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A novel surfactant-assisted ionic liquid pretreatment of sugarcane bagasse for enhanced enzymatic hydrolysis

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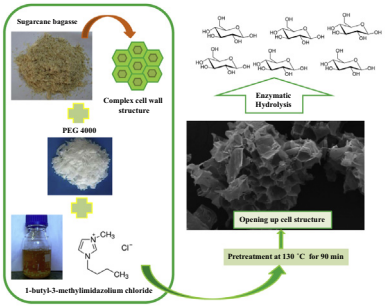
HIGHLIGHTS

- To enhance enzymatic hydrolysis of SCB a novel pretreatment method was presented.
- Tween 80 and PEG 4000 were utilized with ionic liquid for the pretreatment of SCB.
- Surfactants increased lignin removal by 12.5% compared with IL-only pretreated SCB.
- The rate of enzymatic hydrolysis significantly increased by addition of surfactants.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated a novel pretreatment method, as an essential step, for production of second generation bioethanol from sugarcane bagasse (SCB). Effect of tween 80 (TW) and polyethylene glycol 4000 (PEG) on SCB pretreatment was assessed using 1-butyl-3-methyl imidazolium chloride ([BMIM]Cl) as an ionic liquid (IL). Different concentrations of TW and PEG were used to determine the optimum concentration of surfactant for the highest percentage of cellulose conversion. TW and PEG increased lignin removal by 12.5% over the IL-only pretreated sample. The 3% (w/w) PEG showed a significant increase in enzymatic digestibility with an efficiency of 96.2% after 12 h of hydrolysis; this was 23% higher than the efficiency of SCB pretreated with IL. The increase in digestibility of surfactant assisted IL pretreatment method can be attributed to the decrease in cellulose crystallinity, changes in the cellulose lattice, and delignification; which was confirmed by FT-IR, XRD and FE-SEM analysis.

1. Introduction

Lignocelluloses biomass is inexpensive and renewable feedstock for bioethanol production through conversion to fermentable sugars (Li et al., 2010). Pretreatment of lignocelluloses is required to break down the crystalline structure of the cellulose, remove hemicellulose, eliminate or modify lignin to increase cellulose accessibility, and decrease the tenacity of cellulose to enzymatic attack before it can be used as a feedstock for biofuel or other chemical processing (Zhang et al., 2012). The very complex structure of lignocelluloses makes it resistant to enzymatic hydrolysis and decreases the sugar yield of saccharification (Qiu and Aita, 2013).

Pretreatment is the most challenging step in the production of bioethanol because it disrupts the compact and highly-ordered structure of the lignocelluloses (Choi et al., 2013). All pretreatment methods increase the enzymatic digestibility of polysaccharides, but there is a need to develop an efficient process which is economical, requires low capital expenditure, and has a minimum influence on downstream processing. Ionic liquids (ILs) are a promising pretreatment for lignocelluloses because of their unique

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physical and chemical properties. ILs are organic salts composed of an organic cation and, usually, an inorganic anion that exist in liquid form at relatively low temperatures (Mora-Pale et al., 2011; Liu et al., 2012).

Previous studies have examined the pretreatment of lignocellulosic biomass using various ILs (Li et al., 2010; Qiu and Aita, 2013; da Silva et al., 2011; Shafiei et al., 2013), but the combination of surfactants and IL as a pretreating agent has not been studied yet. Surfactants have both hydrophobic and hydrophilic properties and enhance hydrophobic substance removal by decreasing surface tension between the two liquid phases (Qing et al., 2010; Escalante et al., 2005). Due to this property, surfactants are used widely in pulping industry. Mixtures of anionic and nonionic surfactants have shown an improvement of pulp yield because they can increase wettability of the white liquor. Furthermore, they can act as emulsifiers and dissolve the extractives present in wood structure (Zhao et al., 2004). Also this property makes surfactants good prospective additives for pretreatment of lignocellulose. It has been reported that surfactants increase lignin removal by extracting the hydrophobic products of lignin degradation (Kurakake et al., 1994). Lignin hinders enzymatic hydrolysis by unproductive adsorption of cellulose; it impedes enzymatic accessibility to cellulose and hemicellulose, increasing the reaction times for achieving higher sugar concentrations (Qing et al., 2010). It may be beneficial to use the synergistic effect of IL and surfactant for delignification of biomass. The main objective of this research was to investigate the surfactant-assisted IL pretreatment of SCB to increase enzymatic hydrolysis. The structural features of SCB pretreated with IL and surfactant-IL were examined using Fourier transform infrared spectroscopy (FT-IR), X-ray Diffraction (XRD) and Field emission scanning electron microscopy (FE-SEM) analysis. Tween 80 and polyethylene glycol 4000 were used as additive surfactants, and [BMIM]Cl was applied as the solvating IL.

