STUDY ON COMPOSITION, STRUCTURAL AND PROPERTY CHANGES OF OIL PALM FROND BIOMASS UNDER DIFFERENT PRETREATMENTS

LONG WEE LAI[*], MARINI IBRAHIM[*], NASRUDIN MD RAHIM[*], EMI FAZLINA HASHIM[*], MOHD ZAINI YA'COB[*], ANI IDRIS[**] and JUNAID AKHTAR[**]

[*]*Faculty of Engineering and Life Sciences, Universiti Selangor, Bestari Jaya 45600, Malaysia*
[**]*Faculty of Chemical and Energy Engineering, c/o Institute of Bioproducts Development, Universiti Teknologi Malaysia, Skudai 81310, Malaysia*
✉ *Corresponding authors: Long Wee Lai, zki@unisel.edu.my; Ani Idris, ani@cheme.utm.my*

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Two pretreatment methods were used to treat oil palm frond (OPF) biomass. The first was a one-step pretreatment by the alkali-autoclave-chemical (AAC) method, whereas the second was a two-step pretreatment, consisting in the combination of the alkali-autoclave-chemical process and the microwave-alkali (Mw-A) pretreatment. After the pretreatments, the chemical composition of the samples (cellulose, hemicellulose and lignin) was analyzed. Field electron scanning electron microscopy (FESEM) and Fourier transform infrared (FTIR) analyses were performed so as to examine the morphology of raw and treated samples. The crystallinity index of cellulose was also measured using X-ray diffraction (XRD). The results revealed a huge degree of reduction in lignin, up to 93% for the treated OPF sample. Both one- and two-step pretreatments were capable of disrupting the OPF structure efficiently and each pretreatment resulted in a significant difference in composition.

Keywords: lignocellulose pretreatment, alkali-autoclave-chemical, microwave-alkali, oil palm frond, structure characterization, composition determination

INTRODCUTION

Every year, the oil palm industries in Malaysia generate huge amounts of biomass wastes, which include palm kernel shells (PKSs), oil palm empty fruit bunches (OPEFBs), oil palm fronds (OPFs), oil palm trunks (OPTs), which amount to approximately 59 million tonnes.[1] Considering that Malaysia is the world's second largest oil palm producer, with plantation areas amounting to 5.077 x 10^6 hectares, a good biomass waste management is pivotal as these renewable agricultural biomass wastes could be converted to higher value added products using various biotechnology routes.[2] On the other hand, if this bulk biomass is not properly managed, its disposal will eventually cause environmental problems.[3]

Many research articles have reported that agricultural lignocellulosic biomass (including oil palm biomass) can serve as a potential feedstock not only for glucose, but also for other fermentable sugars, which are intermediates to industrially important compounds.[4] This is because the undisrupted lignocellulosic biomass contains high amounts of polymeric carbohydrates, comprising mainly cellulose, hemicellulose and lignin. In order to produce glucose from ligno-biomass, two processes are necessary and inevitable: i. pretreatment to disrupt the complex cellulose-hemicellulose-lignin structure and make cellulose more accessible to enzymes; ii. enzymatic hydrolysis so as to convert the released cellulose into glucose. Lignocellulosic cellulose is embedded in the complex lignocellulosic matrix, which makes its recovery difficult. Besides, its recovery is also impeded by factors such as composition, physical and chemical structure.[5] The presence of lignin and hemicellulose in the lignocellulosic structure contributes to the strength of plant cell walls and also prevents enzymatic degradation of cellulose.[6] Therefore, the pretreatment methods are important as they can maximize cellulose recovery from lignocellulosic materials. Apparently, an

effective pretreatment method can disrupt lignocellulosic materials efficiently with minimum cost (both operating and capital costs), requiring a minimum pre-pretreatment (preparation/handling) step and leading to maximum recovery of all lignocellulosic constituents in a usable form.[7]

Recently, microwave heating has been reported in the pretreatment of lignocellulosic biomass.[8-12] A combination of acid pretreatment with microwave heating was performed on sugarcane bagasse,[8] oil palm trunks[9] and birch wood.[10] In another study, Hamzah *et al*. combined the alkali pretreatment with microwave heating for the treatment of OPEFB.[11] This method has been also used in the pretreatment of OPT and OPF biomass.[6,13] During microwave heating, the microwaves directly heat the biomass by molecular rotation and vibration, thus producing rapid energy-efficient heating.[14] Studies also reported that microwave irradiation enhanced the organic heating reaction by altering the ultrastructure of cellulose, degrading the lignin and hemicellulose, and eventually increased the susceptibility of lignocellulosic biomass.

