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Ozonolysis pretreatment of maize stover: The interactive effect of sample particle size and moisture on ozonolysis process

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HIGHLIGHTS

- Both particle size and moisture content had significant effects on ozonolysis.
- The ratio of free and bond water is a key factor for ozonolysis.
- Glucose yield after enzymatic hydrolysis increased as delignification rate increased.
- Washing ozonolyzed samples made no obvious difference to enzymatic digestibility.

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ABSTRACT

Maize stover was ozonolyzed to improve the enzymatic digestibility. The interactive effect of sample particle size and moisture content on ozonolysis was studied. After ozonolysis, both lignin and xylan decreased while cellulose was only slightly affected in all experiments. It was also found that the smaller particle size is better for ozonolysis. The similar water activity of the different optimum moisture contents for ozonolysis reveals that the free and bound water ratio is a key factor of ozonolysis. The best result of ozonolysis was obtained at the mesh of −300 and the moisture of 60%, where up to 75% lignin was removed. The glucose yield after enzymatic hydrolysis increased from 18.5% to 80%. Water washing had low impact on glucose yield (less than 10% increases), but significantly reduced xylose yield (up to 42% decreases). The result indicates that ozonolysis leads to xylan solubilization.

1. Introduction

In recent years, biorefinery technologies have attracted significant attention due to the worldwide growing problems including oil shortage, pollution and excessive population. Unlike the first generation biofuel made of cereals, the second generation biofuel made from lignocellulosic materials has greater environmental benefits and sustainability (Menon and Rao, 2012). Lignocellulosic ethanol represents the most developed route of the second generation biofuel. Producing lignocellulosic ethanol involves three steps: (a) pretreatment, which enhances the enzymatic saccharification of biomass; (b) enzymatic hydrolysis, which converts structural polysaccharide into fermentable sugars; and (c) fermentation, which produces ethanol and other products using fermentable sugars (Sarkar et al., 2012).

Maize is one of the world's widest planted crops. It has also become the largest crop in China since 2012. Compared to other types of lignocellulosic material, maize stover has low lignification and high carbohydrate content making it an abundant and valuable agricultural residue. However, maize stover is currently underexploited. In China, only less than 10% of maize stover get in the pathway of high added-value processing while almost 40% is left in the field or burned, causing severe waste and pollution. Other crop straws are dealt with the same way. The Chinese government had taken measures to change this situation. According to the document (State Council, 2008), *Opinions on Accelerating the Utilization of Crop Straw*, great efforts will be taken to establish efficient straw collection and multi-grade utilization system. This policy will propel the biorefinery industries based on maize stover and other agricultural residues.

However, there are several limits that challenge biorefinery industrialization. The most crucial one is the low efficiency of enzymatic hydrolysis of structural polysaccharide. Several factors have been proven to account for this, including particle size,

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cellulose crystallinity, degree of polymerization, and lignin content (Hendriks and Zeeman, 2009). Among these factors, the lignin content shows the highest impact on biomass degradability. Ding et al. (2012) found that lignin removal enhances binding of enzymes to the cell walls and the extent of degradation. They concluded that the best pretreatments should maximize lignin removal.

Various pretreatment technologies have been investigated, including physical, chemical and biological approaches (Agbor et al., 2011; Alvira et al., 2010; Galbe and Zacchi, 2012; Hendriks and Zeeman, 2009). Ozonolysis pretreatment is regarded as one of the most promising chemical approaches. Due to its highly selective oxidation of compounds with high-electron-density groups (mainly in lignin), ozonolysis shows high efficiency in delignification and high recovery of carbohydrates. Furthermore, low inhibitor production was also reported (Alvira et al., 2010). Ozonolysis has been tested on many types of biomass, such as wheat straw (Binder et al., 1980; García-Cubero et al., 2009), sugarcane bagasse (Barros et al., 2013; Travaini et al., 2013), and energy grasses (Panneerselvam et al., 2013a,b). In these studies, lignin removal after ozonolysis improved the enzymatic hydrolysis yield. García-Cubero et al. (2009) found that moisture content and biomass type are the two main factors of ozonolysis. They proposed a kinetic model for the ozonation reaction (García-Cubero et al., 2012). Travaini et al. (2013) found only xylitol, lactic, formic and acetic acid as oxidation products. Inhibitors like furfural and 5-hydroxymethylfurfural were not detected.

