Regression analysis on ionic liquid pretreatment of sugarcane bagasse and assessment of structural changes

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Abstract
This study aims to perform a regression analysis which leads to the optimization on the operating conditions of ionic liquid (IL), 1-ethyl-3-methylimidazolium acetate ([EMIM]oAc) pretreatment on sugarcane bagasse (SCB). The structural changes on SCB during pretreatment were also examined. The effects of temperature, time and solid loading on reducing sugar (RS) yield obtained from enzymatic hydrolysis of pretreated SCB were investigated by applying Central Composite Design (CCD) of Response Surface Methodology (RSM). Results from CCD were modeled into a second order polynomial equation and the model shows a good correlation between predicted and experimental values. The optimized condition for [EMIM]oAc pretreatment were 145°C, 15 min and 14 wt% of solid loading with an optimum RS yield of 69.7%. Characterization of SCB was carried out and there were no significant difference between the chemical composition of untreated and [EMIM]oAc-pretreated SCB. Pretreated SCB was found to be porous, less crystalline and favorable to enzymatic hydrolysis as proven by Scanning Electron Microscopy (SEM), X-ray
Powder Diffraction (XRD) analysis and Fourier Transform Infrared (FTIR) analysis. In short, [EMIM]oAc pretreatment shows good performance in improving the RS yield after enzymatic hydrolysis besides giving desirable structural modification on pretreated SCB. These are of great benefit to the subsequent downstream processes.

**Introduction**

The dwindling supply of fossil fuel has generated great efforts in the search for a renewable and sustainable fuel source. In the recent years, biofuel which can be derived from lignocellulosic biomass has been viewed as one of the potential substitutes for fossil fuel [1]. Agricultural residues are the most abundantly found lignocellulosic biomass especially in countries where agricultural activities are dominant. Like many lignocellulosic biomass, these low cost residues are made up of cellulose, hemicellulose and lignin [2]. Among the components, cellulose and hemicellulose are the well-known raw materials for a wide range of value-added products such as fermentable sugars and biofuel [3].

The complex matrix of lignocellulosic biomass unfavorably hinders the effective conversion of agricultural residues into value-added products. Thus, pretreatment process is necessary to alter the structure of biomass by increasing their surface area, removing the lignin content, reducing cellulose crystallinity or depolymerizing the hemicellulose, depending on the pretreatment methods employed [4, 5]. Pretreatment would enhance the sugar yield from enzymatic hydrolysis of pretreated biomass due to the increasing accessibility of enzymes to cellulose and hemicellulose [6].

Recently, ionic liquid (IL) has emerged as one of the promising pretreatment reagent for lignocellulosic biomass. IL exhibits outstanding ability in dissolution of cellulose at considerably mild conditions [7]. Besides, some of the ILs are environmentally benign, attributed to their non-volatile and non-flammable properties [8]. The IL-pretreated biomass was reported to have a reduced content of lignin, lower degree of crystallinity and can be enzymatically hydrolyzed at a faster rate [9-11]. The recyclability of IL has also added a credit to this particular pretreatment method [8, 12].

Optimization of pretreatment process is an essential step in determining the most suitable and economical condition to enhance the yield of products. Many tools such as full factorial
design, Central Composite Design (CCD) and Box-Behnken Design (BBD) were used by the researchers to optimize different pretreatment processes [13-15]. Among the tools, CCD is the most popular design used for fitting second order models and therefore it is extensively applied in the optimization of pretreatment process [16]. Temperature and reaction time are the widely explored optimization parameters by the researchers [10, 13, 15]. Other factors such as concentration of the reagent, pressure and solid loading might also affect the process significantly [15, 17, 18].

To date, there are limited studies on the optimization of ionic liquid pretreatment process. The objective of this work is to perform a regression analysis on the pretreatment of sugarcane bagasse (SCB) by using IL 1-ethyl-3-methylimidazolium acetate ([EMIM]oAc). [EMIM]oAc was determined to be the most suitable pretreatment medium for SCB [19]. SCB was employed due to its relatively high cellulose content of approximately 50% [3]. The effect of pretreatment temperature, time and solid loading on reducing sugar (RS) yield was investigated and the pretreatment process was optimized by applying CCD. The RS yield obtained from enzymatic hydrolysis of pretreated SCB was used as a parameter to evaluate the effectiveness of [EMIM]oAc in pretreating SCB under different operating conditions. The morphological and structural changes of SCB after [EMIM]oAc pretreatment was also examined.

