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Powerful peracetic acid-ionic liquid pretreatment process for the efficient chemical hydrolysis of lignocellulosic biomass

Uju ^{a, b, †*}, Masahiro Goto ^{c, d} and Noriho Kamiya ^{c, d, †*}

^a Department of Aquatic Products Technology, Bogor Agricultural University, Jl. Lingkar Akademik Kampus IPB Darmaga, Bogor 16680, Indonesia

^b Surfactant and Bioenergy Research Center, Bogor Agricultural University, Jl. Pajajaran No.1, Kampus IPB Baranangsiang, Bogor 16144, Indonesia

^c Department of Applied Chemistry, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

^d Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

* Corresponding authors. Tel: +62-251-8622915; Fax: +62-251-8622916

E-mail address: ujusadi@ipb.ac.id (Uju); nori_kamiya@mail.cstm.kyushu-u.ac.jp (N. Kamiya).

[†] These authors contributed equally to this work.

Abstract

The aim of this work was to design a new method for the efficient saccharification of lignocellulosic biomass (LB) using a combination of peracetic acid (PAA) pretreatment with ionic liquid (IL)-HCl hydrolysis. The pretreatment of LBs with PAA disrupted the lignin fractions, enhanced the dissolution of LB and led to a significant increase in the initial rate of the IL-HCl hydrolysis. The pretreatment of Bagasse with PAA prior to its 1-butyl-3-methylimidazolium chloride ([Bmim][Cl])-HCl hydrolysis, led to an improvement in the cellulose conversion from 20 to 70% in 1.5 h. Interestingly, the 1-butyl-3-methylpyridium chloride ([Bmpy][Cl])-HCl hydrolysis of Bagasse gave a cellulose conversion greater than 80%, with or without the PAA pretreatment. For LB derived from seaweed waste, the cellulose conversion reached 98% in 1 h. The strong hydrolysis power of [Bmpy][Cl] was attributed to its ability to transform cellulose I to II, and lowering the degree of polymerization of cellulose.

Keywords: Ionic liquid, hydrolysis, lignocellulosic biomass, peracetic acid

1. Introduction

Lignocellulosic biomass contains cellulose (40–50%), hemicellulose (25%) and lignin (25%), with the relative amounts of these materials varying depending on the type of lignocellulosic biomass (Tadesse and Luque, 2011). In recent years, considerable research interest has been directed towards lignocellulosic biomass as a potential resource for biofuels and biochemical feedstocks. Furthermore, some studies have reported that the global potential of lignocellulosic biomass could reach $1\text{--}50 \times 10^{10}$ ton/year (Alvira et al., 2010; Tadesse and Luque, 2011). In a similar manner to many first generation biofuel feedstocks, such as starch and simple sugars, the high cellulose and hemicellulose contents could be converted to biofuels and biochemical products through chemical and biological reactions. The main advantage of feedstocks derived from lignocellulosic biomass is their low price compared with other biofuel-based feedstocks, including starch-rich and sucrose-rich materials (Alvira et al., 2010). Furthermore, lignocellulosic biomass feedstocks could potentially replace several commercial biofuel and biochemical products, which are currently derived from food-based resources (sugar and starchy materials) such as corn and sugarcane. This is particularly important because the use of food resources for biofuel feedstocks has been blamed for recent rises in global food price as the world's population continues to grow, represent a threat to food security.

There are several challenges associated with the commercialization of lignocellulosic biomass, including the high resistance of this material to being hydrolyzed to mono- and oligosaccharides (Alvira et al., 2010). Cellulose is insoluble in water and most common organic solvents because of the strong hydrogen bonding interactions

between the many glucose units making up its complex structure (Tadesse and Luque, 2011). Furthermore, lignin binds to cellulose and hemicellulose through a series of covalent bonds to form lignin-carbohydrate complexes. The formation of these lignin-carbohydrate complexes makes the structure of the lignocellulosic biomass much more resistant to the physical, biological and chemical treatment processes typically used to break down these structures (Hu and Ragauskas, 2012). To obtain valuable chemical compounds from lignocellulosic biomass with high cellulose conversion, **the processing of lignocellulosic biomass needs** extreme conditions such **as** high pressure, high temperature and high concentration for extended time periods **due to the presence of lignin. For instance, it was reported that the dissolution of softwood and hardwood biomass using ionic liquids required the pretreatment for 24 h at 140-150 °C (Casas et al., 2012).**

