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Eco-Friendly Extraction and Characterization of Cellulose from Oil Palm Empty Fruit Bunches

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Cellulosic fibers in Oil Palm Empty Fruit Bunches (OPEFB) are tightly packed with lignin, hemicelluloses, small depositions of wax, and inorganic elements. In the present work, eco-friendly reagents with low concentrations of 20% (v/v) formic acid and 10% (v/v) of 30% hydrogen peroxide were employed at 85 $^{\circ}$C for the extraction of cellulose from OPEFB. The yield of 64% (w/w) achieved was among the highest ever reported. Based on the XRD, the α-cellulose content was 93.7% with a high crystallinity of 69.9%. The average diameter was 13.5 μm with structural evidence of separated fibrils as investigated by FESEM. The TEM analysis suggested that the material was crystalline and its geometry was a monoclinic structure. The FTIR spectral peaks representing wax and hemicelluloses at 1735 cm^{-1} and 1375 cm^{-1}, respectively, and lignin at 1248 cm^{-1} and 1037 cm^{-1}, were not observed in the extracted OPEFB-cellulose spectra. Based on the TGA results, thermal stability at 325 $^{\circ}$C with a single degradation curve suggests the purity of OPEFB-cellulose.

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INTRODUCTION

In Malaysia, Oil Palm Empty Fruit Bunches (OPEFB) annually generate 19.8 million tonnes of biomass on a wet basis, or 6.93 million tonnes on a dry basis (Foo *et al.* 2011). This provides huge resources for the conversion into value-added products, as the cellulosic biomass is inexhaustible, renewable, biodegradable, recyclable, and a derivatizable biopolymer. OPEFB are lignocellulosic composites, comprised of compactly arranged cellulose, hemicelluloses, and lignin with 44.4% cellulose fraction (Sun *et al.* 1999), out of which as much as 60.6% can be turned into value-added biopolymer (Wanrosli *et al.* 2004).

Naturally, cellulose polymer is a linear chain of anhydro-glucose monomer units connected through 1-4 β-linkages. The cellulose polymer chain is stabilized at the end-terminal with non-reducing and reducing sugar units (Klemm *et al.* 2005). The physical and chemical properties of the cellulose chain can be attributed to reactive –OH groups that reside on positions C-2, C-3, and C-6. The chain is compactly and tightly arranged due to random twisting and the formation of interactive forces: a weaker Van der Waals force and stronger intermolecular and intramolecular H-bonding (Abdullah *et al.* 2011).

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Acidified sodium chlorite ($NaClO_2 + H_3O^+$) is widely used as a standard reagent for the delignification and the extraction of cellulose from wood materials (Wise *et al.* 1946). Chlorite (ClO_2^-) may produce a chlorine radical, $Cl^\bullet$, which reacts and fragments the lignocellulosic material into highly toxic organochlorine. The cellulose extraction from the OPEFB involving pretreatment with toluene/methanol (2:1,v/v) (Sun *et al.* 1999) and ethanol/benzene (1:2) (Fahma *et al.* 2010), with further delignification using chlorite and microcrystalline cellulose preparation (Rosnah *et al.* 2009), have been reported. However, the use of toluene, benzene, and chlorite are not environmentally friendly. There are now concerted efforts to "go-green" by using less solvent at low concentration, using less toxic and non-toxic feedstock selections in combination with physical treatments that are less energy intensive. The delignification of wheat straw with alkali (Sun and Tomkinson 2002), sulphuric acid hydrolysis of OPEFB (Yunus *et al.* 2010), the formic acid/performic acid hydrolysis of jute fiber to isolate microcrystalline cellulose (Jahan *et al.* 2011), and autothermal degradation of *Miscanthus* x *giganteus* to cellulose fiber using formic acid and hydrogen peroxide (Haverty *et al.* 2012), are among the green methods that have been explored.