**Materials and Methods**

**Materials**

Sugarcane bagasse (SCB) was collected from Purecane Manufacturing Sdn. Bhd., Johor, Malaysia. SCB was thoroughly washed and dried. It was ground and sieved into 250 μm – 500 μm prior to use. Ionic liquid (IL) 1-ethyl-3-methylimidazolium acetate ([EMIM]oAc) was purchased from Sigma Aldrich (St. Louis, USA). Cellulase Onozuka R-10 from *Trichoderma viride* (EC 3.2.1.4) was purchased from Merck (Darmstadt, Germany).

**Ionic liquid pretreatment**

Ionic liquid pretreatment was carried out by adding [EMIM]oAc into the test tube which contains SCB. The [EMIM]oAc-SCB mixture was heated in an oil bath (Julabo Labortechnik GmbH, Seelbach, Germany) and subsequently, deionized water was added to regenerate the dissolved cellulose after the reaction. The mixture was briefly centrifuged before subject to
filtration. The retentate which consists of regenerated cellulose and the insoluble residue of SCB was washed by deionized water and acetate buffer solution. Pretreated bagasse was then dried prior to enzymatic hydrolysis, characterization, scanning electron microscopy (SEM), X-ray powder diffraction (XRD) and Fourier transform infrared (FTIR) analysis.

**Enzymatic hydrolysis**

IL-pretreated SCB was hydrolyzed by using cellulase with a loading of 30 FPU/g substrate. Acetate buffer solution of pH 4.8 was used to buffer the mixture of SCB and cellulase enzymes. Enzymatic hydrolysis was conducted at 50 °C for 48 h. After the reaction, the samples were centrifuged and the concentration of RS in supernatant was determined by using DNS method [20, 21]. All experiments were performed in duplicate. The RS yield obtained from enzymatic hydrolysis was computed by applying the equation as suggested by Li et al. [10].

**Experimental design and regression analysis**

Central Composite Design (CCD) of response surface methodology (RSM) was applied in regression analysis to determine the optimum condition of [EMIM]oAc pretreatment. Regression analysis was carried out with the aid of Design Expert 6.0.6 software (STAT-EASE Inc., Minneapolis, USA). The $\alpha$ value of 1 was applied in the experimental design and 20 experimental runs were conducted to optimize the process. The effect of three independent variables, i.e. pretreatment temperature (°C), time (min) and solid loading (wt%) on RS yield were investigated. Selection on the range for each independent variable was based on preliminary studies, i.e. pretreatment temperature from 120°C to 160°C, duration from 15 min to 45 min and solid loading from 3 wt% to 15 wt%.

The experimental response, $Y$ (RS yield, %) was fitted into a second order polynomial equation as stated in equation (1) [22]. Evaluation on the statistical significance of the model developed was done by using analysis of variance (ANOVA) method.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i<j}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2$$  \hspace{1cm} (1)

where, $X_i, X_j = $ coded independent variables; $\beta_0 = $ intercept effect; $\beta_i = $ linear effect; $\beta_{ij} = $ linear-by-linear interaction; $\beta_{ii} = $ quadratic effect.
Characterization of SCB

Analysis of the chemical compositions in SCB was conducted by adopting the methods from National Renewable Energy Laboratory (NREL) [23]. The compositions of cellulose, hemicellulose, acid soluble lignin (ASL), acid insoluble lignin (AIL) and ash in SCB were determined.

SCB (300 mg) was first hydrolyzed with 72% sulfuric acid (H₂SO₄) for 1 h at 30°C. After the reaction, the acid was diluted to 4% concentration by deionized water. The mixture was then autoclaved at 121°C for 1 h. Filtration of the autoclaved solution was carried out and the solid residues remained after filtration was collected to determine the AIL and ash contents in SCB. The compositions of cellulose, hemicellulose and ASL were determined from the filtrate of the autoclaved mixture.

Scanning electron microscopy (SEM)

The morphology of SCB was examined with scanning electron microscope (Quanta 200 FESEM), FEI, USA). The solid samples were mounted on the aluminum sample stubs with double sided carbon tape. Images were acquired with a 10kV acceleration voltage.