The results of several recent studies have shown that ionic liquids (ILs) can be applied to the treatment of lignocellulosic biomass feedstocks. It has been shown that the treatment of lignocellulosic biomass with ILs leads to a variety of interesting effects, including the swelling of its cell walls, the break down of intermolecular hydrogen bonding interactions, enhanced surface area and a reduction in the crystallinity of cellulose (Lee et al., 2009; Singh et al., 2009), all of which make the cellulose more accessible to water. In particular, imidazolium-chloride ILs, such as 1-butyl-3-methyl imidazolium chloride ([Bmim][Cl]) and 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]), have been shown to assist in the enzymatic hydrolysis of cellulosic feedstocks when they are used for the pretreatment of these materials. Furthermore, the combination of ILs with strong acids has been shown to improve the hydrolysis performances of cellulosic feedstocks by increasing

the cellulose conversion or total amount of reducing sugar (Binder and Raines, 2010; Iguchi et al., 2013; Li and Zhao, 2007; Morales-delaRosa et al., 2012). For instance, the hydrolysis reactions of cellulose with [Bmim][Cl] and [Emim][Cl] in the presence of 20 wt% HCl (1.66 M) at 135 and 105 °C produced glucose yields of 99.8 and 100%, respectively (Morales-delaRosa et al., 2012). However, the maximum glucose yield obtained for a lignocellulosic feedstock such as rice straw was only 70% (Binder and Raines, 2010). The lower cellulose conversion of lignocellulosic biomass compared with microcrystalline cellulose is caused by lignin, which inhibits the hydrolysis of cellulose (Lee et al., 2013; C. Wang et al., 2012). In contrast, there have been very few reports pertaining to the acid-mediated hydrolysis of lignocellulosic biomass using pyridinium-type ILs. The potential of pyridinium-chloride ILs as hydrolysis agents has been reported previously (Binder and Raines, 2010; Uju et al., 2013b, 2012). In the previous report, the pretreatment of Avicel and Bagasse with 1-hexylpyridinium chloride ([Hpy][Cl]) without the addition of any other catalyst resulted in cellulose conversion of 19 and 6%, respectively (Uju et al., 2013b). Furthermore, a higher cellulose conversion (9%) was obtained following the pretreatment of Bagasse with 1-butyl-3-methylpyridinium chloride ([Bmpy][Cl]) at 120 °C for 1 h (Uju et al., 2012). Based on these results, we have developed a keen interest in exploring the potential of pyridinium-type ILs for the hydrolysis of lignocellulosic biomass, especially in combination with other hydrolysis methods.

In this study, evaluation of the performance of the pyridinium chloride IL [Bmpy][Cl] for the dissolution and subsequent hydrolysis of lignocellulosic biomass in the

presence of HCl under various experimental conditions was validated. Furthermore, comparison of the performance characteristics of this IL with those of its imidazolium counterpart, [Bmim][Cl] was carried out. To minimize the inhibitory effect of lignin (i.e., its ability to prevent the penetration of the IL into cellulose) and accelerate its hydrolysis performance, we incorporated a peracetic acid (PAA)-pretreatment process. It was envisaged that the introduction of a pretreatment process prior to the dissolution step would reduce the lignin content (Gonçalves et al., 2015), making the structure of the lignocellulosic biomass more porous in nature (Uju et al., 2013a). Therefore, Incorporating PAA pretreatment in the IL-acid hydrolysis process could enhance the conversion of lignocellulosic biomass to sugars. There have been no reports in the literature describing the combination of a PAA-pretreatment process with a [Bmpy][Cl]-HCl hydrolysis. In this paper, the potential agro-industrial residues of Bagasse and Seaweed waste biomass from carrageenan processing (SWBC)(Uju et al., 2015) were selected as suitable hydrolysis feedstocks. The performance characteristics of this combination process were evaluated in terms of the dissolved biomass fraction (DBF) in the ILs and the conversion of cellulose to saccharides (glucose and cellobiose). To develop a deeper understanding of the mechanisms of the cellulose dissolution and hydrolysis steps, we analyzed the regenerated biomass materials by FT-IR, XRD and SEM. The overall experimental design of this study is summarized in Fig. 1.

2. Materials and Methods

2.1. Materials

[Bmpy][Cl] (>98% purity) and [Bmim][Cl] (> 98% purity) were purchased from IoLiTec (Ionic Liquids Technologies GmbH, Heilbronn, Germany) and Kanto Chemical Co., Inc. (Tokyo, Japan), respectively. Peracetic acid (PAA) 40 % (w/w) was supplied by Mitsubishi Gas Chemical Company, Inc. (Tokyo, Japan). SWBC (particle size ~100 mesh with cellulose and lignin contents of 34 and 4%, respectively)(Uju et al., 2015) was obtained from CV Ocean Fresh (Bogor, Indonesia). Bagasse (particle size ~200 μ m with cellulose and lignin contents of 37.7 and 22.5%, respectively)(Gonçalves et al., 2015) was purchased from Toyota Tsusho Corporation (Nagoya, Japan). All of the reagents were used as received without further purification.

