

## Comparison Study of Different Ionic Liquid Pretreatments in Maximizing Total Reducing Sugars Recovery

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A process scheme combining the most suitable ionic liquid pretreatment, followed by solid acid and enzymatic saccharification was used to maximize the reducing sugars recovery from sago waste. Three types of ionic liquids, *i.e.* 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) and 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>]) were evaluated based on their performance in terms of the total reducing sugars recovery, chemical cost, and pretreatment energy requirement. The results showed that all the ionic liquids assisted the saccharification processes by dissolving and depolymerizing the carbohydrates of the sago waste into shorter chain soluble oligosaccharides, as well as disrupting the biomass structure to produce an amorphous pretreated solid residue. The solid acid saccharifications of the prehydrolysates obtained from the [BMIM]Cl pretreatment gave the highest reducing sugars recovery (61-63%) irrespective of the solid acid catalyst employed. On the other hand, enzymatic saccharification of [EMIM][OAc] pretreated solid residues showed the highest reducing sugars recovery (29%). A maximum recovery of 90% reducing sugars was achieved via incorporation of the ionic liquid pretreatment, solid acid and enzymatic saccharifications using [BMIM]Cl, Amberlyst 15 (A15) and *Trichoderma viride* cellulase respectively. This study suggests that the combined sequential process can maximize the reducing sugars recovery from sago waste effectively.

*Keywords:* Sago waste; Ionic liquid pretreatment; Solid acid saccharification; Enzymatic saccharification; Reducing sugars

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### INTRODUCTION

Lignocellulosic biomass is generated in large quantities, especially in countries that are predominantly agricultural. One of the examples of the biomass is sago waste, which is the cellulosic fibrous residue from starch extraction of the sago palm (*Metroxylon sago*). It is a potential biomass for the production of sugars attributing to its high starch content (Chew and Shim 1993) and other carbohydrates such as cellulose and hemicellulose. The sugars produced from these carbohydrates can be further processed into a variety of products including biofuels, single cell protein, antibiotics, and acetone (Abd-Aziz 2002).

Lignocellulosic biomass yields sugars via saccharification. The conventional techniques involved are enzymatic and acid saccharification. In the former process,

enzymes are used as biocatalysts to depolymerize the biomass into sugars. Since enzyme is specific in its action, enzymatic saccharification of biomass does not degrade sugar products (Zhang *et al.* 2006). However, low enzymatic saccharification (Brandberg *et al.* 2004) and incomplete conversion of the biomass (Rinaldi *et al.* 2010) were observed due to the crystalline structure of the biomass. Therefore, physical or chemical pretreatment is required to disrupt the biomass structure prior to enzymatic saccharification for high sugars yield (Galbe and Zacchi 2012).

In acid saccharification, biomass is dissolved and hydrolyzed in mineral acids. The process is usually conducted at an elevated temperature and pressure in order to achieve a satisfactory yield of sugars (Li *et al.* 2008). The severe saccharification conditions unfavorably degrade the sugars to undesirable products such as 5-hydroxymethyl-2-furfural (HMF) and furfural (Kahar *et al.* 2010; Larsson *et al.* 1999; Sreenath and Jeffries 2000; Taherzadeh *et al.* 1999). Acid saccharification also leads to an acid waste disposal problem (Dadi *et al.* 2006; Li and Zhao 2007; Zhang and Zhao 2009) and requires expensive anti-corrosion equipment.

In recent years, solid acid catalyst has been used in saccharification (Kim *et al.* 2005; Lee *et al.* 2013; Yamaguchi *et al.* 2009) to avoid the shortcomings of the conventional saccharification techniques. The action of a solid acid catalyst in saccharification resembles that of a mineral acid, except it is in the solid phase (Hahn-Hägerdal *et al.* 1984), which can be easily recovered from the reaction mixture and reused. However, saccharification of the biomass using solid acid catalysts results in mass transfer limitations between the solid phase of biomass and the catalyst. Therefore, dissolution of the biomass prior to solid acid saccharification is essential. For this purpose, an ionic liquid capable of dissolving carbohydrates can be used (Fort *et al.* 2007; Kilpeläinen *et al.* 2007; Sun *et al.* 2009; Zavrel *et al.* 2009). The dissolved carbohydrate in ionic liquid can be further converted to sugars by solid acid saccharification. Meanwhile, the solid residue of the biomass obtained from ionic liquid pretreatment is more amorphous compared to the untreated biomass and thus would facilitate the enzymatic saccharification to produce high amount of sugars from the biomass (Dadi *et al.* 2006; Li *et al.* 2009; Zhao *et al.* 2009).