Organic acids such as acetic acid and formic acid are eco-friendly reagents, less corrosive, and effective for the pretreatment of lignocellulosic biomass, and they provide a more stable medium for monosaccharide in the aqueous phase as compared to sulfuric acid (Marzialetti *et al.* 2011). Formic acid could be recovered easily after the complete delignification with multiple additions of water, and the process is economical and easy to control at low operating temperature and pressure (Li *et al.* 2012). A mixture of hexane/ethanol and water has been used to remove wax from the rice husk, with further autoclaving and ultrasound treatments for the delignification and purification of cellulose using hydrogen peroxide and acetic acid (Rosa *et al.* 2012). The extraction of cellulose fibers from the OPEFB by hydrogen peroxide with an ultrasound-assisted alkali extraction yields 49% of cellulose (Nazir *et al.* 2012).

The main objective of the present research work was to explore an eco-friendly method for cellulose extraction from the OPEFB for enhanced yield and high crystallinity. The solvents employed were formic acid and hydrogen peroxide at low concentrations. The structural characterization was investigated by Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). The chemical functional group identification was done by Fourier Transmission Infra-red spectroscopy (FTIR). The crystalline properties were studied by X-ray diffractometry (XRD), and the thermal stability of microcrystalline cellulose was investigated by Thermogravimetric analysis (TGA).

EXPERIMENTAL

Materials

Fresh OPEFB samples were collected from the FELCRA Nasaruddin Oil Palm Mill, Bota, Perak, Malaysia and stored at 4 °C. The chemicals, formic acid (96.0% Sigma-Aldrich), sodium hydroxide (pellet), 30% hydrogen peroxide, and ethanol (99.5% Sigma-Aldrich), of ACS grade were used as purchased. To assist in the physical treatment, an autoclave (75X, All American) was used. The commercial microcrystalline cellulose (MCC) (Cotton linter; 20 µm, Aldrich) was used as a standard for comparison.

Percentage of Moisture Content and Ash Contents

The percentage of the OPEFB moisture content was determined based on the standard method (ASTM 2003). Ten grams of the sample (Dragon 303, METTLER-TOLEDO) were placed in the crucible and left in an oven (UNB-400, MEMERT) at 100 $\pm$ 2 ^{o}C for 3 h. Samples in three replicates were then kept in a desiccator until room temperature, before the final weight was noted, and an average percentage was calculated.

For the ash content percentage (ASTM 2007), a dried sample (at 105 ^{o}C) was ground and sieved through a nylon filter (40 mesh, Kexin). Two grams were placed in a clean crucible and sent to a furnace (Carbolite RHF 1600) until a constant temperature of 600 ^{o}C was reached for 1 h. The crucible was left in a desiccator, weighed, and the ash content percentage of three replicates was calculated.

Pretreatment

OPEFB fibers were physically separated and washed several times with 1% washing detergent to remove oil, greases, and sand particles, and rinsed until the freshly used rinse water turned from dark brown to colorless. The fibers were dried at room temperature and cut into 0.2 mm by using power cutting mill (Pulverisette 25). These fibers were considered to be raw OPEFB.

For the treated OPEFB fibers, the raw fibers after washing were dried in an oven (UNB-400, Memert) at 100 $\pm$ 2 ^{o}C until they were at constant weight, and later cut into 0.2 mm size pieces.

The fibers were dewaxed in 200 mL 70% (v/v) ethanol, with a fiber to solvent ratio of 1:10 (g L^{-1}) using soxhlet (FAVORIT) apparatus for six hours. The fibers were collected and washed with deionized water to remove alcohol traces before further drying.

Later, ten grams of dewaxed fibers were suspended in 10% of 100 mL NaOH, followed by 10% of 100 mL H$_2$O$_2$, covered with aluminium foil, and autoclaved (75X, All American) for one hour at 121 ^{o}C and 1.5 bar. The dark brown supernatant was separated from the fibers, followed by several rounds of washing until clear water was observed.

