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Pretreatment of lignocellulosic palm biomass using a solvent-ionic liquid [BMIM]Cl for glucose recovery: An optimisation study using response surface methodology

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ABSTRACT

The potential of oil palm frond (OPF) for fuel ethanol production was investigated in this current work. Pretreatment of lignocellulosic feedstock with the novel solvent ionic liquid (IL) BMIMCl was used to facilitate the conversion of OPF into fermentable sugar (glucose). The pretreatment was accomplished by first subjecting OPF to ionic liquid treatment, followed by regeneration of cellulose using an anti-solvent. Scanning Electron Microscopy showed a significant destruction of biomass structure after pretreatment with IL, which in turn reduced the crystallinity and improved the enzymatic digestibility of the biomass. The effects of several pretreatment variables, such as temperature, retention time and solid loading, were studied using Response Surface Methodology (RSM) based on a factorial Central Composite Design (CCD). These factors were further optimised using RSM. An optimum 100% glucose recovery was found with pretreatment conditions of 80 °C, a 15-min retention time and 10% solid loading.

1. Introduction

Recently, several research studies have found that ionic liquids (ILs) containing anions of chloride, formate, acetate or alkylphosphonate can dissolve cellulose by the formation of strong hydrogen bonds (Zhao et al., 2009). Ionic liquids are organic salts entirely composed of ions (cations and anions) with low melting temperatures. They are green solvents which are characterised by negligible vapour pressure as well as excellent thermal and chemical stability. The use of ILs as solvents has increased recently as a result of a globally growing interest towards green chemistry. Their unique properties make them an attractive alternative to conventional organic solvents in many chemical processes. Their use can provide a concrete solution to the formation of volatile organic carbons (VOCs) in conventional solvents, which are detrimental to the environment (Anderson, Ding, Welton, & Armstrong, 2002).

Ionic liquids, such as 1-allyl-3-methylimidazolium-chloride ([AMIM]Cl), 1-ethyl-3-methylimidazolium-acetate ([EMIM]Ac), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and 1-ethyl-3-methyl imidazolium diethyl phosphate ([EMIM]DEP), have gained increasing interest as media for dissolving cellulose from lignocellulosic biomass in the pretreatment step (Li et al., 2009; Zavrel, Bross, Funke, Büchs, & Spiess, 2009). The dissolved cellulose in IL can be easily regenerated with the addition of an anti-solvent, such as water, ethanol or acetone (Zhu et al., 2006). As reported in the literature, apart from extracting cellulose from the primitive resources, regenerated cellulose exhibits significantly reduced crystallinity and increased porosity, which enhance the digestibility of the material and subsequently result in a higher yield for the overall conversion process (Kuo & Lee, 2009; Li et al., 2009; Zhu et al., 2006).

In the current study, oil palm frond (OPF), a relatively new lignocellulosic biomass resource, was investigated for bioethanol production using ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) as a pretreatment medium. Among the palm oil biomasses produced from Malaysia's palm oil industry, OPF was chosen as the feedstock in this study mainly due to the abundance of OPF generated during the harvesting of fresh fruit bunches. Approximately 51 million tons of OPF was produced in 2008, accounting for 53% of the total palm biomass (Goh, Tan, Lee, & Bhatia, 2010; MPOB, 2009). Unlike other palm biomass, such as palm shells, palm fibres, palm kernels, palm trunks and empty fruit bunches, OPFs are still under-utilised and discarded on the plantation. Hence, OPF has great potential as a sustainable biomass resource for the production of biofuel in Malaysia. In this study, we evaluated the effects of temperature, retention time and solid loadings on the pretreatment

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efficiency. The data were analysed using Design-Expert software and the optimum conditions were determined to maximise glucose recovery from the biomass.

2. Experimental

2.1. Raw materials and chemicals

The oil palm fronds were collected from oil palm cultivation at the Engineering Campus of Universiti Sains Malaysia, Penang, Malaysia. The fronds were dried under the sun before being shredded into pieces. Subsequently, the shredded fronds were sieved to obtain fractions with a particle size less than 1 mm. The ionic liquid 1-butyl-3 methylimidazolium chloride ([BMIM]Cl) was purchased from Merck Sdn Bhd and used as received. Cellulase (Celluclast 1.5 l) and β-glucosidase (Novozym 188), the enzymes used for the hydrolysis of pretreated fronds, were purchased from Science Technics Sdn Bhd. The enzymatic activities of cellulase and β-glucosidase as received from the manufacture were determined to be 958 endoglucanase units (EGU) ml^{-1} and 326 cellobiase units (CBU) ml^{-1}, respectively. All chemicals needed for the characterisation of biomass such as ethanol, sulphuric acid, acetic acid, sodium chlorite and sodium hydroxide were purchased from Fisher Scientific.

