Non-wood Lignocellulosic Biomass for Cellulosic Ethanol Production: Effects of Pretreatment on Chemical Composition in Relation to Total Glucose Yield

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Non-wood lignocellulosic biomass is a promising raw material that can be converted into glucose for cellulosic ethanol production. Since pretreatment could improve the enzymatic saccharification of lignocellulosic biomass, a comparison of enzymatic hydrolysability among different pretreated non-wood biomasses was presented in this study. Results showed that although the non-wood biomasses, namely kapok, EFB and kenaf core fibres were treated by same pretreatment condition, the enzymatic hydrolysability of these pretreated biomasses was different. This was highly related to the changes of chemical composition in the biomasses after pretreatments. Moreover, the total glucose yield (TGY), which was calculated by multiplying the enzymatic saccharification yield with the solid recovery yield of the pretreated biomass, played an important role in evaluating the effectiveness of pretreatment on the enzymatic hydrolysability of biomass. Besides, TGY could indicate the total amount of glucose that could be attained from a basic weight of the untreated biomass. Hence, the study found that besides the enzymatic saccharification yield, the solid recovery yield was also essential for cellulosic ethanol study as it was affected by the loss of the cellulose during pretreatments.

Key Words
Non-wood lignocellulosic biomass, Pretreatment, Enzymatic hydrolysis, Chemical composition, Total glucose yield

1. Introduction

Other than the production of sugar- or starch-based ethanol, cellulose-based ethanol offers a great promise of replacing fossil fuels without causing the dispute between food and fuel supply. In general, cellulose can be derived from wood and non-wood lignocellulosic biomasses. Since non-wood lignocellulosic biomass is abundantly available, cheap and easy to process as well as consists of short growth and harvest periods, it is currently the most promising biomass feedstock for cellulosic ethanol production. Moreover, the cellulose content in non-wood lignocellulosic biomasses such as agricultural residues, native plants and non-wood plant fibres are about 32-56%, 15-50% and 43-96%, respectively, which are compatible or even surpass the cellulose content in wood 1).

Cellulose can be saccharified through enzymatic hydrolysis into glucose and then, fermented to ethanol. Although cellulose is the most abundant component in the plant cell wall, the enzymatic hydrolysis of cellulose is usually hindered by the cross-links among cellulose and other components (hemicellulose and lignin). Therefore, introduction of pretreatment on biomasses prior to the enzymatic hydrolysis is strongly proposed by many researchers to increase the accessibility of enzyme to the cellulose and thus, enhance the hydrolysability of biomass 2) 3). Previous studies mostly focus on the effects of pretreatment parameters on the enzymatic hydrolysability of biomass without further identifying and discussing its effects on the chemical properties of the biomass 4) ~ 6). Thus, the fundamental reason that affected the enzymatic hydrolysability of biomass actually has not been clearly reported. Moreover, the saccharification yield (on the basis of the oven dry weight of pretreated biomass) of the...
enzymatic hydrolysis was mostly used as the only response to indicate the best pretreatment \(^6\) – \(^8\). However, these data obtained cannot reflect the exact glucose yield on the basis of the oven dry weight of the original biomass (total glucose yield) directly. Hence, in this study, the pretreatment performance was evaluated based on total glucose yield, furthermore the effects of pretreatment on the total glucose yield in relation to the changes of chemical composition in the biomass was also investigated.

Three non-wood lignocellulosic biomasses, namely kapok (Ceiba pentandra), oil palm (Elaeis guineensis) empty fruit bunch (EFB) and kenaf (Hibiscus cannabinus) core fibres were chosen in this study due to their distinct physical structures, wherein the kapok fibre is in a tubular structure covered with waxy surface, EFB fibre is in the form of the vascular strand, and the kenaf core fibre structure is analogous to that of hardwood. Nevertheless, their chemical compositions are indeed similar, ranging from 51-56\% cellulose, 44-49\% hemicellulose and 13-20\% lignin \(^9\) \(^10\). Previous studies reported individually that the enzymatic hydrolysability of these non-wood lignocellulosic biomasses are increased remarkably by adopting merely water, acid and alkali pretreatments, even though only parts of the recalcitrant polymers (lignin and hemicellulose) have been removed \(^9\) \(^10\). In this study, the enzymatic hydrolysability of these three biomasses is compared and discussed comprehensively based on the effects of pretreatments on each biomass’s chemical composition.