Extraction of OPEFB-Cellulose

The extraction method was adapted and modified from that developed for jute fibers (Jahan *et al.* 2011). The autoclaved fibers were soaked in a mixture of 20% formic acid and 10% hydrogen peroxide at 1:1 ratio (v/v), and placed in a water bath set at 85 ^{o}C for 2 h. Delignified fibers were then collected after filtration and washed further with 10% formic acid, followed by washing several times with distilled water. The extracted cellulose fibers were light yellow in color and re-suspended in 10% of hydrogen peroxide at pH 11 for 90 min at 60 ^{o}C. The pH was adjusted with 10% of sodium hydroxide.

The white suspension obtained was filtered, washed several times, and the insoluble fraction of cellulose was collected and the yield (w/w) calculated. The α-cellulose content was determined by the TAPPI method (TAPPI 1991).

Characterization

Field Emission Scanning Electron Microscope (FESEM) and Transmission Electron microscope (TEM)

Morphological studies of raw OPEFB and extracted cellulose were carried out with a Field Emission Scanning Electron Microscope (FESEM, Zeiss Supra 55VP). The samples were suspended in isopropyl alcohol (IPA) and sonicated for 1 h with an interval of eight minutes. One drop of the sample was placed on a carbon tap, and allowed to dry for 24 h at room temperature. The samples were then gold coated using a sputtering method to avoid charges, and the accelerated voltage was set to 2 kV. Morphological observations were made, and the mean diameter was determined by selecting ten fibers randomly from the image. An elemental analysis was performed by an energy-dispersive X-ray (EDX) coupled with FESEM. When an electron beam penetrated the sample, the X-ray produced was collected, analyzed, and detected by Silicon drift detectors (SDD), and the X-ray map was recorded.

Further structural studies were made by using a Transmission Electron Micro-scope (TEM, Libra 200 FE, ZEISS). The samples were suspended in IPA and sonicated as described above. One drop of the sample was placed on a carbon coated Cu-grid and allowed to dry at room temperature for 24 h and the observation was recorded. To investigate the crystalline structure, the Fast Fourier Transmission (FFT) image was analyzed by using ITEM (Olympus) software.

Fourier Transmission Infra-Red spectroscopy (FTIR)

The chemical investigation of functional groups in raw OPEFB and extracted cellulose were studied by using Fourier Transmission Infra-red Spectroscopy (Spectrum Perkin Elmer). The sample disc was prepared by mixing and compressing the sample and KBr at a 1:1 ratio. FTIR spectra were produced after fifty times by scanning at a 4 cm^{-1} resolution for transmission wavelength range of 4000 to 450 cm^{-1}.

X-Ray Diffraction (XRD)

The phase behaviour of raw OPEFB and extracted cellulose was elucidated by using an X-ray diffractometer (D8-Advance Bruker-AXS). The analyses and instrument conditions were set at 1.540^{o}A wavelength (CuKα radiation), with a scan speed of 2° per second and a 2θ range of $2\text{-}80^{o}$. The crystallinity index, C_{Ir}, was calculated as follows (Segal *et al.* 1959),

$$C_{Ir} = [(I_{002}\text{-}I_{am}/I_{002})] \times 100 \tag{1}$$

where, I_{002} is the highest peak intensity of the crystalline fractions and I_{am} is the low intensity peak of the amorphous region.

The crystallite size was determined by using Scherrer's equation (Patterson 1939),

$$D = K\lambda/Bcos\theta \tag{2}$$

where, K is the constant 0.91, θ is Bragg's angle, and β is the intensity of the full width at half of the maximum (FWHM) corresponding to a high intensity peak of the diffraction plane 002.