2.2. Glucose and cellobiose analyses

The saccharide content in the reaction mixture was measured by high performance liquid chromatography (HPLC) using an LC-10AT VP system (Shimadzu, Japan) equipped with a Honepak C18 column (4.6 $\times$ 75 mm; J-Oil Mills Inc., Tokyo, Japan). The mobile phase consisted of deionized water containing 0.02% (v/v) trifluoroacetic acid, which was eluted with a flow rate of 1 mL/min. The column temperature was set at 40 °C. A sample volume of 20 μ L was used for each injection **that was linked by fluorescence detector (excitation and emission wavelengths are 305 nm and 360 nm, respectively)**. The saccharide concentration was determined according to the *p*-aminobenzoic ethyl ester derivatization method described by Yasuno et al., (1997).

The conversion of cellulose to glucose or cellobiose was estimated as follows:

Conversion of cellulose to glucose (%) = [glucose produced (mol)/glucose unit in cellulose (mol)] × 100 (1)

Conversion of cellulose to cellobiose (%) = [cellobiose produced (mol)/glucose unit in cellulose (mol)] × 100 (2)

The conversions were calculated based on initial of lignocellulosic biomass because it is difficult to determine the exact constituent of the pretreated lignocellulosic biomass.

2.3. Peracetic acid pretreatment step (Step I)

Fifty milligrams of lignocellulosic biomass feedstock was added to a 1.9% (v/v) PAA solution in water (1050 μ L), and the resulting mixture was heated at 80 °C for 3 h with stirring (250 rpm). The excess water in the PAA-pretreated lignocellulosic biomass was removed by vacuum distillation over a 3 h period a residue, which was lyophilized for 16–24 h.

2.4. Cellulosic feedstocks hydrolysis

The process for the hydrolysis of cellulose was divided into two steps (i.e. dissolution and hydrolysis steps), as shown for the stepwise heating procedure in Fig. 1. The details of this process are as follows.

a. Dissolution step (Step II)

For the dissolution of cellulose, the untreated or PAA-pretreated biomass was heated in 1 g (the volume ~ 1000 μ L) of IL at 120 °C under stirring at 250 rpm for 30 min.

b. Hydrolysis step (Step III)

The hydrolysis reaction was carried out at 100 °C over a 3 h period with stirring at 250 rpm. During this step, the mixture was treated with 315 µL of a 1 M HCl solution, which was gradually added at 0, 20 and 30 min in volumes of 52, 158 and 105 µL, respectively. The hydrolysis reaction was sampled at 30, 60, 90 and 180 min to measure the levels of glucose, cellobiose and several other dissolved saccharides by HPLC. With regard to the addition of the acid catalyst in the dissolution step, the final concentration of HCl was set to 0.2 M.

2.5. Definition of dissolved biomass fractions

To obtain the dissolved biomass fraction (DBF), the IL-feedstock biomass mixture obtained at the end of dissolution step was cooled to room temperature and treated with 5 mL of deionized water to form regenerated biomass. The mixture was centrifuged at 5800 ×g for 15 min to allow for the separation of the DBF from the regenerated biomass. The regenerated biomass was also washed with deionized water (2 × 5 mL) to remove any residual IL. This process involved vigorous shaking and centrifugation. The amount of DBF was calculated using the following equation:

$$\text{DBF (\%)} = \frac{(\text{Mass of dried untreated biomass} - \text{mass of dried regenerated biomass})}{\text{mass of dried untreated biomass}} \times 100 \quad (3)$$

2.6. Structural characterization of the lignocellulosic biomass

FT-IR, SEM and XRD analyses were carried out for the untreated feedstock, PAA-pretreatment feedstock and the un-dissolved feedstock biomass (i.e. regenerated feedstock) during the dissolution step. The details of the different characterization steps are as follows:

a. Scanning electron microscope (SEM) analysis

Prior to being analyzed, all of the samples were spread on a metal-cylinder plate with carbon tape and coated with platinum under vacuum. SEM analyses were conducted on an INSPECT-S50 SEM system (FEI, Hillsboro, Oregon, USA), which was operated with a 10 kV acceleration voltage.

b. X-ray diffraction (XRD) analysis

XRD analysis was carried out on a Rigaku MultiFlex Diffractometer (Rigaku Co., Tokyo, Japan), which was operated at 40 kV and 30 mA ($\lambda = 1.5 \text{ \AA}$). The samples were scanned for diffraction angles (2θ) in the range of 5 to 50° with a step size of 0.02°.

c. Fourier transform infrared (FT-IR) spectroscopic analysis

The cellulose structure was characterized by infrared spectroscopy using a Spectrum 100 Fourier transform-infrared (FT-IR) spectrometer equipped with an attenuated total reflectance unit (Perkin Elmer, Waltham, Massachusetts, USA). Cellulosic feedstock samples were placed on a holder made of tin plate. The FT-IR spectra were collected for wavelengths in the range of 4000 to 800 cm^{-1} with four scans per sample at a resolution of 4 cm^{-1} .