2. Experimental

2.1 Biomass feedstock

Kapok, oil palm EFB and kenaf core fibres were used in this study. Kapok fibre was obtained from Kelantan, Malaysia. The fibres were manually separated from the seed pod and used as received. EFB fibre in loose strand form was obtained from Eco Fibre Sdn. Bhd., Johor, Malaysia. The EFB fibrous strand was washed and then, refined to size of about 2-4 cm by Andritz Sprout Bauer refiner. The kenaf core of variety 36 (V3) with the length of 3-6 mm was provided by National Kenaf and Tobacco Board (NKTB), Malaysia. The chemical compositions of these non-wood biomasses are shown in Table 1 \(^9\) \(^10\).

| Table 2 Pretreatment conditions for kapok, EFB and kenaf core fibres |
|-----------------|----------|---------|-------|
| Pretreatment    | Temperature (°C) | Time (min) | Agent (% v/v) |
| Water           | 150      | 45      | -     |
| Acid            | 120      | 45      | 1.0 H₂SO₄ |
| Alkali          | 120      | 45      | 1.0 NaOH |

2.2 Pretreatment

Pretreatment was conducted in a 4-L stationary stainless steel digester (NAC Autoclave Co. Ltd., Japan), which was fitted with a thermouple temperature controller probe sensor. Water, acid and alkali pretreatment of kapok, EFB and kenaf core fibres were employed in different pretreatment conditions as shown in Table 2. The liquor-to-material ratio was fixed at 12:1. Upon completion of the pretreatment, the pretreated biomasses were washed with tap water until neutral, and spin-dried before stored in the fridge for further use and analysis. The solid recovery yields of these pretreated biomasses were calculated based on oven-dried fibre weight [% of kg-pretreated/kg-untreated].

2.3 Enzymatic hydrolysis

The enzyme was Celluclast 1.5 L (Novozymes A/S Denmark) with the activity of 70 FPU/ml \(^8\). Enzymatic hydrolysis of pretreated biomasses were carried out in 250 ml Erlenmeyer flasks, containing 2.5\% w/v, dry weight substrate in 0.05 M citrate buffer, pH 4.8 and followed by cellulase loading of 70 FPU/g biomass. Then, the flasks were sealed tightly and incubated at 50 °C for 48 h in a shaker incubator at 100 rpm. On completion of incubation, all the samples were boiled for 10 min to stop

| Table 1 Chemical composition of kapok, EFB and kenaf core fibres\(^9\) \(^10\) |
|-----------------|-----------------|-----------------|
| Holocellulose [kg/kg-extractive free biomass] | Kapok fibre | EFB fibre | Kenaf core fibre |
| α-cellulose [kg/kg-holocellulose] | 0.043 ± 0.032 | 0.881 ± 0.01 | 0.818 ± 0.007 |
| β-cellulose [kg/kg-holocellulose] | 0.019 ± 0.006 | <0.001 ± 0.0 | 0.019 ± 0.004 |
| γ-cellulose [kg/kg-holocellulose] | 0.493 ± 0.011 | 0.44 ± 0.002 | 0.472 ± 0.009 |
| Klason lignin [kg/kg-extractive free biomass] | 0.134 ± 0.004 | 0.151 ± 0.001 | 0.203 ± 0.002 |

Table 1 Chemical composition of kapok, EFB and kenaf core fibres\(^9\) \(^10\)

