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Combined alkali and acid pretreatment of spent mushroom substrate for reducing sugar and biofertilizer production

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HIGHLIGHTS

- An integrated process was built for utilization of spent mushroom substrate (SMS).
- Alkali pretreatment of SMS was investigated for the first time.
- Direct recycle of spent alkali liquor to reduce the cost of pretreatment.
- Combined alkali and acid pretreatment–enzymatic hydrolysis process was proposed.
- SMS residue from enzymatic hydrolysis was used for biofertilizer production.

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ABSTRACT

Spent mushroom substrate (SMS) was pretreated with alkaline reagents including potassium hydroxide, lime and ammonia to enhance enzymatic saccharification. Under the best pretreatment conditions (1 M KOH, 80 °C, 90 min; 1 M lime, 80 °C, 120 min; 10 M ammonia, 70 °C, 120 min), the total reducing sugar (TRS) yield reached 258.6, 204.2 and 251.2 mg/g raw SMS, which were respectively 6.15, 4.86, and 5.98 times of untreated SMS. The effects of pretreatment by above alkaline reagents and sulfuric acid on the composition and structure of SMS were evaluated to provide comparative performance data. A new process, combined alkali and acid (CAA) pretreatment followed by enzymatic hydrolysis, was innovatively proposed to improve the cost-effectiveness and avoid environmental problems. The SMS residue after CAA pretreatment–enzymatic hydrolysis process was converted to biofertilizer with *Pichia farinose* FL7 and a cell density of 3.0×10^8 cfu/g in biomass was attained.

1. Introduction

The spent mushroom substrate (SMS) is a lignocellulosic byproduct of the mushroom industry. As estimated, there are about 2 million tons of SMS produced in China each year (Qiao et al., 2011), however, most of the SMS have been burnt for energy, which is neither environment-friendly nor economic. Furthermore, the lack of sustainable utilization strategies has greatly restricted the development of the mushroom industry (Finney et al., 2009). As a kind of lignocellulosic materials, SMS could be a source of reducing sugars for producing biofuels and other value-added biomaterials (White et al., 2008; Kaparaju et al., 2009). However, pretreatment by removing lignin and hemicellulose and breaking down the cellulose crystalline structure is required to overcome the problems caused by biomass recalcitrance for an effective cellulose conversion process. (Sun and Cheng, 2002; Himmel et al., 2007).

Sulfuric acid has been used to pretreat SMS by Qiao et al. (2011) and Kapu et al. (2012). The sulfuric acid pretreatment method involves high process temperature, which leads to high energy input and may also cause degradation of useful sugars and formation of fermentation inhibitors (Oliva et al., 2006; Panagiotou and Olsson, 2007). Compared to acid pretreatment strategy, alkali pretreatment generally proceeds under lower temperatures and pressures and its efficiency depends on the nature of the biomass feedstock, especially the lignin content (McMillan, 1994). Sodium hydroxide, ammonia and lime have been widely employed as alkaline pretreatment agents in lignocellulosic biomass and have been proved efficient at mild conditions (McIntosh and Vancov, 2010; Wu et al., 2011; Kim and Lee, 2005; Ko et al., 2009; Chang et al., 1997; Xu et al., 2010). In this study, we reported reducing sugar yields from SMS using three different alkaline reagents including potassium hydroxide (KOH), ammonia and lime under mild conditions for

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the first time. The effects of pretreatment by above alkaline reagents and sulfuric acid on the composition and structure of SMS were also evaluated to provide comparative performance data.

Costs and pollution are the main problems during the process of converting lignocellulose to reducing sugars. For conventional acid or alkali pretreatment–enzymatic hydrolysis process, the solid–liquid mixture has to be separated by filtration after pretreatment and then the solid fraction must be washed to remove the remaining alkali or acid. Furthermore, buffer is required for enzymatic hydrolysis to maintain a stable pH environment. All these procedures increase the cost of the pretreatment process. Moreover, the discharge of the spent pretreatment liquid will cause environmental problems such as water and soil contamination. In this research, in order to overcome these deficiencies, a cost-effective and zero-emission process, combined alkali and acid (CAA) pretreatment followed by enzymatic hydrolysis, was innovatively proposed to perform on SMS for reducing sugar production. In CAA pretreatment–enzymatic hydrolysis process, the resulting slurries after alkali and acid pretreatment respectively were mixed together to practice enzymatic hydrolysis. Therefore, this method without filtration, washing, buffer addition and spent liquid emission would have a good economic and environmental prospect.

