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Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment ☆

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Abstract

Barley hull, a lignocellulosic biomass, was pretreated using aqueous ammonia, to be converted into ethanol. Barley hull was soaked in 15 and 30 wt.% aqueous ammonia at 30, 60, and 75 °C for between 12 h and 11 weeks. This pretreatment method has been known as "soaking in aqueous ammonia" (SAA). Among the tested conditions, the best pretreatment conditions observed were 75 °C, 48 h, 15 wt.% aqueous ammonia and 1:12 of solid:liquid ratio resulting in saccharification yields of 83% for glucan and 63% for xylan with 15 FPU/g-glucan enzyme loading. Pretreatment using 15 wt.% ammonia for 24–72 h at 75 °C removed 50–66% of the original lignin from the solids while it retained 65–76% of the xylan without any glucan loss.

Addition of xylanase along with cellulase resulted in synergetic effect on ethanol production in SSCF (simultaneous saccharification and co-fermentation) using SAA-treated barley hull and recombinant *E. coli* (KO11). With 3% w/v glucan loading and 4 mL of xylanase enzyme loadings, the SSCF of the SAA treated barley hull resulted 24.1 g/L ethanol concentration at 15 FPU cellulase/g-glucan loading, which corresponds to 89.4% of the maximum theoretical yield based on glucan and xylan.

SEM results indicated that SAA treatment increased surface area and the pore size. It is postulated that these physical changes enhance the enzymatic digestibility in the SAA treated barley hull.

Keywords: Bioenergy; Lignocellulosic biomass; Aqueous ammonia; Lignin removal; SAA

1. Introduction

There has been growing interest in using barley grain as a feedstock for fuel ethanol. Barley is currently being used for ethanol production in Europe but not in the US. Barley has some advantages as a corn substitute for ethanol production outside the Corn Belt, particularly on the East Coast, the upper Midwest, and the Northwest (USDA-NASS, 2006). North America grows approximately 14% of the world annual production of barley (Kim and Dale, 2004). Most fuel ethanol in the US is corn-based. Hence most production facilities are located in the Corn Belt, not on either of the coasts where demand for ethanol is high (Hsu, 1996). Barley hull, obtained as a low-value by-product of barley (starch) ethanol facilities, represents a pre-collected source of lignocellulosic biomass that could be utilized on site for "cellulosic" ethanol production. Transportation of biomass to an ethanol production facility is one of the main costs in most cellulosic ethanol production systems (Mahmudi, 2005; Searcy1 et al., 2007). Using a pre-collected form of biomass, as in barley hull, could result in transportation and energy cost savings.

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Another difficulty in utilization of biomass is to maintain year-round supply of feedstock. Agronomically, winter barley fits extremely well into a three-crop two-year rotation with corn and soybeans. Barley is harvested earlier than wheat, allowing a farmer to plant (double crop) soybeans earlier, which can result in good maturity and high yields of the soybeans harvested later that same year. Barley grows well in many areas where corn does not; therefore, it may become a financially cost-effective ethanol feedstock for these regions (USDA-NASS, 2006). Furthermore, the composition of barley hull indicates that about 70% of the weight is carbohydrate which could potentially be utilized for ethanol production.

For fuel ethanol production, pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock, due to its recalcitrant nature. Part of the effect of pretreatments is the removal of lignin, a constituent that is known to inhibit saccharification enzymes and fermentative microorganisms (Chang and Holtzapple, 2000; Mooney et al., 1998; Schwald et al., 1988; Cowling and Kirk, 1976). The barley hull is also quite abrasive on processing equipment and makes up a considerable amount of a hulled barley kernel, up to 10–15% of the grain weight. A pretreatment that can reduce the rigidness of this material is therefore desired.