3. Results and discussion

3.1. Effect of peracetic acid pretreatment

As shown in the **Supplementary information, Fig. S1A**, the pretreatment of the different lignocellulosic biomass materials with PAA led to noticeable difference in their physical appearances. For example, the treatment of the lignocellulosic biomass with PAA at 80 °C for 3 h led to a change in its color. The SWBC feedstock became white in color following its treatment with PAA. In the case of Bagasse, the color changed from brown-yellowish to pale yellow following its treatment with PAA (**Supplementary information, Fig. S1A a-b**). These changes in the appearances of the biomass materials to brighter colors were attributed to some of the chromophoric groups of the lignin being removed by oxidation (Zhao et al., 2011). The PAA-pretreated lignocellulosic biomass materials were then dried. Notably, the pretreated materials were bulkier than the untreated lignocellulosic biomass materials (**Supplementary information, Fig. S1A c**), which implied that the PAA-pretreatment process led to an increase in the surface area of the pretreated materials. SEM, XRD and FT-IR analyses were also conducted on the untreated and PAA-pretreated biomass materials to develop a better understanding of these differences. The results of these analyses are shown and discussed in the section 3.3

3.2. Dissolution process

The dissolution step in the IL-acid hydrolysis of lignocellulosic biomass is one of the most important factors for obtaining a high cellulose conversion (Morales-delaRosa et al., 2014,

2012; C. Wang et al., 2012). The complete dissolution of cellulose makes it easier for the acid catalyst (H^+) to access its β -glucosidic bonds (Li and Zhao, 2007), leading to an increase in the efficiency of the hydrolysis process.

Samples of the untreated and PAA-pretreated biomass materials were added to an IL, and the resulting mixtures were heated at 120 °C for 30 min with stirring at 250 rpm. Notably, [Bmim][Cl] immediately melted and formed viscous mixtures with the different biomass materials, whereas [Bmpy][Cl] needed around 1 min to melt and form IL-biomass mixtures. The longer time required for [Bmpy][Cl] to form IL-biomass mixtures was attributed to its higher melting (95 °C) compared with [Bmim][Cl] (73°C) (Heinze et al., 2005). At the end of the dissolution process (30 min heating), the mixtures generally existed as homogenous IL-biomass mixtures that were brown to dark brown in color (Supplementary information, Fig. S1B). The homogenous nature of these biomass mixtures suggested that most of the biomass fraction had dissolved. The heating of the different biomass materials in [Bmim][Cl] led to the formation of swollen fractions following the precipitation of the materials from water. Notably, the swollen fractions were larger for the biomass feedstocks without PAA pretreatment. Furthermore, the PAA-pretreated material precipitated from the SWBC mixture formed a tough gel-like structure that could not be broken down, even after vigorous vortex mixing.

The dissolved biomass fractions (DBFs) were analyzed to allow for the quantification of the dissolution step. The DBFs were calculated based on the difference between the mass of the untreated regenerated biomass and the mass of the dried untreated biomass, as stated in equation 3. The regenerated biomass materials obtained from the

[Bmpy][Cl] dissolution were readily dried following a short period (16 h) of freeze drying. In contrast, the regenerated biomass material from the [Bmim][Cl] dissolution had to be lyophilized for a longer period of time (24 h) to be dried completely. This difference in the drying times was attributed to the absorption of the [Bmim][Cl] into the regenerated biomass. As shown in Fig. 2A, the DBFs obtained after the heating of the biomass materials with [Bmpy][Cl] were much higher than those obtained from the heating of the same materials in [Bmim][Cl]. Furthermore, the PAA pretreatment of the biomass materials dissolved in [Bmpy][Cl] enhanced the DBF values. The maximum DBF value obtained following the heating of the biomass materials with [Bmpy][Cl] was 87%, whereas the maximum value obtained with [Bmim][Cl] under same conditions was only 38%. The greater capability of [Bmpy][Cl] to dissolve cellulosic biomass has been reported previously (Heinze et al., 2005). According to this study, [Bmpy][Cl] dissolved 2 to 3 times more Avicel and Spruce sulfite pulp than [Bmim][Cl].