Biofertilizers have been found to enhance crop yield and promote the sustainable development of agriculture industry. In our previous work, SMS was converted to biofertilizer using a stress-tolerant phosphate-solubilizing *Pichia farinose* FL7, which significantly improved the growth of soybean in pot experiments, demonstrating a tremendous potential in agricultural application (Zhu et al., 2012). However, 1.5% corn flour was added to provide nutritions during the biofertilizer preparation process, which increased production costs considerably. In this research, the SSF medium was prepared mainly from SMS residue after CAA pretreatment–enzymatic hydrolysis process, which was added as nutrients as well as a solid carrier for the growth of *P. farinose* FL7. The SMS hydrolysates containing reducing sugars served as a carbon source for *P. farinose* FL7 growth was added to the SSF medium. This design of SSF medium not only reduced the production costs greatly, but could supply phyto-essential elements remaining in SMS hydrolysates, such as ammonium, sulfate and potassium, which further enhanced the biofertilizer efficacy in promoting plant growth.

Therefore, the objectives of this research were to: (1) evaluate the TRS released from SMS with alkali pretreatment followed by enzymatic hydrolysis, (2) study the composition and structure change of SMS after alkali pretreatment, (3) investigate the efficacy of CAA pretreatment–enzymatic hydrolysis process and (4) determine the feasibility of cultivating *P. farinose* FL7 using SMS residue and hydrolysates after CAA pretreatment–enzymatic hydrolysis process for biofertilizer production. To the best of our knowledge, this is the first research reporting biofertilizer production using biomass residue from enzymatic hydrolysis.

2. Methods

2.1. Materials

SMS, the substrate after harvesting *Pleurotus ostreastus*, was obtained from Tianjin, PR China. The mushroom substrate used for planting *P. ostreastus* was composed of field hay, wheat straw, corn cobs and cotton seed hulls. SMS was ground to a particle size of 800 μm after drying and then stored in air-tight container at 4 °C for further use. Cellulase (NS50013) and xylanase (NS50014) preparations were kindly supplied by Novozymes A/S (Beijing, PR China). Enzyme activities as described by supplier are 70 FPU (Filter Paper Unit)/g and 600 XU (Xylanase Unit)/g respectively. All other chemicals used in this study were of analytical grade and purchased from Sigma Chemical Company (Shanghai, PR China).

2.2. Pretreatment

To evaluate the effect of pretreatment parameters (temperature, time, and alkali concentration) in alkali pretreatment, a $4 \times 4 \times 4$ factorial design was applied. KOH, ammonia and lime at temperatures of 50, 60, 70 and 80 °C in a static water bath were used to pretreat milled SMS samples at a solid loading of 10% (w/v) with pretreatment times of 30, 60, 90 and 120 min respectively. KOH and lime at concentrations of 0.25, 0.5, 0.75 and 1 M and ammonia at concentrations of 1, 5, 10 and 15 M were investigated. After alkali pretreatment, the samples were adjusted to room temperature and filtered through 0.2 μm nylon filter. Pretreated solids were washed with deionized water until the filtrate registered a neutral pH and dried at 105 °C for compositional analysis or enzymatic hydrolysis experiment.

The reuse of the spent KOH pretreatment liquid was conducted at 80 °C for 90 min with the solid loading of 10% (w/v). The filtrate was saved for pretreating the next batch of SMS at the same conditions. The reuse process was conducted four times.

The dilute sulfuric acid pretreatment of SMS was performed likewise except that an oil bath was employed. According to previous study (Qiao et al., 2011), the dilute sulfuric acid pretreatment was carried out under the optimal condition: temperature of 120 °C, concentration of 4%, pretreatment time of 120 min and solid to liquid rate of 1:16. All pretreatment experiments were carried out in triplicates.

2.3. Enzymatic hydrolysis

Three grams of alkali pretreated samples (dried at 105 °C for 6 h) were mixed with the 20 FPU cellulase and 200 XU xylanase per g solids, 60 ml of 50 mM acetate buffer (pH 4.8) was added, and the samples were incubated at 40 °C in a shaker bath for 72 h. After enzymatic hydrolysis, the samples were filtered through a filter paper and sugar analysis was performed on the supernatant.

The enzymatic hydrolysis of the dilute sulfuric acid pretreated SMS was performed under the same conditions. The CAA pretreatment–enzymatic hydrolysis process was detailed in Section 3.6. All enzymatic hydrolysis experiments were carried out in triplicates.

2.4. Analytical methods

2.4.1. Composition and sugar analysis

The lignin content of raw and pretreated SMS were determined according to National Renewable Energy Laboratory (NREL) standard methods Sluiter et al. (2008). Cellulose and hemicellulose content was determined by high performance liquid chromatography (HPLC) analysis using a Bio-Rad Aminex HPX-87P column and a refractive index detector (SHODEX). Sugars were analyzed at 65 °C using 0.00004% H_2SO_4 as the mobile phase (0.6 ml/min). Total reducing sugar (TRS) were determined using 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). All analyses were performed in triplicates.

2.4.2. Scanning electron microscopy (SEM) analysis

The structural differences in the lignocellulosic morphology of untreated and pretreated SMS were taken by scanning electron microscope (Hitachi S-4800, Tokyo, Japan). All images were taken at a magnification of 500×. The specimens to be coated were mounted on a conductive tape and coated with gold palladium using a JEOL-JFC-1200 fine coater and observed using a voltage of 10 kV.