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Surfactant-assisted pretreatment and enzymatic hydrolysis of spent mushroom compost for the production of sugars

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ABSTRACT

Spent mushroom compost (SMC), a byproduct of commercial mushroom cultivation, poses serious environmental problems that have hampered the growth of this important agro-industry. In an effort to develop new applications for SMC, we explored its use as a feedstock for bioethanol production. SMC constitutes approximately 30% w/w polysaccharides, 66% of which is glucan. Following dilute-acid pretreatment and enzymatic hydrolysis, both in the presence of PEG 6000, 97% of glucan and 44% of xylan in SMC were converted into the corresponding monosaccharides. Incorporation of PEG 6000 reduced the cellulase requirement by 77%. Zwittergent 3–12 and 3–14 also significantly increased the efficacy of acid pretreatment and enzymatic hydrolysis. The use of SMC in bioethanol production represents a potential mitigation solution for the critical environmental issues associated with the stockpiling of the major byproduct of the mushroom industry.

1. Introduction

The conversion of lignocellulosic biomass into biofuel has received considerable scientific and public interest, because biofuels provide an attractive alternative for avoiding the environmental, economic, and political drawbacks associated with petroleum usage (Himmel et al., 2007; Lynd et al., 2008). An alluring aspect of biofuel production is its reliance on low-cost byproducts and residues from a broad range of industries as sources of feedstock. Consequently, there is strong interest in the development of more efficient methods to produce ethanol and other useful chemicals from diverse lignocellulosic substrates.

Spent mushroom compost (SMC) is a lignocellulosic byproduct of the commercial mushroom (*Agaricus bisporus*) industry, where the production of each kilogram of mushroom biomass generates approximately five kilograms of SMC (Finney et al., 2009). The total US production of agaric mushrooms in 2009 was approximately 240,000 tonnes (USDA, 2010), which translates into more than one million tonnes of SMC annually. Commercial mushrooms are cultivated using a substrate consisting of various mixtures of wheat straw, hay, corncobs, seed hulls and meal, horse manure, chicken manure, inorganic fertilizer, calcium sulfate, peat moss, and ground limestone. After mushroom harvest, the residual substrate is pasteurized and either discarded or sold as SMC. The US mushroom industry incurs an estimated cost of US$7 million annually for SMC disposal (pers, commun, Glenn Cote, Laurel Valley Farms). However, the rate of SMC production far exceeds its demand for existing applications. Hence, field storage of SMC leads to significant environmental problems, including the leaching of nitrates and phosphorous that can lead to eutrophication in water resources (Finney et al., 2009) and public health issues associated with the attraction of flies and other insects (Derikx et al., 1990; Kaplan et al., 1995). The lack of sustainable disposal strategies is a major limiting factor in the growth of the mushroom industry (Finney et al., 2009).

To address the daunting disposal issue, SMC has been explored for a diversity of potential commercial applications, including an amendment for plant growth media (Medina et al., 2009; Ribasa et al., 2009) and a bioremediation agent (Chiu et al., 2009). There has been relatively little interest however, in examining the suitability of SMC as a renewable feedstock for fuels and chemicals. Here, one experimental approach entailed the combustion of SMC to generate power (Finney et al., 2009; McCahey et al., 2003; Williams et al., 2001), while another involved an ammonia-based (AFEX) pretreatment combined with enzymatic conversion into fermentable sugars (Balan et al., 2008; Dale et al., 2007). However, to date, none of the above applications has provided a sustainable solution to the stockpiling of SMC.

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Lignocellulosic biomass, such as SMC, is a relatively inert, complex material requiring pretreatment prior to enzymatic conversion into simple sugars. Owing to its efficacy and economy, dilute sulfuric acid pretreatment of lignocellulosic biomass has been widely studied (Sun and Cheng, 2005). Adoption of this method increased the enzymatic digestibility of a number of feedstocks, including corn stover, switchgrass, poplar, rye straw, and Bermuda grass (Lloyd and Wyman, 2005; Schell et al., 2003; Sun and Cheng, 2005). Yet, current dilute-acid pretreatment processes result in the degradation of sugars and formation of compounds that can inhibit enzymatic hydrolysis and fermentation (Almeida et al., 2007; Sineiro et al., 1997).

The use of surfactants as cellulase synergists in the enzymatic hydrolysis of substrates, ranging from pure cellulose to lignocellulosic biomass, is well documented (Eriksson et al., 2002; Kaar and Holtzapple, 1998; Qing et al., 2010). Additionally, several groups (Qi et al., 2010; Qing et al., 2010), including ours, are investigating the use of surfactants to improve the dilute-acid pretreatment process. Herein, we describe a dilute-acid pretreatment protocol for the conversion of SMC into sugars as well as demonstrate the effect of surfactants on increasing the efficiency of both acid pretreatment and enzymatic hydrolysis of SMC.