The autoclave method has been also employed in many pretreatments of lignocellulosic biomass and it is commonly performed at specific conditions of 121 °C, 0.12 MPa and 15 min. Such harsh conditions are able to unwind the encrusted lignocellulosic matrix. In addition, like the microwave pretreatment, the autoclave method is conducted together with some chemical solutions (alkali, acid or water), so as to intensify the efficiency of the reaction process. Some examples include the use of 1% dilute sulfuric acid on corncob biomass and 3.6% sulfuric acid on corn fiber during autoclaving.[15,16] Saleh *et al*. autohydrolysed OPF fibers using autoclave conditions to facilitate the separation of hemicelluloses, whereas Shamsudin *et al*. performed the steam pretreatment on OPEFB to enhance its digestibility for sugars production.[17,18]

In this work, the AAC and Mw-A pretreatments, which are chemical pretreatment methods, were carried out with a view to use the treated OPF as substrate for simultaneous saccharification and fermentation (SSF). The acid/Mw-acid treatment was avoided because when the acid solution together with the biomass are heated to a high temperature, the free sugars (xylose) released under such conditions degrade to furfural, and glucose to hydroxymethyl furfural (HMF). Both compounds are known to be inhibitors to cell metabolism and may have detrimental effects on the simultaneous saccharification and fermentation of the pretreated biomass.[19]

Previous studies on oil palm lignocellulosic biomass have shown that different pretreatment methods resulted in different cellulose and hemicellulose recovery and lignin removal.[6,20] The OPEFB biomass was pretreated using sequentially a two-step pretreatment (Mw-A + AAC), as described by Hamzah *et al*.,[20] and a one-step pretreatment (Mw-A) for OPF biomass.[6] The research work performed on OPF using one-step AAC or a combination of two steps AAC + Mw-A is rather limited. Hence, in this present study, OPF was treated using the one-step and consequently the two-step pretreatment in order to reveal the influence of these pretreatment processes on the chemical composition, structure and morphology of OPF biomass. Apart from that, the results of this investigation can contribute to providing a complementary research database and a deeper insight into the outcomes of both one-step and two-step sequential pretreatment processes.

EXPERIMENTAL

Raw material

The oil palm frond (OPF) samples were obtained from FELDA palm oil plantation, Tenggaroh, Johor, Malaysia. The 3-4 inches sliced fibers were initially washed using tap water to remove unwanted dirt. They were then dried under sunlight, prior to grinding using a disk mill (FFC-15, China). The ground biomass samples were further sieved using a Restuch sieve shaker (AS 200 basis, Germany) so as to ensure particle sizes below 1.0 mm. A small particle size ensures an increased OPF surface area, thus favoring enzyme digestibility in subsequent experiments.

Chemical reagents

The chemical reagents used were of analytical grade. Acetic acid (CH_3COOH), sodium hydroxide (NaOH), potassium bromide (KBr) and sodium hypochlorite (NaClO) solution were obtained from Merck (M) Sdn. Bhd.

Alkali-autoclave-chemical (AAC) pretreatment

An amount of 200 ml of 2.5 M NaOH was poured into a beaker containing 20.0 g of oil palm frond biomass. The mixture was then placed in an autoclave (ALP, CL-32LDP, Japan). The autoclave conditions were set to 121 °C and 0.12 MPa for a

period of 15 min. Upon completion, the slurry was cooled to room temperature and then filtered.[21] The autoclave filtrate was washed with tap water (4 x 1000 ml) followed by distilled water (4 x 500 ml). The second step was performed by adding 200 ml of fresh NaClO into the autoclave treated biomass. The pH of the solution was measured using a bench top pH meter (Thermo-Scientific Orion 2-star, Singapore) and adjusted to an acidic level (pH 3.5) using CH_3COOH. The solution was then filtered-off and the acid-treated biomass was further washed using tap water (4 x 1000 ml) followed by distilled water (4 x 5000 ml). The AAC-treated oil palm biomass was then dried in an oven at 70 °C and kept for subsequent analysis.

Alkali-autoclave-chemical (AAC) pretreatment followed by microwave-alkali (Mw-A) pretreatment

The OPF biomass was first subjected to the alkali-autoclave-chemical (AAC) treatment, as mentioned in the previous section. The NaOH was added to the AAC-treated biomass in the ratio of 10:1 and then heated in the microwave. The SINEO's microwave (MAS-II, China) was used to conduct the Mw-A pretreatment under normal atmosphere pressure. The conditions were set as follows: temperature, 80 °C; microwave power, 700 W and duration, 60 min.[11,20] Once the microwave heating was completed, the NaOH solution was discarded and the slurry was washed using tap water (4 x 1000 ml) followed by distilled water (4 x 500 ml). The AAC + Mw-A treated OPF was dried in the oven prior to subsequent analysis.