In view of the dissolution and structural disruption properties of the ionic liquid, the main objective of the present work is to capitalize the dual-function ionic liquid to maximize the recovery of reducing sugars from sago waste. A process scheme combining ionic liquid pretreatment, solid acid saccharification, and enzymatic saccharification was applied. The effects of different ionic liquids on sugars recovery in the prehydrolysates (liquid products of ionic liquid pretreatment) and pretreated solid residues (solid products of ionic liquid pretreatment) were respectively investigated by applying solid acid and enzymatic saccharification. A most suitable ionic liquid was then selected in terms of total reducing sugars recovery, pretreatment energy, and chemical cost.

## EXPERIMENTAL

### Materials

Sago waste was ground and sieved to a particle size between 250  $\mu\text{m}$  to 500  $\mu\text{m}$ . The ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and *Trichoderma viride* cellulase with an activity of 1 U/mg were purchased from Merck (Germany). The chemicals 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), 1-ethyl-3-methylimida-

zolium diethyl phosphate ([EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>]), the solid acid catalysts Amberlyst 15 (A15), Amberlite IR120, and Nafion NR50 were purchased from Sigma-Aldrich (USA). All other chemicals were obtained from commercial sources and are of analytical grade.

### Characterization of Sago Waste

Starch content in sago waste was determined according to the procedure described by Megazyme in Total Starch Assay Procedure K-TSTA 07/11. Cellulose, hemicellulose, and lignin contents were characterized based on the procedure in the National Renewable Energy Laboratory (NREL) Technical Report NREL/TP-510-42618 (Sluiter *et al.* 2011).

#### *Determination of starch content of sago waste*

A test tube containing 100 mg of sago waste was added with 0.2 mL of ethanol (80%, v/v). The sample was stirred on a vortex mixer to aid substrate dispersion. 2 mL of 2 M potassium hydroxide was added to the test tube and placed in an ice bath over a magnetic stirrer for approximately 20 min, after which 8 mL of sodium acetate buffer (1.2 M, pH 3.8), 0.1 mL of thermostable  $\alpha$ -amylase, and 0.1 mL of amyloglucosidase were added to the reaction mixture and incubated for 30 min at 50 °C with intermittent mixing. The content of the test tube was diluted with ultrapure water (Arium<sup>®</sup> 611UF, Sartorius, Germany) to a total volume of 100 mL before it was centrifuged (Centrifuge 5810R, Eppendorf, Germany). The composition of starch was determined from the clear aliquot using glucose oxidase/peroxidase (GOPOD) reagent. The starch-free solid residue was collected via filtering the remaining of the solution and it was washed and dried prior to analyses for cellulose, hemicellulose and lignin content.

#### *Determination of cellulose, hemicellulose, and lignin content of sago waste*

Starch-free solid residue of 300 mg was hydrolyzed with 3 mL of 72% sulfuric acid in a water bath at 30 °C for 1 h. The reaction mixture was diluted by 84 mL of ultrapure water and autoclaved at 121 °C for 1 h. After that, it was cooled to room temperature before being subject to vacuum filtration through a filtering crucible. The liquid sample was collected to determine the cellulose and hemicelluloses content; while the solid residue remaining after filtration was washed several times with ultrapure water prior to lignin determination.

### Ionic Liquid Pretreatment of Sago Waste

A solution of 4% (w/v) sago waste was prepared by adding 1 mL of ionic liquid into a test tube that contained 40 mg of sago waste. The mixture in the test tube was heated at 100°C in an oil bath (MC, Julabo, Germany) for 3 h and samples were taken periodically. The sample was mixed with 5 mL of ultrapure water and centrifuged to separate the liquid portion (hereafter called prehydrolysate) from the pretreated sago waste solid residue. A portion of the prehydrolysate (1.5 mL) was analyzed for its reducing sugars by 3,5-dinitrosalicylic acid (DNS) assay (Miller 1959), and the remaining prehydrolysate was subjected to solid acid saccharification. The pretreated solid residue was washed with ultrapure water and dried prior to enzymatic saccharification and Fourier transform infrared spectroscopy (FT-IR) analysis. The experiments were conducted with ultrapure water as control, and all the experiments were performed in triplicate.