2.2. Design of experiments

The effect of three independent variables (pretreatment temperature, retention time and solid loading) on the response (percent glucose recovery) was studied using a factorial Central Composite Design (CCD) of Response Surface Methodology (RSM), which is a collection of mathematical and statistical techniques for designing experiments, analysing the effects of variables, developing models and optimising the process variables for the optimum response (Ferreira, Duarte, Ribeiro, Queiroz, & Domingues, 2009). The experimental data were fit using a low-order polynomial equation to evaluate the effect of each independent variable to the response, which was later analysed to determine the optimum process conditions. In this study, a polynomial quadratic equation, as shown in Eq. (1), was employed:

$$y = \beta_o + \sum_{i=1}^{3} \beta_i \chi_i + \sum_{i=1}^{3} \beta_{ii} \chi_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ii} \chi_i \chi_j \quad (1)$$

where y is the response, x_i and x_j are independent variables, β_o is the constant coefficient, β_i is the ith linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the ijth interaction coefficient. For fitting second-order models, CCD is one of the most commonly used response surface designs (Ferreira et al., 2009). CCD consists of 2^k factorial points, $2k$ axial points ($\pm\alpha$), and 6 centre points, where k is the number of independent variables. Each of the variables were investigated at five coded levels (−2, −1, 0, 1, 2), as listed in Table 1, and the complete experimental design matrix for this study is shown in Table 2. A total of twenty experiments, including eight for factorial design, six for axial points and six repetitions at the central point, were performed.

Table 1
Levels of the pretreatment condition variables tested in the CCD.

Variable	Coding	Unit	Levels				
			−2	−1	0	1	2
Temperature	*A*	°C	60	68	80	92	100
Retention time	*B*	Min	15.0	24.1	37.5	50.9	60.0
Solid loading	*C*	% (w/w)	2.00	3.62	6.00	8.38	10.00

2.3. Statistical analysis

The CCD experimental results were analysed using Design-Expert software version 6.0.6, from STAT-EASE, Inc., Minneapolis, USA. Each coefficient in Eq. (1) was calculated and the possible interaction effects of the process variables on the response were obtained. Their significance was checked by variance analysis (ANOVA). Three-dimensional plots were drawn using the same program to illustrate the effects of independent variables on the response. Lastly, two additional replicates were conducted to verify the validity of the predicted optimum values by the program.

Table 2
Experimental design matrix of CCD and corresponding results (glucose recovery).

Run	Experimental variables			Glucose recovery (% g glucose/g cellulose)
	Temperature, *A* (°C)	Retention time, *B* (min)	Solid loading, *C* (%, w/w)	
1	68	24.1	3.62	36.09
2	92	24.1	3.62	57.93
3	68	50.9	3.62	47.79
4	92	50.9	3.62	44.00
5	68	24.1	8.38	40.10
6	92	24.1	8.38	100.00
7	68	50.9	8.38	43.46
8	92	50.9	8.38	75.45
9	60	37.5	6.00	25.12
10	100	37.5	6.00	38.56
11	80	15.0	6.00	63.64
12	80	60.0	6.00	50.81
13	80	37.5	2.00	47.85
14	80	37.5	10.00	96.01
15	80	37.5	6.00	33.68
16	80	37.5	6.00	50.33
17	80	37.5	6.00	43.05
18	80	37.5	6.00	45.15
19	80	37.5	6.00	45.64
20	80	37.5	6.00	42.26
Untreated OPF	–	–	–	23.50

2.4. Experimental procedures

2.4.1. Pretreatment

Solutions containing fronds with different solid loadings (2–10 wt%) were prepared by mixing the shredded fronds with [BMIM]Cl in 50-ml conical flasks. The pretreatment was carried out in a water bath shaker with an agitation rate of 170 rpm at various temperatures and for different periods of time. At the end of the pretreatment step, 10 ml of deionised water was added as an anti-solvent to the frond/IL solution to precipitate cellulose. Shaking was continued until the temperature cooled down to room temperature. The resulting mixture was briefly centrifuged, and the supernatant was removed. The precipitate (IL-treated biomass) was washed thoroughly with anti-solvent and subsequently centrifuged. The washing and centrifuging steps were repeated until a colorless supernatant was obtained. The IL-treated biomass was then collected and oven-dried for subsequent enzymatic hydrolysis. Arora et al. (2010) reported that colorless supernatant is the key observation to ensure complete removal of IL from biomass as in their study, IL was not detected in the colorless supernatant using Fourier Transform Infrared (FTIR) measurement.