Thermogravimetric Analysis (TGA)

The thermal stability of the raw OPEFB and extracted cellulose was studied by using TGA (Pyris 1, Perkin Elmer). Ten mg of sample (dried) was kept under TGA observation at a set temperature range of 30 to 700 ^{o}C in a nitrogen environment. The constant nitrogen flow was maintained at 30 cm^{3}min^{-1}, followed by a furnace flow rate of 150 cm^{3}min^{-1}, before the samples were run.

RESULTS AND DISCUSSION

Extraction of OPEFB-cellulose

The percentage of the moisture and ash contents of the raw OPEFB fibers were 9.9% and 3.8%, respectively, and they were comparable to a previous report (Faruk *et al.* 2012). The ethanol extracts by the soxhlet method were 3%, containing oil, waxes, fats, and resin, and they were comparable to a previous study (Fahma *et al.* 2010). The α-cellulose content of the isolated OPEFB cellulose was 93.7%, which is comparable to the reported value of 97.1% (Leh *et al.* 2008), but higher than 62.3% (Wanrosli *et al.* 2011). Further delignification with performic acid effectively removed the lignin and the hemicelluloses fraction, and produced a total cellulose yield of 64% (w/w) on a dry weight basis, which was higher than the previously reported yield at 44.4% (Sun *et al.* 1999) and 60.6% (Wanrosli *et al.* 2004) using chlorite and sodium hydroxide, respectively. The moisture, ash, and ethanol extractive fraction vary with the environmental abiotic and biotic stresses, while the cellulosic content varies with the plant species, growth factors, and the choice of reagent during the extraction.

High cellulose content suggests that the method developed was efficient. A pretreatment in an autoclave produces high energy steam that dislocates fibrillar arrangements and assists with the penetration of sodium hydroxide and hydrogen peroxide for partial oxidation. Formic acid, with its major contributors, is very stable in an aqueous medium. A resonance system (A=B–X$^-$) may be generated in the molecule with the release of protons (H$^+$), and stability is gained by shifting electrons from the negatively charged oxygen to a single bond and turns it into a multiple bond. On the other end, the multiple bond between the C=O shifts onto a connecting oxygen atom and the oxygen bearing the negative charge. The to and fro multiple shifting of electrons through the single bond produces equivalent structures, or major contributors, to give maximum stability. This may form performic acid by accepting an oxygen atom from hydrogen peroxide.

Stable formic acid provides the medium for the interaction and dissolution of the non-crystalline part of cellulose, while the crystalline region is stable to the acid attack, thus increasing the crystallinity. The synergistic effects remove the non-lignin parts, hemicelluloses, and lignin (by partial oxidation), converting the hemicelluloses to the corresponding salt forms, and the lignin to respective carboxylic acids. These will be removed as a water soluble fraction, leaving behind the crystalline cellulose that is insoluble and suspended in an aqueous medium. The decomposition products of reacting mixture are CO_2, H_2O, and O_2 which may be less hazardous than the chloro-organic compounds generated with the use of chlorite, whilst the CO_2 is recovered from agro-resources.