X-ray powder diffraction (XRD) analysis

XRD diffractogram of SCB were obtained by using D8 Advance X-Ray Diffractometer (Bruker AXS, USA). The samples were scanned over a range of 10-80° (2θ) with a step size of 0.02° and step time of 1 s. The scans were done at 40 kV and 40 mA under ambient temperature. Crystallinity index (CrI) of SCB was computed from equation (2) [24].

\[
CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100
\]

where, \( I_{002} \) = intensity for crystalline portion of SCB at about 2θ = 22°; \( I_{am} \) = intensity for amorphous portion of SCB at about 2θ = 18°.

Fourier transform infrared (FTIR) analysis

FTIR-FIR spectrometer (Perkin Elmer, Massachusetts, USA) was employed to examined the chemical structures of SCB. The dried solid samples were mixed with potassium bromide
(KBr) and the mixture was pressed into a disc. The samples were then scanned in the range of 4000-450 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\).

**Results and discussion**

**Regression analysis**

**Analysis of variance (ANOVA)**

Table 1 presents the experimental design matrix and the RS yield obtained from [EMIM]oAc-pretreated SCB. A second order polynomial equation as shown in equation (1) was used to fit the response. The coefficients $\beta_0$, $\beta_i$, $\beta_{ij}$ and $\beta_{ii}$ of equation (1) were identified by using Design Expert 6.0.6 software. The final equation in terms of the coded factors after elimination of the insignificant model terms was demonstrated in equation (3).

\[
Y = 69.73 - 1.66A - 1.25B - 0.70C - 13.63A^2 - 5.91AB + 2.04AC
\]

where, $A$ = coded factor of temperature; $B$ = coded factor of time; $C$ = coded factor of solid loading.

<table>
<thead>
<tr>
<th>Run</th>
<th>Temperature $A$ ($^\circ$C)</th>
<th>Time $B$ (min)</th>
<th>Solid loading $C$ (wt%)</th>
<th>RS yield $Y$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140</td>
<td>30</td>
<td>3</td>
<td>69.9</td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>30</td>
<td>15</td>
<td>69.0</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>30</td>
<td>9</td>
<td>57.3</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>45</td>
<td>15</td>
<td>50.1</td>
</tr>
<tr>
<td>5</td>
<td>140</td>
<td>30</td>
<td>9</td>
<td>69.5</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>45</td>
<td>3</td>
<td>66.0</td>
</tr>
<tr>
<td>7</td>
<td>160</td>
<td>15</td>
<td>3</td>
<td>62.1</td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>15</td>
<td>15</td>
<td>51.3</td>
</tr>
<tr>
<td>9</td>
<td>140</td>
<td>15</td>
<td>9</td>
<td>69.0</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>30</td>
<td>9</td>
<td>58.8</td>
</tr>
<tr>
<td>11</td>
<td>160</td>
<td>15</td>
<td>15</td>
<td>59.9</td>
</tr>
<tr>
<td>12</td>
<td>120</td>
<td>45</td>
<td>15</td>
<td>58.1</td>
</tr>
<tr>
<td>13</td>
<td>140</td>
<td>45</td>
<td>9</td>
<td>67.4</td>
</tr>
</tbody>
</table>
The results based on analysis of variance (ANOVA) for the reduced quadratic model (equation (3)) are shown in Table 2. At 90% confidence level, $A$, $A^2$, $AB$ and $AC$ were the significant model terms and this indicates that the variation in temperature regardless of its linear, quadratic or interaction terms has the largest impact on RS yield. Conversely, solid loading ($C$) has the smallest effect on RS yield as indicated by its largest Probability $F$-value. Apart from that, the model with a $F$-value of 28.69 implied that this model was statistically significant under 90% confidence level. The lack of fit of this model is found to be insignificant relative to the amount of variation in the response in replicated design points. In addition, the coefficient of determination, $R$-squared value of this model was 0.9298. All three criteria mentioned above indicated that there was a high correlation between the predicted and experimental RS yields.