Carbohydrates:

| Glucose [kg/kg-holocellulose] | Kapok fibre | EFB fibre | Kenaf core fibre |
|-------------------------------|-------------|-----------|------------------|
| Xylose [kg/kg-holocellulose]  | 0.388 ± 0.0  | 0.389 ± 0.007 | 0.266 ± 0.006 |
| Others sugar\(^a\) [kg/kg-holocellulose] | 0.065 ± 0.002 | 0.049 ± 0.001 | 0.079 ± 0.004 |

\(^a\) Others sugar: arabinose, mannose, galactose
the enzymatic reaction and subsequently, centrifuged at 39.2 km/s² (4000 G) for 10 min to remove the unhydrolyzed residues. The supernatant (reducing sugar in hydrolyzates) was stored frozen (-4 °C) until used for sugar analysis by High Performance Liquid Chromatography (HPLC).

2.4 Chemical composition analysis

The air-dried pretreated non-wood biomasses (kapok, EFB and kenaf core fibres) were milled into small size (< 2.0 mm) by IKA® MF10 basic microfine grinder drive for chemical composition analysis. The chemical composition analyses were performed as previously described in Tye et al. Kloss lignin content (TAPPI 222 cm-02), holocellulose content (Wise et al., 1946), cellulose (alpha and beta cellulose) and hemicellulose (gamma-cellulose) contents (Japanese Standard Method JIS 8101) as well as Carbohydrate content (TAPPI 249 cm-00) were used for carbohydrate analysis by gas chromatography (GC). The GC was equipped with a flame ionization detector (FID). A 30 m × 0.25 mm × 0.25 μm DB-225 column (J&W Scientific, Folsom, CA, USA) was used and the GC oven was programmed at 220 °C with a holding time of 30 min. Helium was used as carrier gas with flow rate of 25 ml/min, split ratio of 50:1, and sample size injected was 1 μl.

Reducing sugar in the enzymatic hydrolyzates was determined by HPLC. An Agilent Tech. 385-ELSD of HPLC system was equipped with a 30 mm × 7.7 mm Hi-Plex Ca column. Distilled-deionised water was used as the eluent with a flow rate of 0.6 ml/min. After the supernatant was filtered using a 0.22 μm syringe filter, 20 μl of the sample was injected. The glucose content was calculated according to calibration curve of standard glucose solutions.

3. Results and Discussion

3.1 Effects of water pretreatment

The chemical composition and enzymatic hydrolysis of water pretreated biomasses are summarized in Table 3 and Fig. 1. After water pretreatment, the total glucose yields of all the biomasses were different. Water pretreated EFB fibre attained the highest total glucose yield (24.9%) followed by kapok fibre (18.2%) and kenaf core fibre (3.4%). Since water pretreatment was using water alone as