Compositional analysis

The compositional analyses for AAC and AAC + Mw-A treated OPF are described as follows. The verification procedure was performed in duplicate. In order to ensure the reproducibility of the results, the relative percentage difference (RPD) was set to less than 5%, revealing that the fiber analysis was reliable.[22] The Soxhlet extraction process was performed to produce the extractive fraction of the OPF sample.[23] The extracted sample was used to determine Klason's lignin.[24] The holocellulose content was quantified as the sodium chlorite delignified residue.[25] The TAPPI test method in a slightly modified version was employed to determine α-cellulose content.[26] Meanwhile, the hemicellulose amount of the OPF sample was calculated as the difference between holocellulose and cellulose.[25]

Surface analysis using FESEM

The physical variation in AAC and AAC + Mw-A treated OPF biomass was captured under FESEM. Images of the internal and external surfaces were taken at a magnification of 500X (JSM-6071F, Jeol USA Inc.). The specimens were mounted on aluminum stubs using double sided adhesive tapes and sputter coated with a thin layer of gold powder at 200 Å before analysis and then observed using a voltage of 5.0 kV.

Spectroscopy measurement by FTIR

The changes in the treated samples as regards functional groups were determined using FTIR spectroscopy. The spectra were recorded in the range of 4000-370 cm^{-1} (Pelkin Elmer 2000, USA). Pellet discs were prepared by mixing dried biomass sample with KBr spectroscopic grade salt in a granite mortar. The pellet (10 mm diameter x 1 mm thickness) was then pressed at 1 MPa for about 10 min before reading the spectra.

Crystallinity index measurement by XRD

A Rigaku X-ray Diffractometer (D/Max 2500, Japan) was used to test and measure the crystalline index of AAC and AAC + Mw-A treated samples. Before the measurement, the sample was packed tightly in a rectangular glass cell with a dimension of 15 x 10 mm and thickness of 1.5 mm. X-ray beams at 40 kV and 30 mA were then charged to the sample, using Cu Kα radiation (λ = 1.54184 Å), a grade range between 5-40° and a step size of 0.05°.

The crystallinity of cellulose was calculated according to the empirical method proposed by Segal *et al*., as shown in Equation 1:[27]

$$\text{CrI, \%} = [(I_{002} - I_{18^\circ}) / I_{002}] \times 100 \quad (1)$$

where CrI is the crystalline index, I_{002} is the maximum intensity of the (002) lattice diffraction and I_{18° is the intensity diffraction at 18°, 2Θ degree.

RESULTS AND DISCUSSION

Chemical composition analysis

Table 1 shows the compositions of the various constituents in treated OPF. These pretreatment methods (AAC and AAC + Mw-A) have a significant effect on the composition of the treated oil palm frond samples. The cellulose content in AAC + Mw-A treated OPF was slightly increased, *i.e.* 2.34% or equivalent to 42.86 g/100 g biomass; while for OPF biomass treated by AAC pretreatment a lower value was recorded – of 32.40 g/100 g biomass, which is 22.64% less than for the untreated one.

In our previous work, when the OPF was microwave-alkali treated (Mw-A), the cellulose content increased to 64.42%.[6] This could indicate that the Mw-A method was more efficient in releasing cellulose, compared to the AAC and AAC + Mw-A pretreatment. In the presence of the 2.5 M NaOH solution under microwave irradiation, the biomass was heated

rapidly due to the oscillation in the molecules. Such rapid oscillations can disrupt the inter- and intra-molecular hydrogen bonds embedded within the matrix of lignin and hemicellulose, thus releasing cellulose. Similar findings were reported for Mw-A treatment on oil palm empty fruit bunch (OPEFB), where the amount of cellulose was significantly enhanced.[11] Other researchers also obtained similar results when treating lignocellulosic biomass using microwave heating, where the microwave-pretreated biomass showed a higher content of cellulose than the untreated one.[5,28]

The hemicellulose content in AAC-treated and AAC + Mw-A treated OPF was of 63.29 and 54.54 g/100 g biomass, respectively, when compared to Mw-A treated (11.84 g/100 g biomass) or untreated (33.61 g/100 g biomass) samples. The results revealed that different pretreatment methods resulted in samples with different chemical composition. Mw-A pretreatment yields higher cellulose content, whereas the AAC and AAC + Mw-A pretreatments yield higher hemicellulose content.

