Influence of surfactant-free ionic liquid microemulsions pretreatment on the composition, structure and enzymatic hydrolysis of water hyacinth

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HIGHLIGHTS

- Excellent surfactant-free IL microemulsion pretreatment was demonstrated.
- The delignification of 38.9–63.6% was observed through the designed pretreatment.
- A majority of the cellulose and most of the hemicellulose were retained.
- The maximum reducing sugar yield of pretreated water hyacinth was 563.7 mg/g.
- The maximum hydrolysis yield of pretreated water hyacinth was 86.1%.

ARTICLE INFO

ABSTRACT

This study investigated the pretreatment performance of surfactant-free ionic liquid microemulsions (ILMs) on water hyacinth. Pretreatment effects were evaluated in terms of lignocellulosic composition, structure and enzymatic hydrolysis. Analysis of the regenerated water hyacinth indicated that the content of the lignocellulosic composition changed, and the surface became more porous. After being pretreated with ILM[a] (mass ratio of toluene: ethanol: 1-ethyl-3-methylimidazolium acetate ([Emim]Ac) = 0.35:0.3:0.35) at 70 °C for 12 h, the maximum delignification of 63.6% was observed. The cellulose of the water hyacinth was well protected and retained during the pretreatment process. After being enzymatically hydrolyzed for 48 h, the reducing sugar yield of the water hyacinth pretreated with ILM[a] at 70 °C for 6 h was 563.7 mg/g, and its hydrolysis yield (86.1%) was nearly four and a half times of that of the untreated one (20.2%). In conclusion, the designed surfactant-free ILMs exhibit promising potential application in biomass pretreatment.

1. Introduction

Lignocellulosic biomass can be used as renewable feedstock in bioconversion to produce fermentable sugars that can be further utilized in bioethanol production (Jung et al., 2013). Water hyacinths (WH) are aquatic weeds found in tropical and subtropical regions that, given their high propagation rate, are believed to pose serious problems to water bodies (Mukhopadhyay and Nandi, 1998). For this reason, the conversion of WH to fuels has received considerable attention in recent decades (Kumar et al., 2009). WH have a greater lower cellulose content than wood and straw (Sánchez, 2009). The lignin composed of three primary phenyl propane units acts as a glue that binds the cellulose and the hemicellulose, giving WH remarkable resistance against the degradation of lignocellulosic biomass (Weerachanchai et al., 2012). However, hydrolysis processes are made difficult and uneconomical by the crystalline structure and available surface area of WH, suggesting that pretreatment should be performed to overcome these restrictions and improve bio-digestibility (Gao et al., 2013b).

Researchers have proposed many physical, chemical, and biological pretreatment methods, such as steam explosion, ammonia fiber explosion, acid hydrolysis, alkaline hydrolysis, organosolv, and irradiation (Lee et al., 2009). However, these methods are limited by several drawbacks, such as high toxicity, waste generation, extreme conditions, high energy requirements, restricted industrial application, and fermentable sugar inhibition (Yang et al., 2010). At present, the low volatility, designability, and excellent solvency of ionic liquids (ILs) render them as ideal green solvents for biomass pretreatment processes (He et al., 2014). In addition, ILs can be recycled and reused with only slight

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loss of effectiveness (Weerachanchai and Lee, 2014). The sugar conversion and lignin extraction content of rice straw reportedly increased significantly after being pretreated with [Emim]Ac at 120 °C for 24 h (Weerachanchai et al., 2012). However, most types of ILs contain high viscosities, making them unsuitable for industrial applications. A possible solution to overcome this problem is to lower the viscosity of pretreatment solvents by using mixtures of ILs and organic solvents. For example, mixtures of [Emim]Ac and 40–60 vol% dimethylacetamide provided similar values of sugar yield and extracted lignin content as compared with those of [Emim]Ac (Weerachanchai and Lee, 2013). In our previous research, Gao et al. (2013a) added dimethyl sulfoxide (DMSO) as a co-solvent to reduce the viscosity of ILs. Consequently, compared with the cellulose content of the untreated WH, that of the regenerated WH increased by 27.9%, and 49.2% of lignin was removed after the WH were pretreated with 1-butyl-3-methylimidazolium chloride ([Bmim]Cl)/DMSO at 120 °C for 120 min.

Ionic liquid microemulsions (ILMs) have a relatively high temperature independence; as such, ILM systems are broadly applied in many fields. After a combined pretreatment of quadrol and anhydrous ILMs, the hemicellulose and the non-crystalline region of the cellulose were broken down and eliminated completely. The loose and highly accessible structure caused the glucose yield of the untreated masson pine to increase from 35.8% to 78.2% after pretreatment (Li et al., 2014). Microemulsions stabilized by amphisolvents, which can be solubilized both in water and oil phases, are called surfactant-free microemulsions (SFMEs) because of the absence of traditional surfactants. Freeze-fracture electron microscopy observations conducted by Xu et al. (2013) have confirmed that all three microstructure types existed in 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF$_4$)-toluene-ethanol. That study was the first to report on non-aqueous IL SFMEs, which have special applications in separation and reaction engineering because of their waterless and surfactant-free nature (Xu et al., 2013).