The ability of an IL to dissolve cellulose is determined by the nature of its cationic and anionic components. Chloride is one of the strongest anions for cellulose dissolution. The free chloride ions of an IL can disrupt the intra- and intermolecular hydrogen bonding networks of cellulose (Kilpeläinen et al., 2007; H. Wang et al., 2012). Furthermore, Zhao et al., (2012) reported that the interaction energy between the free chloride anions and cellulose in [Bmpy][Cl] was stronger than that of the chloride ions in [Bmim][Cl], making cellulose much more soluble in [Bmpy][Cl] than it was in [Bmim][Cl]. With regard to the cationic components of ILs, imidazolium and pyridinium cations can disrupt the π - π interactions of lignin because of their aromatic character (Kilpeläinen et al., 2007).

Furthermore, Basa, (2010) showed that [Bmpy][Cl] exhibited a higher potential for $n\text{-}\pi/\pi\text{-}\pi$ interactions than [Bmim][Cl]. In terms of the different biomass feedstocks used in the current study, SWBC gave slightly higher DBF values than Bagasse, especially in the absence of a PAA pretreatment process, which suggested that SWBC was easier to dissolve than Bagasse. However, these results were not surprising because the presence of lignin could prevent the IL from penetrating the cellulose during the dissolution process (Kilpeläinen et al., 2007; Xiang et al., 2003). The higher lignin content would therefore result in a reduction in the dissolution of the biomass.

HPLC analysis was used to identify the different mono- and disaccharide components of the DBFs. As shown in Fig. 2B, the dissolution of the biomass materials in [Bmpy][Cl] or [Bmim][Cl] resulted in the release of several simple sugars, such as cellobiose and glucose. During the dissolution of Bagasse, [Bmpy][Cl] released cellobiose 7.9% and glucose 7.8%. A similar trend was also observed for the dissolution of SWBC. The total cellulose conversions for both forms of the sugar reached around 16%. The ability of [Bmpy][Cl] to liberate glucose and cellobiose was consistent with the results of a previous report (Uju et al., 2012). The cellulose conversions of the lignocellulosic biomass materials dissolved in [Bmim][Cl] were lower than those obtained in [Bmpy][Cl], with both biomass materials affording cellulose conversions of less than 2.5% in [Bmim][Cl]. Notably, the effect of the PAA pretreatment was much more pronounced for the biomass materials pretreated in [Bmpy][Cl] than it was for those pretreated in [Bmim][Cl].

HPLC chromatograms were also recorded for samples taken during the different treatment processes to determine the presence of any other dissolved compounds

(Supplementary information, Fig. S2). The results of this analysis not only revealed peaks corresponding to glucose and cellobiose, but also revealed several other peaks with retention times of less than 4.8 min. These peaks showed low molecular weights that were consistent with oligosaccharides. Furthermore, the general pattern of these chromatograms was similar to that obtained following the dilute acid hydrolysis of these material (Xiang et al., 2003) in that the amounts of the different oligosaccharides decreased with increasing degree of polymerization (DP). [Bmpy][Cl] produced a higher concentration of oligosaccharides than [Bmim][Cl], which implied that [Bmpy][Cl] was more capable of depolymerizing cellulose than [Bmim][Cl] (Heinze et al., 2005). Notably, the amount of oligosaccharides increased following the pretreatment of the biomass material with PAA. These results were supported by FT-IR analysis, which showed that the PAA pretreatment process promoted the depolymerization process. Furthermore, the oligosaccharides released from the biomass would be readily hydrolyzed during the subsequent IL-HCl hydrolysis step.

3.3. Structural analyses of regenerated biomass

To develop a better understanding of the effects of the PAA pretreatment and IL dissolution steps on the subsequent IL-HCl hydrolysis, the characterizations of regenerated biomass materials obtained during the dissolution step were carried out by SEM, XRD and FT-IR spectroscopy.

3.3.1 Characteristic of PAA pretreated biomass

SEM micrographs of the untreated and PAA-pretreated biomass materials are shown in the Supplementary information, Fig. S3. The SEM images of the untreated Bagasse showed that it existed as a rigid, ordered structure. The pretreatment of Bagasse with PAA resulted in a disorganized structure compared with the native form. This result indicated that some of the lignin fraction had been removed from the Bagasse, because lignin is responsible for the highly compact structure of plant cell walls (Kilpeläinen et al., 2007; Tadesse and Luque, 2011).