The significant reduction of hemicellulose content may be attributed to the ability of microwave irradiation to depolymerize the hetero-polysaccharides sugar building blocks into oligosaccharides.[6] The microwave heating transfers and induces heat directly to the lignocellulosic biomass, such as OPF and eventually disrupts the structure of the hetero-polysaccharides sugar building blocks of hemicellulose.[29] Unlike the Mw-A pretreatment, the AAC and AAC + Mw-A methods resulted in higher hemicellulose content, which is unlikely to be a problem when using the treated biomass as a substrate for simultaneous saccharification and fermentation, as Xu *et al*. reported that a higher level of hemicellulose in NaOH and sulfuric acid treated *Miscanthus* had no effect on the lignocellulosic crystallinity, but significantly enhanced biomass digestibility.[30] Hence, the AAC and AAC + Mw-A treated OPF samples with higher hemicellulose content should neither present resistance nor impede the subsequent enzymatic experiment.

The lignin content was also examined after the pretreatments. In AAC and AAC + Mw-A pretreatments, the treated OPF samples contained (g/100 g biomass): 1.51 and 0.90 lignin, respectively. There was a high reduction in the lignin content, at least of 92.69-95.64% for both AAC and AAC + Mw-A pretreated samples, whilst the Mw-A treated samples experienced only a 17.97% reduction, compared to the untreated ones. The AAC and AAC + Mw-A pretreatments apparently achieved higher lignin removal due to the higher autoclave temperature and pressure, plus the sudden change of pH (from alkaline to acidic). Meanwhile the Mw-A treated OPF biomass was only exposed to 80 °C microwave heating at normal atmosphere pressure. Hence, more lignin was eliminated in the case of AAC and AAC + Mw-A pretreatments. Thus, when lignin needs to be removed effectively, the two sequential steps: AAC and AAC + Mw-A pretreatment could be used since it allowed the removal of most of the lignin, and the absence of lignin in the treated samples would eventually improve the enzyme hydrolysis rate. This is because lignin shields the cellulose chains and adsorbs the enzymes and its presence is a major obstacle for efficient hydrolysis.[31]

Surface analysis by Field Emission Scanning Electron Microscopy (FESEM)

The FESEM images of one-step AAC and two-step AAC + Mw-A treated OPF are presented in Figure 1 (a-d). It is observed that the OPF biomass samples treated using AAC and AAC + Mw-A pretreatments have rough, bumpy and rugged surfaces (Figs. 1a and 1c).

Table 1
Chemical composition of raw and treated OPF biomass (g/100 g biomass)

Pretreatment	Cellulose	Hemicellulose	Lignin	Extractives
Raw[6]	41.88 ± 1.34	33.61 ± 1.67	20.65 ± 0.19	3.86 ± 0.20
Mw-A[6]	68.86 ± 1.15	11.84 ± 0.94	16.94 ± 1.53	2.36 ± 1.32
AAC	32.40 ± 3.05	63.29 ± 3.08	1.51 ± 0.00	2.80 ± 0.04
AAC + Mw-A	42.86 ± 0.48	54.54 ± 0.23	0.90 ± 0.10	1.70 ± 0.06

All measurements were duplicated and with the relative percentage difference below 5%[22]
Mw-A: Microwave-alkali pretreatment, AAC: Alkali-autoclave-chemical pretreatment[6,22]

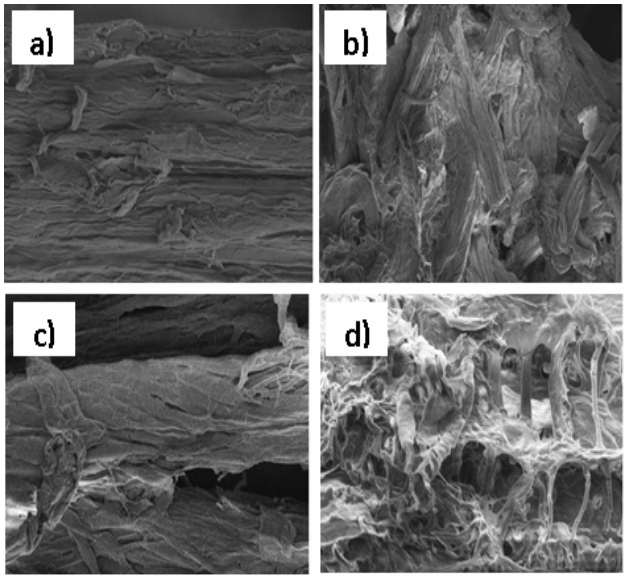

Figure 1: FESEM images at 500X magnification and 5.0 kV of treated oil palm frond: (a) AAC surface, (b) AAC internal structure, (c) AAC + Mw-A surface and (d) AAC + Mw-A internal structure (AAC: Alkali-autoclave-chemical pretreatment; Mw-A: Microwave-alkali pretreatment)

