Assessment of Cellulosic Biomass Saccharification by Molten Brönsted Acidic 1-Ethyl-3-Methylimidazolium Hydrogen Sulphate ([EMIM][HSO_4]) *via* Kinetic Studies

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Ionic liquids have been employed to deconstruct and fractionate lignocellulosic biomasses because of their capacity to dissolve cellulose. However, there is limited literature reporting the use of ionic liquids in biomass saccharification, which mostly involves the addition of acid or water that conceals the true action of ionic liquid in saccharification. This article assesses the performance of molten Brönsted acidic 1-ethyl-3-methylimidazolium hydrogen sulphate ([EMIM][HSO_4]) in saccharifying three agricultural biomasses, namely sago *hampas*, sugarcane bagasse, and rice husk, *via* saccharification kinetics. At 100 °C, [EMIM][HSO_4] saccharification of the biomasses achieved equilibrium reducing sugar yields at various durations (sago *hampas*, 3 h; sugarcane bagasse, 1 h; rice husk, 5 h). The kinetic rate constant was obtained from model fitting, indicated that [EMIM][HSO_4] showed a preference for saccharifying less recalcitrant sugarcane bagasse (37.9%) than sago *hampas* (7.0%) and rice husk (1.1%). Compared to H_2SO_4 saccharification, reducing sugar yields of [EMIM][HSO_4] were consistently lower. The difference in yields might be attributed to the hydrous/anhydrous state of reaction and limited availability of component ions of the ionic liquid for dissolution and saccharification. This study demonstrates the feasible technical aspects of applying [EMIM][HSO_4] to saccharify agricultural biomasses, which may lead to economic feasibility, recyclability, and cost effectiveness of ionic liquids in saccharification.

Keywords: Ionic liquid; Simultaneous dissolution and saccharification; Kinetic modeling; Sago hampas; Sugarcane bagasse; Rice husk

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INTRODUCTION

Saccharification is an essential step in the bioconversion of lignocellulosic biomass to fermentable sugars. There are two major routes involved, *via* the use of enzymes and hydrolytic chemical reagents. Between the two saccharification strategies, enzymatic saccharification has limitations that need to be overcome for more efficient biomass conversion. Because of the complexity of the lignocellulosic structure, most of the biomass is resistant to enzymatic saccharification. The intertwined lignocellulosic structure impedes the access of saccharifying enzymes to cellulose and hence hampers the saccharification process (Chandra *et al*. 2007). To improve the enzymatic saccharification process, a prerequisite pretreatment is necessary to disrupt the recalcitrant structure of the biomass (Chang *et al*. 1998; Shafiei *et al*. 2010). Physicochemical pretreatment is frequently

employed for this purpose. Most of the time, a physical pretreatment such as size reduction alone is insufficient to improve the performance of enzymatic saccharification. It has to be complemented by a chemical pretreatment using acid or base solution (Bhandari *et al.* 1984). It is also worth noting that biomass can be saccharified by acid treatment alone to achieve near-theoretical yields (Moe *et al.* 2012). Nevertheless, acid saccharification has a disadvantage, whereby high acid concentrations result in the formation of sugar degradation products such as furfural from pentoses and 5-hydroxymethyl furfural from hexoses.

The emergence of non-volatile and non-flammable ionic liquids has innovated biomass pretreatment strategies. The unique characteristics of ionic liquids explain their application in the fractionation of lignocelluloses (Zhu *et al.* 2006; Pezoa *et al.* 2010). Fractionation of lignocelluloses is initiated by dissolution of cellulosic material, followed by regeneration and precipitation of dissolved cellulose in the presence of polar solvent (Zhu *et al.* 2006).

Many previous reports have described the use of ionic liquids for pretreating lignocelluloses, where the dissolved cellulosic material is regenerated before it is saccharified enzymatically (Li *et al*. 2010; Pezoa *et al*. 2010; Ang *et al*. 2012). However, there are limited reports describing the application of ionic liquids in saccharifying lignocelluloses. Moreover, these reported processes involve the addition of a diluted acid or water, which camouflages the true action of the ionic liquid (Amarasekara and Owereh 2009; Wang *et al.* 2015). Therefore, this study attempted the possibility of saccharifying lignocelluloses using a molten ionic liquid.