### Solid Acid Saccharification of the Prehydrolysate

The prehydrolysate obtained from the ionic liquid pretreatment was saccharified with 10% (w/v) solid acid catalyst in the oil bath at 120 °C for 1.5 h. The solid acid catalysts under investigation were A15, Amberlite IR120, and Nafion NR50. Once the solid acid catalyst in the reacted sample was separated, the hydrolysate (liquid product from solid acid saccharification) was taken to determine its reducing sugars using DNS assay.

### Enzymatic Saccharification of the Pretreated Solid Residue

The pretreated solid residue was buffered with 1 mL of 50 mM, pH 4.8 acetate acid-sodium acetate buffer solution, followed by the addition of *Trichoderma viride* cellulase at a concentration of 30-50 FPU/g substrate for enzymatic saccharification. The reaction was carried out in a water bath at 50 °C for 48 h. The reacted mixture was centrifuged and the supernatant (liquid product from enzymatic saccharification) collected was analyzed for its reducing sugars content using DNS assay.

### Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

FTIR spectrophotometry (Spectrum RX1, Perkin Elmer, USA) was used to examine the crystallinity of the pretreated sago waste solid residues and the untreated sago waste. The spectra were measured with a resolution of 1 cm<sup>-1</sup> over the range between 400 and 4000 cm<sup>-1</sup>. The crystallinity of the biomass was determined by measuring its two infrared ratios using Equation (1) (Nelson and O'Connor 1964):

$$\text{Total crystallinity index (TCI)} = \alpha_{1378} / \alpha_{2900} \quad (1)$$

The higher TCI value indicates that the biomass has a higher crystallinity and ordered structured.

### Computation of Reducing Sugars Recovery

The total reducing sugars recovery of the process scheme was the sum of reducing sugars recovery from the hydrolysate and supernatant. Reducing sugars recovery from the hydrolysate refers to the percentage of reducing sugars that can be recovered from the carbohydrates in sago waste after having the prehydrolysate saccharified with solid acid catalyst. It is calculated using Eq. 2,

$$\text{Reducing sugars recovery from the hydrolysate (\%)} = \frac{w_{rs,h}}{(1.11(w_s+w_c)+1.14w_h)} \times 100 \quad (2)$$

where  $w_{rs,h}$  represents the weight of reducing sugars in the hydrolysate,  $w_s$ ,  $w_c$ , and  $w_h$  represent the weight of starch, cellulose, and hemicellulose in sago waste, respectively. The values 1.11 and 1.14 are the multiplication factors that convert carbohydrates to equivalent sugars (Sluiter *et al.* 2011). While, reducing sugars recovery from the supernatant is the percentage of reducing sugars that can be recovered from the carbohydrates in sago waste when the pretreated sago waste solid residue is subjected to enzymatic saccharification. Equation 3 was applied to compute the reducing sugars recovery from the supernatant.

Reducing sugars recovery from supernatant (%) =

$$w_{rs,s} / (1.11(w_s+w_c)+1.14w_h) \times 100 \quad (3)$$

where  $w_{rs,s}$  represents the weight of reducing sugars in the supernatant.

### Computation of Energy Requirement for Ionic Liquid Pretreatment

The energy required for pretreatment was determined according to the first law of thermodynamics as shown in Eq. 4,

$$Q = \Delta U = m \int_{T_i}^{T_f} C_p \, dT \quad (4)$$

where  $Q$  is the heat transferred to the system,  $\Delta U$  is the change in the internal energy,  $m$  is the mass of the ionic liquid,  $C_p$  is the specific heat capacity of the ionic liquid, and  $T_i$  and  $T_f$  are respectively the initial and final absolute temperatures. Molar heat capacities of the ionic liquids are the sum of their cations and anions molar heat capacities that can be determined by using Eq. 5 (Soriano *et al.* 2010).

$$C_{p, ion} \text{ (J mol}^{-1} \text{ K}^{-1}\text{)} = A + BT + CT^2 \quad (5)$$

The symbols  $A$ ,  $B$ , and  $C$  represent the empirical parameters, and  $T$  is the absolute temperature.