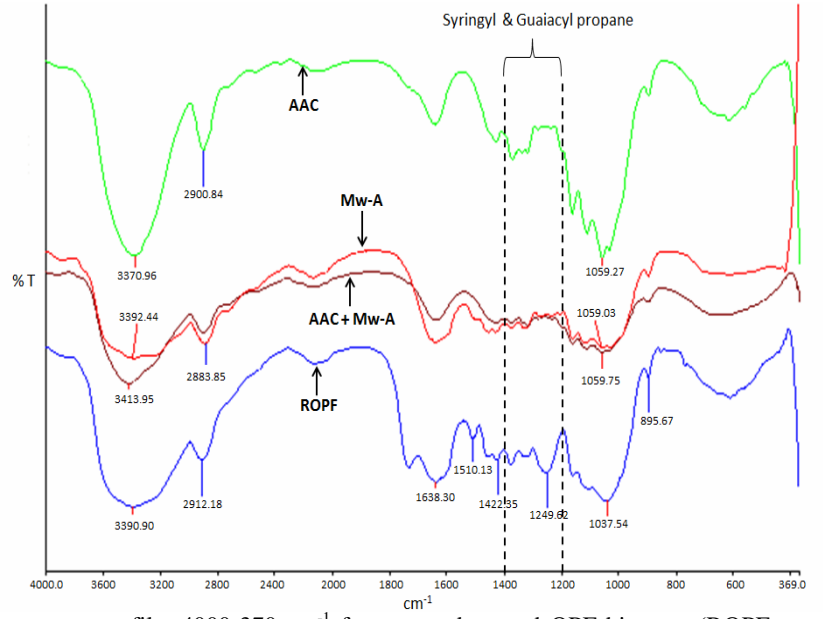

Figure 2: FTIR spectrum profile: 4000-370 cm^{-1} for raw and treated OPF biomass (ROPF: raw oil palm frond,[6] AAC: alkali-autoclave-chemical pretreatment, AAC + Mw-A: alkali-autoclave-chemical + microwave-alkali pretreatment, Mw-A: microwave-alkali pretreatment[6])

On the other hand, Figures 1b and 1d display cracks, tiny cavities, disorganized or irregular lignocellulosics in the internal structure. Figure 1d shows the shredded and skeletal internal structure of the two-step sequential AAC + Mw-A pretreatment, which depicts a higher disruption.

The results clearly reveal that the types of pretreatment used: AAC and AAC + Mw-A have a significant influence on the structure and composition of OPF. The degree of disruption of the oil palm lignocellulosic structure resulted in the modification of its composition and this

hypothesis is supported by the change in the chemical composition, as tabulated in Table 1.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to probe the chemical framework changes of raw and treated OPF biomass, as shown in Figure 2. The absorption band at 3413-3370 cm^{-1} represents the H-bond stretching of OH groups and that at 2912-2883 cm^{-1} refers to C-H expanding.[32] In general, the lignin building block can be discerned at frequencies within the region of 1509, 1464, 1422 cm^{-1}, as described in an earlier work.[33] In addition, the lignin derived from oil palm empty fruit bunch consists of syringyl propane (S) and guaiacyl propane (G) units, which contain one and two methoxy groups, respectively.[33] Based on Figure 3, the typical lignin behaviors are clearly observed in raw OPF biomass, particularly at 1422 cm^{-1} for syringyl propane unit and at 1249 cm^{-1} for guaiacyl propane units. The existence of guaiacyl in the lignin structure restricts fiber swelling and eventually impedes enzyme accessibility more so than syringyl. Hence, the disappearance of these bands for the treated OPF biomass samples represents the destruction of guaiacyl and syringyl propane units. The absorbencies at 1059-1037 cm^{-1} indicates disrupted crystalline region for raw and treated OPF samples. These bands illustrate the shattering of H-bonds in the treated samples.[34]

The raw OPF shows a sharp band at 895 cm^{-1}, which reflects the spectrum of cellulose attributed to the β-glycosidic linkage between the sugar units.[35] However, this band is not observed in all treated OPF samples, namely Mw-A, AAC and AAC + Mw-A. The subtle band in the treated samples might be due to the coverage of cellulose by hemicellulose or lignin.[8] Table 2 demonstrates the typical infrared band frequencies and FTIR spectra of wood components in units of wavenumber, cm^{-1}.[36,37]