| Table 3 Water pretreated kapok, EFB and kenaf core fibres |
|---------------------------------|-----------------|-----------------|
| Liquor pH | 4.11 | 3.61 | 4.52 |
| Solid recovery yield          | [kg/pretreated/kg/untreated] | a | 0.775 ± 0.039 | 0.497 ± 0.018 | 0.657 ± 0.015 |
| Holocellulose [kg/kg-pretreated] | b | 0.815 ± 0.017 | 0.711 ± 0.015 | 0.640 ± 0.006 |
| α-cellulose [kg/kg-holocellulose] | c | 0.690 ± 0.002 | 0.798 ± 0.006 | 0.809 ± 0.006 |
| β-cellulose [kg/kg-holocellulose] | d | 0.02 ± 0.0004 | 0.015 ± 0.001 | 0.027 ± 0.00 |
| γ-cellulose [kg/kg-holocellulose] | e | 0.35 ± 0.0 | 0.187 ± 0.005 | 0.164 ± 0.006 |
| Kloss lignin [kg/kg-pretreated] | f | 0.173 ± 0.022 | 0.162 ± 0.013 | 0.401 ± 0.009 |
| [kg/kg-untreated] | g | 0.134 ± 0.017 | 0.08 ± 0.006 | 0.263 ± 0.006 |
| Carbohydrates (GC analysis): |
| Glucose [kg/kg-holocellulose] | h | 0.739 ± 0.007 | 0.813 ± 0.007 | 0.848 ± 0.005 |
| Xylose [kg/kg-holocellulose] | i | 0.222 ± 0.002 | 0.137 ± 0.002 | 0.113 ± 0.002 |
| Others sugar a [kg/kg-holocellulose] | j | 0.04 ± 0.005 | 0.049 ± 0.005 | 0.039 ± 0.007 |
| Calculated Total Glucose Content b [kg/kg-pretreated] | k | 0.602 ± 0.018 | 0.578 ± 0.017 | 0.543 ± 0.009 |
| Recovered Glucose Content c [kg/kg-untreated] | l | 0.467 ± 0.012 | 0.287 ± 0.019 | 0.357 ± 0.014 |
| Enzymatic hydrolysis: |
| Theoretical glucose concentration d [g/L] | m | 15.1 ± 0.5 | 14.5 ± 0.5 | 13.6 ± 0.2 |
| Glucose yield (HPLC analysis) [g/L] | n | 5.9 ± 0.1 | 12.5 ± 1.3 | 13 ± 0.0 |
| Glucose yield based on: |
| [kg/kg-(glucose in pretreated)] e | o | 0.391 ± 0.004 | 0.862 ± 0.062 | 0.096 ± 0.001 |
| [kg/kg-pretreated] f | p | 0.233 ± 0.004 | 0.500 ± 0.052 | 0.052 ± 0.0 |
| [kg/kg-untreated] g | q | 0.182 ± 0.003 | 0.249 ± 0.026 | 0.034 ± 0.0 |

a Others sugar: arabinose, mannose, galactose
b Glucose content (GC test) × Holocellulose
c Calculated total glucose content × Solid recovery yield
d Initial sample concentration (25g/L) × Calculated total glucose content
e = Theoretical glucose yield = Glucose yield (g/L) / Theoretical glucose concentration (g/L) × 100
f = Enzymatic saccharification yield = glucose yielded (g/L) / Initial sample concentration (25 g/L)
g = Total glucose yield = Enzymatic saccharification yield × solid recovery yield
medium for pre-hydrolysis reaction, the changes of fibre chemical composition were mainly due to the removal of hemicellulose content \(^1\).

Although pretreated kenaf core fibre showed a remarkably hemicellulose (\(\gamma\)-cellulose) removal (about 82.2% of hemicellulose removal), its enzymatic hydrolysability was the lowest (Tables 1 and 3). This phenomenon was possibly due to the high lignin content in the remaining fibre. The increase of lignin content could be revealed by the depolymerization and condensation of dissolved lignin during pretreatment. In general, lignin could be depolymerized through the homolytic cleavage of the \(\alpha\)-O-bond and \(\beta\)-O-bond of lignin in an acidic condition. However, when an elevated reaction temperature and prolonged reaction time were applied, the condensed fragments of dissolved lignin in the liquor would precipitate onto the fibre \(^1\). In comparison to pretreated kenaf core fibre, it was found that there was only a slight elimination of hemicellulose (or xylose) for pretreated kapok fibre, which was about 52.5% (or 56.1%) (Tables 1 and 3). However, the total glucose yield of the latter was relatively higher than that of the former. This result was probably due to the low lignin content remaining in the pretreated kapok fibre. It was believed that the residual lignin in the fibre absorbed the enzyme and resulted in the reduction of enzyme activity during hydrolysis \(^1\). Hence, the high lignin amount remaining in the kenaf core fiber caused a limitation in the improvement of fibre hydrolysability.