In this study, the potential application of the Brönsted acidic hydrogen sulphate-based ionic liquid [EMIM][HSO_4] in simultaneous dissolution and saccharification of agricultural biomass such as sago *hampas*, sugarcane bagasse, and rice husk was assessed. The selected ionic liquid possesses the same conjugate base (HSO_4^-) as sulphuric acid (H_2SO_4); thus, it was hypothesised that [EMIM][HSO_4] is capable of performing simultaneous dissolution and saccharification of dissolved cellulosic materials.

Empirical modeling of the time-profile of reducing sugar yield was performed to evaluate the kinetics of ionic liquid saccharification relative to that in sulphuric acid saccharification. Chemical and structural analyses using Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were also performed to support the modelling results and to study the mechanism of anhydrous ionic liquid saccharification.

EXPERIMENTAL

Preparation of Biomasses

The agricultural biomasses under investigation, sago *hampas*, sugarcane bagasse, and rice husk, were collected from local sources in Malaysia. These biomasses were washed and dried at 55 °C before being ground to approximately 30-mesh size (500 μm) in a single batch to ensure consistency throughout the experiments; they were then stored in a dry cabinet prior to use.

The compositions of the biomasses, as previously determined, are presented in Table 1.

Table 1. Composition of Agricultural Biomass

	Composition (%, w/w)				Reference
	Starch	Cellulose	Hemicellulose	Lignin	
Sago *hampas*	37	40	13	5	Lee *et al.* (2014)
Sugarcane bagasse	- *	41	30	21	Yoon *et al*. (2011)
Rice husk	- *	53	5	20	Ang *et al*. (2011)
* Data not available					

Saccharification of Biomasses

An ionic liquid 1-ethyl-3-methylimidazolium hydrogen sulphate ([EMIM][HSO_4]) and concentrated sulphuric acid (H_2SO_4) were purchased from Merck (Germany). The [EMIM][HSO_4] was used without dilution throughout the experiments, whereas the concentrated H_2SO_4 was diluted to prepare 1% (v/v) H_2SO_4 solution.

The simultaneous dissolution and saccharification of the lignocellulosic biomass was conducted using a 4% (w/v) biomass loading in [EMIM][HSO_4]. The mixture in a test tube (13 × 100 mm) was heated to 100 °C ± 1 °C in an oil bath (Julabo MC (v.2), Germany) for 5 h. At the end of the heating, it was cooled to an ambient temperature in a cooling bath before an equal volume of deionised water was added to dilute the reaction mixture. After centrifugation at 3500 rpm for 20 min, the supernatant portion was separated for reducing sugar content determination. The reducing sugar yield time-profile was constructed through a series of experiments under the same conditions with different heating times, *i.e.* from 1 h to 5 h at one hour intervals. The residual biomasses were washed and dried for FT-IR and SEM analyses. For comparison purposes, the saccharification experiments were repeated using 1% (v/v) H_2SO_4 under the same conditions.

Analytical Methods

The total reducing sugar content of the supernatants was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959; Ghose 1987). The absorbance of the reacted DNS solution was determined at 540 nm, and the relative total reducing sugar concentration was computed from a standard calibration curve constructed with anhydrous glucose (Merck, Germany). The reducing sugar yield was calculated using Eq. 1.

$$\text{Reducing sugar yield, \%} = \frac{\text{Total reducing sugar content (mg)}}{\text{Total carbohydrate fraction (mg)}} \times 100\% \quad (1)$$

In Eq. 1, the 'total carbohydrate fraction' is the summation of cellulose, hemicellulose, and/or starch content in the biomass before saccharification. The term 'total carbohydrate fraction' was used instead of 'total biomass' because only the carbohydrate fraction in the biomass can be saccharified to reducing sugars.

The samples for FT-IR analysis were prepared by mixing each sample of dried biomass residues with spectroscopic grade potassium bromide and pressing of the mixture into discs. The measurements were recorded in the range of 450 to 4000 cm^{-1} with a nominal resolution of 4 cm^{-1} at room temperature using an FT-IR spectrometer (Perkin Elmer, Spectrum 400, USA). The measurement background was recorded with an empty cell. The spectra are illustrated as the relative transmittance percentage (%) of the wavenumber (cm^{-1}).

