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Statistical optimization of operating conditions for supercritical carbon dioxide-based pretreatment of guayule bagasse

*Narayanan Srinivasan, Lu-Kwang Ju**

Department of Chemical and Biomolecular Engineering, The University of Akron, 200 E Buchtel Commons, Akron, OH 44325-3906, USA

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ABSTRACT

A central composite design (CCD) was used to find the optimal temperature, pressure, moisture and duration for the supercritical CO_2-based pretreatment of guayule, a desert shrub for commercial production of hypoallergenic latex. The pretreatment involved: adding water to the biomass, raising system temperature, pressurizing with supercritical CO_2, holding the system for a period of time, and exploding the biomass by rapidly opening the vent valve. The pretreated biomass was then hydrolyzed at 30 °C for 72 h in an enzyme solution with 5% (w/v) solid loading. The yields of released glucose and pentose were determined and used as the CCD response variables. Statistical analysis of results led to the following recommended condition: 175 °C, 26.2 MPa (3800 psi), 60% moisture and 30 min. The corresponding glucose and pentose yields were 56% and 61%. X-ray diffraction analysis was done to get the crystallinity index of the bagasse before and after pretreatment. Scanning electron microscopy was also used to examine the structural changes caused by pretreatment. These characterizations indicated that at milder conditions, the pretreatment exposed cellulose and hemicellulose for subsequent enzyme attack; at harsher conditions, the pretreatment destroyed cellulose crystallinity and gave higher glucose yields. To isolate the explosion effect (caused by instantaneous pressure drop, ΔP) from the reaction (pressure) effect, a series of experiments was made with the bagasse pretreated at the same condition (27.6 MPa) but exploded against different back pressures. The explosion needed to be severe enough (with $\Delta P > 17$ MPa) to give a sugar yield of over 50%.

1. Introduction

Using lignocellulosic biomass as biorefinery feedstock for production of ethanol [1], butanol [2,3], and other chemicals [4,5] via fermentation typically requires the polymeric carbohydrates (cellulose and hemicellulose) to be broken down to small sugar molecules (e.g., glucose and xylose) prior to the fermentation. To enhance the saccharification, particularly if by the gentler enzyme hydrolysis, the biomass needs to be pretreated to loosen the structure and increase the accessibility and susceptibility of cellulose and hemicellulose [6,7]. In our earlier study a supercritical CO_2-based method was evaluated for its feasibility to pretreat waste guayule biomass (bagasse) [8]. Guayule (*Parthenium argentatum* Gray) [9,10] has attracted considerable interest due to its use in commercial production of hypoallergenic latex and high-quality, multipurpose resins [11–14]. However, latex and resins account for no more than 20% of the guayule dry weight. The rest of the

* *Corresponding author*. Tel.: +1 330 972 7252; fax: +1 330 972 5856.
E-mail addresses: LukeJu@UAkron.edu, ju@uakron.edu (L.-K. Ju).
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Nomenclature

CCD	central composite design
CrI	crystallinity index, as described in Eq. (1)
D	duration of supercritical pretreatment, min
$\widehat{D}$	dimensionless pretreatment duration in Eqs. (5) and (6), $=(D-30)/10$
DNS	dinitrosalicylic acid
ESEM	Environmental Scanning Electron Microscope
FPU	filter paper unit, a measure of cellulase enzyme activity
I_{am}	X-ray diffraction intensity at $2\theta = 18.7°$
I_{002}	X-ray diffraction intensity at $2\theta = 22.6°$
M	moisture, weight % of water in bagasse
$\widehat{M}$	dimensionless moisture in Eqs. (5) and (6), $=(M-60)/10$
P	pressure, MPa
$\widehat{P}$	dimensionless pressure in Eqs. (5) and (6), $=(p-27.6)/3.45$
ΔP	pressure drop between the reaction pressure and the venting pressure, MPa
SAS	Statistical Analysis System
SEM	scanning electron microscopy
T	temperature, °C
$\widehat{T}$	dimensionless temperature in Eqs. (5) and (6), $=(T-165)/35$
XRD	X-ray diffraction

plant material, already chopped, milled and extracted [15], is potentially a good source of waste biomass for biorefinery.