2.4.2. Enzymatic hydrolysis

The enzymatic hydrolysis of IL-treated fronds was carried out in the presence of 50 mM sodium citrate buffer (pH 4.8). Enzymes were loaded in the amounts of 60 FPU/g substrate and 200 CBU/g substrate for Celluclast 1.5 l and Novozym 188, respectively. The reaction mixture (30 ml) was placed into a 50-ml conical flask and incubated at 48 °C for 48 h in a water bath shaker with an agitation rate of 150 rpm. Finally, the reaction mixture was filtered to separate the leftover biomass from the liquid fraction. The liquid fraction was analysed for soluble sugars (glucose) by high-performance liquid chromatography.

2.4.3. Analytical method

The glucose concentration of the solution for the enzymatic hydrolysis of IL-pretreated frond was analysed using a Shimadzu liquid chromatograph equipped with a LiChroCART® 250-4 Purospher® STAR NH_2 column (efficiency >50000 N/m and pore size of 12 nm) and a refractive index detector (Shimadzu RID-10A). The column was calibrated with a series of standard D-glucose solutions of known concentration ranging from 0.001 to 0.010 g/ml. The centrifuged and filtered samples were injected onto the HPLC with an injection volume of 20 μl. A 75% aqueous acetonitrile solution was used as the mobile phase with a flow rate of 1.0 ml/min, and the oven temperature was set at 40 °C. By assuming a 10% loss of liquid solution during the 48 h of enzymatic hydrolysis, the percent glucose recovery was defined as the weight of glucose found in the liquid divided by the weight of cellulose contained in the biomass, as illustrated in Eq. (2).

$$\%\,\text{glucose recovery} = \frac{\text{Glucose (g/ml)} \times 27\,\text{ml Buffer solution}}{0.15\,\text{g Biomass} \times 0.2508\,\text{g/g Cellulose}} \times 100\% \quad (2)$$

2.5. Characterisation of untreated and pretreated palm frond

2.5.1. Composition analysis

2.5.1.1. Determination of extractives. The palm frond samples were extracted with deionised water and then ethanol respectively by Soxhlet extraction apparatus according to the laboratory analytical procedure for determination of extractives in biomass (Ehrman, 1994). Samples were washed with deionised water and dried overnight at 80 °C prior to analysis. In the first step of analysis, the extraction of water-soluble compounds, such as inorganic material, non-structural sugars and nitrogenous material, was carried out for 8 h using 200 ml of deionised water. Upon completion, the sample was carefully removed from the extraction thimble and dried to constant weight. The mass loss during extraction was measured as the water extractives content. Another Soxhlet extraction followed, but 95% ethanol (200 ml) was used for 24 h to remove chlorophyll, waxes and other minor components. The ethanol extractives content of the palm fronds was measured as the mass loss during ethanol extraction.

2.5.1.2. Determination of moisture and ash content. The palm frond samples were dried overnight at 80 °C to determine the percentage of moisture. The samples were thereafter heated to 550 °C in a muffle furnace for 2 h to determine the ash content.

2.5.1.3. Determination of acid-insoluble lignin. The lignin content in the raw and pretreated oil palm fronds was determined by using two-step acid hydrolysis as described in the standard NREL laboratory analytical procedure (LAP) 003 (Templeton & Ehrman, 1995). First, 3 ml of a 72% (w/w) H_2SO_4 solution was added to 0.3 g of sample; hydrolysis was allowed for 2 h in a water bath shaker at 30 °C and 150 rpm. Upon completion, 84 ml of deionised water was added to dilute the acid to a final concentration of 4% (w/w). The reaction mixture was then transfer to a reactor for second step hydrolysis at 121 °C/2 atm for 1 h. The solid residue remaining after two-step hydrolysis was dried overnight to constant weight and further placed in a furnace at 550 °C for 2 h to determine the ash content. The content of acid insoluble lignin was then corrected for the ash content.