The SEM micrographs were obtained using a Carl Zeiss AURIGA® CrossBeam® focused ion beam-scanning electron microscope (FIB-SEM) workstation (Zeiss,

Germany). The samples were affixed onto aluminium specimen stubs using double-sided adhesive conductive carbon tape and viewed without metal coating at a 10-kV accelerating voltage under low vacuum mode.

Kinetics of Biomass Saccharification using Acid and Ionic Liquid

The performance of ionic liquid and acid saccharifications was assessed *via* kinetic studies. The saccharification kinetics was determined by modeling the time-profile of reducing sugar yields using a simple empirical exponential model (Thompson 2011), as shown in Eq. 2,

$$Y = Y_{sat}(1 - e^{-bt}) \quad (2)$$

where Y is the reducing sugar yield (%, w/w), Y_{sat} is the equilibrium reducing sugar yield (%, w/w), b is the rate constant for saccharification of cellulosic materials (h^{-1}), and t is the heating duration (h). The constants Y_{sat} and b for biomass saccharification using [EMIM][HSO_4] and H_2SO_4 were determined by fitting Eq. 2 with their experimental reducing sugar yield profiles using the MATLAB Curve Fitting Toolbox, Version 2.1 (The MathWorks, Inc., United States).

RESULTS AND DISCUSSION

[EMIM][HSO_4] Saccharification and Kinetic Modelling

The experimental time-profile of normalised reducing sugar yields (Y/Y_{sat} *versus* t) fit well to the empirical model, with adjusted R-square values falling in the range of 0.75 to 1.0, as illustrated in Fig. 1. The kinetics of [EMIM][HSO_4] and H_2SO_4 saccharifications were evaluated based on their rate constants b, as described in Eq. 2. The rate constant b for each biomass in [EMIM][HSO_4] saccharification closely resembled H_2SO_4 saccharification, which suggests that the ionic liquid were governed by analogous mechanisms in biomass saccharification to the acid. The saccharification kinetics for both [EMIM][HSO_4] and H_2SO_4 varied only with the type of biomass. Among the biomasses, sugarcane bagasse was saccharified at the fastest rate, as indicated by its highest rate constant b values (2.2 to 3.6 h^{-1}), followed by sago *hampas* (b = 0.7 to 0.8 h^{-1}) and rice husk (b = 0.3 to 0.4 h^{-1}). Biomass with a higher rate constant b such as sugarcane bagasse signifies that it is more easily saccharified compared to the other two biomasses. The lower saccharification rate of rice husk was primarily a result of its recalcitrant nature (Feng *et al.* 2004), whereas that for sago *hampas* was due to the shielding of cellulose by starch, which hinders the accessibility of [EMIM][HSO_4] during saccharification (Lappalainen *et al.* 2013).

The plots in Fig. 1 show that sago *hampas* achieved equilibrium reducing sugar yield, Y_{sat}, at the third hour for both [EMIM][HSO_4] and H_2SO_4 saccharification, whereas sugarcane bagasse and rice husk achieved equilibrium reducing sugar yields at the first and fifth hour, respectively. There was no sign of sugar degradation for all the biomass saccharifications using the ionic liquid and acid, except for [EMIM][HSO_4] saccharification of sugarcane bagasse beyond three hours (Fig. 1b). This observation is in contrast to saccharification with H_2SO_4, where there was no degradation of reducing sugars.