Table 2
Typical infrared band frequencies and FTIR spectra of wood components

Infrared band,[36] cm^{-1}	Assignment of common functional groups	Infrared band,[37] cm^{-1}	Assignment of wood components
3500-3200	O-H (H-bond)		
2840-2690	C-H (aldehyde C-H)		
1740-1720	C=O (saturated aldehyde)	1735	C=O in xylans (hemicelluloses)
1695-1630	C=O, C=C	1647	Absorbed OH at conjugate C=O
1500-1450	C=C (in ring) (2 bands)	1505	Aromatic skeletal vibration in lignin
		1421	CH deformation in lignin and carbohydrate
1300-1000	C-O	1235	Syringyl ring and CO stretch in lignin and xylan
		1371	CH deformation in cellulose and hemicelluloses
		1319	CH vibration in cellulose, CO vibration in syringyl derivative
		1155	C-O-C vibration in cellulose and hemicellulose
		1030	C-O vibration in cellulose and hemicellulose
900	C-H	897	CH deformation in cellulose

Crystallinity index measurement by X-Ray Diffraction (XRD)

The X-ray diffraction profiles of AAC and AAC + Mw-A treated oil palm frond biomass are depicted in Figures 3a and 3b, respectively. Generally, the major diffraction peaks of cellulose occur within 2θ scale ranging between 22° and 23° as the primary peak, whereas the secondary peak is in the range of 16° to 18°.[38] Figure 3 (a-b) shows the tangible peaks within the mentioned ranges for both pretreatment samples, which confirm the presence of

amorphous and crystalline structure of the cellulose constituent. Liu *et al*. reported that the maximum intensity of the 002 lattice diffraction (I_{002} peak intensity) represents the primary peak and is categorized as the diffraction intensity of crystalline regions; whereas the secondary peak constitutes the diffraction intensity of the amorphous area.[39]

Figures 3a and 3b display a certain degree of narrowing exclusively of the primary peak, indicating a reduction in the crystallinity structure. The variation in the width of the crystalline peak reflects intensity transformation in the cellulose molecular hydrogen bonding. The hydrogen bonds in AAC and AAC + Mw-A treated samples were disrupted owing to autoclave heating, sudden change of pH and microwave heating. This can be explained by the rapid heating caused by microwave irradiation, which effectively enhanced the splitting effect of NaOH on the crystalline cellulose chain and maximized the conversion to the amorphous state.[39]

Table 3
Intensity and crystallinity index (%) of raw, treated OPF biomass

Material	Intensity, cps		Crystallinity index, %
	Primary peak, I_{002}	Secondary peak, I_{18}	
Raw[6]	2150	1350	37.21
Mw-A[6]	2631	1468	44.20
AAC	235	127	45.96
AAC + Mw-A	265	108	59.25

Mw-A: Microwave-alkali pretreatment, AAC: Alkali-autoclave-chemical pretreatment

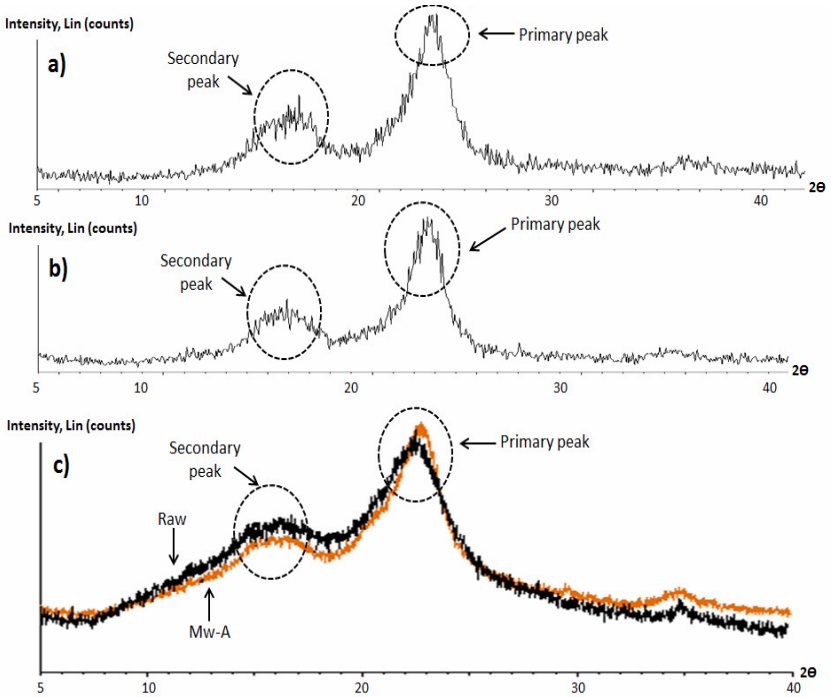

Figure 3: XRD pattern within 2Θ scale ranging from 5 to 40° of treated OPF biomass: (a) AAC, (b) AAC + Mw-A and (c) raw and Mw-A[6] (AAC: Alkali-autoclave-chemical pretreatment; Mw-A: Microwave-alkali pretreatment; [Ref: 6])

Moreover, the 2.5M NaOH aqueous solution used in the experiment behaved as an intra-crystalline swelling agent, which was able to penetrate and swell both the accessible

crystalline and the amorphous zones.[40] Thus the destruction of the cellulose crystalline structure occurred and fibril sequences in cellulose were distorted. Consequently, microfibrils emerged out of the connected structure and became fully exposed, thus improving the external surface and porosity of the cellulose.

