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Combined pretreatment using ozonolysis and ball milling to improve enzymatic saccharification of corn straw

Feng Shi [a,b,c,*], Heji Xiang [a,b], Yongfu Li [d]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China
[c] Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi 214122, China
[d] National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China

HIGHLIGHTS

- Combination of OZ and BM for the more effective pretreatment of corn straw.
- BM after OZ resulted in obviously higher saccharification yield than mere OZ or BM.
- OZ after BM sometimes resulted in higher saccharification yield than mere OZ or BM.
- Cellulase loading for effective hydrolysis decreased after OZ and/or BM treatment.

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ABSTRACT

Two clean pretreatments, ozonolysis (OZ) and planetary ball milling (BM) were applied separately and in combination to improve the enzymatic hydrolysis of corn straw. Pretreatment of corn straw by OZ and BM alone improved the enzymatic hydrolysis significantly, primarily through delignification and decrystallization of cellulose, respectively. When combined, OZ–BM and BM–OZ pretreatments made the enzymatic hydrolysis more efficient. The glucose and xylose yield of corn straw treated with OZ for 90 min followed by BM for 8 min (OZ90–BM8) reached to 407.8 and 101.9 mg/g-straw, respectively under cellulase loading of 15 FPU/g-straw, which was fivefold more than that of untreated straw. Under much lower cellulase loading of 1.5 FPU/g-straw, the glucose and xylose yield of treated straw OZ90–BM8 remained at 416.0 and 108.4 mg/g-straw, respectively, while the yield of untreated straw decreased. These findings indicate that the combined OZ–BM can be used as a promising pretreatment for corn straw.

1. Introduction

The growing demand for energy is leading to the gradual depletion of conventional fossil fuels. Therefore, exploitation of nonpetroleum-based sources of energy is important. Ethanol is the primary renewable liquid fuel with very good performance (Datta et al., 2011). Ethanol can be blended with petrol or used as neat alcohol in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization (Hahn-Hagerdal et al., 2006). Current production of bioethanol relies on ethanol from starch and sugars, but there has been considerable debate regarding its sustainability. In this context, bioethanol produced from lignocellulosic materials is considered to be a promising alternative because lignocellulosic raw materials are abundant and less expensive than conventional agricultural feedstock, such as corn and sugarcane. The use of lignocellulose to produce bioethanol generally involves three main steps: (1) pretreatment, to break down the recalcitrant structures of lignocelluloses; (2) enzymatic hydrolysis, to hydrolyze polysaccharides (i.e., cellulose and hemicellulose) into fermentable sugars; and (3) fermentation, to convert sugars into ethanol (Huang et al., 2011). Pretreatment is perhaps the single most crucial step as it has a large impact on all the other steps in the overall conversion scheme in terms of cellulose digestibility, fermentation toxicity, stirring power requirements, energy demand in the downstream processes, and waste water treatment demands (Galbe and Zacchi, 2007).

Abbreviations: BM, ball milling; OZ, ozonolysis; AIL, acid insoluble lignin; ASL, acid soluble lignin; WSF, water soluble fraction; HPLC, high performance liquid chromatography; NREL, National Renewable Energy Laboratory; CrI, crystallinity index; TL, total lignin.

* Corresponding author at: State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, China. Tel./fax: +86 510 85329236.

E-mail address: shifeng@jiangnan.edu.cn (F. Shi).

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In recent years, various pretreatment methods have been developed, including biological, chemical, and physical methods, as well as a combination of these methods. In biological pretreatment processes, such microorganisms as brown-, white-, and soft-rot fungi are used to degrade lignin and hemicelluloses, but the rate of degradation is generally very low (Sun and Cheng, 2002). Chemical methods, such as alkali pretreatments and acid pretreatments, have been extensively studied and demonstrated to be effective for breaking the recalcitrant structures of lignocelluloses. However, these methods have a detrimental effect on the environment. Additionally, utilization of these chemicals requires alkali- or acid-resistant equipment and a neutralization step. Furthermore, several types of inhibitors may form and thus inhibit the microbial growth and fermentation, which results in less yield and productivity of ethanol (Taherzadeh and Karimi, 2007).

Mechanical treatments, such as ball milling (BM), are effective in reducing particle size and crystallinity of lignocellulosic substrates (Howsmon and Marchessault, 1959; Silva et al., 2012), which greatly improves the accessibility of biomass cellulose. This treatment has the advantage of being environmentally friendly as no chemical is used; however, it is an energy-intensive process (Hendriks and Zeeman, 2009; Alvira et al., 2010) and therefore needs to be used in combination with other treatment methods to save energy and reduce costs. In addition, BM cannot remove lignin, the one that acts as a physical barrier to restrict the access of cellulases to cellulose, as well as irreversibly adsorb cellulases (Berlin et al., 2006; Zhao et al., 2012).