Morphological Characterization

FESEM

Figures 1 a-c show that the raw OPEFB is a lignocellulosic composite with compact fibrillar packing. The external surface suggests an irregular heavy deposition of wax, hemicelluloses, lignin, and other inorganic components (Na, Mg, K, Ca, S, Al, Si) as illustrated by EDX spectra (not shown). The extracted cellulose (Figs. 1 d-f) show an isolated fibril, having an average diameter of 13.5 μm, and it was separated from each other, suggesting the complete removal of cementing wax, hemicelluloses, and lignin. The external surface showed a mixture of a smooth surface with scars, highly likely due to the removal of inorganic particles such as silica and the metal components. This was supported by EDX spectra (data not shown), which showed only carbon and oxygen remaining.
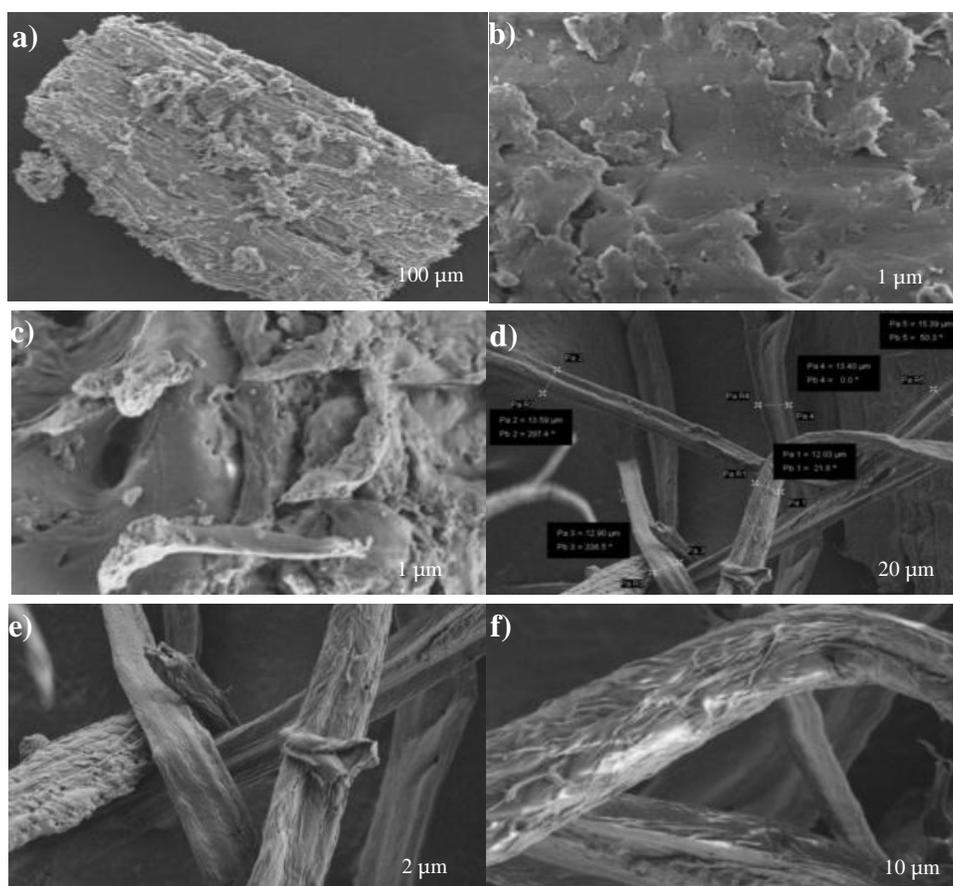

Fig. 1. FESEM micrographs of (a-c) raw OPEFB; (d-f) extracted OPEFB-cellulose

TEM

The raw OPEFB (Figs. 2a and 2b) show an unexposed crystalline region surrounded by amorphous hemicelluloses and lignin with solid round elemental particles, most probably silica or inorganic elements. The extracted cellulose image suggests the presence of a large crystalline region and a small amount of an amorphous region (Fig. 2c). One part of the image was selected and cropped for FFT (Fast Fourier Transmission) (Fig. 2d), where the crystalline arrangement of cellulose can be observed. The diffraction pattern was recorded (Fig. 2e), and the inter-planar distances were calculated (Fig. 2f)

