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Pretreatment of guayule biomass using supercritical carbon dioxide-based method

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ABSTRACT

Guayule, a desert shrub harvested for commercial production of hypoallergenic latex and resins constitutes <20% of the biomass. Converting the remaining bagasse to biorefinery feedstock for value-added products is an optimal economic option. A supercritical CO_2-based process had been developed previously for resin extraction. In this study, the feasibility of including a supercritical CO_2-based bagasse pretreatment method was evaluated. The pretreatment involved: adding water to the bagasse, raising system temperature, pressurizing using supercritical CO_2, holding the system for a period of time, and exploding the bagasse. The pretreated biomass was subjected to enzyme hydrolysis. The yields of released sugars were used as pretreatment effectiveness indicators. Supercritical method outperformed other methods and gave much higher overall sugar yields for guayule (as high as 77% for glucose and 86% for total reducing sugars through both pretreatment and hydrolysis, as compared to 50% for glucose and 52% for total sugars with the dilute-acid pretreatment and 36% for glucose and 52% for total sugars with the delignification pretreatment). The enzymatic hydrolyzates were tested on the cellulase-producing fungus *Trichoderma reesei* Rut C-30. No inhibitory/toxic effects were apparent in terms of cell growth, sugar consumption, and cellulase and xylanase production. The supercritical CO_2-based method was found to be very promising for pretreatment of waste biomass as the feedstock for subsequent enzymatic hydrolysis and fermentation to produce value-added bioproducts.

1. Introduction

Biomass is regarded as an alternative energy source to fossil fuels (Jones and Semrau, 1984). In 2000 the energy derived from biomass corresponded to ~10% of the global energy supply (Goldemberg and Programme, 2000). Corn, rice straw and switch grass have been evaluated extensively as renewable biomass substrates for making ethanol (Sun and Cheng, 2002). However, the use of corn and rice straw could cause the food and cattle feedstock price to rise (Drake et al., 2002; Bhat and Bhat, 1997; Zheng et al., 2005). It is desirable to explore other biomass sources from non-food-related industrial crops.

Guayule (*Parthenium argentatum* Gray) is such an industrial crop currently grown for commercial production of hypoallergenic latex (Cornish, 1998; Schloman et al., 1996). The increased occurrence of life-threatening allergic reactions to *Hevea* rubber products has compelled the rapid development of guayule latex as the safe alternative (Drew et al., 2004). In addition, guayule resins have strong termiticidal and fungicidal properties, and have been formulated in preservatives for wood products as well as in coatings (Bultman et al., 1991; Gutierrez et al., 1999; Nakayama et al., 2001; Thames et al., 1996). Large quantities of guayule plants are expected to be harvested, estimated at 5 million tons per year by 2015, for meeting the demand of hypoallergenic latex. Since the latex and resins account for no more than 20% of the guayule dry weight, substantial quantities of waste bagasse are generated. The waste guayule biomass is a good candidate as non-food-related biorefinery feedstock.

Not much work has been done to evaluate the waste biomass of guayule as biorefinery feedstock. There have been minimal attempts so far on saccharifying guayule bagasse, using mineral acid (Schloman et al., 1983) and a zinc chloride-based cellulose solvent (Gong et al., 1999), have achieved limited success. Schloman et al. (1983) found that the dilute-acid hydrolysis of guayule bagasse did not give a good yield of fermentable sugars (Schloman et al., 1983). Reagents such as zinc chloride are good cellulose solvents but not well suited for large-scale process operations because they are toxic, hazardous, corrosive or expensive (Gong et al., 1999). There is a need for a better process which can overcome the disadvantages mentioned and at the same time give good yields of fermentable sugars.

Combining a mild pretreatment and a subsequent enzymatic hydrolysis is considered to have the highest potential for reducing the cost of producing fuel ethanol and enabling biorefinery development (Chandra et al., 2007; Deng et al., 2007). This process preserves more sugars and requires less stringent detoxification of the hydrolysate generated (Adsul et al., 2005; Chandra et al., 2007).

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The purpose of pretreatment is to enhance the accessibility and susceptibility of the cellulose and, if pertinent, the hemicellulose to enzymatic hydrolysis. This purpose is normally achieved by loosening and/or removing hemicellulose and/or lignin (Bhat and Bhat, 1997; Chandra et al., 2007).

In this study we evaluated the feasibility of a supercritical CO_2-based pretreatment method for guayule bagasse. Supercritical extraction has been considered as a preferred method (over the conventional solvent extraction) for collecting guayule resins and rubber, and patent-pending procedures for these supercritical extractions have been developed (Cornish et al., 2007). Potentially the supercritical pretreatment method can be integrated into the supercritical extraction process without incurring substantially higher capital costs.

The supercritical CO_2-based pretreatment is similar to the steam explosion pretreatment in principle. In steam explosion, the biomass is exposed to high temperature steam (commonly about 190 to 240 °C) for the water molecules to penetrate the plant cell structure (Shimizu et al., 1998). After a residence time of ~3–8 min, the pressure is released suddenly. The water molecules rush out in an explosive fashion causing the lignocellulose structure to rupture, resulting in higher availability of cellulose to the enzymes in the subsequent hydrolysis step (Laser et al., 2002). The high temperature steam, however, causes significant hemicellulose destruction and generates compounds inhibitory to microbial metabolism. Water wash can be used to remove the inhibitory compounds but it also results in significant loss of sugars derivable from the hemicellulose. In the supercritical CO_2-based pretreatment, the (more) inert CO_2 molecules are presumably to cause the (physical) explosion of biomass structure without appreciable (chemical) hydrolysis.