2.5.1.4. Determination of holocellulose and α-cellulose. Holocellulose, which is the sum of hemicellulose and cellulose in the biomass, was determined according to the method reported by Teramoto, Lee, and Endo (2009). First, 0.5 g of the extracted fronds was treated with 30 ml of deionised water containing 0.04 ml of acetic acid and 0.4 g of sodium chlorite ($NaClO_2$) for 1 h at 75 °C. Subsequently, 0.04 ml of acetic acid and 0.2 g of $NaClO_2$ were added to the mixture every 1 h for 3 h. The residue was filtered, washed with deionised water and acetone, and then dried overnight to constant weight. The holocellulose content was determined from the solid residue obtained after the reaction with $NaClO_2$ in acetate buffer.

The cellulose content of the oil palm fronds was determined as the amount of α-cellulose insoluble in a 17.5% NaOH aqueous solution. The analysis was done by shaking 0.2 g of previously obtained holocellulose in a flask with 5 ml of a 17.5% NaOH aqueous solution at 30 °C for 40 min using a water bath shaker. Next, 5 ml of deionised water was added to the mixture and shaking was continued for 5 min before the mixture was filtered. The remaining residue was then washed with 8 ml of 10% acetic acid and boiling water. Finally, the α-cellulose residue was dried overnight and weighed. The hemicellulose content was calculated as the difference between the holocellulose and cellulose contents.

2.5.2. Scanning electron microscopy (SEM)

The effect of pretreatment on the morphology of the palm fronds was observed with field emission scanning electron microscopy (FESEM, Supra 35 VP-24-58, Germany) at an acceleration voltage of 5.0 kV. Samples were mounted on aluminium sample stubs and sputter coated with a thin layer of gold before analysis.

3. Results and discussion

3.1. Composition of untreated and pretreated oil palm fronds and scanning electron microscopy

The chemical compositions of various potential feedstocks for fuel ethanol production are shown in Table 3, including the oil palm fronds used in this study. The various types of biomass have different contents of lignin, hemicellulose and cellulose. Woody

Table 3
Compositions of various lignocellulosic biomasses (wt%).

Component	OPF	Switchgrass[a]	Eucalyptus[b] (dry basis)	Rice husk[c] (dry basis)	Pine[b] (dry basis)
Lignin	18.46	16.30	27.71	17.60	27.67
Holocellulose	49.13	57.70	62.57	56.00	66.45
Hemicellulose	24.06	21.10	13.07	20.90	21.90
Cellulose	25.08	36.60	49.50	35.10	44.55
Water extractives	5.17	5.19	4.27	–	2.88
Ethanol extractives	5.90	7.27		–	
Moisture	9.26	8.50	–	–	–
Ash	11.66	5.00	1.26	12.10	0.32

[a] Suryawati et al. (2009).
[b] Balat and Balat (2009).
[c] Goh et al. (2010).

materials such as eucalyptus and pine contain a higher percentage of cellulose and lignin content compared to herbaceous feedstock, which is characterised by slightly lower lignin and cellulose contents. The OPF is herbaceous and was determined to contain 18% lignin, 24% hemicellulose and 25% cellulose in the raw biomass.

By considering only three constituents (lignin, hemicellulose, cellulose) in the biomass, untreated oil palm fronds gives 27, 36 and 37 wt%, whereas pretreated oil palm fronds show 27, 34, and 39 wt% of lignin, hemicellulose and cellulose, respectively. The result shows that treating biomass with IL, in which cellulose was initially dissolved and subsequently precipitated out by adding antisolvent, has negligible influence on the biomass composition. Similar observations were reported by Samayam and Schall (2010), who studied the pretreatment of poplar and switchgrass using the IL 1-ethyl 3-methyl imidazolium acetate (EmimOAc). Although the composition of lignocellulosic biomass was not altered by IL pretreatment as in other pretreatment methods, the structure of raw frond changes significantly after IL treatment, as indicated by the SEM images obtained for fresh and IL-treated oil palm fronds (Fig. 1).

Micrographs (A), (B) and (C) in Fig. 1 are SEM images for the cross-sectional area of fresh OPF at 3000× magnification and the surface of fresh OPF at 3500× and 300× magnification, respectively. The SEM images for pretreated oil palm fronds using BMIMCl with selected pretreatment conditions are shown in Fig. 1D–F. The images clearly demonstrate that IL pretreatment could alter the biomass structure through dissolution and regeneration of cellulose. The web structure of the fronds (Fig. 1A) was significantly changed, giving rise to a conglomerate texture (Fig. 1D) after IL treatment. Conglomeration also happened on the surface of the fronds, as shown in Fig. 1F. Moreover, there were some irregular cracks found on the surface of the pretreated frond compared to the raw frond (Fig. 1B and E). These alterations were attributed to the breakdown of biomass natural structures during the dissolution of cellulose from biomass into IL, leaving a highly homogeneous morphology on the treated frond. With the disruption of the structure and a reduction in crystallinity, the IL-treated oil palm fronds became more susceptible to enzymatic attack in the subsequent hydrolysis process and was supported by glucose recovery from enzymatic hydrolysis of fresh OPF (without pretreatment), as listed in Table 2. A mere 23.5% conversion of cellulose into glucose is expected if the feedstock is directly used for hydrolysis without pretreatment; 100% conversion can be obtained if IL pretreatment precedes hydrolysis.