The supercritical CO_2-based pretreatment method used in our earlier study involved the following steps: adding a small amount of water to the bagasse loaded in the reactor, raising the system temperature, pressurizing using supercritical CO_2, holding the system for a period of time, and exploding the bagasse by rapidly opening the valve to release the pressure [8]. The pretreated biomass was then subjected to enzyme hydrolysis at 30 °C for 72 h and the yields of released glucose and total reducing sugars measured. The results confirmed the feasibility of the supercritical CO_2-based method in preparing guayule bagasse for the subsequent enzymatic hydrolysis. Unlike the commonly used dilute acid pretreatment, the supercritical method did not incur appreciable loss/hydrolysis of cellulose and hemicellulose and did not require neutralization or water wash of the pretreated biomass.

Conceptually four operation factors may be important to the performance of the supercritical pretreatment. These factors are: temperature, pressure, moisture content of biomass, and the pretreatment (reaction) duration. In the previous feasibility study the pretreatment was only done at 6 conditions [8]. In this study the pretreatment was done at 26 conditions, chosen according to the factorial design, and the response surface analysis [16,17] was performed to determine the optimal pretreatment condition.

In addition to the sugar yields from enzyme hydrolysis of pretreated bagasse, X-ray diffraction (XRD) analysis was used to assess the effect of pretreatment on the crystallinity of bagasse pretreated under different conditions, similar to the previous work of Zheng et al. [18]. Lowering cellulose crystallinity is known to enhance the performance of enzyme hydrolysis [19,20]. Scanning electron microscopy (SEM) was also used to examine the changes of bagasse structure caused by the pretreatment. Observing the plant material structures such as lignin sheath and cellulose and hemicellulose fibers with SEM had been reported in the literature [21].

Furthermore, the "explosion" (rapid pressure release) effect was shown important to the outcome of the supercritical pretreatment process in our previous study [8]. Hypothetically, larger amounts of CO_2 would enter into the bagasse structure under the supercritical conditions, with the penetration being assisted by the water absorbed in bagasse [18,22]. When the vent valve is rapidly opened, CO_2 expands and rushes out of the biomass, rupturing the bagasse structure and exposing the cellulose and hemicellulose for subsequent enzyme hydrolysis. Accordingly, the pretreatment effectiveness was expected to be affected by the magnitude of the pressure drop (ΔP) between the reaction pressure and the venting pressure employed. Nonetheless, the magnitude of ΔP needed for an adequate "explosion" effect was unknown. To study this latter factor in isolation, a set of experiments were done by using the same pressure in the supercritical reactor but different back pressures in the vent vessel (into which the reactor fluid was vented). In real production processes the CO_2 collected in the vent vessel would be repressurized and used for the supercritical reactor. A lower ΔP (i.e., a higher P in the vent vessel) may be more economic in minimizing the energy required for repressurization.

The objectives of this work were to optimize the pretreatment conditions in the reactor and the (back) pressure in the vent vessel, and to examine the effects of supercritical pretreatment on the crystallinity and structures of guayule bagasse (and their correlation with the outcome of subsequent enzyme hydrolysis).

2. Materials and methods

2.1. Materials

Guayule plant material was provided by Yulex Corporation (Carlsbad, CA). The harvested shrub was chopped, the leaf stream removed, and the branches wet milled for collection of latex as the primary product. The remaining biomass, referred to as "bagasse", could then be extracted for resin and/or remaining rubber. The bagasse that had been extracted for resin and rubber by the supercritical extraction procedures [15] at Yulex Corporation was transported to our lab for use in this optimization study. The bagasse was in the form of rod-shaped dry powders of about 100–300 μm in diameter and 300–2000 μm in length. The bagasse sample had the following composition [8]: 21% (±3%) cellulose, 13% (±1%) hemicellulose, 54% (±2%) acid insoluble materials, 0.7% (±0.1%) acid soluble lignin, and 11% (±3%) other (unaccounted for) materials.

The enzyme solution (Spezyme CP) used in the study for enzyme hydrolysis of pretreated bagasse was obtained from