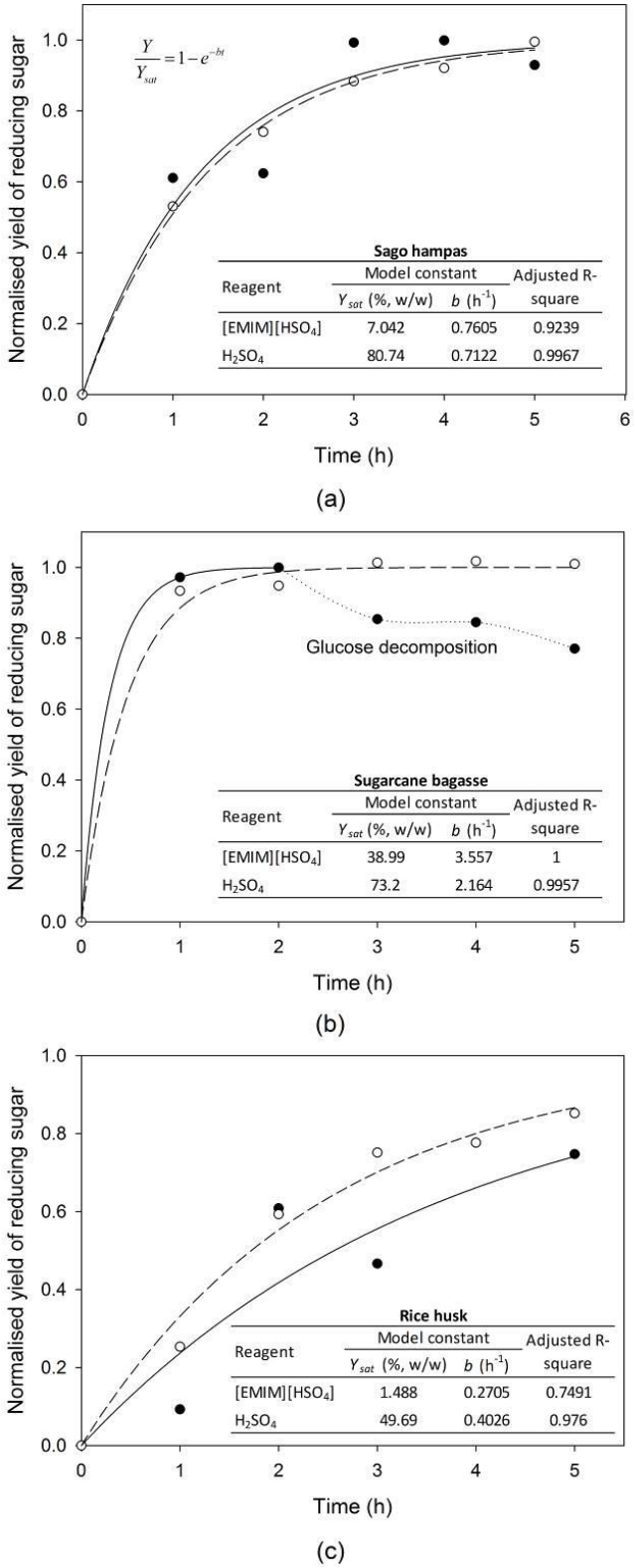

Fig. 1. Saccharification kinetics of (a) sago *hampas*, (b) sugarcane bagasse, and (c) rice husk using [EMIM][HSO_4] (●) and H_2SO_4 (○) based on empirical exponential model: (—) fitted yield for [EMIM][HSO_4]; (– –) fitted yield for H_2SO_4

The reduction in reducing sugar yield of [EMIM][HSO_4]-saccharified sugarcane bagasse might be due to the degradation of reducing sugars. Therefore, the reducing sugar yields of sugarcane bagasse after the third hour onward where the degradation of sugars was observed were not included in model fitting for [EMIM][HSO_4] saccharification. In general, [EMIM][HSO_4] saccharification gave lower reducing sugar yields compared to H_2SO_4 saccharification, as presented in Fig. 1. This could be due to insufficient component ions of the ionic liquid available for dissolution and saccharification of the biomasses. The performance of [EMIM][HSO_4] saccharification can be enhanced by increasing the numbers of its component ions by increasing the ionic liquid-biomass ratio. The reducing sugar yields of H_2SO_4 saccharification corresponded to the biomass total carbohydrate content, which are in descending order of sago *hampas* (71.3%, w/w) > sugarcane bagasse (68.3%, w/w) > rice husk (42.3%, w/w) under their respective optimal heating durations. This observation supports the non-specific hydrolytic action of H_2SO_4 in biomass saccharification. On the other hand, the reducing sugar yields of [EMIM][HSO_4] saccharification in sugarcane bagasse was the highest at 37.9% (w/w), followed by sago *hampas* (7.0%, w/w) and rice husk (1.1%, w/w). This shows that the ionic liquid exhibited specificity in dissolution and hydrolysis of cellulosic biomass. The specificity of an ionic liquid in dissolution of cellulosic biomass is attributed to the specific properties of its anion and/or cation (MacFarlane *et al.* 2006). The concomitant reactions of cellulose dissolution and saccharification were initiated by the anions, such as the hydrogen sulphate ion in [EMIM][HSO_4]. The preference of [EMIM][HSO_4] in dissolving and saccharifying less recalcitrant cellulosic biomass was similar to another ionic liquid, 1-butyl-3-methylimidazolium hydrogen sulphate ([BMIM][HSO_4]), which has the same anion (Grasvik *et al.* 2014).