The operating conditions of the supercritical CO_2-based pretreatment method used in this study were chosen according to two previous studies (Kim and Hong, 2001; Zheng et al., 1995, 1998). Tsao and coworkers (Zheng et al., 1995, 1998) focused on how the explosion (created by the rapid release of pressurized CO_2) affected the cellulose crystallinity and the glucose or ethanol yield from the subsequent enzymatic hydrolysis or simultaneous saccharification and fermentation. They carried out the studies with cellulosic materials, such as Avicel (a commercial product of pure cellulose), recycled paper, and sugarcane bagasse after the hemicellulose was removed by dilute-acid hydrolysis. Kim and Hong (2001) did not observe the explosion effects and attributed their results more to the hydrolysis effects presumably caused by the interaction of supercritical CO_2 and water inside the lignocellulose (e.g., forming carbonic acid capable of mild hemicellulose hydrolysis). Because of the different focuses of the two studies, Tsao and coworkers did not report careful control of the moisture contents in cellulose and employed lower temperatures (35–80 °C) while Kim and Hong carefully evaluated the effects of moisture contents (0–73% of the dry biomass weight) and used higher temperatures (112–165 °C) in their study. It should be noted that Tsao and coworkers clearly demonstrated the importance of keeping the temperature higher than the supercritical temperature of CO_2 (31.1 °C).

To potentially achieve both the explosion and the hydrolysis effects, the following conditions were chosen to be used in this study: moisture content – 50–75%, pressure – 2500–4000 psi, temperature – 100–200 °C, and pretreatment duration – 30–60 min. The performance of the supercritical CO_2-based pretreatment at the chosen conditions was compared with dilute-acid pretreatment and delignification pretreatment, as described later in Section 2. The potential generation of inhibitory compounds by the supercritical CO_2-based pretreatment was tested with the cellulase-producing *Trichoderma reesei* Rut C-30 culture (Tsao and Chiang, 1983).

2. Methods

2.1. Materials

Guayule samples were provided by Yulex Corporation (Carlsbad, CA). Briefly, the company chopped the harvested shrub and then separated the branches from the leaf stream. The branches went through wet milling to collect the primary product, latex, from the homogenate. The remaining biomass from the wet milling was referred to as the "bagasse," which could subsequently be extracted for resins and/or remaining rubber. Two types of bagasse were used in this study. Both were in the form of rod-shaped dry powders of ~100–300 μm in diameter and 300–2000 μm in length. (In addition, a leaf-stream sample, in even finer rods of ~50 μm in diameter and 100–2000 μm in length, was also analyzed for cellulose and hemicellulose contents). One, hereafter referred to as Bagasse 1, had been subjected to some extents of removal of both resins and rubber by supercritical extraction procedures; the other, Bagasse 2, only to rubber extraction. The resins and rubber extractions were done by Supercritical Solutions LLC (Allentown, PA) according to the established procedures (Cornish et al., 2007). Spezyme CP from Genencor (Palo Alto, CA) was used for the enzymatic hydrolysis. Prior to the use in this study the commercial enzyme solution was measured to contain 32.3 FPU/ml of cellulase activity, 26.7 IU/ml of β-glucosidase activity, and 12.4 U/ml (=208 nkat/ml) of xylanase activity. *T. reesei* Rut C30 (NRRL 11460) was used in the study for potential inhibitory effects of hydrolysate. The fungus was obtained from the United States Department of Agriculture, Agricultural Research Service Culture Collection (Peoria, IL). The culture maintenance and inoculum preparation have been described elsewhere (Zhang et al., 2007).

2.2. Methods

2.2.1. Dilute-acid pretreatment

A one-stage dilute-acid pretreatment method, developed by Tennessee Valley Authority (Lee et al., 1999; Vlasenko et al., 1997), was used. Dry biomass, 10% w/v, was pretreated for 5-min retention time at 180 °C using 0.75% H_2SO_4. The slurry was filtered to collect the solids and the hydrolysate (filtrate) separately. The solids were further washed with water. The hydrolysate was over-limed to pH 11 and then neutralized with sulfuric acid.

2.2.2. Delignification pretreatment

The delignification was done by Tennessee Valley Authority (TVA), similar to the dilute-acid pretreatment. Dry biomass (10%) was treated with an inorganic delignin chemical at 180 °C for 30 min. The pretreated solids were collected and washed by filtration. The filtrate was discarded.

2.2.3. Supercritical CO_2-based pretreatment

The procedure was chosen to potentially achieve both the explosion and hydrolysis effects. Fifteen grams of a bagasse sample, predetermined for moisture content (4.4–6.7%), were placed inside a closed reactor (500 ml) along with water (amount calculated to give the investigated water content). The reactor, 17.5 cm in height, 4 cm in the inner diameter, and 6 cm in the outer diameter, was made of stainless steel with alloy steel cap (High Pressure Equipment Company, Erie, PA). The reactor temperature was raised to the studied level, with the reactor wall completely covered by a coil of oil heated to the set temperature. Carbon dioxide was then pumped (ISCO 260D syringe pump, ISCO Inc., Lincoln, NE) into the reactor and the reactor pressure held at the chosen level for a particular duration. (The water content, temperature, pressure, and duration used in different experiments are given in