Ammonia as a pretreatment reagent has many advantages for an effective delignification as well as swelling of biomass. Pretreatment methods using aqueous ammonia have been studied for the purpose of ethanol production (Kim and Lee, 2007, 2006, 2005a, 2005b; Kim et al., 2003). Among them, the soaking in aqueous ammonia (SAA) at low temperature retains the hemicellulose in the solids by minimizing the interaction with hemicellulose during treatment, which was reported as a feasible approach to increase the fermentation yield and simplify the bioconversion scheme (Kim and Lee, 2007, 2005b). Retained xylan can usually be hydrolyzed to fermentable pentoses by most commercial cellulase and xylanase mixtures (Kim and Lee, 2007, 2005b). In the study, barley hull was soaked in 15–30 wt.% aqueous ammonia for extended periods of time at 30–75 °C. This study was focused on evaluation of the SAA method as a pretreatment method of barley hull for the production of ethanol and the effect of additional xylanase on SSCF reaction of SAA-treated barley hull. Enzymatic saccharification was conducted to evaluate the pretreated hull's potential for bioconversion to fuel ethanol and/or for use as a ruminant (dairy and beef cattle) feed component with enhanced digestibility.

2. Methods

2.1. Materials

Nomini, a six-row winter hulled barley grown and harvested in Virginia in 2004, was used in this study. The barley was dehulled by a roller mill and aspirator resulting in a purified hull fraction which contained only about 12.1 wt.% of residual starch. Separated barley hull was put through a two-step enzymatic destarching process to remove the residual starch in order to avoid interference with glucose from cellulose during experiments. It is probable that in a future commercial process, separated hull would be subjected to the destarching process, and then the recovered residual starch would eventually be combined with the main starch liquefaction stream. Enzymes and the conditions for the treatment used were as follows:

- Amylase (Spezyme Fred, Genencor International Inc. Lot #107-02285-003), pH 6.0, 80 °C, 60 min.
- Glucoamylase (Optidex L-300, Genencor International Inc. Lot #105-01232-001), pH 4.5, 55 °C, 60 min.

Cellulase enzyme, GC-220 (Genencor International Inc., Lot #301-04232-162), was obtained from Genencor International. The average activity of the enzyme was 45 filter paper unit (FPU)/mL and the protein content was 184 mg/mL. Activity of β-glucosidase (Novozyme 188 from Novo Inc., Lot No. 11K1088) was 750 CBU/mL. Xylanase enzyme, Multifect-Xylanase (Genencor International Inc) was used for the SSCF tests and the protein content was 40 mg/mL.

Avicel® PH-101, microcrystalline cellulose (MCC), was purchased from Sigma–Aldrich (Sigma Cat. No. 11365, Lot No. 1094627-54804207). Avicel was used as a reference in the enzymatic digestibility test for cellulose, because it is nearly pure cellulose (~97%) and has no lignin with uniform quality in any batch.

Recombinant *Escherichia coli* ATCC® 55124 (KO11) was employed for the SSCF tests. LB medium (Sigma Cat. No. L-3152) was used for the growth of KO11, which contained 1% tryptone, 0.5% yeast extract, 1% NaCl, and 40 mg/L chloroamphenicol.

2.2. Experimental setup and operation

For the pretreatment using SAA, destarched barley hull was treated with 15 or 30 wt.% of aqueous ammonia in screw-capped laboratory bottles (pyrex bottles) at 30–75 °C for 12 h to 77 days with no agitation. Solid-to-liquid ratio of 1:12 was applied. After soaking, the solids were separated by filtering, washed with deionized (DI) water until its pH was around 7.0, and then subjected to the solid compositional analyses and enzymatic digestibility tests. Acid insoluble lignin, carbohydrate content, and digestibility were all determined following NREL Chemical Analysis and Testing Standard Procedure (NREL, 2004).

2.3. Digestibility tests

The enzymatic digestibility of barley hull was determined in duplicate according to the NREL Chemical Analysis and Testing Standard Procedure (NREL, 2004). The conditions of the enzymatic digestibility tests were 50 °C