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Short Communication

Impact of surfactant type for ionic liquid pretreatment on enhancing delignification of rice straw

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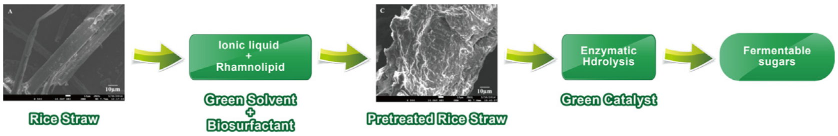
HIGHLIGHTS

- Compared with different types of surfactant combined with IL pretreatment.
- The highest cellulose conversion was obtained with IL + bio-surfactant pretreatment.
- Improved the hydrophilic ability by IL + bio-surfactant pretreatment.
- IL + bio-surfactant is an effective and environmental pretreatment for rice straw.

GRAPHICAL ABSTRACT



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ABSTRACT

This work describes an environmentally friendly method for pretreating rice straw by using 1-Allyl-3-methylimidazolium chloride ([AMIM]Cl) as an ionic liquid (IL) assisted by surfactants. The impacts of surfactant type (including nonionic-, anionic-, cationic- and bio-surfactant) on the ionic liquid pretreatment were investigated. The bio-surfactant + IL-pretreated rice straw showed significant lignin removal (26.14%) and exhibited higher cellulose conversion (36.21%) than the untreated (16.16%) rice straw. The cellulose conversion of the rice straw pretreated with bio-surfactant + IL was the highest and the lowest was observed for pretreated with cationic-surfactant + IL. Untreated and pretreated rice straw was thoroughly characterized through SEM and AFM. In conclusion, the results provided an effective and environmental method for pretreating lignocellulosic substrates by using green solvent (ionic liquid) and biodegradable bio-surfactant.

1. Introduction

The production of bioethanol and biobutanol from abundant lignocellulosic biomass would decrease greenhouse gas emissions by replacing the use of fossil fuels and would improve energy security and rural economy. Effective production of sugars from lignocellulosic biomass is one of the greatest impediments in biofuel production (Singhvi et al., 2014). Pretreatment by physical, chemical or biological means is a well-investigated process for ethanol production from lignocellulosic materials. A pretreatment method that can facilitate lignin removal and increase enzymatic access to cellulose is required to ensure favorable conversion efficiency. Effective pretreatment can reduce the downstream unit operation costs (Yang and Wyman, 2008). Recently, ionic liquids (ILs) have received growing attention as green solvents for lignocellulose pretreatment because of the unique physical and chemical properties (Yang and Fang, 2015). Studies examining the pretreatment of lig-

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nocellulosic biomass by using various ILs have shown that ILs can reduce the crystallinity of cellulose, with partial hemicelluloses and lignin removal (Financie et al., 2016), without generating degradation products inhibitory to enzymes or fermenting microorganisms (Lee et al., 2009). IL pretreatment methods are less energy demanding, easier to handle, and more environmentally friendly than other pretreatment methods are (Elgharbawy et al., 2016). However, the residual lignin and hemicellulose in the IL-pretreated lignocellulosic biomass significantly affect enzymatic hydrolysis (Moniruzzaman and Ono, 2012). A surfactant pretreatment method was recently proposed for improving enzymatic hydrolysis efficiency and reducing the amount of enzyme required. Surfactants have both hydrophobic and hydrophilic properties as well as enhance hydrophobic substance removal by reducing surface tension between two liquid phases (Qing et al., 2010). The effects of different surfactants on lignocellulosic substrates treated with ammonium hydroxide (Cao and Aita, 2013), dilute acid (Kapu et al., 2012), and alkali (Pandey and Negi, 2015) have been reported; however, studies evaluating the effect of surfactants on IL-pretreated lignocellulosic biomass remain limited.