## RESULTS AND DISCUSSION

### Composition of Sago Waste

The chemical composition of sago waste obtained from the characterization study was compared with results from analysis of other types of lignocellulosic biomass that are usually used in sugars production, as shown in Table 1. Different biomass has varying starch, cellulose, hemicellulose, and lignin content. On average, 50 to 70% dry weight of the biomass shown is comprised of carbohydrates (*i.e.* starch, cellulose, and hemicellulose). However, sago waste used in this study was found to contain as much as 90% of carbohydrates, which indicates that it is a favorable substrate for sugars production.

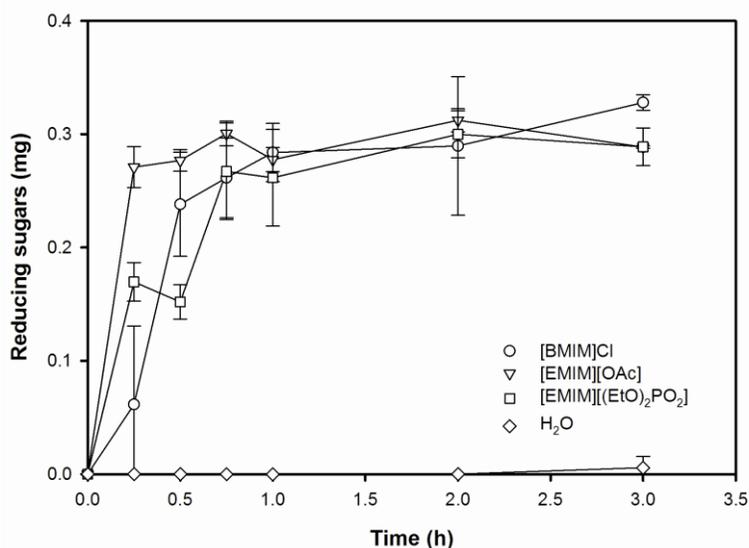
**Table 1.** Chemical Composition of Various Lignocellulosic Biomass

Lignocellulosic Biomass	Starch	Cellulose	Hemicellulose	Lignin	Reference
Sago waste	36.5 ± 1.2	40.0 ± 2.2	13.1 ± 0.1	4.5 ± 0.1	This study
Corn stover	-	34.5	27.7	17.8	Kumar <i>et al.</i> (2011)
Rice husk	-	53.2	4.6	19.7	Ang <i>et al.</i> (2011)
Sugarcane bagasse	-	41.6	25.1	20.3	Kim and Day (2011)
Switchgrass	-	38.9	28.9	31.7	Kumar <i>et al.</i> (2011)
Wheat straw	-	35.0	22.3	15.6	Petersen <i>et al.</i> (2009)

## Ionic Liquid Pretreatment

[BMIM]Cl, [EMIM][OAc], and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] ionic liquids were used to dissolve the sago waste in this study. One of the factors that contributes to the choice of an ionic liquid is the good dissolution capability of the ionic liquid which can be predicted by the Kamlet-Taft beta ( $\beta$ ) parameter. [BMIM]Cl and [EMIM][OAc] have a high  $\beta$  value of 0.84 (Fukaya *et al.* 2006) and  $\sim 1.1$  (Doherty *et al.* 2010), respectively, which indicates good biomass dissolution. As for [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>], it has a low viscosity that is expected to impart minimal mass and phase transfer limitations, and thus lead to better dissolution performance (Li *et al.* 2009; Mora-Pale *et al.* 2011). Therefore, [BMIM]Cl, [EMIM][OAc], and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] ionic liquids were used to dissolve the sago waste in this study.