It should be noted here that the focus of this entire study, from ionic liquid pretreatment to subsequent enzymatic hydrolysis, is mainly on the cellulose fraction instead of the two other main components in biomass which are lignin and hemicellulose. This is because the crystallinity of cellulose can be easily reduced using the pretreatment method used in this study and hence being hydrolysed into glucose whereas most of the lignin and hemicellulose content remain intact in the OPF at the end of the process. Furthermore, insignificant amount of xylose was detected in the product mixture using HPLC indicated that hemicellulose is not being hydrolysed by the cellulase (Celluclast 1.5 l), but due to heat treatment in the process.

3.2. Model development

The experimental data shown in Table 2 were used to determine the regression coefficients of the second-order polynomial equation using Design-Expert software and the following models that describe the glucose recovery in terms of coded parameters and actual parameters were obtained:

$$\text{Glucose recovery} = 40.20 + 9.70 \times A - 3.29 \times B + 11.29 \times C + 5.56 \times B^2 + 10.76 \times C^2 - 6.69 \times A \times B + 9.23 \times A \times C \quad (3)$$

$$\text{Glucose recovery} = 98.2131 + 0.4359 \times \text{Temperature} + 0.7907 \times \text{Time} = 44.1797 \times \text{Solid Loading} + 0.03106 \times \text{Time}^2 + 1.9016 \times \text{Solid loading}^2 - 0.04208 \times \text{Temperature} \times \text{Time} + 0.3263 \times \text{Temperature} \times \text{Solid loading} \quad (4)$$

where A, B and C are the pretreatment temperature, time and solid loading, respectively. To test the significance of the developed model, analysis of variance (ANOVA) was performed and the results are presented in Table 4. A model is considered significant if its p-value (also known as the 'Prob > F' value) is lower than 0.05, indicating only a 5% chance that a 'Model F-value' could occur because of noise. The 'Prob > F' values were also used to evaluate the significance of the effects of each linear, quadratic and interaction term on the response. Because the 'Prob > F' value for the model is very low (less than 0.0001), the model equation adequately describes the response (Table 4). In addition, the 'Prob > F' values for each model term suggest that A, C, B^2, C^2, AB and AC are the model terms that have significant effects on the glucose yield. However, the linear effect of time (B) on glucose yield is not significant because its 'Prob > F' value is greater than 0.1. Although not significant, this factor could not be excluded from the model in order to retain model hierarchy because the quadratic effect of retention time (B^2) is significant.

The lack of fit test, which was used to determine the adequacy of the model, indicates an insignificant lack of fit with an F-value of 3.12. At the same time, the coefficient of determination (R^2) of the model was 0.8838, implying a high correlation between the