XRD analysis (Supplementary information, Fig. S4A) of untreated Bagasse and SWBC materials showed that they exhibited cellulose I characteristics, as exemplified the presence of a strong diffraction peak around 22° and a much weaker peak around 16.5° . The pretreatment of these materials with PAA led to a decrease in the intensity of the peak around 22° , which indicated that there had been a reduction in the crystallinity of the cellulose. Moreover, FT-IR analysis (Supplementary information, Fig. S4B) showed that the untreated Bagasse contained peaks at 1608 and 1465 cm^{-1} , which are indicative of vibrations in the aromatic skeletal of lignin and CH-deformations in the lignin and carbohydrate structures (Zhao et al., 2008). In contrast, these peaks only appeared as small shoulders in the FT-IR spectrum of the Bagasse pretreated with PAA, although this material did still contain two bands at 1515 and 840 cm^{-1} , which were attributed to the aromatic C–H groups of lignin (Tibolla et al., 2014). These findings were in agreement with those of the SEM analysis, which showed that the pretreatment of the biomass with PAA resulted in the removal of some lignin. Gonçalves et al., (2015) reported that the treatment of biomass with PAA at 80°C for 3 h led to a 28% decrease in the amount of

acid-insoluble lignin but had very little impact on the level of soluble lignin. Furthermore, Zhao et al., (2008) reported that the removal of some lignin led to an increase in the surface area of the pretreated biomass. It was envisaged that the removal of some lignin would accelerate the dissolution and hydrolysis of the biomass materials during the subsequent processing steps.

In the case of the SWBC biomass, there were very few changes in the FT-IR spectra of the biomass materials recorded before and after the PAA pretreatment process. However, the peaks in the FT-IR spectrum of the untreated material at 1041 and 1059 cm^{-1} , which were assigned to the C–O bonds at C6 and C–O at C3, respectively, shifted to 1039 and 1057 cm^{-1} in the PAA-pretreated material. This phenomena have also been reported in several other studies (Liu et al., 2007; Uju et al., 2012) and is indicative of a reduction in the DP. Moreover, (Isogai and Atalla, 1998) reported that cellulose materials with lower DP values dissolve much more readily than those with higher DP values.

3.3.2 Characteristics of regenerated biomass

The results of the SEM analysis showed that the dissolution of the different biomass materials with [Bmim][Cl] resulted in the swelling of the biomass fractions, and that more swollen fractions were obtained following the pretreatment of the biomass materials with PAA (Supplementary information, Fig. S5A and B, upper). In contrast, the dissolution of the different biomass materials with [Bmpy][Cl] resulted in the fragmentation of the regenerated biomass materials, with smaller fragments being observed for the regenerated biomass materials pretreated with PAA prior to their dissolution (Supplementary information, Fig.S5A and B, bottom). SWBC showed different swelling and fragmentation

characteristics to Bagasse, which implied that SWBC was more susceptible to the PAA pretreatment process.

As shown in the Supplementary information, Fig. S6A, the dissolution of the biomass materials with [Bmpy][Cl] had a much greater effect on their structural characteristics than the other IL tested in this study. The XRD patterns of the two biomass materials treated with [Bmpy][Cl] showed diffraction patterns that were consistent with cellulose II (Iguchi et al., 2013; Miyata and Miyafuji, 2014; Uju et al., 2012). Furthermore, this diffraction pattern was observed more clearly when the dissolution step was preceded by PAA pretreatment, as indicated by the stronger doublet peaks observed around 20° and 22°, as well as the peaks at 12°. When the Bagasse feedstocks were dissolved in [Bmim][Cl], significant changes were observed only in the diffraction pattern for the biomass material that was been pretreated with PAA prior to its dissolution. For example, the peaks around 16° disappeared completely following the pretreatment of the Bagasse feedstock with PAA, and the peaks around 22° became much less pronounced. However, the center of peak remained unchanged. Similar changes in the diffraction pattern were also observed for the SWBC feedstock both with and without the pretreatment of the feedstock with PAA. These results indicated that the pretreatment of the biomass with PAA led to a decrease in the crystallinity of its cellulose. Furthermore, the higher lignin content of the Bagasse biomass meant that it was harder to reduce the crystallinity of its cellulose compared with SWBC.

The FT-IR spectra of the different materials are shown in the Supplementary information, Fig. S6B-C. analysis of the different bands was divided into two ranges (i.e.,

3550–3200 and 1700–800 cm^{-1}). The results revealed that the lignocellulosic biomass materials dissolved in [Bmpy][Cl] all produced cellulose II regardless of whether or not they were pretreated with PAA, based on the peaks observed at 3492 and 3445 cm^{-1} . These peaks indicated the existence of intramolecular hydrogen bonding interactions between the hydroxyl groups of the cellulose II crystals and the specific O(3)H...O(5) intramolecular hydrogen bonds, respectively (Šíroký et al., 2010). This finding effectively confirmed the XRD results. The most significant change observed in the FT-IR spectra of the biomass materials dissolved in [Bmim][Cl] occurred in the range of 3550–3200 cm^{-1} , where the central peak shifted to a higher wavenumber. This shift was indicative of a change in the hydrogen bonding network within the cellulose structure (Uju et al., 2013b; Zhou et al., 2012). Notably, a small peak was observed at 3447 cm^{-1} in the FT-IR spectrum of the SWBC feedstock pretreated with PAA, which indicated that small fractions of cellulose II had been formed.