Chemical and Structural Analyses

The residues from [EMIM][HSO_4] and H_2SO_4 saccharifications were further investigated by means of FT-IR spectroscopy, to detect changes in chemical composition, and by FIB-SEM to examine changes in surface structure attributed by the reagents. Figure 2 shows the FT-IR spectra of saccharification residues and their respective untreated biomasses. When compared to the untreated biomasses, all the residues of [EMIM][HSO_4] and H_2SO_4 saccharifications showed insignificant changes in the spectra, except for the rice husk residue of H_2SO_4 saccharification. It is postulated that [EMIM][HSO_4] saccharifies the less recalcitrant biomasses such as sugarcane bagasse and sago *hampas* with similar mechanisms as in H_2SO_4 saccharification. The findings complimented the rate constant *b* in the kinetic modelling of [EMIM][HSO_4] saccharification. The spectrum of rice husk residue from H_2SO_4 saccharification had obvious composition enrichment in cellulosic/hemicellulosic materials and lignin contents, which was illustrated by the intensification of the band in the region of approximately 1035 cm^{-1}, which reflects C-O stretching in biomass (Fig. 2c). The observation was in agreement with the inorganic acid-enriched cellulose/hemicellulose contents in rice husk by leaching silica from the rice husk (Feng *et al.* 2004).

All the residues from [EMIM][HSO_4] saccharification were comparably more amorphous than their untreated biomasses and residues from H_2SO_4 saccharification. The increase in amorphous character can be observed from the band broadening of the spectra in the region between 800 and 950 cm^{-1}. These findings are supported by the FIB-SEM micrographs, which show more porous structures of the residues (Fig. 3).