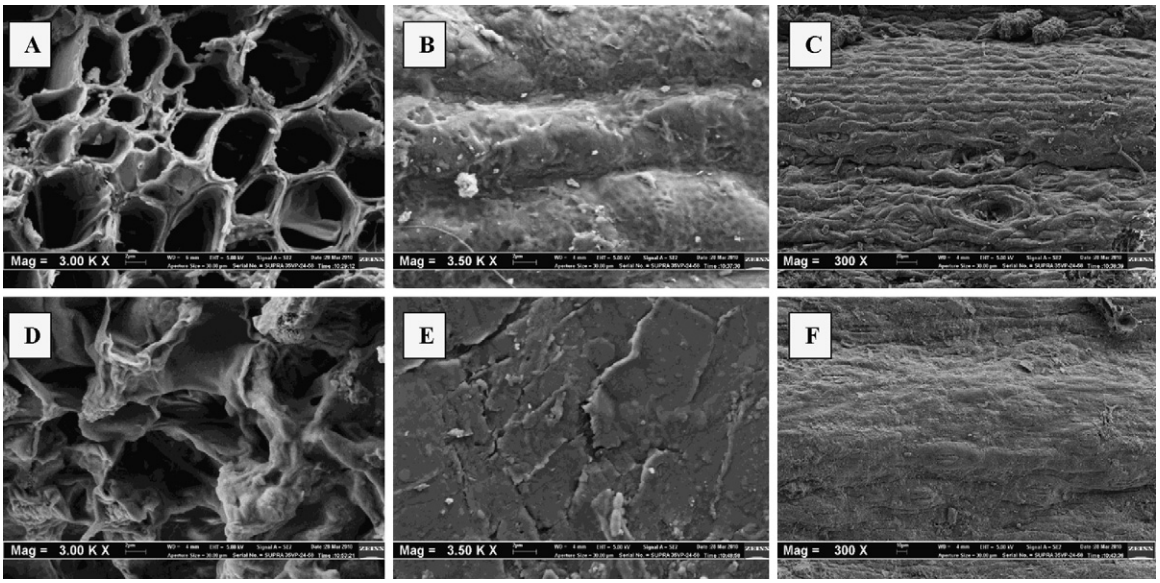

Fig. 1. SEM images of oil palm fronds: (A) Raw frond (cross-sectional), (B) Raw frond (surface 3500×), (C) Raw frond (surface 300×), (D) IL-pretreated frond (cross-sectional), (E) IL-pretreated frond (surface 3500×), (F) IL-pretreated frond (surface 300×).

Table 4
ANOVA table for the quadratic model.

Source	Sum of squares	DF	Mean square	*F*-value	Prob > *F*	
Model	6208.63	7	886.95	13.03	<0.0001	Significant
A	1286.20	1	1286.20	18.90	0.0009	
B	148.24	1	148.24	2.18	0.1657	
C	1741.03	1	1741.03	25.59	0.0003	
B^2	449.73	1	449.73	6.61	0.0245	
C^2	1684.27	1	1684.27	24.75	0.0003	
AB	358.54	1	358.54	5.27	0.0405	
AC	681.59	1	681.59	10.02	0.0081	
Residual	816.55	12	68.05			
Lack of fit	664.59	7	94.94	3.12	0.1141	Not significant
Pure error	151.97	5	30.39			

DF—Degree of freedom.

observed and predicted values, as shown in Fig. 2. Therefore, only 11.62% of the total variation cannot be explained by the model. All of these statistical tests showed that the developed model was suitable for representing the data and able to provide a good description of the relationship between the process variables and response. Furthermore, the quadratic model is adequate for predicting glucose recovery under different pretreatment conditions within the range used in this study.

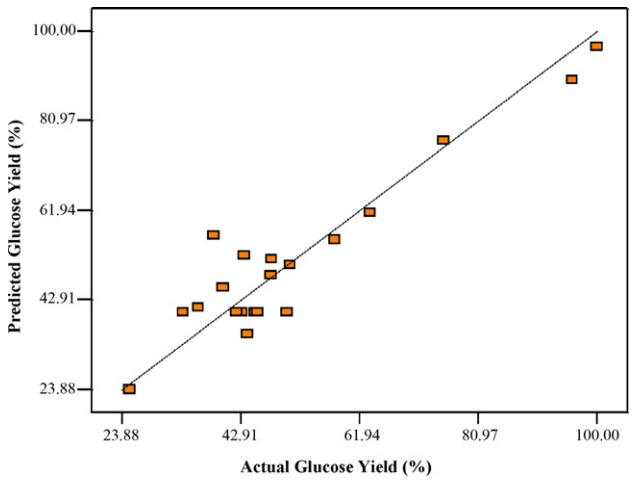

Fig. 2. Predicted vs. actual glucose yield.

3.3. Effect of three pretreatment variables on glucose recovery

The effects of the three pretreatment process variables (temperature, retention time and solid loading) on glucose recovery were analysed using RSM. Three-dimensional response surface and contour plots were generated to investigate the interactive effects of any two variables on the response by evaluating two variables at a time while holding the other one constant at central level. A three-dimensional plot can give a clearer geometrical representation of the nature and extent of the interaction between the variables and response within the experimental range studied.

3.3.1. Effect of pretreatment temperature and retention time

The effect of pretreatment temperature and retention time on glucose recovery in enzymatic hydrolysis with a constant solid loading level of 6 wt% is depicted in Fig. 3. With shorter pretreatment retention times, the percent glucose recovery increased almost linearly from 26.2% to 96.7% as the pretreatment temperature increased from 60 °C to 100 °C. However, when the retention time was increased to 60 min, the percent glucose recovery decreased slightly as the temperature increased. Therefore, we concluded that the pretreatment temperature and time are significant factors influencing the efficiency of the IL pretreatment process.