Nasirpour et al. (Nasirpour et al., 2014) investigated a novel and improved pretreatment method for sugarcane bagasse and demonstrated the effectiveness of adding surfactants before IL pretreatment. The authors used two kinds of nonionic surfactants (Tween 80 and polyethylene glycol 4000) as the additive surfactants and 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) as the solvating IL. In our pervious study, the effectiveness of surfactants (sodium dodecyl sulfate and cetyl trimethyl ammonium bromide) as additives for biomass pretreatment with [BMIM]Cl in a single system was evaluated (Chang et al., 2016). However, the different types of surfactant would cause various synergistic effects. Therefore, the objective of the present study was to investigate the effectiveness of rice straw pretreated by [AMIM]Cl combined with different types surfactant (including nonionic-, anionic-, cationic- and biosurfactant) in lignin removal and enhancing enzymatic hydrolysis. Composition analysis, field emission scanning electron microscopy (FE-SEM) and atomic force microscopy (AFM) analysis methods were applied to identify the physicochemical changes before and after pretreatment.

2. Materials and methods

2.1. Substrate, IL, and surfactants

Straw was obtained from 5-month-old japonica rice plants cultivated on the experimental farm located on the Academia Sinica campus, Taipei, Taiwan. The rice straw was dried, ground to powder, and stored in a sealed plastic bag at room temperature. [AMIM]Cl was purchased from Shanghai Monils Chem. Eng. Sci. & Tech. Co. Ltd., China. Sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB) were purchased from China Titans Energy Technology Group Co. Ltd., China, and Sigma-Aldrich, USA, respectively. Polyethylene Glycol Octylphenol Ether (TritonX-100) and rhamnolipid were purchased from Shanghai Aladdin Co. Ltd., China.

2.2. Surfactant-assisted IL pretreatment

CTAB, TritonX-100, SDS or rhamnolipid was combined with [AMIM]Cl for pretreating rice straw. [AMIM]Cl (5 g), rice straw (40 mesh; 0.5 g), and 1% (W/W, based on the weight of [AMIM]Cl) surfactants were mixed in 50-mL vials and heated at 110 °C for 1 h with continuous stirring. After complete dissolution, 45 mL of deionized water was added to precipitate carbohydrate-rich materials. The mixture was centrifuged at 7000 rpm for 15 min, and the supernatants were stored. The carbohydrate-rich materials were washed with deionized water until the pH of the washing water was 7 and stored for enzymatic hydrolysis after drying. Untreated and [AMIM]Cl-pretreated rice straw samples were used as controls. The solid recovery and composition of the pretreated biomass were also analyzed.

2.3. Chemical composition analysis

The presence of cellulose, lignin, and holocellulose was determined using the 20% nitric acid–ethanol, 72% sulfuric acid, and sodium chlorite methods, respectively (Jung et al., 2015).

2.4. Enzymatic hydrolysis of rice straw

Enzymatic saccharification of pretreated and untreated rice straw was performed in 25-mL conical flasks. For each sample, 0.1 M sodium citrate buffer (pH 4.8) was added to an equivalent amount of 2.5% dry material. To prevent bacterial growth, 100 μL of 0.02% sodium azide was added. The substrates were hydrolyzed using a cellulase complex (Novozyme NS220086) at 50 FPU/g rice straw and β-glucosidase (Novozyme NS221118) at 40 CBU/g rice straw at 50 °C in a horizontal shaker incubator (150 rpm) for 72 h. The reducing sugar was determined through the 3, 5-dinitrosalicylic acid method. Cellulose conversion was calculated as follows:

$$\text{Cellulose conversion } (\%) = \frac{\text{RS} \times 0.9}{\text{HOLO}} \times 100 \tag{1}$$

where RS is the reducing sugar produced through enzymatic hydrolysis and HOLO is the holocellulose in pretreated biomass.

2.5. SEM

The morphologies of untreated and pretreated rice straw were examined on an S-3400N (II) scanning electron microscope (Hitachi, Japan). After freeze-drying, the samples were carefully mounted on the powder sample stubs by using a double-sided carbon tape, and a 30-nm-thick conductive gold coating was applied to the surface.