Table 2: ANOVA results for the reduced quadratic model

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>DF$^a$</th>
<th>Mean square</th>
<th>$F$-value</th>
<th>Probability $&gt; F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1289.84</td>
<td>6</td>
<td>214.97</td>
<td>28.69</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>A</td>
<td>27.56</td>
<td>1</td>
<td>27.56</td>
<td>3.68</td>
<td>0.0774</td>
</tr>
<tr>
<td>B</td>
<td>15.63</td>
<td>1</td>
<td>15.63</td>
<td>2.09</td>
<td>0.1724</td>
</tr>
<tr>
<td>C</td>
<td>4.90</td>
<td>1</td>
<td>4.90</td>
<td>0.65</td>
<td>0.4333</td>
</tr>
<tr>
<td>$A^2$</td>
<td>928.88</td>
<td>1</td>
<td>928.88</td>
<td>123.97</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>AB</td>
<td>279.66</td>
<td>1</td>
<td>279.66</td>
<td>37.32</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>AC</td>
<td>33.21</td>
<td>1</td>
<td>33.21</td>
<td>4.43</td>
<td>0.0553</td>
</tr>
<tr>
<td>Residual</td>
<td>97.41</td>
<td>13</td>
<td>7.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>47.11</td>
<td>8</td>
<td>5.89</td>
<td>0.59</td>
<td>0.7615</td>
</tr>
</tbody>
</table>

$^a$ DF: Degrees of Freedom
The model developed was further tested for its validity. Three additional experiments were carried out and the experimental RS yields were compared with the predicted RS yields. The results obtained were summarized in Table 3. Error was computed based on the differences between the predicted and the experimental values. As shown in Table 3, the predicted RS yields agree reasonably well with the experimental RS yield with less than 2% error.

Table 3: Model validity check

<table>
<thead>
<tr>
<th>Pretreatment conditions</th>
<th>Predicted RS yield (%)</th>
<th>Experimental RS yield (%)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 °C</td>
<td>30 min</td>
<td>15 wt%</td>
<td>55.8</td>
</tr>
<tr>
<td>140 °C</td>
<td>30 min</td>
<td>6 wt%</td>
<td>68.1</td>
</tr>
<tr>
<td>120 °C</td>
<td>45 min</td>
<td>9 wt%</td>
<td>62.4</td>
</tr>
</tbody>
</table>

Model analysis

Based on the quadratic model developed, the response surface graphs and interaction graphs were plotted by varying two variables at their experimental ranges while keeping one variable constant at zero level. Figure 1 illustrates the three-dimensional response surface plot and the interaction plot of the effect of pretreatment temperature and time on RS yield. The three-dimensional response surface plot obtained was best described as a stationary ridge system because the RS yield was found to be constant along the ridge [22]. Temperature exhibits a quadratic effect on the RS yields whereas pretreatment duration shows a linear effect on the yields as demonstrated in Figure 1.

Improvement in RS yield was observed at a longer pretreatment duration when lower temperature, i.e. 120°C was applied. In contrast, prolonged pretreatment duration would lead to reduction in RS yield when pretreatment was carried out under higher temperature (>135°C) [Figure 1(b)]. The decreasing RS yield at higher temperature and duration might be...
contributed by the depolymerization of polysaccharides into their respective monomers [25]. Cellulose and hemicellulose in SCB might have undergone certain degree of depolymerization under these extremes conditions. According to Miyafuji et al. [25], this phenomenon was more apparent at higher pretreatment temperature. As a result, depolymerization and RS loss might be the contributing factors to the lower RS yield attained.

![Figure 1: (a) Three-dimensional response surface plot of the effect of pretreatment temperature and time on RS yield, solid loading = 9 wt%, and (b) interaction plot between pretreatment temperature and time on RS yield, solid loading = 9 wt%.](image)

The rate of dissolution was governed by temperature and thus, higher temperature would accelerate the dissolution of SCB’s cellulose into [EMIM]oAc. Consequently, more cellulose in SCB was dissolved and thus, a larger amount of regenerated cellulose can be obtained.
when SCB was pretreated at a higher temperature. The regenerated cellulose has a relatively amorphous structure that is more accessible by cellulase enzyme as agreed by Zhao et al. [11]. Moreover, higher temperature can reduce the viscosity of [EMIM]oAc and this has contributed to an improved mixing during the pretreatment process [26]. Hence, a shorter time (15 min) is sufficient to enhance RS yield when higher pretreatment temperature (>135°C) was applied.

On the other hand, SCB and [EMIM]oAc tend to form a coagulate when the mixture was heated to a higher temperature for a prolonged duration in this study. It was difficult to regenerate the dissolved SCB from the coagulate. Significant amount of [EMIM]oAc residual might still present in the pretreated SCB even after thorough washing. This ionic liquid residual might cause cellulase inactivation and consequently lead to a decrease in RS yield [11]. In addition, Teramoto et al. [27] reported that overcooking of biomass occurred at high temperature and prolonged pretreatment time. The overcooked biomass with burnt surface imposed a negative effect on enzymatic digestion.

