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Short Communication

Long term storage of dilute acid pretreated corn stover feedstock and ethanol fermentability evaluation

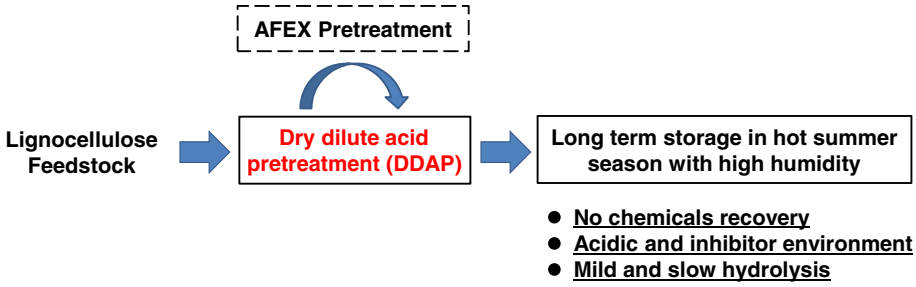
Jian Zhang [1], Shuai Shao [1], Jie Bao *

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

HIGHLIGHTS

- Dry dilute acid pretreatment was applied to long term storage of lignocellulose.
- 3 months' storage led no negative changes of physical property and hydrolysis yield.
- Additional merits were found by dry dilute acid pretreatment for long term storage.

GRAPHICAL ABSTRACT



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ABSTRACT

This study reported a new solution of lignocellulose feedstock storage based on the distributed pretreatment concept. The dry dilute sulfuric acid pretreatment (DDAP) was conducted on corn stover feedstock, instead of ammonia fiber explosion pretreatment. Then the dry dilute acid pretreated corn stover was stored for three months during summer season with high temperature and humidity. No negative aspects were found on the physical property, composition, hydrolysis yield and ethanol fermentability of the long term stored pretreated corn stover, plus the additional merits including no chemicals recovery operation, anti-microbial contaminant environment from stronger acid and inhibitor contents, as well as the mild and slow hydrolysis in the storage. The new pretreatment method expanded the distributed pretreatment concept of feedstock storage with potential for practical application.

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1. Introduction

Long time preservation of lignocellulose biomass without microbial contamination and dry matter loss is crucially important for developing supply chain of feedstock to large scale biofuels production (Eranki et al., 2011; Jin et al., 2013; Liu et al., 2013). Commonly practiced storage methods include dry storage and wet storage. Dry storage is to store the dried (moisture content less than 10%) lignocellulose bales outdoors, but considerable drying cost, solid matter loss, and fire risk were the negative aspects of dry storage (Liu et al., 2013). Wet storage is to store the newly collected and moist lignocellulose biomass similar to ensiling method of animal forage (Cui et al., 2012; Digman et al., 2010), in which endogenous microflora such as lactic acid bacteria consume oxygen and generate organic acids to create an anaerobic and acidic environment to prevent further microbial growth and facilitate long term storage (Pakarinene et al., 2011). However, bale wrapping and disassembling, as well as mixing of bacteria broth or alkali or acidic chemicals with solid lignocellulose are the technical barriers for large scale applications of wet storage, besides the soluble sugars loss.

* Corresponding author. Tel./fax: +86 21 64251799.
E-mail address: jbao@ecust.edu.cn (J. Bao).
[1] These authors are equally contributed to this work.

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Different from the regular storage methods, Dale and his colleagues used biorefinery techniques to store lignocellulose feedstock. One option is to store the liquefied hydrolysate after lignocellulose pretreatment and enzymatic hydrolysis (Jin et al., 2013), but this method is under the risk of microbial contamination especially in summer seasons and tropical areas. The second option is to store the pretreated lignocellulose feedstock by distributing pretreatment operations near collection locations, instead of storage in the central biorefinery plants (Eranki et al., 2011; Bals and Dale, 2012). Ammonia fiber expansion (AFEX) pretreatment was applied to the distributed storage by taking advantage of pretreated feedstock on anti-contamination and densification. This distributing storage provided a practical solution for long term storage of lignocellulose.

In this study, we applied a different pretreatment method, the dry dilute sulfuric acid pretreatment (DDAP), into the distributed pretreatment concept for lignocellulose feedstock storage (Zhang et al., 2011; He et al., 2014). A three months' storage of the dry dilute sulfuric acid pretreated corn stover was conducted during the summer season of Shanghai, a typical southern city of China with high temperature and humidity. The changes in physical property, composition, enzymatic hydrolysis yield and ethanol fermentability were evaluated during long term storage. The positive result with additional merits supported the new distributed storage method based on dry dilute sulfuric acid pretreatment. This study expanded distributed pretreatment concept for feedstock storage with improved application potentials.

2. Methods

2.1. Raw materials

Corn stover was obtained from Dancheng County, Henan Province, China, in fall 2013. Corn stover was water-washed to remove the field dirts, air-dried, and milled using a beater pulverizer to pass through the 10-mm diameter apertures. The milled corn stover was sealed in plastic bags and stored at room temperature until used.