Gaseous ozone is a strong oxidizing agent with very high reactivity toward compounds having double bonds and functional groups with high electron densities, such as those present in lignin. Delignification by ozonolysis (OZ) pretreatment of several agricultural residues, such as wheat straw and rye straw, has been demonstrated to be effective and therefore can increase the yield in following enzymatic hydrolysis (Garcia-Cubero et al., 2009). Meanwhile, OZ has several advantages, such as (1) on site generation, thereby avoiding problems associated with chemical supply and storage; (2) reaction at ambient temperature and pressure; (3) no formation of inhibitory compounds that can interfere with subsequent enzymatic hydrolysis and fermentation (Vidal and Molinier, 1988).

In this study, to pretreat corn straw effectively and cleanly, a novel approach based on OZ in combination with planetary BM was evaluated and its effect on enzymatic hydrolysis of corn straw was analyzed. Pretreatment of corn straw by planetary BM alone resulted in a great reduction in particle size, a great increase of available surface area of corn straw, and a great decrease of crystallinity of cellulose, whereas pretreatment of corn straw by OZ alone resulted in significant removal of lignin. Although enzymatic hydrolysis of corn straw pretreated by OZ or BM alone was improved, the combination of OZ and BM made the pretreatment and enzymatic hydrolysis more efficient. Pretreatment of corn straw by OZ for 90 min followed by BM for 8 min (OZ90–BM8) resulted in the highest glucose yield (407.76 mg/g-straw) and nearly highest xylose yield (101.87 mg/g-straw), indicating it as the optimal pretreatment strategy tested. Meanwhile, the cellulase loading for optimal enzymatic hydrolysis of corn straw decreased considerably after OZ and/or BM pretreatment.

2. Methods

2.1. Raw materials

The corn straw was harvested from Henan province in China in November 2011. After collection, the straw was dried at room temperature and later stored at 4 °C. Prior to experimentation, the corn straw was cut by a grinder and subsequently passed through a 0.6-mm mesh. The resulting particles were regarded as the raw materials for ball milling and ozonolysis pretreatment. The chemical composition of the initial raw material was analyzed by the methods described in Section 2.5 and was determined to be 31.8% glucan, 19.6% xylan, 17.6% acid insoluble lignin (AIL), 2.2% acid soluble lignin (ASL), and 11.3% ash.

2.2. Ozonolysis pretreatment

The corn straw was ozonized in a fixed bed reactor (glass column 50 cm in height and 2.7 cm in diameter) under room conditions. At the beginning of each test, 10 g of corn straw (dry weight) was adjusted to the humidity of 50% (*w*/*w*) and allowed to equilibrate for 8 h. Subsequently, ozone was charged at 60 mg/L, with a gas flow rate of 60 L/h for 30, 45, 60, 75, 90, 105, and 120 min. The ozonated straws obtained were designated as OZ30, OZ45, OZ60, OZ75, OZ90, OZ105, and OZ120. An ozone generator (CF-G-3-20g, Guolin, China) supplied with industrial grade oxygen was used to produce the ozone. Ozone concentrations in the reactor inlet and outlet were measured on site by two UV ozone monitors (IDEAL-2000, Aidier, China). The amount of ozone consumed was calculated from the inlet and outlet ozone concentrations and was expressed as the consumption amount per gram of dry straw. After pretreatment, the ozonated straw was washed with distilled water in a 1:20 (*w*/*v*) mass of dry materials and then filtered under vacuum to remove potential inhibitors. The resulting solid residues were dried at 40 °C for enzymatic hydrolysis immediately or for the following BM pretreatment. The filtrate was dried at 105 °C and used to quantify the water-soluble fraction (WSF) of ozonated straw.

2.3. Ball milling pretreatment

The corn straw were dried in vacuum at 40 °C for 12 h before milling and later subjected to a planetary ball miller (XXM, Jiaxing, China) equipped with two jars (150 mL each) as reported previously (Shi et al., 2014) with several modifications. Briefly, 6.0 g of samples was added to one jar and milled with 120 g of zirconia balls (8 mm in diameter) at 139.5 rad/s for 1, 2, 4, 8, and 12 min. The milled material obtained were designated as BM1, BM2, BM4, BM8, and BM12 and used directly for enzymatic hydrolysis or for the next BM pretreatment.

2.4. Enzymatic hydrolysis

Enzyme hydrolysis was performed under corn straw concentration of 5% (*w*/*w*) and an enzyme load of 15 FPU Celluclast 1.5 L cellulase (Novozyme, China) supplemented with 10 CBU cellobiase (Ruiyang, China) per gram of corn straw in 50 mM sodium citrate buffer (pH 4.8). An excess of cellobiase was used to prevent cellobiose accumulation and 40 μg/mL of tetracycline was added to prevent microbial contamination during the digestion. Reactions were conducted at 50 °C for 24 h with rotary shaking (150 rpm). After hydrolysis, the aliquot was heated at 95 °C for 15 min and centrifuged. The supernatant was then filtered through a 0.2-μm syringe filter and analyzed by high performance liquid chromatography (HPLC) to measure sugar content.

2.5. Component and sugar analysis

The composition of cellulose, hemicellulose, AIL, ASL, and ash were determined using the procedure described by National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). Monomeric sugars including glucose and xylose in the enzymatic and acidic hydrolysates were analyzed by HPLC (Chromaster, Hitachi,