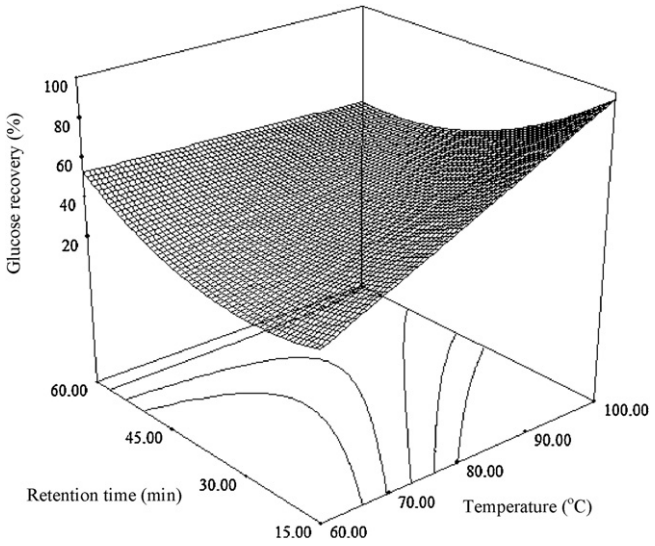

Fig. 3. Three-dimensional response surface plot for the effects of pretreatment temperature and retention time on glucose recovery at a constant solid loading of 6 wt%.

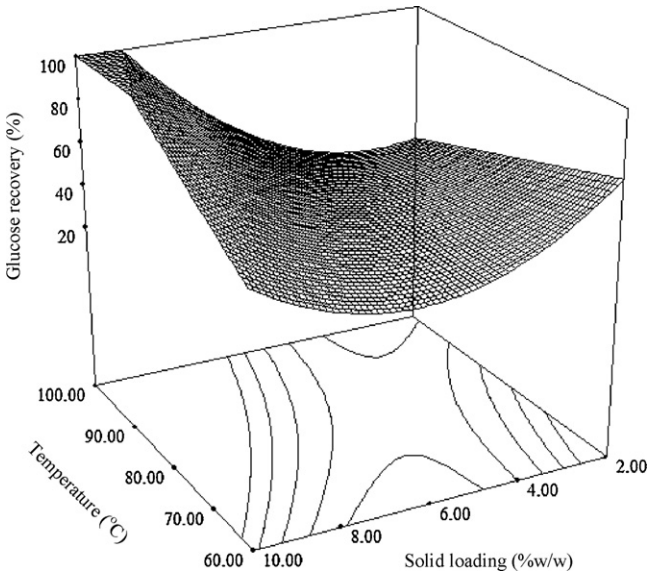

Fig. 4. Three-dimensional response surface plot for the effects of pretreatment temperature and solid loading on glucose recovery at a constant retention time of 38 min.

When using a short retention time, the solubility of cellulose from the fronds into ILs can be accelerated by increasing the temperature. The viscosity of the IL BMIMCl is correlated to its temperature; the viscosity of BMIMCl is reduced at higher temperature and therefore favours the swelling and subsequent dissolution of cellulose (El Seoud, Koschella, Fidale, Dorn, & Heinze, 2007; Kuang, Zhang, & Wang, 2007). Thus, at high temperatures, more cellulose can be easily extracted from the biomass that then can be hydrolysed to glucose. Similar findings on the effect of temperature towards pretreatment of lignocellulosic biomass using IL as a solvent have been reported elsewhere (Li et al., 2009; Xie, Li, & Zhang, 2005).

Nevertheless, the above explanation does not apply when the pretreatment time was prolonged to 60 min. As shown in Fig. 3, when a 60-min retention time was used, the percent glucose recovery decreased from 53.0% to 47.7% as the pretreatment temperature increased from 60 °C to 100 °C. Although increasing the pretreatment temperature could promote swelling and subsequent dissolution of cellulose, cellulose degradation may occur when exposing the reaction mixture to higher operating temperatures for prolonged retention times (Sun & Chen, 2008). Degradation of cellulose during pretreatment consequently lowers the final glucose recovery. In addition, the degraded products will adversely affect the subsequent hydrolysis process, such as by inhibiting the cellulase activity, resulting in a slower hydrolysis rate and reduced glucose release (Kuo & Lee, 2009).