2.2. Strains and enzymes

Ethanol fermenting strain *Saccharomyces cerevisiae* DQ1 (stored in China General Microbial Collection Center, Beijing, China, with registration number CGMCC 2528) was used for ethanol production during SSF. The fungus strain *Amorphotheca resinae* ZN1 (CGMCC 7452) was used for degrading inhibitors from dry dilute sulfuric acid pretreatment of corn stover (Zhang et al., 2010a).

The cellulase enzyme Youtell #6 was purchased from Hunan Youtell Biochemical Co., Yueyang, Hunan, China. The filter paper activity of Youtell #6 was 135 FPU/g determined using the NREL protocol LAP-006 (Adney and Baker, 1996), the cellobiase activity was 344 CBU/g, and the protein content was 90 mg per gram of cellulase reagent determined by Bradford method.

Sulfuric acid with a purity of 95.0–98.0% (w/w) used in the pretreatment was purchased from Shanghai Lingfeng Chemical Reagent, Shanghai, China; Standard samples of acetic acid was purchased from Sinopharm Chemical Reagents, Shanghai, China. Furfural and 5-hydroxymethylfurfural (HMF) were purchased from J&K Scientific, Beijing, China.

2.3. Dry dilute sulfuric acid pretreatment and biodetoxification operations

Corn stover was pretreated using dry dilute sulfuric acid pretreatment (DDAP) according to Zhang et al. (2011) and He et al. (2014). Briefly, corn stover and dilute sulfuric acid solution were co-currently fed into the 20 L pretreatment reactor at a solid/liquid ratio of 2:1 (w/w) and helically stirred at 50 rpm. The operation was kept at 175 °C for 5 min with sulfuric acid of 2.5 g per 100 g solid lignocellulose. The solids content of the pretreated material was about 50% (w/w) with pH 2.0 and no wastewater was generated.

The pretreated corn stover material was detoxified via solid state biodetoxification according to Zhang et al. (2010a) by hydrolysis and fermentation operations. Briefly, the pretreated corn stover was neutralized with 20% (w/w) $Ca(OH)_2$ to pH of 5–6, and then inoculated with *A. resinae* ZN1 spores. Biodetoxification lasted for 48 h at 28 °C with sterilized aeration at 1.0 vvm and periodical mixing by helical impeller agitation every 12 h. The solids content of biodetoxified corn stover was still around 50% (w/w). Corn stover composition after biodetoxification showed no obvious change with the freshly pretreated feedstock.

2.4. Enzymatic hydrolysis and ethanol fermentability tests

The stored corn stover material was assayed in two aspects: (1) enzymatically hydrolysis evaluation on its hydrolysis performance; (2) simultaneous saccharification and ethanol fermentation (SSF) evaluation on its fermentability performance.

Enzymatic hydrolysis assay of the pretreated corn stover feedstock was carried out according to the protocol of NREL LAP-009 (Brown and Torget, 1996). 0.5 g of the pretreated corn stover (dry base) and 10 mL of deionized water were loaded into a 100 mL flask to prepare the slurry in 0.1 M citrate buffer containing 2.5% (w/w) solids and pH was finely adjusted to 4.8 by adding 5 M NaOH solution. 0.08 mL of cycloheximide (10 mg/mL in deionized water) was added to avoid the microbial contamination. 20 FPU/g DM (dry pretreated corn stover matter) of cellulase was added and the hydrolysis lasted for 72 h at 50 °C and 150 rpm in a water-bath shaking incubator.

Ethanol fermentability assay was carried out in a 5 L helical ribbon stirrer agitated bioreactor as described in Zhang et al. (2010a). Briefly, the pretreated and biodetoxified corn stover was loaded into the bioreactor to reach 25% (w/w) solids content. 15 FPU/g DM of cellulase enzyme was added and the pre-hydrolysis was carried out for 12 h at 50 °C, then the temperature was reduced to 37 °C and the seed cells of *S. cerevisiae* DQ1 were inoculated into the bioreactor at 10% inoculation ratio (v/v) to start the simultaneous saccharification and fermentation step (SSF). Samples were taken periodically for analysis of ethanol and glucose.

2.5. Long term storage of pretreated corn stover feedstock

Freshly pretreated corn stover was fed into commonly used disposable polyvinylchloride (PVC) bags with only loosely closure without thermal sealing of the open ends. Each bag contained approximately 2 kg of pretreated corn stover then placed on terrace of the 13th floor of the research building #18, Xuhui campus, East China University of Science and Technology, Shanghai, China. The storage started on June 16, 2014 and ended on September 15, 2014 (91 days). Every two bags were withdrawn at the 15th day (July 1, 2014), 29th day (July 15, 2014), 59th day (August 14, 2014), and 91st day (September 15, 2014), respectively, for composition, hydrolysis and ethanol fermentation assays. The fresh pretreated corn stover immediately after pretreatment was taken as the control of storage.

2.6. Analysis

The bulk density of corn stover was tested using the methods discussed in Hoover et al. (2014). A 261 mL container was used (instead of the 100 mL container). The bulk density was calculated based on the dry matter of the corn stover.

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