The crystallinity index of cellulose was used to interpret the variation in its structure. A high crystallinity index value reflects a low crystalline structure, whereas a low crystallinity index reflects a high crystalline structure. The crystallinity index values for treated OPF biomass by AAC and AAC + Mw-A methods are of 45.96% and 59.25%, respectively, as depicted in Table 3. As expected, the untreated biomass has a low crystallinity index, of 37.21%, which reflects its crystalline structure. Meanwhile, all pretreatment methods, including microwave-alkali alone (44.20%), reduce the crystalline structure of oil palm biomass, which is an advantage for the further fermentation process. However, crystallinity is not the only factor that influences cellulose accessibility to enzymes; other factors, such as lignin/hemicellulose contents and distribution, porosity and particle size, also influence cellulose accessibility to enzymes.[41]

The highly amorphous structure was only achievable using the two-step (AAC + Mw-A) pretreatments, where a crystalline index of 59.25% was attained. The ability of the pretreatment process to disrupt the crystalline structure is crucial because it influences the efficiency of the subsequent process, which involves enzyme hydrolysis and further fermentation. The conversion of the crystalline region into a more amorphous structure makes enzyme accessibility to the substrate easier, and thus results in higher product yields. Binod *et al*. stated that the enzymes can rapidly digest the "easy amorphous" material, compared to the "difficult crystalline" cellulose.[5]

Although all the pretreatment methods can disrupt the crystalline structure of lignocellulose, its selection is very much dependent of the type of lignocellulose and its structure. Highly crystalline lignocellulose will require harsher methods, compared to lignocelluloses with lower crystallinity.

CONCLUSION

The AAC and AAC + Mw-A pretreatments were successfully performed on the lignocellulosic OPF, which contributed to a significant reduction of lignin and an increase in the cellulose content. The reduction in the lignin content is an advantage for the following simultaneous saccharification and fermentation (SSF) process. The AAC + Mw-A pretreatment produced a slightly higher amount (24%) of cellulose than the AAC method, and such increment is encouraging as cellulase enzyme has more substrate (cellulose) to digest and the accessibility to cellulose is made easier due to the reduction in the lignin content. The results are also in good agreement with the crystallinity index, *i.e.* the AAC + Mw-A treated sample had a higher CrI value (59.25%) than the AAC treated sample (45.96%). The higher CrI value indicates more amorphous cellulose, which would enhance the enzyme hydrolysis reaction. The FESEM images revealed that the surfaces of the treated OPF were rough with cavities and disordered internal structures, indicating these pretreatment methods have disruptive effects on the biomass samples. The FTIR spectra profiles indicated stretching of the H-bond for all the pretreated OPF biomass, which demonstrated the structural variation caused by the chemical treatment and microwave heating or by their combination. The XRD pattern showed that the microwave-alkali method had significant effects on the crystallinity of the OPT. In short, the two-step sequential pretreatment (AAC + Mw-A) can be considered as a good pretreatment method, with a great potential of scaling up for industrial applications.

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REFERENCES

[1] E. Chen and G. Danapal, Malaysia's oil palm biomass conundrum, http://www.greenprospectsasia.com/ content/malaysia%E2%80%99s-oil-palm-biomass-conundrum (accessed January 25, 2013).

[2] Oil palm planted area. Economic and Industry Development Division Report, http://bepi.mpob.gov.my/images/area/2012/Area_summary.pdf (accessed July 18, 2013).

[3] Y. H. Chong, S. H. Ng and C. P. Leh, *Cellulose Chem. Technol.*, **47**, 277 (2013).

[4] J. Akhtar, A. Idris and R. A. Aziz, *Appl. Microbiol. Biot.*, **98**, 987 (2014).