Figure 2 illustrates the three-dimensional response surface plot and the interaction plot of the effect of pretreatment temperature and solid loading. Apparently, the interactive effect of pretreatment temperature and solid loading (AC) was weaker than the interactive effect of pretreatment temperature and time (AB). This is confirmed by the lower $F$-value of $AB$ term (37.32) as presented in Table 2.

Decrease in RS yield was observed when solid loading increased from 3 wt% to 15 wt% under pretreatment temperature of 120°C [Figure 2(b)]. High solid loading imposed limitation in mass and heat transfer [15]. Hence, further increase in solid loading did not improve the RS yield when lower pretreatment temperature (120°C) was employed. However, this is not true when applying temperature higher than approximately 147°C in [EMIM]oAc pretreatment. The chemical equilibrium of the reaction might have shifted to favor more cellulose dissolution at a higher temperature. As a result, a higher solid loading constituted to an improved RS yield in this case.
Optimum condition for [EMIM]oAc pretreatment

The optimum condition for [EMIM]oAc pretreatment was determined by using the numerical optimization feature of Design Expert software. As discussed in the previous section (Model analysis), stationary ridge system offers flexibility in locating the optimum condition for the pretreatment process. The RS yield was found to be constant along the ridge as shown in Figure 1 and 2 when temperature duration and solid loading applied. Hence, operating conditions with shorter reaction time and higher solid loading which offer a lower operating cost was considered as the optimum. The optimized condition attained was 145°C, 15 min
and 14 wt% with a prediction of 71.0% RS yield. The experimental RS yield obtained under
optimized pretreatment condition was 69.7%. The 1.3% difference between predicted and
experimental values implies that the predicted RS yield correlated well with the experimental
data.

Several researchers have attempted to optimize the process of ionic liquid pretreatment for
various lignocellulosic biomass [10, 15, 28]. The findings of this study and previous works
are summarized in Table 4. Severity factor, \( \log R_0 \) was computed as a measure of the severity
of a particular pretreatment method. Pretreatment that operates under a higher severity
(higher temperature and longer duration) impose a higher operating cost. From Table 4, the
severity factor of this study is comparable to that reported by Dadi et al. [28]. Apart from that,
this study was able to pretreat a larger quantity of solid under optimum condition compared
to the works of Li et al. [10], Dadi et al. [28] and Tan et al. [15]. This is particularly
beneficial and cost saving if the subsequent process of pretreatment is solid state fermentation
or enzymatic hydrolysis.

Table 4: Optimized operating condition of ionic liquid pretreatment for different substrates

<table>
<thead>
<tr>
<th>Author</th>
<th>Types of experimental design</th>
<th>Substrate and IL used</th>
<th>Optimum pretreatment condition</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. (2009) [10]</td>
<td>Single factor at a time</td>
<td>Wheat straw, 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM]DEP)</td>
<td>- 130°C - 30 min - 40% solid loading</td>
<td>- 54.8% RS after enzymatic hydrolysis for 12 h - (^{a})log ( R_0 ) = 1.78</td>
</tr>
<tr>
<td>Tan et al. (2011) [15]</td>
<td>Central Composite Design</td>
<td>Oil palm frond, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl)</td>
<td>- 80°C - 15 min - 10% solid loading</td>
<td>- 100% cellulose conversion to glucose after 48 h of enzymatic hydrolysis - (^{a})log ( R_0 ) = 0.59</td>
</tr>
<tr>
<td>Dadi et al. (2006) [28]</td>
<td>Single factor at a time</td>
<td>Cellulose powder, [BMIM]Cl</td>
<td>- 130°C - 2 h - 5% solid</td>
<td>- Approximately 95% cellulose conversion to</td>
</tr>
</tbody>
</table>
The application of IL pretreatment has brought significant improvement in the RS yield as computed from the results obtained from enzymatic hydrolysis [10, 15, 28]. A higher RS yield was obtained in this study under optimum condition compared to the yield from [EMIM]DEP-pretreated wheat straw shown by Li et al. [10]. On the other hand, the results from the current study are found to be in good agreement with Tan et al. [15] confirming the ability of ionic liquid in pretreating biomass at a shorter time (15 min) and higher solid loading (>10%) although Tan et al. [15] applied a lower temperature in the pretreatment process.