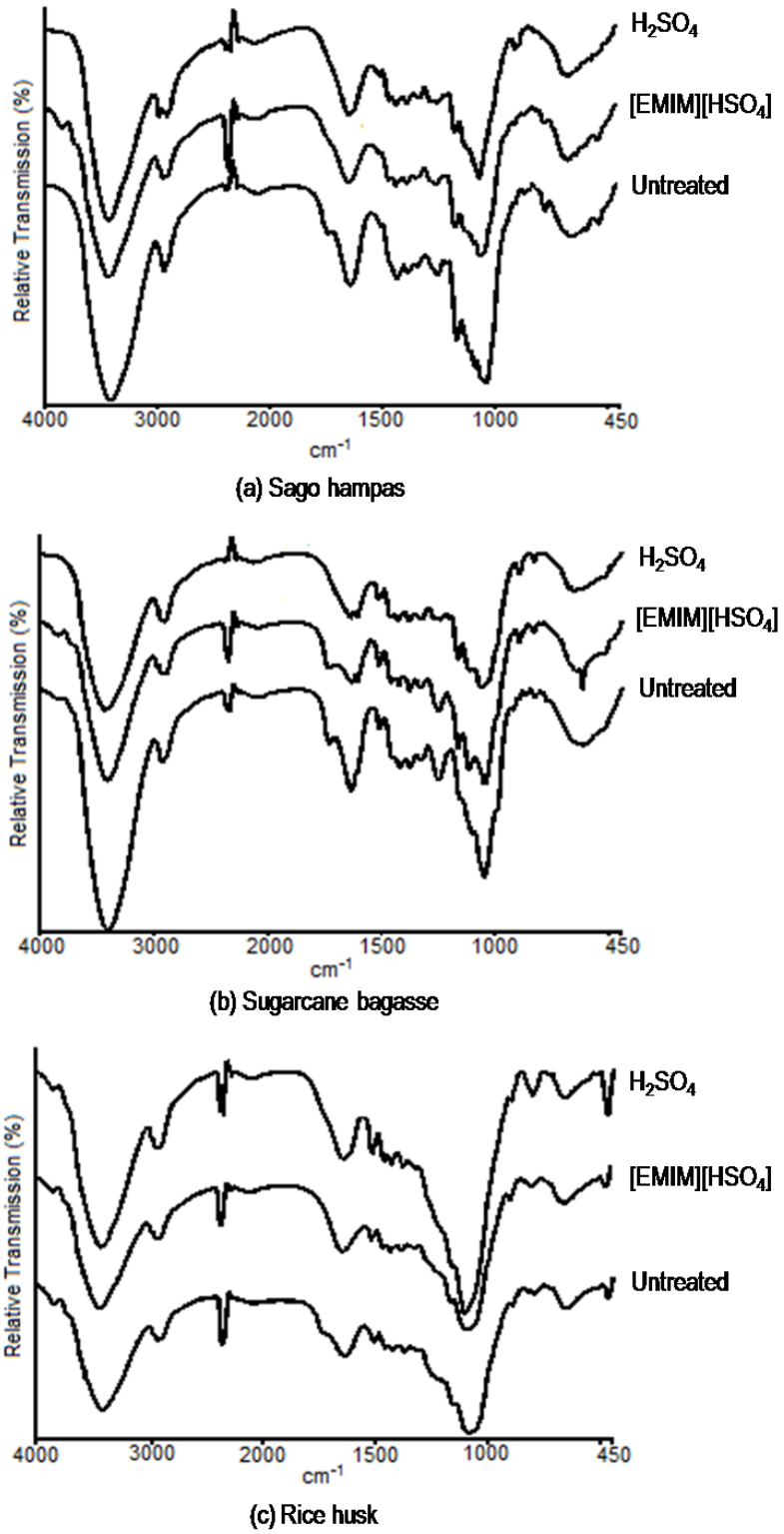

Fig. 2. FT-IR spectra of residues from H_2SO_4 and [EMIM][HSO_4] saccharification compared with untreated biomass

Even though the action of the Brönsted acidic [EMIM][HSO_4] is similar to that of H_2SO_4, the ionic liquid saccharified biomass in an aqueous-free phase. In H_2SO_4 saccharification, water moistens the biomasses and makes the ionised protons available for the hydrolysis of cellulose and hemicellulose (Xiang *et al*. 2003). Conversely, it is difficult for the viscous ionic liquid (with a relative density of 1.367 g cm^{-3}) and the large anion molecule to penetrate and hydrolyse the biomasses in aqueous-free conditions. Therefore, biomass needs to be dissolved in ionic liquid before it can be saccharified to reducing sugars. Ionic liquid dissolution of biomass caused severe structure disruption and formation of micropores throughout the residual surface (Ang *et al*. 2012). Comparing the FIB-SEM micrographs of residues from [EMIM][HSO_4] and H_2SO_4 saccharifications, residues of [EMIM][HSO_4] saccharification exhibited a higher degree of surface shrinkage and micropores formation when compared with the residues from H_2SO_4 saccharification.