2.6. AFM

AFM (Bruker Dimension FastScan, Bruker Co. Ltd., Germany) was used to observe the variation of roughness of the untreated, [AMIM]Cl -treated, and [AMIM]Cl + 1% rhamnolipid treated rice straw samples. Signal error and topography images were taken with a scan rate of 2.00 Hz using standard peak force-mode. Images treatment was analyzed by a Nanoscope Analysis v 1.40 software. The surface roughness includes root mean square roughness (Rq) and average roughness (Ra), are defined as follows (Mendez-Vilas et al., 2007):

$$\text{Rq} = \frac{1}{N}\sum_{i=1}^{N} |Z_i - \bar{Z}| \tag{2}$$

$$\text{Ra} = \sqrt{\frac{\sum_{i=1}^{N} (Z_i - \bar{Z})^2}{\text{N}}} \tag{3}$$

where Zi is the height of the *ith* point with respect to the lowest one in the image and N is the total number of points comprised in the image.

3. Results and discussion

3.1. Effect of surfactants in [AMIM]Cl pretreatment on rice straw composition

The rice straw compositions of untreated and pretreated with IL only and surfactants-IL are summarized in Table 1. The biomass losses of the rice straw pretreated with IL only, [AMIM]Cl + 1% CTAB, [AMIM]Cl + 1% TritonX-10, [AMIM]Cl + 1% SDS, and [AMIM]Cl + 1% rhamnolipid were 15.92%, 15.59%, 16.99%, 16.09%, and 18.94%, respectively. These biomass losses may have resulted from the removal of partial hemicellulose or lignin after the rice straw was pretreated. Here, the IL, [AMIM]Cl + 1% CTAB, [AMIM]Cl + 1% TritonX-10, [AMIM]Cl + 1% SDS, and [AMIM]Cl + 1% rhamnolipid pretreatment led to a lignin removal of 21.48%, 19.44%, 24.63%, 28.21%, and 26.14%, respectively, compared with untreated rice straw. In most of the cases (except for the CTAB group), the addition of surfactant increased lignin removal because the surfactants acted as delignifying agents (Sindhu et al., 2013) through hydrophobic interactions with lignin (Qing et al., 2010). Adding 1% SDS generated the highest lignin removal, enhancing it by 8.58% compared with IL only pretreatment. For the [AMIM]Cl + 1% rhamnolipid pretreatment, the hemicellulose content was 18.07% compared with 24.15% and 21.48% for the untreated and IL only pretreatment, respectively. Hemicellulose removal increases the internal surface area, increases the enzymatic access to cellulose, and reduces nonproductive binding of cellulase with hemicelluloses sugar, thus enhancing the enzymatic saccharification of cellulose (Jeoh et al., 2007). Minimizing cellulose loss during the pretreatment processes is crucial and directly associated with the total reducing sugar yield. After IL only, [AMIM]Cl + 1% CTAB, [AMIM]Cl + 1% TritonX-10, [AMIM]Cl + 1% SDS, and [AMIM]Cl + 1% rhamnolipid pretreatment, 41.65%, 41.29%, 40.15%, 41.37% and 42.56% of the cellulose was retained in the rice straw, respectively.