When ionic liquid was applied to sago waste at a temperature of 100 °C, a series of reactions were initiated. First, the carbohydrates in sago waste were dissolved in the ionic liquid via the breaking down of the extensive network of hydrogen bonds in the carbohydrate (Feng and Chen 2008). The dissolved carbohydrate subsequently was depolymerized to oligosaccharides via hydrolysis of glycosidic bonds that linked the sugar monomers. These oligosaccharides can be further saccharified to sugars monomers, which is confirmed by the presence of reducing sugars in all the prehydrolysates obtained from the ionic liquid pretreatment of sago waste, as illustrated in Fig. 1.

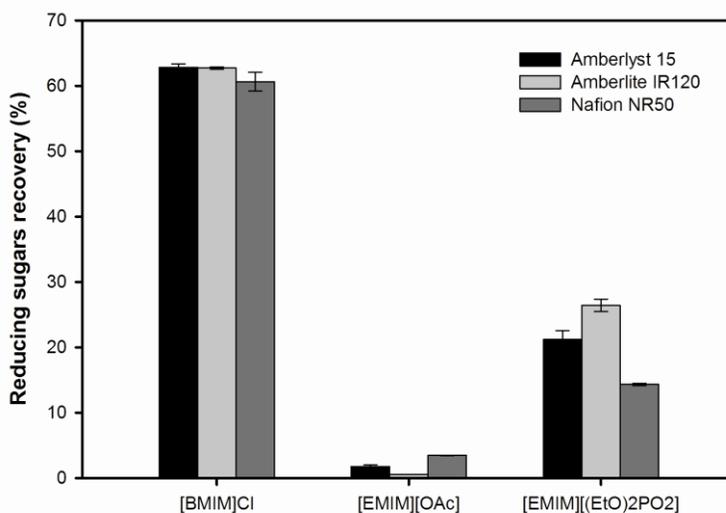


**Fig. 1.** Amount of reducing sugars in prehydrolysates after ionic liquid pretreatments of sago waste.

The figure shows that the reducing sugars content for all the prehydrolysates increased gradually until reaching a plateau after 1 h of pretreatment. The amounts of reducing sugars produced by all the ionic liquid pretreatments were very low as in the range of 0.06 to 0.33 mg. This was as expected because ionic liquid pretreatment is aimed to dissolve the biomass rather than to produce sugars. In spite of that, the presence of sugars in the prehydrolysates indicated the depolymerization capability of the ionic liquids, as they could break down the carbohydrates polymers into its smaller constituents of sugars monomers.

## Solid Acid Saccharification of the Prehydrolysate

Having the prehydrolysates saccharified with solid acid catalyst, the amount of reducing sugars produced was greatly improved in comparison with their respective prehydrolysates, as shown in Fig. 2. This is because, solid acid catalyst provided an acidic environment by releasing its protons through ion exchange at the catalyst sulfonic groups ( $\text{SO}_3\text{H}$ ) with alkylimidazolium ions of the ionic liquid (Dwiatmoko *et al.* 2010), which has favored the saccharification process. The relatively high reducing sugars yield obtained from the solid acid saccharification signifies the formation of oligosaccharides during the ionic liquid pretreatment of the sago waste. Only when oligosaccharides were present in the prehydrolysates was the subsequent solid acid saccharification able to produce a higher amount of reducing sugars.