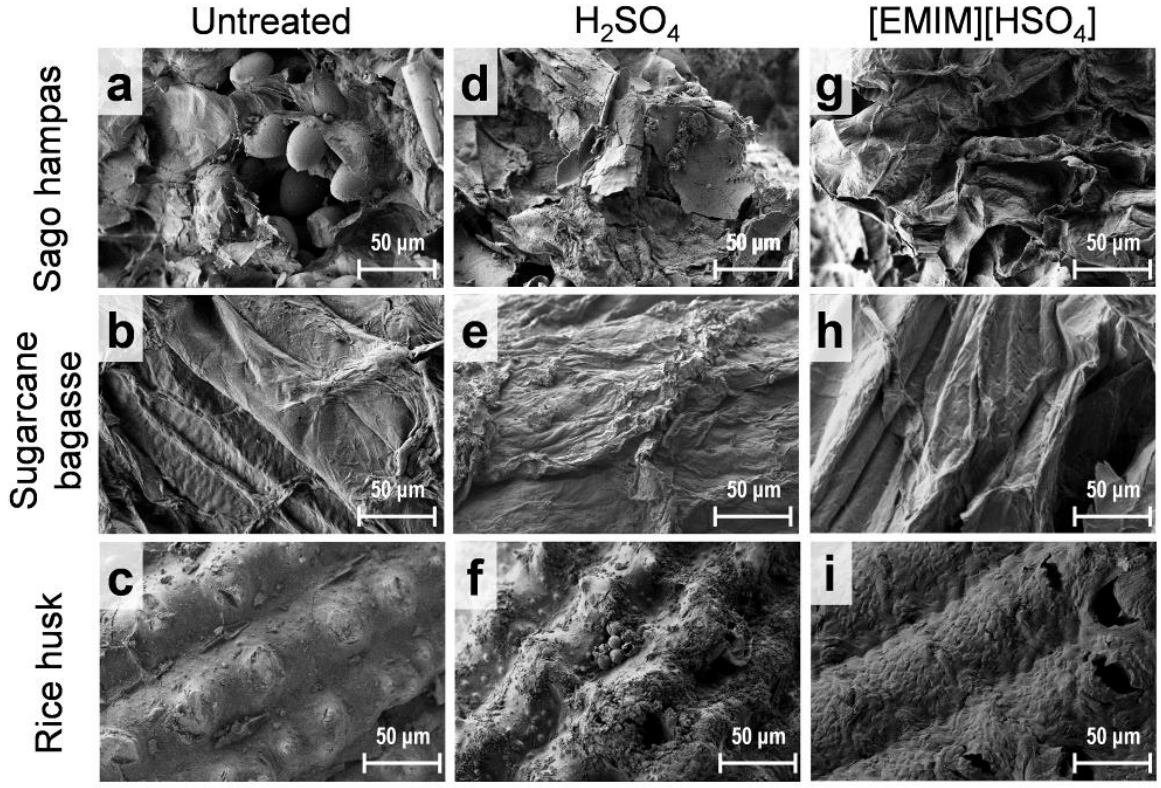

Fig. 3. FIB-SEM micrographs of untreated biomasses and their residues after saccharification using H_2SO_4 and [EMIM][HSO_4]

CONCLUSIONS

1. Both [EMIM][HSO_4] and H_2SO_4 saccharifications demonstrated similar kinetics for all three cellulosic biomasses. In addition, [EMIM][HSO_4] significantly increased their amorphous character with enhanced surface structure, and it showed preference in saccharifying less recalcitrant biomasses such as sugarcane bagasse and sago *hampas* compared to rice husk. This signifies the potential of the ionic liquid to be used interchangeably with H_2SO_4 in saccharifying cellulosic biomasses.

2. Although [EMIM][HSO_4] saccharification had lower reducing sugar yields, its kinetics suggested that the saccharification efficiency could be improved by increasing the ionic liquid-biomass ratio.
3. The application of molten [EMIM][HSO_4] in cellulosic biomass saccharification is feasible.

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REFERENCES CITED

Amarasekara, A. S., and Owereh, O. S. (2009). "Hydrolysis and decomposition of cellullose in Bronsted acidic ionic liquids under mild conditions," *Industrial and Engineering Chemistry Research* 48(22), 10152-10155. DOI:10.1021/ie901047u

Ang, T. N., Yoon, L. W., Lee, K. M., Ngoh, G. C., Chua, A. S. M., and Lee, M. G. (2011). "Efficiency of ionic liquids in the dissolution of rice husk," *BioResources* 6(4), 4790-4800. DOI:10.15376/biores.6.4.4790-4800

Ang, T. N., Ngoh, G. C., Chua, A. S. M., and Lee, M. G. (2012). "Elucidation of the effect of ionic liquid pretreatment on rice husk via structural analyses," *Biotechnology for Biofuels* 5, 67. DOI:10.1186/1754-6834-5-67

Bhandari, N., Macdonald, D. G., and Bakhshi, N. N. (1984). "Kinetic studies of corn stover saccharification using sulphuric acid," *Biotechnology and Bioengineering* 26(4), 320-327. DOI:10.1002/bit.260260405

Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., and Saddler, J. N. (2007). "Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics," *Advances in Biochemical Engineering/Biotechnology* 5(5), 904-910. DOI:10.1007/10_2007_064

Chang, V. S., Nagwani, M., and Holtzapple, M. T. (1998). "Lime pretreatment of crop residue bagasse and wheat straw," *Applied Biochemistry and Biotechnology* 74(3), 135-159. DOI:10.1007/BF02825962