[Emim]Ac is an excellent solvent that can dissolve cellulose (Zavrel et al., 2009). Ethanol can be utilized to solubilize lignin and obtain treated cellulose that is suitable for enzymatic hydrolysis (Zhao et al., 2009). Moreover, ethanol pretreatment is less toxic than other pretreatment methods, and it can be easily recovered through distillation. In the present study, surfactant-free ILMs of [Emim]Ac, ethanol, and toluene were used as pretreatment solvents of WH. Pretreatment performances were evaluated in terms of the composition, structure, and enzymatic hydrolysis yield of WH.

2. Methods

2.1. Materials

WH stems were obtained from the Yingzhou Ecological Park in Guangzhou. These stems were washed and chopped to a length about 1 cm, dried at 50 °C for 48 h, and powdered. IL 1-ethyl-3-methyimidazolium acetate ([Emim]Ac) (purity > 99%) was purchased from Center of Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics, China. Cellulase from *Trichoderma reesei* (800 EGU/g) and beta-D-Glucosidase (300 CBU/g) were purchased from Sigma–Aldrich, Inc.

2.2. Phase diagram construction

The phase diagram of [Emim]Ac-ethanol-toluene ternary system was determined by direct observation. Mixtures with different weight ratios of [Emim]Ac to toluene were prepared in dry test tubes at 30 °C. Then titrating the mixtures by ethanol under moderate agitation. The phase boundary was determined by observing the transition from turbidity to transparency. Repeated this experiment at 50 °C and 70 °C. The component content was expressed as the weight fraction in the ternary phase diagram.

2.3. Pretreatment and regeneration of WH

In this study, 3.0 g of WH was added to 250 mL round-bottom flask and mixed with 60.0 g solvent. The solutions were heated and stirred in an oil bath pan at varied temperatures from 30 °C to 70 °C and incubated for 3, 6 or 12 h. After the pretreatment at a constant temperature was over, 30 mL ethanol was added to the pretreatment system. This mixture was vigorously stirred for 10 min and separated by vacuum filtration. The filtrate was collected and used in the ionic liquid recovery, while the precipitate was washed for three times with deionized water. Finally, the solid sample was dried at 50 °C for 48 h and further used for the enzymatic hydrolysis.

2.4. Enzymatic hydrolysis of WH

200 mg pretreated or untreated WH was mixed with 30 mL acetate buffer (pH 4.8, 50 mM) in a 50 mL flask. Cellulase (0.01 mL) and beta-D-Glucosidase (0.01 mL) were added to each sample. Enzymatic hydrolysis was carried out at 50 °C and 150 rpm for 48 h in an incubating shaker. 1.0 mL samples were taken at 1, 3, 6, 12, 24 and 48 h, respectively, and analyzed for reducing sugars by the 3,5-dinitrosalicylic acid (DNS).

2.5. Analytical methods

Cellulose, hemicelluloses and lignin contents of pretreated and untreated WH samples were determined according to NREL procedures (Sluiter et al., 2008). Delignification with pretreatments was calculated through the following equation:

$$\text{Degree of delignification } (\%) = 1 - L \cdot Y_T / L_0 \quad (1)$$

where L and L_0 are the lignin contents (%) of pretreated and raw WHs, and Y_T is the total solid recovery (%) after pretreatment.

Untreated and pretreated WH samples were measured by scanning electron microscopy (SEM) and Fourier transformed IR (FTIR). The crystallinities of pretreated and untreated WHs were measured using a D8 Advance X-ray diffractometer (XRD). The XRD data of samples was used to calculate the crystallinity index (CrI) as follows:

$$\text{CrI} = (I_{002} - I_{am}) / I_{002} \times 100\% \quad (2)$$

where I_{002} is the maximum intensity of the 002 lattice diffraction at near 22°, and I_{am} is the intensity diffraction at suitable locations for the amorphous background at near 18°.

Yield of reducing sugars from cellulosic materials was calculated as follows:

$$\text{Reducing sugars yield(mg/g)}$$
$$= [\text{Reducing sugar concentration}](\text{mg/L})$$
$$\times [\text{hydrolysis liquid volume}](\text{L})/[\text{WH dry weight}](\text{g}) \quad (3)$$

The hydrolysis yield of samples were calculated as follows:

$$\text{Hydrolysis yield}(\%)$$
$$= [\text{Reducing sugar concentration}](\text{mg/L})$$
$$\times [\text{hydrolysis liquid volume}](\text{L})/(\text{cellulose content}(\text{mg}) \times 1.11$$
$$+ \text{hemicellulose content}(\text{mg}) \times 1.14)\text{in loaded WH} \times 100\%$$
$$\quad (4)$$