suggesting the equivalence to a monoclinic crystal system. In a unit cell monoclinic structure, all of the lengths must be different ($a \neq b \neq c$). The image shows only $\beta = \gamma$ ($\approx 90^o$), and $\beta \neq 90^o$ angles (Fig. 2f). The crystalline structure of the cellulose chains and the interaction between the consecutive chains are due to the H-bonding or Van der Waals forces (Zugenmaier 2007). The monoclinic structure of the extracted cellulose suggests the stability of the cellulose chain through H-bonding (Avolio *et al*. 2012).
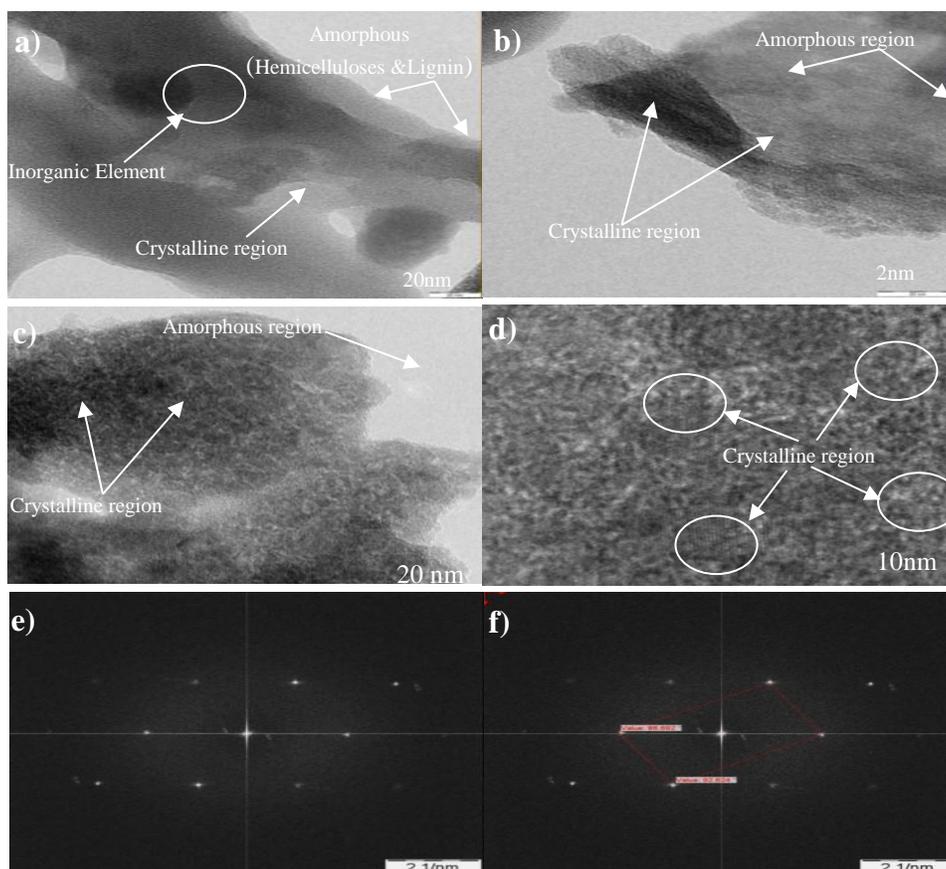

Fig. 2. TEM micrograph of (a & b) raw OPEFB; (c & d) extracted cellulose; (e) the diffraction pattern; (f) the monoclinic pattern and its measurement

Chemical Characterization

FTIR

The FTIR spectra (Figs. 3a, b, and c) show the transmittance peaks at 3400 cm^{-1} and 2906 cm^{-1}, which were attributable to cellulose –OH and C-H groups stretching vibrations, respectively (Khalil *et al*. 2001). Similarly, the peaks at 1432, 1375, and 1326 cm^{-1} present in the raw OPEFB are associated with the bending vibrations of -CH$_2$, C-H, and C-O of cellulose, and they were also present in the extracted cellulose and the commercial MCC. The water absorption peak was observed at 1644 cm^{-1}, and the peak at 895 cm^{-1} assigned to the β-gycosidic linkage (Le Troedec *et al*. 2008) was present in all of the spectra. However, the peak at 1735 cm^{-1} in the raw OPEFB spectra (Fig. 3a) attributable to a waxy C=O acetyl group of hemicellulose ester or carbonyl ester of the *p*-coumaric monomeric lignin unit, and the peak at 1248 cm^{-1} due to the C-O-C of aryl-alkyl ether in lignin (Alemdar and Sain 2008; Nazir *et al*. 2012) were not present in the