Due to the substrate-specific property of ILs, researchers have been adopting different types of ILs in pretreating their respective substrates as presented in Table 4. There are several benefits to operate the process by using [EMIM]oAc compared to [BMIM]Cl and [EMIM]DEP. Firstly, [EMIM]oAc is a non-toxic, non-corrosive and biodegradable solvent. [EMIM]DEP is corrosive and [BMIM]Cl is toxic and harmful to the environment [8]. Furthermore, ILs with 1-butyl-3-methylimidazolium based cation ([BMIM]) is also proven to be poorly biodegradable [29]. In addition, [EMIM]oAc is less viscous and it exists in liquid form at room temperature. Hence, it is easier to handle [EMIM]oAc compared to [BMIM]Cl which is in crystalline solid form under room temperature.

![Table 4](image-url)
Structural analysis

Characterization of SCB

Besides the enhancement in RS yield, the structural and morphological changes in SCB after [EMIM]oAc pretreatment were also investigated. An effective pretreatment method should be able to retain a high amount of cellulose and hemicellulose in the biomass after pretreatment. This is a vital criterion to enhance the RS yield from the pretreated biomass via enzymatic hydrolysis. Hence, characterization of untreated and pretreated SCB was carried out to quantify the amount of cellulose, hemicellulose and lignin in the biomass. As Table 5 shows, no significant variation was identified in the chemical composition of SCB before and after [EMIM]oAc pretreatment. Similar observation was found in the report by Tan et al. [15]. [EMIM]oAc however has removed a large amount of other compounds in SCB such as extractives and acetyl content during pretreatment.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Untreated SCB (wt%)</th>
<th>[EMIM]oAc-pretreated SCB (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>41.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>30.1</td>
<td>32.1</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>6.8</td>
<td>8.7</td>
</tr>
<tr>
<td>(ASL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid insoluble lignin</td>
<td>14.4</td>
<td>12.3</td>
</tr>
<tr>
<td>(AIL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lignin</td>
<td>21.2</td>
<td>21.0</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Others</td>
<td>7.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

By applying the optimum pretreatment condition of this study, [EMIM]oAc was ineffective in delignification of SCB. The ASL content in SCB increased slightly whereas AIL decreased after [EMIM]oAc pretreatment. This indicates that though lignin removal is not obvious, the structure of lignin was modified to a more acid soluble type after pretreatment. Lee et al. [30] also supported that high degree of lignin removal is not necessary to enhance the enzymatic digestibility of biomass. In short, [EMIM]oAc mainly altered the crystallinity of cellulose rather than remove the lignin content in SCB during pretreatment.
Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) analysis

Figure 3 shows the SEM images of untreated and [EMIM]oAc-pretreated bagasse under different magnifications. There was no apparent difference in the morphology of untreated and pretreated SCB when they were both examined under lower magnification (180×). However, a relatively more porous structure can be observed for [EMIM]oAc-pretreated SCB when higher magnification (1600×) was applied. [EMIM]oAc-pretreated bagasse has a more irregular and corrugated surface with thinner fibers compared to the untreated SCB as illustrated in Figure 3(1) and (c).

![SEM images of sugarcane bagasse](image)

A more crystalline structure was confirmed by XRD analysis with the occurrence of a sharper peak at $2\theta = 22^\circ$ in the untreated SCB as compared to [EMIM]oAc-pretreated SCB [31]. Substantial reduction in crystallinity index ($CrI$) of the pretreated SCB after [EMIM]oAc pretreatment was also clearly indicated. The $CrI$ of pretreated bagasse was merely 29.8 compared to the untreated SCB with a $CrI$ value of 39.3. Lower crystallinity index signifies that a larger amount of amorphous cellulose was present in the pretreated biomass [32]. This provides a larger surface area accessible by cellulase enzymes. Hence, an enhanced RS yield was expected after enzymatic hydrolysis of the IL-pretreated bagasse.
The enhancement of RS yield was observed in a previous preliminary study [19] whereby the untreated bagasse produced 34.5% RS compared to [EMIM]oAc-pretreated bagasse which has yielded 69.7% under optimized condition. Several investigators have also reported that the structure of the biomass became more porous, less crystalline and susceptible to enzymatic attack after IL pretreatment [9, 15, 32]. Generally, regardless of the type of biomass, its structure will be altered in such a way favorable to enzymatic hydrolysis and microbial attack, after IL pretreatment.