Comminution is an essential step before other processes. It aims to increase the specific surface area and reduce the bulk volume. Studies have shown that individual application of comminution in a specific range of meshes hardly improves hydrolysis (Chang et al., 1997; Chang and Holtzapple, 2000; Elshafei et al., 1991). Hence, focus has been shifted to the combination of comminution with other pretreatment methods (Barros et al., 2013; Chundawat et al., 2006; Miura et al., 2012; Souza-Correa et al., 2014). Barros et al. (2013) investigated the combination of wet disk milling (WDM) and ozonolysis and found that ozonolysis before WDM results in higher saccharification yield than the reverse and reduces the WDM energy consumption. Souza-Correa et al. (2014) compared the ozonation process on substrates with a range of particle sizes. They found that the smaller particle size led to better ozonation result.

In this study, the interactive effect of sample moisture content and particle size on ozonolysis of maize stover was investigated from the perspectives of reaction dynamics, compositional variation and hydrolysis yield. A series of samples with a wide range of particle sizes and moisture contents were ozonolyzed in a fixed bed reactor under fixed conditions. The untreated sample (raw) and completely delignified sample (CDL) were set as blank control and positive control, respectively. To each experimental test, the breakthrough ozone concentration versus reaction time was measured, which revealed a dynamic profile of the ozonolysis reaction. The major components, such as lignin and structural carbohydrates, and enzymatic hydrolysis yield were analyzed to monitor the impact of ozone oxidation on samples. Water activity (A_w) of each experimental test was determined to evaluate the free and bound water ratio.

2. Methods

2.1. Raw material

Maize stover was collected in Liaoning province, China. It was sundried and stored at 4 °C. The leaves, ears and rotten parts were removed then grinded with a laboratory impact mill. The grinded material was sieved with a set of standard sieves: 20, 40, 80, 150, 300 mesh. Thus five fractions obtained from the sieves were named as 20/40, 40/80, 80/150, 150/300 and −300, respectively. The 20/40 refers to the fraction from between 20 mesh and 40 mesh sieves, and so on for the other fractions. Among the five fractions, 20/40, 80/150 and −300 was used as raw sample in this study. The major components of these three fractions were determined (Fig. 2), shown similar value to each other.

2.2. Complete delignified samples (CDL)

Complete delignified samples (CDL) were prepared using 20/40, 80/150 and −300 raw samples. Acid chlorite was used to remove lignin in the raw samples (Ding et al., 2012). The acid chlorite solution contains 0.1 N HCl and 10% $NaClO_2$. Raw samples were added in the 1% (w/v) solution under magnetic stirred at room temperature for 12 h. The mixture was neutralized and vacuum filtrated. The solid residue was washed and freeze dried to obtain the CDL.

2.3. Ozonolysis treatment

The ozonolysis reaction was performed in a glass column reactor (6 cm in diameter and 22.5 cm in height) at constant room temperature, 25 °C. The column was vertically placed. The gas flowed from bottom to top. Ozone monitors were used to detect the ozone concentration in gas flow before and after the reactor. Ozone was generated by an ozone generator (CF-G-3-20g, QingDao GuoLin industry Co., Ltd) with pure oxygen source. The flow rate and ozone concentration maintained at 1.0 L/min and 60 mg/L during the reaction, respectively.

The samples of 20/40, 80/150 and −300 was used in the treatment. Each of the samples was adjusted to the moisture contents of 30%, 45%, 60% and 75% by adding distilled water before ozonolysis. The reactor was loaded with 10 g raw material (dry basis) and the reaction time is 1 h for all tests.

2.4. Compositional analysis

The moisture content was measured by a moisture analyzer (Ohaus MB23). The water extractives were determined by gravimetric analysis before and after water wash. After water wash, insoluble components such as cellulose, xylan and lignin were determined following the NREL LAP "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter et al., 2012). The sugar analysis was conducted through high performance liquid chromatography (Agilent 1260 infinity) equipped with differential refraction detector (RID). An Aminex HPX-87H (7.8 × 300 mm, Bio-Rad, USA) column was used at 60 °C with 5 mM H_2SO_4 as eluent, and the flow rate of eluent is 0.6 ml/min.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis was performed on both raw and pretreated maize stover, with or without washing, to verify the differences of enzymatic degradation. The hydrolysis was performed in 20 ml 0.1 M pH 4.8 citrate buffer with 5% biomass (dry basis) suspended in it. The mixture was put in a 100 ml autoclavable glass bottle with a sealing cap to ensure no evaporate losses during incubating. Two enzymes were used in hydrolysis. One was Novozyme Celluclast 1.5 L with the activity of 33 FPU/ml. Another was cellobiase from Jiangsu Ruiyang Biotech CO., LTD, which has an activity of 60 IU/g. The dose of these two enzymes was 15 FPU and 5 IU per gram of cellulose, respectively. The reaction was incubated at 50 °C for 48 h with a shaking speed of 120 rpm. After incubation, the mixture was centrifugated and the supernate was collected for sugar analysis by HPLC with the same conditions as described in Section 2.2.