Several changes were observed in the FT-IR spectra of the biomass materials in range of 1800–800 cm^{-1} , which attributed to changes in the structures of the lignin and cellulose. The FT-IR spectra of the Bagasse feedstock contained lignin peaks at 1608, 1515 and 840 cm^{-1} , which disappeared following a combination of the PAA pretreatment and IL dissolution steps. For example, the FT-IR spectrum of the PAA-pretreated Bagasse showed a peak at 840 cm^{-1} , which disappeared completely in the FT-IR spectrum of the Bagasse subjected to the PAA-IL combination. These results therefore indicated that an IL could be used to enhance the removal of lignin when it was used in combination with a PAA pretreatment process.

Furthermore, dramatic changes in the cellulose peaks were observed at 1052, 1038 and 899 cm^{-1} following the PAA-IL combination, which were indicative of changes in the C–O vibrations associated with the C(3)...O(3)H, C–O stretching and C–O valence vibrations at C(6), respectively (Šíroký et al., 2010). The FT-IR spectra of the biomass materials pretreated with PAA or a combination of PAA pretreatment with [Bmpy][Cl] revealed that the peak around 1058 cm^{-1} became much clearer and that it shifted to 1062–1064 cm^{-1} compared with the untreated materials. This sharpening and shifting of this peak was consistent with the transformation of the cellulose from cellulose I to cellulose II (Šíroký et al., 2010). The transformation of cellulose caused the structural change from parallel to antiparallel chains, and the van der Waals interaction between hydrogen bonded sheets became weaker (Wada et al., 2010).

3.3. Chemical hydrolysis Process

Finally, the IL-HCl hydrolysis reactions of these materials were investigated at 100 °C after the dissolution step. It is noteworthy that hydrolysis reactions of this type should be conducted at temperatures below 120 °C to reduce energy expenditure and minimize losses resulting from the degradation of glucose. Although [Bmpy][Cl] has a melting point around 95 °C, the biomass-[Bmpy][Cl] mixture existed as a liquid under the hydrolysis conditions at 100 °C. Furthermore, the stepwise addition of a total volume of 315 μL of 1 M HCl over 30 min at the beginning of the hydrolysis, led to a significant reduction in the viscosity of the liquid.

The cellulose conversions of the different lignocellulosic biomass materials to

glucose and cellobiose increased sharply during 30–60 min (Fig. 3). Furthermore, both IL-HCl methods reached a cellulose conversion of more than 70% following hydrolysis times in the range of 60–90 min. Extending the hydrolysis time beyond 90 min led to a decrease in the cellulose conversion, with considerable decreases observed for a hydrolysis time of 180 min. This observation was attributed to the degradation of glucose to low molecular weight products such as hydroxymethylfurfural, furfural and levulinic acid (Tadesse and Luque, 2011), as indicated by the formation of a dark solution.

The results for the hydrolysis of the different lignocellulosic biomass materials with [Bmpy][Cl]-HCl showed that this system allowed for fast and effective hydrolysis of the substrate. During the early phase of hydrolysis (30 min), the cellulose conversions reached 34–42%. The Bagasse material subjected to a PAA pretreatment process showed a slightly higher cellulose conversion than that of the untreated material after a short hydrolysis time. Furthermore, the cellulose conversion for the [Bmpy][Cl]-HCl hydrolysis reached 81–89% after 90 min, regardless of whether or not the biomass had been pretreated with PAA. The high cellulose conversion with [Bmpy][Cl]-HCl hydrolysis was ascribed to the transformation of cellulose I to cellulose II followed by the depolymerization of the pretreated biomass, which has been reported in previous literatures (Iguchi et al., 2013; Miyata and Miyafuji, 2014; Uju et al., 2012). Ni et al., (2015) reported that cellulose II materials with low DP values showed a high rate of cellulose conversion because of the weakly hydrogen bonded short cellulose chains. As a result, they could be readily hydrolyzed to water soluble sugars. In the present study, the dissolution of lignocellulosic biomass with [Bmpy][Cl] afforded more water-soluble cellulose and oligosaccharide

fractions, making it easier to hydrolyze the resultant materials during the subsequent chemical hydrolysis step.