2. Methods

2.1. Feedstock and surfactants

SMC was generated at the Mushroom Test Demonstration Facility, The Pennsylvania State University (University Park, PA) using the standard commercial protocol for cultivating a hybrid off-white strain of *A. bisporus* (Pecchia et al., 2002; Schroeder et al., 1981). Briefly, a mixture of wheat straw, switch grass, horse manure, distiller's grain, poultry manure, soybean meal, and water were composted using a 6-day bunker Phase I and 6-day tunnel Phase II. A 52-day cropping cycle, which consisted of a 14-day compost colonization period, 17-day case-hold period, and 21-day mushroom-harvest period, was culminated by a post-crop steam pasteurization of SMC at 140 °C for 12 h. Batches of SMC were obtained within 24 h after cool down to ambient temperature and then stored at −80 °C.

Surfactants evaluated in this study were Zwittergent 3–12 or *N*-Dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate, Zwittergent 3–14 or *N*-tetradecyl-*N*,*N*-dimethyl-3-amino-1-propane sulfonate, CHAPS or 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, Tween 20 or polyoxyethylene (20) sorbitan monolaurate, and PEG 6000 or polyethylene glycol 6000 (Sigma Chemical Co., St. Louis, MO).

2.2. Feedstock preparation and characterization

SMC was milled to pass through a 2 mm screen and then washed with water. The total solids content of the resultant slurry was determined according to the National Renewable Energy Laboratory (NREL) Analytical Procedure: determination of total solids in biomass and total dissolved solids in liquid process samples (Sluiter et al., 2008). The composition of raw SMC and dilute-acid pretreated SMC solids (refer to Section 2.3) was determined by the NREL.

2.3. Dilute-acid pretreatment

Acid pretreatment was performed at various temperatures for 40 min with 1% w/w H_2SO_4 in a custom-made 2 l stirred-tank Parr reactor (Parr Instruments Company, Moline, IL) at 5% w/w solids loading and agitation rate of 70 rpm. As indicated, specified amounts of the surfactants were added to the acid pretreatment reaction mixtures. After acid pretreatment, the solid and liquid fractions were recovered by filtration through a 100-mesh sieve. The solids were neutralized by washing with water until the washings had a pH of approximately 6 and the total solids content of the resultant slurry was determined as described in Section 2.2. Prior to separation by Dionex ICS 3000 ion exclusion chromatography, the liquid fractions from the pretreatment process were neutralized and filtered through 0.2 μm PTFE filters (Thermo Fisher, Rochester, NY) (Sluiter et al., 2006). Separation was performed at 30 °C using CarboPac PA20 guard (3 × 30 mm) and analytical (3 × 150 mm) columns. Concentrations of glucose, xylose, and other sugars in the samples were determined as described by Rezaei et al. (2008).

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis reactions were conducted according to the NREL Analytical Procedure: enzymatic saccharification of lignocellulosic biomass (Selig et al., 2008), with minor modification. In experiments performed to evaluate the effect of different surfactants on acid pretreatment of SMC, each enzymatic hydrolysis reaction contained 50 mg of SMC by dry weight in a 5 ml final volume. The material was digested with various loadings of cellulase (NS50013, Novozymes North America Inc., Franklinton, NC) and β-glucosidase (Novozymes NS50010) at 1.75 CBU per 1 FPU (Yang and Wyman, 2006). Cellulase loadings ranged 0.4 to 13.6 mg protein/g pretreated SMC solids, depending on the pretreatment and hydrolysis conditions. Sodium azide was added to a 2 mM final concentration as a microbial inhibitor. The reactions were carried out in 50 mM sodium acetate pH 4.8 for up to 120 h at 50 °C with 70-rpm end-over-end agitation. The incubation time of 120 h was chosen since we have found it to be optimal for maximizing levels of SMC conversion and surfactant-cellulase synergy during SMC hydrolysis (data not shown). The release of reducing sugar was determined by the PAHBAH assay (Lever, 1972). Selected samples were also analyzed by HPLC following standard methods (Sluiter et al., 2006). The same general experimental protocol was followed in evaluating cellulase synergism of different surfactants using 100 mg of substrate in a 5 ml final volume. Cellulase synergy of an additive was calculated as follows: % Synergy = [(% conversion of glucan with additive − % conversion of glucan without additive)/(% conversion of glucan without additive)] × 100.

3. Results and discussion

3.1. Dilute-acid pretreatment of SMC

In order to assess the potential of SMC as a feedstock for sugar production, the polysaccharide composition and the lignin content were determined at the NREL. The findings of this analysis indicated 19.4% glucan, 8.0% xylan, 1.1% arabinan, 0.8% galactan, and 28.5% lignin on a dry weight basis. Although lower than the analyses reported for other feedstocks, such as corn stover (34.4% glucan and 22.8% xylan) (Weiss et al., 2009), SMC contained significant amounts of available sugars (~30% by dry weight) for conversion into ethanol. Moreover, its abundance and significant disposal cost for the mushroom industry provided a compelling rationale to evaluate the usefulness of SMC as a feedstock.

Digestion of raw (non-pretreated) SMC with mixtures of cellulase and β-glucosidase, even at loadings up to 6 FPU/g of dry SMC over a 120 h reaction time, resulted in the conversion of less than 2% of the glucan into glucose, clearly illustrating the recalcitrant nature of raw SMC towards enzymatic hydrolysis (data not shown). In general, microbial pretreatment of lignocellulosics, especially with fungal