3.3.2. *Effect of pretreatment temperature and solid loading*

Fig. 4 contains the response surface plot showing the effect of pretreatment temperature and solid loading on glucose recovery at a fixed retention time of 38 min. Surprisingly, with a maximum solid loading of 10 wt%, the recovery of glucose increased substantially with increasing pretreatment temperature up to 100 °C; moreover, the opposite trend was observed when solid loading was reduced to 2 wt%. These results suggested that increasing the IL-to-biomass ratio during pretreatment does not enhance the final glucose yield, but instead reduced the glucose recovery from 61.7% to 41.3% as the temperature increased.

For most of the cases reported in literature, a high solid-to-liquid ratio could ruin the effect of pretreatment that increases the enzymatic accessibility of biomass as higher solid loading will result in mixing problems and further cause heat and mass transfer limitation in the system. These are mainly due to low solid liquefaction and nonuniformity of the system at high solid-to-liquid ratios. As reported by Kootstra, Beeftink, Scott, and Sanders (2009) and Noureddini and Byun (2010) in their studies on the pretreatment of wheat straw and distillers' grains, respectively, high solid loading can also promote the formation of inhibitory compounds, such as furfural, which will inhibit subsequent cellulose hydrolysis.

Thus, generally it can be concluded that the efficiency of pretreatment can be improved by reducing the solid-to-liquid ratio; however, our results with IL-pretreated OPF showed the opposite trend. This may be partly due to the viscosity of the IL BMIMCl that is significantly higher than that of mediums used for other pretreatment methods. For example, water, which has lower viscosity, was used as the solvent for both dilute acid and ammonia fibre expansion (AFEX) pretreatment. The viscosity of IL led to problems with mixing and mass diffusion during pretreatment. The problems could not be diminished, even with the lowest solid loading. Instead, a higher biomass concentration under agitation, allowing for more frequent contact and collision between the biomass particles, caused impinging on the biomass surface, inadvertently promoting cellulose dissolution from the biomass matrix. While not proven, this explanation might account for the seemingly opposite results obtained. The overall heat transfer within the system is also enhanced by frequent collision between concentrated solid particles and is believed to be another factor that could lead to better glucose recovery from the hydrolysis process. Therefore, apart from reducing the viscosity of IL by increasing the pretreatment temperature as discussed in the previous section, increasing the solid loading is another promising way to improve the pretreatment efficiency.

Likewise, substantially raising the biomass loading can increase the economic feasibility of the biorefinery process as a high substrate concentration has the potential to reduce energy demands as well as equipment size during pretreatment. Increasing biomass loading also leads to a more concentrated product stream, in turn improving the efficiency of downstream processing while also decreasing the capital and operating costs (Kim et al., 2008; Kootstra et al., 2009).

3.4. Optimisation and confirmation experiments

In the process, a lower temperature, shorter time and lower amount of IL are preferred to yield optimum glucose recovery. Although a 100% conversion of cellulose contained in oil palm fronds to glucose was achieved using the conditions shown in Table 2, however, the pretreatment temperature of 92 °C may have been too high. Therefore, Design Expert software was used to predict the optimal conditions for the IL pretreatment. The optimum pretreatment temperature, retention time and solid loading were found to be 80 °C, 15 min and 10%, respectively. A 13% reduction in temperature, 38% reduction in retention time and 19% increase of solid loading, when comparing the optimum conditions with the experimental conditions (Run 6, Table 2) that gave the highest yield, was achieved by the optimisation. To validate the optimum conditions, two replicate confirmation experiments were conducted, yielding 100% conversion, a result consistent with the predicted values. These data show that the experimental value obtained was in good agreement with the value calculated from the model developed using the software. Therefore, the model is useful for predicting glucose recovery as well as optimisation of the experimental conditions.

4. Conclusion

The conversion of oil palm frond (OPF) into reducing sugar through IL pretreatment and enzymatic hydrolysis was presented in this study. The results from this study demonstrated that OPF which contain 28% cellulose (based on dry matter) can be an alternative feedstock for the production of bioethanol provided it is properly pre-treated with ionic liquid such as BMIMCl. Our results indicated that the final glucose yield was enhanced by pretreatment temperature and solid loading. On the contrary, glucose recovery was not increased with longer retention times, potentially because of cellulose degradation from exposing the reaction mixture to heat treatment during the prolonged retention time. Lastly, RSM was also used to optimise the pretreatment conditions for maximum percent glucose recovery. The optimum conditions were determined to be 80 °C, 15 min retention time and 10% solid loading.

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