Feng, Q., Yamamichi, H., Shoya, M., and Sugita, S. (2004). "Study on the pozzolanic properties of rice husk ash by hydrochloric acid pretreatment," *Cement and Concrete Research* 34(3), 521-526. DOI:10.1016/j.cemconres.2003.09.005

Ghose, T. K. (1987). "Measurement of cellulase activities," *Pure and Applied Chemistry* 59(2), 257-268. DOI:10.1351/pac198759020257

Grasvik, J., Winestrand, S., Normark, M., Jonsson, L. J., and Mikkola, J. P. (2014). "Evaluation of four ionic liquids for pretreatment of lignocellulosic biomass," *BMC Biotechnology* 14, 34. DOI:10.1186/1472-6750-14-34

Lappalainen, K., Karkkainen, J., and Lajunen, M. (2013). "Dissolution and depolymerization of barley starch in selected ionic liquids," *Carbohydrate Polymers*

93, 89-94. DOI:10.1016/j.carbpol.2012.04.011

Lee, K. M., Ngoh, G. C., Chua, A. S. M., Yoon, L. W., Ang, T. N., and Lee, M. G. (2014). "Comparison study of different ionic liquid pretreatments in maximizing total reducing sugars recovery," *BioResources* 9(1), 1552-1564. DOI: 10.15376/biores.9.1.1552-1564

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H. V., Auer, M., Vogel, K. P., Simmons, B. A., and Singh, S. (2010). "Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification," *Bioresource Technology* 101(13), 4900-4906. DOI:10.1016/j.biotech.2009.10.066

MacFarlane, D. R., Pringle, J. M., Johansson, K. M., Forsyth, S. A., and Forsyth, M. (2006). "Lewis base ionic liquids," *Chemical Communications* 37(18), 1905-1917. DOI:10.1039/B516961P

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry* 31(3), 426-428. DOI:10.102/ac60147a030

Moe, S. T., Janga, K. K., Hertzberg, T., Hagg, M. B., Oyaas, K., and Dyrset, N. (2012). "Saccharification of lignocellulosic biomass for biofuel and biorefinery applications - A renaissance for the concentrated acid hydrolysis?" *Energy Procedia* 20, 50-58. DOI:10.1016/j.egypro.2012.03.007

Pezoa, R., Cortinez, V., Hyvarinen, S., Reunanen, M., Hemming, J., Lienqueo, M. E., Salazar, O., Carmona, R., Garcia, A., Murzin, D. Y., Mikkola, J. P. (2010). "The use of ionic liquids in the pretreatment of forest and agricultural residues for the production of bioethanol," *Cellulose Chemistry and Technology* 44(4-6), 165-172.

Shafiei, M., Karimi, K., and Taherzadeh, M. J. (2010). "Pretreatment of spruce and oak by *N*-methylmorpholine-*N*-oxide (NMMO) for efficient conversion of their cellulose to ethanol," *Bioresource Technology* 101(13), 4914-4918. DOI:10.1016/j.biotech.2009.08.100

Thompson, J. R. (2011). *Empirical Model Building: Data, Models, and Reality*, John Wiley and Sons, Hoboken, NJ. ISBN:978-0-470-46703-9

Wang, G., Zhang, S., Xu, W., Qi, W., Yan, Y., and Xu, Q. (2015). "Efficient saccharification by pretreatment of bagasse pith with ionic liquid and acid solutions simultaneously," *Energy Conversion and Management* 89, 120-126. DOI:10.1016/j.enconman.2014.09.029

Xiang, Q., Lee, Y.Y., Pettersson, P. O., and Torget, R. W. (2003). "Heterogeneous aspects of acid hydrolysis of α-cellulose," *Applied Biochemistry and Biotechnology* 105-108, 505-514. DOI: 10.1385/ABAB:107:1-3:505

Yoon, L. W., Ngoh, G. C., May Chua, A. S., and Hashim, M. A. (2011). "Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification," *Journal of Chemical Technology and Biotechnology* 86(10), 1342-1348. DOI:10.1002/jctb.2651

Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Ding, Y., and Wu, G. (2006). "Dissolution of cellulose with ionic liquids and its application: A mini review," *Green Chemistry* 8(4), 325-327. DOI:10.1039/B601395C

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