In contrast, pretreated EFB fibre showed the best enzymatic hydrolysability among the three biomasses, which was most possibly due to the large removal of both amorphous (hemicellulose and lignin) fractions from the fibre. From Tables 1 and 3, about 83.0% and 47.0% of hemicellulose and lignin have been eliminated. Although EFB fibre could attain up to 86% of cellulose-to-glucose conversion yield (theoretical glucose yield), it resulted in the most severe cellulose degradation and dissolution during water pretreatment, wherein its recovered glucose content (total cellulose recovered after pretreatment which calculated on the basis of untreated biomass) was the least (28.7%) among the water pretreated biomasses.

### 3.2 Effects of acid pretreatment

For acid pretreatment, kapok fibre attained the highest total glucose yield (31.0%), followed by EFB fibre (26.0%) and kenaf core fibre (1.3%) (Table 4). Similar to the water pretreatment, acid pretreatment also mainly hydrolyzed the hemicellulose (or xylose) fraction of the fibre. According to the results presented in Table 4, pretreated kenaf core fibres achieved the lowest total glucose yield, even though it presented the ability of high hemicellulose elimination. Therefore, it was believed that the substantially high lignin content remained in the kenaf core fibre due to the depolymerization and condensation of dissolved lignin during acid pretreatment had restricted the enzymatic hydrolysis, as discussed earlier.

Although pretreated EFB fibre presented a higher hemicellulose and lignin removal than the pretreated kapok fibre did, the total glucose yield of the former was about 5% lower (Table 4). This phenomenon was due to the loss...
Table 4 Acid pretreated kapok, EFB and kenaf core fibres

|                | Kapok fibre | EFB fibre | Kenaf core fibre |
|----------------|-------------|-----------|-----------------|
| Liquor pH      | 0.77        | 0.81      | 0.86            |
| Solid recovery yield | [kg-pretreated/kg-untreated] | a 0.800±0.029 | 0.623±0.018 | 0.663±0.030 |
| Chemical composition |             |           |                 |
| Holocellulose  | [kg/kg-pretreated] | b 0.810±0.008 | 0.703±0.017 | 0.634±0.009 |
| α-cellulose    | [kg/kg-holocellulose] | c 0.704±0.012 | 0.887±0.007 | 0.776±0.009 |
| β-cellulose    | [kg/kg-holocellulose] | d 0.141±0.025 | <0.001±0.0  | 0.041±0.004 |
| γ-cellulose    | [kg/kg-holocellulose] | e 0.156±0.013 | 0.113±0.006 | 0.183±0.006 |
| Klasson Lignin | [kg/kg-pretreated] | f 0.151±0.021 | 0.220±0.001 | 0.402±0.021 |
| Carbohydrates (GC analysis): |             |           |                 |
| Glucose        | [kg/kg-holocellulose] | h 0.843±0.005 | 0.883±0.003 | 0.854±0.007 |
| Xylose         | [kg/kg-holocellulose] | i 0.144±±0.0 | 0.096±0.002 | 0.085±0.001 |
| Others sugar   | [kg/kg-holocellulose] | j 0.018±0.0003 | 0.021±0.001 | 0.061±0.006 |
| Calculated Total Glucose Content | [kg/kg-pretreated] | k 0.683±0.011 | 0.621±0.017 | 0.541±0.032 |
| Recovered Glucose Content | [kg/kg-untreated] | l 0.546±0.029 | 0.387±0.022 | 0.359±0.024 |
| Enzymatic hydrolysis |      |           |                 |
| Theoretical glucose concentration | [g/L] | m 171±0.3 | 15.5±0.4 | 13.5±0.3 |
| Glucose yield (HPLC analysis) | [g/L] | n 9.7±0.6 | 10.4±0.7 | 0.5±0.0 |
| Glucose yield based on | [kg/kg-(glucose in pretreated)]* | o 0.508±0.026 | 0.671±0.029 | 0.037±0.001 |
|                     | [kg/kg-pretreated] | p 0.383±0.024 | 0.416±0.028 | 0.020±0.001 |
|                     | [kg/kg-untreated] | q 0.310±0.019 | 0.260±0.018 | 0.013±0.001 |