The lowest cellulose conversion was obtained for the hydrolysis of the untreated Bagasse with [Bmim][Cl]-HCl. The maximum cellulose conversion achieved for this method was 20% following a 60 min hydrolysis. Notably, further increases in the hydrolysis time did not lead to an improvement in the cellulose conversion. However, the pretreatment of the biomass with PAA prior to the hydrolysis resulted in an increase in the cellulose conversion to 67 and 70% following hydrolysis times of 60 and 90 min, respectively. This improvement in the cellulose conversion was attributed to the PAA pretreatment process, which disrupted the cell wall structure (Supplementary information, Fig. S5) and removed some of the lignin (Supplementary information, Fig. S6B-C). These results therefore demonstrated that the pretreatment of lignocellulosic biomass with PAA is an effective strategy for obtaining higher levels of cellulose conversion from the IL-acid hydrolysis of lignocellulosic biomass materials with rigid, lignin-rich structures. In the case of the SWBC feedstock, the cellulose conversion of the material hydrolysis with [Bmim][Cl]-HCl without the PAA pretreatment process was 81%. In contrast, the material pretreated with PAA prior to the dissolution step gave a cellulose conversion of 98% after only 60 min of hydrolysis.

The effectiveness of PAA and ionic liquid pretreatment for enzymatic saccharification with different ionic liquid and feedstock was reported by Uju et al.(2013a). The PAA and [Emim][OAc] pretreatments converted 75% of cellulose of Pine wood by enzymatic saccharification for 25 h. Compared to the enzymatic saccharification and other

chemical hydrolysis of biomass, the present process can be conducted in shorter time with lower HCl concentration. For further processing to sugars available for subsequent fermentation to bioethanol, the hydrolysate can be treated by ion-exclusion chromatography to separate the sugars and the other impurities (Binder and Raines, 2010).

3.5 Proposed mechanism of lignocellulosic biomass hydrolysis in IL-HCl

To analyze the individual effects of the IL and the HCl hydrolysis reaction, a series of control experiments involving the replacement of the HCl or IL with deionized water using the Bagasse biomass without PAA pretreatment **were conducted**. All of these experiments were conducted under the standard conditions with the IL-HCl hydrolysis as previously described. The results showed that the use of water (i.e., 0 M HCl) with [Bmpy][Cl] led to a cellulose conversion that was 35 times higher than that achieved under the same conditions using [Bmim][Cl]. This cellulose conversion achieved under these conditions was also 5 times greater than that achieved under conventional hydrolysis conditions using 0.2 M HCl (**Fig. 4**). These results showed that [Bmpy][Cl] itself exhibited considerable hydrolytic activity towards lignocellulosic biomass. In this way, [Bmpy][Cl] provided rapid access to water-soluble cellulose and oligosaccharide units during the dissolution step, which were readily hydrolyzed in the subsequent hydrolysis step. In the case of [Bmim][Cl], a high cellulose conversion could be obtained via the hydrolysis of a suitable feedstock with a lower cellulose crystallinity, because it would contain weaker hydrogen bonding networks.

Based on the characteristics of lignocellulosic biomass and the results obtained throughout this study, the following strategy **is** a general guide for obtaining a high cellulose conversion to sugars using the present method. Most importantly, the steps must be conducted in the following order: (1) degradation of the lignin network by a PAA pretreatment process; (2) preparation of water accessible and preferably water soluble cellulose fractions by selecting a suitable pyridinium-type IL; and (3) incubation for a suitable period of time with a suitable amount of acid for the final hydrolysis step.

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Conclusions

The use of an IL-HCl hydrolysis in combination with a PAA pretreatment process has been validated. PAA pretreatment not only enhanced the dissolution of biomass, but also led to an increase in the initial rate of its hydrolysis, as well as an improvement in its cellulose conversion following a [Bmim][Cl]-HCl hydrolysis. In contrast, [Bmpy][Cl] exhibited good activity towards the hydrolysis of lignocellulosic biomass because it could transform cellulose I to cellulose II with a much lower DP value. [Bmpy][Cl]-HCl hydrolysis could therefore be employed for the efficient hydrolysis of lignocellulosic biomass.

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Figure captions

Fig. 1. Overall scheme for this experimental procedure (PAA = peracetic acid, IL = ionic liquid, DBF = dissolved biomass fraction). The black-shaded quadrangles show the stream of hydrolysis methods.

Fig. 2. Dissolved biomass fraction (DBF) (A), and Cellulose conversion of biomasses (B) pretreated by IL with or without prior PAA pretreatment. (PAA(–) = without PAA pretreatment, PAA(+) = with PAA pretreatment). Data indicate the mean values $\pm$ SD of three independent experiments.

Fig. 3. Ionic liquid-HCl hydrolysis reactions of Bagasse and SWBC at 120 °C. (PAA(–) = without PAA pretreatment, PAA(+) = with PAA pretreatment). Data are the mean values $\pm$ SD from three independent experiments.

Fig. 4. Comparison of different hydrolysis methods using HCl 0.2 M, [Bmim][Cl] + water, and [Bmpy][Cl] + water. The hydrolysis was carried out at 120 °C for 1 h on the Bagasse material that had not been subjected to a PAA pretreatment process.

Fig. 1

Fig. 2

Fig. 3

Fig. 4