2. Methods

2.1. Feedstock and materials

Sugarcane bagasse was supplied by the Iranian Research Organization for Science and Technology (IROST). Samples were ground in a cutter mill (Moulinex, AR1044) and passed through sieves of mesh size 30 and 70. Ionic liquid [BMIM]Cl was purchased from Sigma–Aldrich. Celluclast 1.5 L (cellulase from *Trichoderma reesei*) and Novozyme 188 (cellubiase from *Aspergillus niger*) were the commercial enzymes used were purchased from Sigma–Aldrich.

2.2. Experimental and analytical procedures

2.2.1. Characterization of SCB

The chemical composition of the cellulose, hemicellulose, total lignin, and ash in the SCB of untreated and pretreated SCB was determined as recommended in National Renewable Energy Laboratory procedures 001–004 (Sluiter et al., 2008). The SCB was hydrolyzed with 72% H_2SO_4 (w/w) for 2 h at 30 °C. The acid was diluted to 4% H_2SO_4 (w/w) by the addition of deionized water. The mixture was further hydrolyzed in an autoclave for 1 h at 121 °C. The suspension was filtered using Whatman filter paper 52 and the solid biomass remaining was used to determine the percentages of acid-insoluble lignin and ash. The composition of the cellulose, hemicellulose, and acid-soluble lignin was also determined from the filtrate.

2.2.2. Surfactant assisted ionic liquid pretreatment

The solid biomass subjected to pretreatment was 0.5 g pre-milled SCB based on dry weight. All samples were soaked in surfactant overnight to assure sufficient penetration of the liquid into the SCB solids. The IL was then added to the sample tubes until the ratio of IL to solid content was 10:1. The mixture was heated in an oil bath to 130 °C for 90 min.

Following pretreatment, 5 ml deionized water was added to the reaction mixture. The solution was mixed and centrifuged (Vifion, VF550) at 12,000 rpm for 10 min. To remove the residual IL from the regenerated SCB, the biomass was washed 3 times with 10 ml deionized water. The wet regenerated SCB was then freeze-dried for 24 h prior to enzymatic hydrolysis and further analysis.

2.2.3. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) was carried out using a Perkin Elmer system (Massachusetts, USA) to determine the chemical structure of the untreated and pretreated SCB. The samples were mixed with potassium bromide (KBr) and then pressed uniformly into a disc. The samples then were scanned at a range of 4000–450 cm^{-1} with a spectral resolution of 2 cm^{-1}. The rubber band correction method was used for base line correction following the spectrum minima (Singh et al., 2009).

2.2.4. X-ray diffraction analysis

X-ray diffraction (XRD) patterns of the untreated and pretreated SCB were obtained using a Philips X'Pert MPD diffractometer (Netherlands). A Co kα tube was used for radiation. The SCB samples were attached to microscopic slides with double-sided tape. Scans were collected at 40 kV and 40 mA with a wave length of 1.79 Å and a step size of 0.02. Scanning was conducted in a 2θ range of 2–60°. The crystallinity index (CrI) and crystallite size (L) were determined from the XRD data and calculated using the peak height and Scherrer method:

$$\mathrm{CrI} = \frac{I_{002} - I_{\mathrm{AM}}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the intensity of the crystalline portion of the biomass at approximately $2\theta = 22.5°$; and I_{AM} is the peak amorphous portion at approximately $2\theta = 16.6°$ for the Cu kα tube (Kumar et al., 2009):

$$L = \frac{k\lambda}{B_S \cos\theta} \quad (2)$$

where k is the Scherrer constant; λ is the X-ray wave length (Å); θ is the diffraction angle; and B_s (radian) is the width of the peak at half maximum height (Danilchenko et al., 2002).

2.2.5. Field emission scanning electron microscopy (FE-SEM)

Field emission scanning electron microscopy images of the untreated and pretreated SCB were taken using a Hitachi F4160 (Japan, Tokyo), operating at 30 kV. FE-SEM was used to monitor the morphological changes of the SCB after pretreatment. After freeze-drying, the untreated and pretreated SCB was carefully attached to adhesive carbon tubes and a 30 nm thick conductive coating of gold was applied to the surface.

2.2.6. Enzymatic hydrolysis

Enzymatic saccharification was carried out using 2.5% (w/v) of pretreated and untreated SCB in 0.1 M citrate buffer at pH 4.8. To prevent bacterial growth, 0.02% sodium azide was applied. The substrates were hydrolyzed using Celluclast 1.5 L at 50 FPU/g substrate and Novozyme 188 at 40 CBU/g substrate. Samples were collected at 0, 3, 6, 12, 24, 48 and 72 h and centrifuged (B. Braun A15) at 13,500 rpm. Concentration of reduced sugars was measured using DNS (3,5-dinitrosalicylic acid) (Brown and Torget, 1996). All experiments performed in duplicate.