment (LMAA) method was developed. The main objective of this study was to design an effective pretreatment process with anhydrous ammonia that can significantly reduce energy input and ammonia consumption. Pretreatment of biomass with low moisture using gaseous ammonia leads to short exposure time and can be carried out under ambient conditions, therefore, low capital costs are projected. In addition, it has been speculated that ammoniation can also supply assimilable nitrogen (up to 1.2 weight percent (wt.%) of dry biomass) for microbial growth in the fermentor using the treated biomass as substrate (Taylor et al., 2008).

In this study, various pretreatment conditions for the LMAA method were explored. Among the various reaction conditions, reaction time and temperature were optimized to maximize the ethanol yield in the subsequent fermentation. To establish the correlation between these factors and ethanol yield, response surface methodology was applied. The effects of xylanase and residual ammonia on the SSCF of LMAA-treated biomass were also investigated.

2. Methods

2.1. Materials

2.1.1. Feedstock

Corn stover was harvested from central Iowa in 2009 and air-dried at ambient temperature. The corn stover was ground and screened to a nominal size of 9–35 mesh. The composition of the corn stover was 38.7 wt.% glucan, 23.3 wt.% xylan, 2.1 wt.% galactan, 4.5 wt.% arabinan, 17.1 wt.% lignin (acid insoluble + acid soluble), 1.5 wt.% sucrose, 1.2 wt.% ash, and 11.6 wt.% other extractives. The procedure for compositional analysis is described in the Analytical section.

2.1.2. Enzymes

Cellulase GC 220 (Lot #301-04232-162) and Multifect-xylanase (Lot #301-04021-015) were provided by Genencor, a Danisco Division. The average activities of cellulase (GC-220) and xylanase (Multifect) were 45 filter paper unit (FPU)/ml and 8000 Genencor xylanase unit (GXU)/ml, respectively. The β-glucosidase enzyme, Novozyme 188 (Novo Inc., Lot #11K1088), was purchased from Sigma–Aldrich (St. Louis, MO). Activity of Novozyme 188 was 750 cellobiase units (CBU)/ml.

2.1.3. Microorganism

Recombinant *E. coli* KO11 (ATCC_ 55124) was purchased from the American Type Culture Collection (ATCC) and used for the simultaneous saccharification and cofermentation (SSCF) experiments. *E. coli* KO11 was maintained on LB (Luria–Bertani) solid medium (Sigma, Cat. #L-3152) which consisted of 5 g/l yeast extract, 10 g/l tryptone, and 5 g/l NaCl, supplemented with 15 g/l agar (Sigma Cat. #B0128234), 2 g/L dextrose (Fisher Cat. #D16), and 40 mg/l chloramphenicol (Sigma Cat. #C-0378). The culture was transferred monthly. To prepare the plates, the media were autoclaved at 121 °C for 15 min and allowed to cool to about 50 °C. Dextrose and chloramphenicol then were added and the media were poured onto the plates and allowed to solidify. The plates were kept refrigerated at 4 °C.

2.2. Experimental setup and operation

2.2.1. Moisturization

The moisture content of air-dried corn stover was ~8%. In order to adjust the moisture content of corn stover to the various target levels (30, 50, and 70 wt.%), additional water was added and the corn stover was steeped for 24 h. Each sample was ammoniated, pretreated, and dried under same conditions. Residual ammonia content in each sample was measured and then the SSCF was conducted.

2.2.2. Ammoniation

Steeped corn stover was placed in the sealed batch reactor (2.9 inch (8.1 cm) internal diameter × 6.5 inch (18.5 cm) length, 690 ml internal volume). An ammonia gas cylinder with single stage gas regulator was connected to the bottom of the reactor