[5] P. Binod, K. Satyanagalakshmi, R. Sindhu, K. U. Janu, P. K. Sukumaran *et al*., *Renew. Energ*., **37**, 109 (2012).
[6] L.-W. Lai and A. Idris, *Bioresources*, **8**, 2792 (2013).
[7] B. R. Ewanick, in "Bioalcohol Production: Biochemical Conversion of Lignocellulosic Biomass", edited by W. Keith, Woodhead Publishing Series in Energy 3, 2010, pp. 1-23.
[8] W. H. Chen, Y. J. Tu and Y. J. Sheen, *Appl. Energ*., **88**, 2726 (2011).
[9] S. Khamtib, P. Plangklang and A. Reungsang, *Int. J. Hydrogen Energ*., **36**, 14204 (2011).
[10] S. Zhou, L. Liu, B. Wang, F. Xu and R. C. Sun, *Process Biochem*., **47**, 1799 (2012).
[11] F. Hamzah, A. Idris, R. Rashid and S. J. Ming, *J. Appl. Sci*., **9**, 3086 (2009).
[12] J. Akhtar, C. L. Teo, L. W. Lai, N. Hassan, A. Idris *et al*., *Bioresources*, **10**, 588 (2015).
[13] L. W. Lai, A. Idris and N. M. Yusof, *Malaysian J. Fundam. Appl. Sci*., **10**, 59 (2014).
[14] C. O. Kappe, "Practical Microwave Synthesis for Organic Chemists: Strategies, Instruments and Protocols", Wiley, 2009, Chapter 1.
[15] H. Ling, K. Cheng, J. Ge and W. Ping, *New Biotechnol*., **28**, 673 (2011).
[16] T. Guo, A.-Y. He, T.-F. Du, D.-W. Zhu, D.-F. Liang *et al*., *Bioresour. Technol*., **135**, 379 (2013).
[17] S.-H. Saleh, M. A. M. Noor and A. Rosma, *Bioresour. Technol*., **102**, 1234 (2011).
[18] S. Shamsudin, U. K. M. Shah, H. Zainudin, A.-A. Suraini, S. M. M. Kamal *et al*., *Biomass Bioenerg*., **36**, 280 (2012).
[19] H. Christos, R. Cynthia and P. P. George, *Appl. Biochem. Biotech*., **57/58**, 443 (1996).
[20] F. Hamzah, A. Idris and K. S. Tan, *Biomass Bioenerg*., **35**, 1055 (2011).
[21] L. W. Lai, C. L. Teo and A. Idris, in *Procs. International Conference on Industrial Engineering and Management Science*, Shanghai, September 28-29, 2013, pp. 156-162.
[22] P. J. Soest, J. B. Robertson and A. Lewis, *J. Dairy Sci*., **74**, 3583 (1991).
[23] TAPPI test method, (1997) T 204 cm-97.
[24] TAPPI test method, (2002) T 222 om-02.
[25] Y. Teramoto, S. H. Lee, T. Endo, *Bioresour. Technol*., **100**, 4783 (2009).
[26] TAPPI test method, (2009) T 203 cm-09.
[27] L. Segal, J. J. Creely, A. E. Martin and C. M. Conard, *Text. Res. J*., **29**, 786 (1959).
[28] S. Zhu, Y. Wu, Z. Yu, J. Liao and Y. Zhang, *Process Biochem*., **40**, 3082 (2005).
[29] A. Ebringerova, *Macromol. Symp*., **232**, 1 (2006).
[30] N. Xu, W. Zhang, S. Ren, F. Liu, C. Zhao *et al*., *Biotechnol. Biofuels*, **5**, 58 (2012).
[31] J. Henning, B. K. Jan and F. Claus, *Biofuel. Bioprod. Bioref*., **1**, 119 (2007).
[32] L. L. Wang, G. T. Han and Y. M. Zhang, *Carbohyd. Polym*., **69**, 391 (2007).
[33] J. X. Sun, X. F. Sun, R. C. Sun, F. Paul and S. B. Mark, *J. Agric. Food Chem*., **51**, 6719 (2003).
[34] N. Sun, M. Rahman, Y. Qin, M. L. Maxim, H. Rodriguez *et al*., *Green Chem*., **11**, 646 (2009).
[35] M. Sekkal, V. Dincq, P. Legrand and J. P. Huvenne, *J. Mol. Struct*., **349**, 349 (1995).
[36] R. William, Michigan State University, http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm (accessed July 10, 2013).
[37] K. K. Pandey and A. J. Pitman, *Int. Biodeter. Biodegrad*., **52**, 151 (2003).
[38] W. H. Chen, Y. J. Tu and H. K. Sheen, *Int. J. Energ. Res*., **34**, 265 (2010).
[39] J. G. Liu, Q. H. Wang, S. Wang, Z. Dexun and K. Sonomoto, *Biosyst. Eng*., **112**, 6 (2012).
[40] W. Shujun, Y. Jinglin, C. Haixia and P. Jiping, *Food Hydrocolloid*., **21**, 1217 (2007).
[41] P. Sunkyu, O. B. John, E. H. Michael, A. P. Philip and K. J. David, *Biotechnol. Biofuels*, **3**, 10 (2010).