3.2. Effect of different pretreatment types on enzymatic saccharification

To compare the effects of different pretreatment types on enzymatic saccharification, enzymatic hydrolysis was performed at enzyme loadings of 50 FPU/g substrate and 40 CBU/g substrate at 50 °C and 150 rpm. Fig. 1 displays the percentage of cellulose conversion for untreated and IL only-, [AMIM]Cl + 1% CTAB-, [AMIM]Cl + 1% TritonX-10-, [AMIM]Cl + 1% SDS-, and [AMIM]Cl + 1% rhamnolipid-pretreated rice straw. IL pretreatment significantly increased cellulose conversion efficiency compared with untreated rice straw. In the surfactant combined with ionic liquid groups, the cellulose conversion of the rice straw pretreated with biosurfactant was the highest, anionic surfactant was the second highest, and the lowest was observed for pretreated with cationic surfactant. Adding 1% rhamnolipid enhanced cellulose conversion to 36.21% at 72 h compared with 28.25% for IL only pretreatment. Thus, adding rhamnolipid during [AMIM]Cl pretreatment could promote the enzymatic hydrolysis of rice straw. In the present study, rhamnolipid enabled more delignification, resulting in the binding of less nonproductive enzymes to lignin and more hydrophilic biomass surface interactions, thereby facilitating the dissolution of enzymes in water to access the biomass surface. Rhamnolipid is a bio-surfactant and superior to synthetic surfactants for several reasons: it is easily biodegradable with very low toxicity (having higher EC50 values); it can operate in extreme conditions such as temperature, pH, and salinity; and it is sustainably produced. Therefore, [AMIM]Cl + rhamnolipid provide an environmentally friendly method for pretreating rice straw by using green solvent (ionic liquid) and biodegradable biosurfactant.

3.3. FE-SEM

Supplemental data Fig. S1 displays the images of the FE-SEM performed on untreated and [AMIM]Cl only-, and [AMIM]Cl + 1% rhamnolipid -pretreated rice straw. The morphologies of the untreated and pretreated rice straw differed. The untreated rice straw had highly ordered and rigid structures, whereas the cellulose surfaces became rougher, more porus, and agglomerated after pretreatment (Fig. S1(B) and (C)). Although IL treatment displayed different surface conditions depending on the regenerated cellulose and lignin removal degree, the typical cell wall structure of the fibers remained intact. By contrast, the [AMIM]Cl + 1% rhamnolipid -pretreated rice straw exhibited considerable cell wall structural transformation. These alterations may be attributed to the disruption of the structure and reduction in crystallinity of cellulose. Accessibility of cellulolytic enzymes to cellulose is a major factor influencing enzymatic saccharification. Our morphological investigation revealed a significant increase in the porosity and surface area after pretreatment, resulting in improved enzymatic saccharification.

3.4. AFM characterization

AFM can be used to examine samples at molecular or even atomic resolutions in three dimensions. It also has the advantages of in situ measurements in atmospheric or controlled environments. Using software "Nanoscope Analysis (Version 1.40)" to process the raw images and calculate the root mean square roughness (Rq), the average roughness (Ra) and Surface area (Sa). The results were showed in Table 2. The untreated rice straw appeared flat and even with a relatively well-oriented ultrastructure. After the [AMIM]Cl and [AMIM]Cl + 1% rhamnolipid pretreatment, the surface structure of rice straw were observed to be greatly rougher in comparison with the untreated sample. Moreover, the structure surface of pretreated rice straw were uneven and non-uniformly covered with visible microfiber fragments, indicating the fiber surface was modified after pretreatment. This phenomenon was in accordance with SEM images in Fig. S1. It is evident that after the pretreatment many substances (i.e., lignin, hemicelluloses and other extractive) were removed from the rice straw. [AMIM]Cl and [AMIM]Cl + 1% rhamnolipid pretreatment can disrupt the structure of rice straw, and modify its morphology surface, result-

Table 1
Chemical composition of untreated and pretreated rice straw.

Pretreatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Recovered solides (%)
Untreated	35.36 ± 0.01	24.15 ± 0.05	25.98 ± 0.04	100.00
[AMIM]Cl	41.65 ± 0.01	21.48 ± 0.01	20.40 ± 0.16	84.08
[AMIM]Cl + 1% CTAB	41.29 ± 0.07	22.64 ± 0.03	20.93 ± 0.03	84.41
[AMIM]Cl + 1% TritonX-100	40.15 ± 0.09	21.05 ± 0.01	19.58 ± 0.26	83.01
[AMIM]Cl + 1% SDS	41.37 ± 0.06	21.12 ± 0.06	18.65 ± 0.30	83.91
[AMIM]Cl + 1% rhamnolipid	42.56 ± 0.02	18.07 ± 0.04	19.19 ± 0.16	81.06