extracted cellulose and the commercial MCC (Figs. 3b and 3c). The peaks at 1161, 1137, and 1105 cm^{-1} attributable to the deformation of the C–H rocking vibration and the C–O–C pyranose were more prominent in Figs. 3b and 3c (Kargarzadeh *et al.* 2012). These spectral changes are characteristics of cellulose, which further demonstrates the removal of hemicelluloses and lignin.
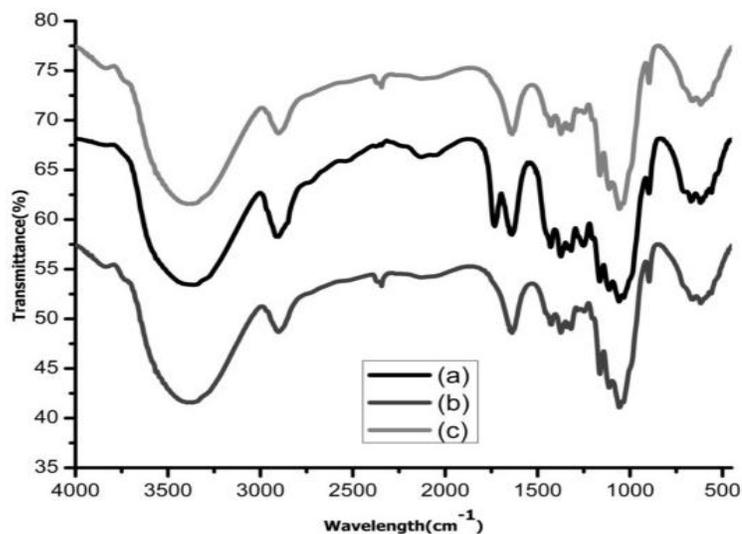

Fig. 3. FTIR of (a) raw OPEFB, (b) extracted OPEFB-cellulose, (c) commercial MCC

XRD

Raw OPEFB fibers are composites of amorphous (hemicellulose, lignin) and crystalline (cellulose) structures (Fig. 4a); hence the small peak intensity as the cellulose fibers may still be entrapped in amorphous hemicellulose and lignin.
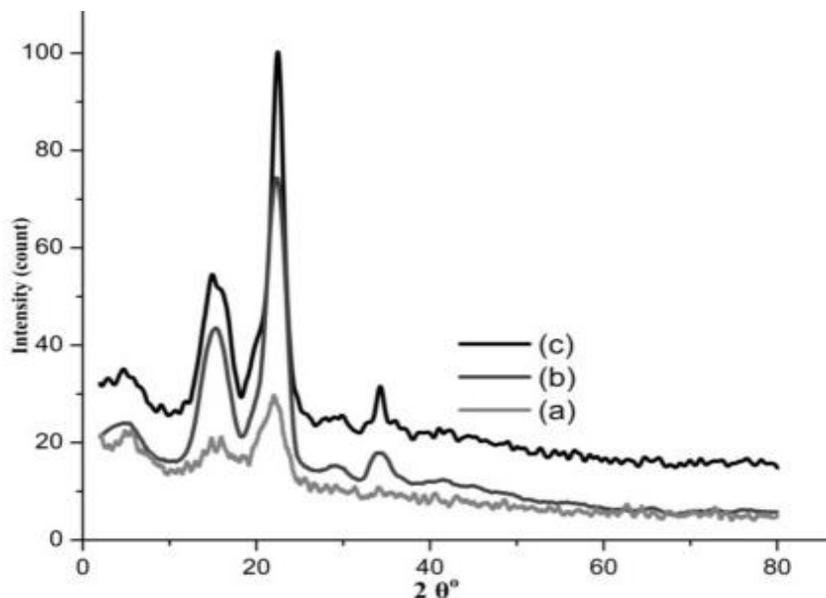