**Fig. 2.** Reducing sugars recovery from solid acid saccharification of ionic liquid pretreated sago waste.

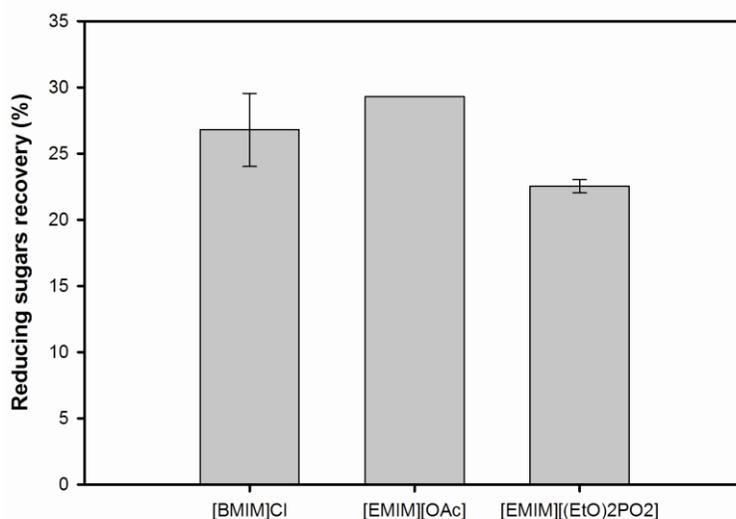
It can be seen from Fig. 2 that the reducing sugars recovery in the [BMIM]Cl hydrolysate was at least three times higher than that of [EMIM]((EtO)<sub>2</sub>PO<sub>2</sub>) and [EMIM][OAc], regardless of the type of solid acid catalyst used in the saccharification. The vast differences in reducing sugars produced might be attributed to the degree of compatibility between the ionic liquid and the solid acid catalyst employed. Unlike [BMIM]Cl, both [EMIM][OAc] and [EMIM]((EtO)<sub>2</sub>PO<sub>2</sub>) contain weakly basic anions of acetate and phosphonic. Both the acetate and phosphonic anions are easily protonated to acetic acid and phosphonic acid, respectively by the solid acid catalyst (Rinaldi *et al.* 2010). Protonation of these basic anions considerably reduces the amount of protons available for saccharification. Therefore, less reducing sugars was detected in [EMIM][OAc] and [EMIM]((EtO)<sub>2</sub>PO<sub>2</sub>) hydrolysates as compared to that in [BMIM]Cl hydrolysate.

It can be also observed from Fig. 2 that [BMIM]Cl hydrolysates obtained from various solid acid saccharifications gave rise to almost the same reducing sugars recovery. Despite the similar saccharification efficiencies of the solid acid catalysts, they are different in term of physical properties and price. Amberlite IR120 is the most susceptible to physical attributions such as continuous wetting and drying due to its gel structure (Kunin *et al.* 1962).

On the other hand, A15 and Nafion NR50 are physically more stable and can withstand prolonged heating up to 120-140 °C and 280 °C, respectively (Harmer and Sun 2001). Although Nafion NR50 can withstand prolonged heating at a higher temperature, it is much more expensive compared to A15. In view of the above, A15 was chosen as the solid acid catalyst for subsequent work to maximize the recovery of reducing sugars from the ionic liquid pretreated sago waste.

### Enzymatic Saccharification of the Pretreated Solid Residue

The undissolved carbohydrates in the ionic liquid pretreated solid residues were saccharified enzymatically to recover reducing sugars. Enzymatic saccharification was applied instead of solid acid saccharification to overcome the mass transfer limitation induced by solid-solid contact. As shown in Fig. 3, enzymatic saccharification of all the ionic liquid pretreated solid residues yielded 20 to 30% of reducing sugars. The highest reducing sugars yield was obtained via enzymatic saccharification of [EMIM][OAc] pretreated solid residue, followed by [BMIM]Cl and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] pretreated solid residues. However, the differences in yield were insignificant especially between the [EMIM][OAc] and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] pretreated solid residues.



**Fig. 3.** Reducing sugars recovery from enzymatic saccharification of ionic liquid pretreated sago waste.

The crystallinity indices of the biomass after the pretreatments were determined as presented in Table 2. Compared to the untreated sago waste, the crystallinities of all the pretreated solid residues were reduced. Among the pretreated solid residues, [EMIM][OAc] had the highest total crystallinity index. Again, the differences in crystallinity index among the pretreated solid residues were insignificant, which explains well the insignificant difference in reducing sugars recoveries between the pretreated solid residues.

**Table 2.** Total Crystallinity Index (TCI) and Lignin Content of the Untreated Sago Waste and Pretreated Solid Residues

Sago Waste Sample	Total Crystallinity Index (TCI)	Lignin content * (mg)
Untreated sago waste	1.06	13.5 ± 0.4
[BMIM]Cl pretreated solid residues	0.99	12.0 ± 1.5
[EMIM][OAc] pretreated solid residues	1.02	9.0 ± 0.6
[EMIM][(EtO) <sub>2</sub> PO <sub>2</sub> ] pretreated solid residues	0.98	9.9 ± 0.9

\* The lignin contents in all the samples were measured for 300 mg of sago waste samples and reported in absolute values for comparison purpose.