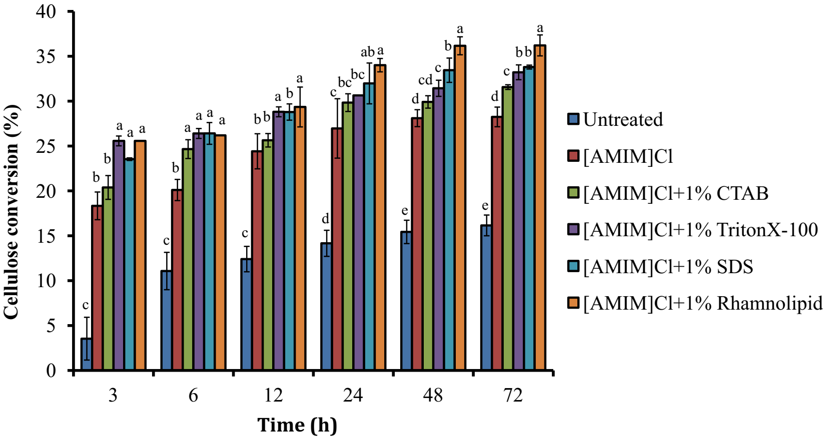

Fig. 1. Profiles of enzyme hydrolysis of regenerated rice straw. Hydrolysis condition: 1 mg/mL regenerated rice straw, 50 FPU/g substrate and 40 CBU/g substrate, 50 °C with 150 rpm. Each value is expressed as mean ± S.D. (n = 3). Means within the same group with different letters are significantly different at $P \leqslant 0.05$.

Table 2
AFM surface roughness factor of untreated and pretreated rice straw.

	Square roughness (Rq)	Average roughness (Ra)	Surface area (SA, nm^2)
Untreated	1.3	0.9	1,040,248
[AMIM]Cl	13.6	11.3	1,741,972
[AMIM]Cl + 1% rhamnolipid	18.0	16.3	3,021,592

ing in exposure of microfiber fragments, which could increase enzymatic accessibility to cellulose and the bioconversion efficiency into ethanol production (Liu et al., 2015).

It showed that compared with untreated, the roughness Rq and Ra were significantly increased (Table 2). The Surface area was increased from 1,040,248 nm^2 to 1,741,972 nm^2 and 3,021,592 nm^2, respectively, after pretreatment. It was revealed that the [AMIM]Cl and [AMIM]Cl + 1% rhamnolipid pretreatment caused a dramatic change of surface roughness and area. Surface roughness might play a very relevant role in the processes of enzyme molecule adhesion to biomass surfaces.

With the presence of surface viscous damping or adhesion, the shift in the phase of the oscillation reflects the variation in substrate surface stiffness (Liu et al., 2009). Any boundaries with material discontinuity caused by the difference in surface properties can be reflected by phase shift. Therefore, AFM phase imaging is very effective for differencing materials with different hydrophilicity. The AFM tip can adhere strongly to hydrophilic areas that hence appear light in color (Pang et al., 2012). In the phase contrast image, there are much brighter areas in [AMIM]Cl + 1% rhamnolipid pretreated sample; however, the brightness decreases and disperses in [AMIM]Cl pretreated sample, meaning that the surface of [AMIM]Cl + 1% rhamnolipid pretreated is more hydrophilic than that of [AMIM]Cl pretreated. Carbohydrates are generally more hydrophilic than lignin or extractives. Result shows that the bio-surfactant improved the hydrophilic ability of rice straw substrates.

4. Conclusions

This study compared the different types of surfactant combined with IL pretreatment. The cellulose conversions are listed in the order as follows: bio-surfactant-IL > anionic surfactant-IL > nonionic surfactant-IL > cationic surfactant-IL > IL. The IL + 1% rhamnolipid-pretreated rice straw showed significant lignin removal (26.14%) and exhibited higher cellulose conversion (36.21%) than the untreated (16.16%). AFM images showed that pretreatment caused a dramatic change of surface roughness. Moreover, bio-surfactant improved the hydrophilic ability of rice straw.

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