* a, b, c, d, e, f, g = Table 3

of cellulose during acid pretreatment of EFB fibre and thus, lowering the recovered glucose content (38.7%). Since a decrease of solid recovery yield (62.3%), reflecting in the dissolution of cellulose was observed from the pretreated EFB fibre, a lower total glucose yield was expected, even though EFB fibre showed better enzymatic saccharification than kapok fibre (Table 4). As a result, biomass with high enzymatic saccharification yield was not adequate to indicate the effectiveness of the pretreatment as part of the cellulose might be degraded and eliminated together with the spent liquor during pretreatment. Hence, in order to obtain a high total glucose yield, a high enzymatic saccharification yield was not necessary.

### 3.3 Effects of alkali pretreatment

Among the alkali pretreated biomasses, kapok fibre showed the highest total glucose yield (33.0%), followed by EFB fibre (26.9%) and kenaf core fibre (11.3%). In comparison to water and acid pretreatments, alkali pretreatment was more effective for lignin solubilisation. Based on the results (Table 1 and Table 5), pretreated kenaf core fibre exhibited the relatively higher removal of hemicellulose content (64.4%) than the other two fibres. Moreover, pretreated kenaf core fibre was also capable of removing lignin, in which it removed about 28.1% of the lignin content. Surprisingly, it showed the lowest enzymatic saccharification yield as well as total glucose yield in this study. Therefore, it was believed that in addition to the changes of chemical composition, the changes in the physical structure of the fibres might be one of the factors that affected the enzymatic accessibility and hydrolysability of fibres. Hence, further study was required.

It could be seen that pretreated kapok and EFB fibre were capable of removing about 56.8% and 25.8% of hemicellulose, respectively. However, the former did not exhibit good lignin removal compared to the latter; wherein the amounts of lignin removal were about 8.2% and 63.6%, respectively (Tables 1 and 5). Although alkali pretreatment could remove both hemicellulose (the most) and lignin (the least) from the kapok fibre, the pretreated kapok fiber attained a low theoretical glucose yield (77.4%) in comparison to EFB fiber (90.0%) (Table 5). Thus, this finding indicated that the removal of lignin was more important than the removal of hemicellulose for an alkali pretreatment to enhance the enzymatic accessibility and hydrolysability of fibre.

Nevertheless, the total glucose yield of the alkali pretreated EFB fibre was lower than that of the similarly pretreated kapok fibre (Table 5). This phenomenon was due to higher cellulose degradation and dissolution during alkali pretreatment of EFB fibres as indicated by recovered glucose content), which ended up with lower solid recovery yield (67.9%). Hence, besides enzymatic saccharification yield, solid recovery yield of pretreatment was also one of the crucial...
Although the chemical compositions of these non-wood biomasses were indeed similar, the effects of pretreatment on these different biomasses were varied. Regardless of water, acid or alkali pretreatments, even the same pretreatment condition caused the chemical composition of the pretreated biomasses to change differently. Consequently, their enzymatic hydrolysability and total glucose yield were also distinct. Since the original untreated biomasses were basically chemically similar, the different pretreatment effects might be due to the distinct physical structures of biomasses. Thus, further investigations of these biomasses are needed. Furthermore, to obtain a high total glucose yield, besides achieving a high enzymatic saccharification yield, the biomasses must also exhibit negligible cellulose degradation.

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4. Conclusion
Although the chemical compositions of these non-wood biomasses were indeed similar, the effects of pretreatment on these different biomasses were varied. Regardless of water, acid or alkali pretreatments, even the same pretreatment condition caused the chemical composition of the pretreated biomasses to change differently. Consequently, their enzymatic hydrolysability and total glucose yield were also distinct. Since the original untreated biomasses were basically chemically similar, the different pretreatment effects might be due to the distinct physical structures of biomasses. Thus, further investigations of these biomasses are needed. Furthermore, to obtain a high total glucose yield, besides achieving a high enzymatic saccharification yield, the biomasses must also exhibit negligible cellulose degradation.

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