The other factor that can affect reducing sugars recovery in enzymatic saccharification of pretreated solid residues is the degree of delignification of the biomass after the pretreatments. Lee *et al.* (2009) and Wu *et al.* (2011) reported that effective delignification of the biomass is able to improve the sugars yield. Table 2 presents the amount of lignin in all the ionic liquid pretreated solid residues. The lowest lignin content in the residue after [EMIM][OAc] pretreatment is consistent with the finding of the highest yield of reducing sugars in the sample. Delignification is important, as irreversible adsorption of enzyme on the biomass was prevented (Ooshima *et al.* 1986; Zhu *et al.* 2006). The absorption of enzyme onto lignin lowers the saccharification rate and hinder recycling of enzyme, leading to a lower reducing sugars yield.

As mentioned earlier, reducing sugars recoveries of all the enzymatic saccharified pretreated solid residues were in the range of 20 to 30%. The amount of reducing sugars produced from enzymatic saccharification significantly affect the total reducing sugars production especially in process scheme with [EMIM][OAc] and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] pretreatments. As previously discussed, in the case of the less compatible [EMIM][OAc] and [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>] due to protonation of their anions in A15, solid acid saccharification of their prehydrolysates did not yield a high amount of reducing sugars. In these cases, most of the reducing sugars produced were contributed by the enzymatic saccharification of their pretreated solid residues. The findings suggest that the undissolved carbohydrates in the solid residue of the ionic liquid-pretreated sago waste can be enzymatic saccharified to maximize the reducing sugars recovery from sago waste.

### Process Scheme Evaluation for Maximum Total Reducing Sugars Recovery

In this integrated process, [BMIM]Cl-pretreated sago waste gave the highest reducing sugars yield of 90% after having both of its liquid and solid products saccharified with A15 solid acid catalyst and *Trichoderma viride* cellulase, respectively. The total reducing sugars recovery in the present work is higher compared to the other process schemes. For instance, an optimum yield of 62% was reported in acid saccharification of sago waste using 1.5 M sulfuric acid at 90 °C for 2 h (Kumoro *et al.* 2008). The authors also reported enzymatic saccharification of sago waste with 6 AGU mL<sup>-1</sup> glucoamylase that produced an optimum yield of 56% at 60 °C and 30 min.

The combined sequential pretreatment and saccharification process introduced in this study apparently has a better sugars recovery potential as ionic liquid pretreatment enables the complete utilization of biomass via its dual function role to depolymerize the sago waste and disrupt its structure. Besides providing the highest total reducing sugars recovery from sago waste, pretreatment using [BMIM]Cl is the most economical for

application because it has the cheapest cost among the ionic liquids and has the lowest energy demand during the pretreatment reaction. As shown in Table 3, [BMIM]Cl pretreatment requires the least amount of energy, followed by treatment with [EMIM][OAc] or [EMIM][(EtO)<sub>2</sub>PO<sub>2</sub>]. These collectively suggest the feasibility of applying [BMIM]Cl to dissolve and pretreat sago waste prior to solid acid and enzymatic saccharifications to maximize the recovery of reducing sugars.

**Table 3.** Energy Requirement for the Ionic Liquid Pretreatments

Ionic Liquid Pretreatment	Energy Required per g of Sago Waste (kJ)
[BMIM]Cl	3.04
[EMIM][OAc]	3.72
[EMIM][(EtO) <sub>2</sub> PO <sub>2</sub> ]	3.87

## CONCLUSIONS

1. The present study demonstrates that ionic liquids can have a dual function, *i.e.* biomass dissolution and structural disruption, and that this facilitates the maximum recovery of reducing sugars from sago waste.
2. A maximum recovery of 90% reducing sugars can be achieved by incorporating [BMIM]Cl pretreatment into A15 saccharification and *Trichoderma viride* cellulase saccharification.
3. [BMIM]Cl pretreatment is the most economically feasible in view of the lowest chemical cost and the least pretreatment energy.
4. The compatibility of the ionic liquid and the solid acid catalyst is essential to ensure the effectiveness for reducing sugars recovery from the hydrolysates.
5. The findings collectively suggest that the ionic liquid pretreatment facilitates an efficient conversion of lignocellulosic biomass to reducing sugars, where it could be further converted into valuable biofuels and other biochemical products.

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