Fig. 4. X-ray diffractogram of (a) raw OPEFB, (b) extracted OPEFB-cellulose, (c) commercial MCC

With the extracted cellulose (Fig. 4b), a single high intensity peak at 22.5^{o} (002), comparable to the commercial MCC (Fig. 4c), suggests that the depolymerization of hemicellulose and delignification had been successfully achieved. The sharp high intensity peak describes the crystalline nature of the material, and the intensity value shows the amount of crystalline structure. The diffractogram of the extracted cellulose showed a high intensity crystalline peak I_{002} at $2\theta=22.5$, and an amorphous peak with an intensity I_{am} at $2\theta=16^{o}$ corresponding to the crystallographic plane (101), similar to results reported earlier (Johar *et al.* 2012; Sheltami *et al.* 2012). These characteristic peaks were attributed to cellulose based on the JCPDS data (03-0289). The crystallinity index and the crystallite size of raw fibers were calculated as 43.9% and 31.5 nm, respectively, and for the extracted cellulose, they were 70% and 9.8 nm, respectively, corresponding well with a previously reported value of 69% (Jonoobi *et al*. 2011).

TGA

Lignocellulosic material is a composite structure of different types of components, and it will degrade below 400 ^{o}C with wax, pectin, and hemicelluloses degraded at 180 ^{o}C, cellulose at around 300 ^{o}C, and lignin at 400 ^{o}C (Johar *et al*. 2012). TGA of the raw OPEFB (data no shown) showed around 10% of water losses at 110 ^{o}C, with low temperature degradation of hemicelluloses, wax, and pectin material ranging from 150 to 230 ^{o}C. After 230 ^{o}C, the sharp change was due to cellulose degradation before becoming stable at 350 ^{o}C, followed by a slow decomposition phase of lignin. In OPEFB cellulose, two phases of thermal degradation could be observed, the thermal degradation of the OPEFB-cellulose with water loss below 200 ^{o}C, and the depolymerization of the cellulose chain between 200 to 390 ^{o}C. The decomposition phases attributed to hemicelluloses (>180 ^{o}C) and lignin (>390 ^{o}C) were absent in celluloses. The sharp weight loss with linearity of the thermal curve from 325 ^{o}C and 350 ^{o}C was comparable to that of commercial MCC. Thermal weight loss curve suggests high purity of the extracted MCC, which would have otherwise produced different phases should there be any impurities. The thermal stability of the OPEFB-cellulose (325 ^{o}C) was higher than that of wheat straw (296 ^{o}C) and soy hulls (290 ^{o}C) (Alemdar and Sain 2008). The residual mass around 25% at 700 ^{o}C for the raw OPEFB may be due to the formation of ash with enriched lignin carbon compounds accumulated as char (Fahma *et al.* 2011). The residues of 5% char may be produced as a result of aromatization of fragmented cellulose C–C, C—O bonds at temperatures higher than 400 ^{o}C (Jahan *et al.* 2011).

CONCLUSIONS

1. A physico-chemical method was developed for the extraction of the OPEFB-cellulose using a low concentration of formic acid (20%) and hydrogen peroxide (10%), which yielded 64% of cellulose with an α-cellulose content of 93.7% and 70% crystallinity.

2. FESEM observation showed that the morphology of the extracted cellulose appeared as separate fibrils with crystallite parallel chains. The TEM diffraction pattern elucidated the unitary crystalline structure of cellulose in a monoclinic crystal pattern.

3. FTIR studies demonstrated the removal of wax, lignin, and hemicelluloses functionalities. XRD had proven the crystalline nature of the cellulose extracted, and the TGA analysis indicated the purity in terms of thermal stability achieved at 325 ^{o}C, which was confirmed and validated by comparing the results with commercially available MCC.

ACKNOWLEDGEMENTS

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