**FTIR analysis**

FTIR analysis was applied to examine the changes in chemical structures of SCB after [EMIM]oAc pretreatment. The spectra of [EMIM]oAc-pretreated and untreated SCB were found to be similar as illustrated in Figure 4. The bands at 3330 cm⁻¹ and 2905 cm⁻¹ which represent the hydroxyl (O-H) group [31] and CH stretching [33] were present in both untreated and pretreated SCB. The presence of these bands might be attributed to the cellulose, hemicellulose and lignin content in SCB confirming the appearance of lignocellulosic matrix in this biomass [34].

As compared to hemicellulose and lignin, [EMIM]oAc pretreatment has the greatest impact toward the cellulose content. The spectra at 1425 cm⁻¹ which represent the CH₂ scissoring motion in crystalline cellulose diminished after pretreatment [32]. Whereas the absorbance of amorphous cellulose at 897 cm⁻¹ [32] turned out to be more intense after [EMIM]oAc pretreatment. Amorphous cellulose as reported by Kuo and Lee [32] is more susceptible to enzymatic attack due to its more porous and irregular structure along with the increment of accessible surface area by enzymes. This outcome suggests that the cellulose content in SCB was altered into a more amorphous structure that contributed to a greater improvement in RS yield after enzymatic hydrolysis of [EMIM]oAc-pretreated bagasse.

Lignin structure characterized by the peaks at 1600 cm⁻¹ and 1515 cm⁻¹ undergone some slight changes after the pretreatment. A reduction in the intensity of 1515 cm⁻¹ band coupled with the disappearance of 1600 cm⁻¹ band was observed in pretreated SCB. Adel et al. [35] suggested that these findings might be the results of depolymerization of lignin during pretreatment. The depolymerization of lignin might lead to the increment in ASL content in [EMIM]oAc-pretreated SCB as reported in Section ‘Characterization of SCB’. Other lignin
peaks at 1634 cm\(^{-1}\), 1375 cm\(^{-1}\) and 1328 cm\(^{-1}\) which explained the OH bending of adsorbed water [33], CH deformation [33] and syringyl units in lignin [36] were remained in the pretreated SCB. This indicates that lignin still present in the pretreated SCB in a considerable amount.

Hemicellulose was most prominently represented by the band ar around 1732 cm\(^{-1}\). Zhao et al. [37] suggested that this band describes the stretching behavior in C=O groups in the acetyl content of hemicellulose. This particular peak was retained in [EMIM]oAc-pretreated SCB and it signifies that [EMIM]oAc pretreatment did not cause the removal of hemicellulose content during the process. The results obtained from FTIR analysis correlates well with the characterization, SEM and XRD examinations. Moreover, FTIR analysis also confirmed the increase of the amorphous cellulose content in pretreated SCB along with the structural changes in the lignin content of SCB.

Figure 4: FTIR spectra of [EMIM]oAc-pretreated and untreated SCB. (a) 3330 cm\(^{-1}\), (b) 2905 cm\(^{-1}\), (c) 1732 cm\(^{-1}\), (d) 1634 cm\(^{-1}\), (e) 1600 cm\(^{-1}\), (f) 1515 cm\(^{-1}\), (g) 1425 cm\(^{-1}\), (h) 1375 cm\(^{-1}\), (i) 1328 cm\(^{-1}\), and (j) 897 cm\(^{-1}\).

Conclusion

This study examined the effect of temperature, time and solid loading on the RS yield from [EMIM]oAc-pretreated bagasse by using Central Composite Design. Good correlations between the experimental and predicted RS yields were found. The optimum pretreatment
condition of 145°C, 15 min and 14 wt% solid loading has given 69.7% of RS. Although changes in the chemical composition of [EMIM]oAc-pretreated SCB was not apparent, the pretreated SCB has a more porous and less crystalline structure which is a desired feature for subsequent solid state fermentation and enzymatic hydrolysis step. The content of amorphous cellulose increased significantly after [EMIM]oAc pretreatment as confirmed by FTIR analysis. In conclusion, the good performance of [EMIM]oAc in enhancing the RS yield and modifying the structure and morphology of the pretreated SCB has rendered this pretreatment process feasible. Due to the non-corrosive property of [EMIM]oAc, cost saving can also be achieved in the process without the usage of expensive corrosion resistant equipment. The aspect of reusability of [EMIM]oAc is worth investigating to improve the cost effectiveness of the entire pretreatment process.

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References


