EXTRACTION AND CHARACTERIZATION OF CELLULOSE MICROFIBRILS FROM AGRICULTURAL WASTES IN AN INTEGRATED BIOREFINERY INITIATIVE

M. NURUDDIN, A. CHOWDHURY, S. A. HAQUE, M. RAHMAN,[*] S. F. FARHAD,[**] M. SARWAR JAHAN[*] and A. QUAIYYUM

Department of Applied Chemistry and Chemical Technology, Dhaka University, Dhaka
[*]Pulp and Paper Research Division, BCSIR Laboratories, Dhaka
[**]Industrial Physics Division, BCSIR Laboratories, Dhaka

Received January 25, 2011

The compositional characteristics of rice straw, wheat straw, corn stalks and dhaincha were established in terms of lignin, cellulose and hemicellulose contents, to assess the suitability of agricultural wastes for the extraction of microfibrils. Cellulose microfibrils were prepared by sulphuric acid hydrolysis from these raw materials, which had previously been submitted to a fractionation process by formic acid/peroxyformic acid/peroxide. Cellulose, amounting to 37-43%, lignin (16-20%), hemicelluloses (20-33%) and other compounds were extracted. The extracted cellulose microfibrils were characterized by Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), thermogravimetric analysis (TGA) and X-ray diffraction. All cellulose microfibril samples showed good thermal stability. The extracted microfibrils presented triclinic structure.

Keywords: microfibrils, rice straw, wheat straw, corn stalks, dhaincha, biorefinery, formic/peroxyformic acid fractionation, characterization

INTRODUCTION

As known, agriculture produces significant amounts of wastes, which contain high quantities of organic matter. The agricultural wastes produced in a particular period of the year pose potential pollution problems. Therefore, an efficient utilization of such agricultural wastes is of great importance not only for minimizing the environmental impact, but also for obtaining a higher profit. In fact, according to the biorefinery concepts now in use,[1-3] these organic wastes have a considerable potential as renewable feedstocks for the production of fuel grade ethanol, chemicals and materials.

Rice, wheat and corn are the main agricultural products in South-East Asia. In 2009, the South-East Asian countries produced 194826, 159 and 36655 thousand metric tonnes of rice, wheat and maize, respectively,[4] which generated higher amounts of wastes. Dhaincha (*Sesbania aculeata*) is a crop generally cultivated for its nutritive value for the soil, during the monsoon seasons, almost throughout all Bangladesh as it grows well in loamy, clayey, black and sandy soil. The main solid wastes are the stalks made of lignified tissue. These stalks could be an interesting biomass for the fractionation of lignin, cellulose and hemicelluloses, as potential sources of biomaterials.[3,5-6]

Microcrystalline cellulose (MCC) is widely used especially in the food, cosmetic and medical product industries, where it is expected to act as water retainer, suspension stabilizer, or as a reinforcing agent for various products, such as medical tablets. MCC is obtained at an industrial scale through the hydrolysis of wood cellulose and of cotton cellulose, using dilute mineral acids. MCC preparation from wheat and cereal straws,[7] jute,[8] soybean husk,[9] flax fibres and flax straw,[10] sugar cane bagasse,[11] mulberry barks[12] and peel of pear fruits,[13] involving cellulose extraction by different methods, with the sacrifice of lignin and

hemicelluloses, has been studied. Nevertheless, lignin and hemicelluloses also possess a huge potential for green products. The development of the biorefinery concept involves the complete utilization of all biomass processing streams in the most efficient and beneficial way.

As a cheap and readily available organic solvent, formic acid appears as a potential chemical agent for biomass fractionation.[1,14-15] In this process, lignin is dissolved in formic acid due to the cleavage of the lignin β-*O*-4 bonds,[16] while hemicellulose degrades into mono- and oligosaccharides, leaving solid cellulose in the residue. When water is added to the liquor, lignin is precipitated and separated from the liquor. After pulping, formic acid can be easily recovered for reuse by distillation.

The scope of the present study, following the same process concept, includes:

1) corn stalks, rice straw, wheat straw and dhaincha fractionation into cellulose, hemicelluloses and lignin, by formic acid and peroxyformic acid treatment processes;

2) subsequent transformation of the cellulose fraction into microcrystalline cellulose (MCC) through strong acid hydrolysis;

3) characterization of the prepared MCC samples by Thermogravimetric Analysis (TGA), Furrier Transform Infrared (FTIR), X-ray detraction (XRD) and Scanning Electron Micrograph (SEM) techniques.

EXPERIMENTAL

Materials

Dhaincha, corn stalks, rice straw and wheat straw were collected from the farm area of the Dhaka District, in Bangladesh. All chemicals used in the experiment, of reagent grade, were provided by E Mark, Germany.

The lignin (T211 om83) and pentosan (T223) contents of these raw materials were analysed by Tappi Test Methods. Holocellulose was determined by treating the extractive-free wood meal with a NaClO2 solution. The pH of the solution was maintained at 4, by adding CH3COOH-CH3COON as a buffer, α-cellulose being determined by treating holocellulose with 17.5% NaOH.

Formic acid (FA) treatment

The dhaincha, corn stalks, rice straw and wheat straw were refluxed with formic acid on a hot plate, under the following conditions:

- 90% (v/v) formic acid concentration;
- 120 min reaction time at boiling temperature;
- liquor-to-fibre ratio: 8 (vol/fibre).

On completion of the reaction time, the fibres were filtered in a Büchner funnel and washed with fresh formic acid, followed by hot distilled water.

Peroxyformic acid treatment

The formic acid treated mass was further delignified with peroxyformic acid (PFA), at 80 °C. PFA was prepared by mixing 90% formic acid with 4% H_2O_2. The reaction was carried out in a thermostatic water bath. After 120 min, the fibres were filtered off and washed with an 80% formic acid solution.

Bleaching

Bleaching experiments of unbleached pulp were carried out at 10% pulp concentration. pH was adjusted to 11, by adding the required amount of sodium hydroxide (NaOH). The hydrogen peroxide charge was of 4% (on fibre material). Other conditions: 80 °C and 1 h. The pulp yield was determined gravimetrically. The α-cellulose content in the bleached pulps were determined according to Tappi Test Methods (T 203 om-88).

MCC preparation

The other MCC sample was produced from the bleached pulp, by the procedure of Dong *et al*.,[17] under the following conditions: 64% w/w sulphuric acid solution for 5 h at 45 °C, with constant stirring. Hydrolysis was stopped by adding a large volume of water to the reaction mixture. The resulting mixture was washed with cold water and repeatedly centrifuged. The residue was then dried in a vacuum oven to constant weight for 48 h and ground by a Wiley mill into fine powder.

Lignin and sugar isolation

The lignin dissolved during delignification with formic and peroxyformic acid was precipitated by adding water to the concentrated spent liquor (5 times more than the volume of formic acid). The residue was filtered in a Büchner funnel, washed with water and vacuum dried over P_2O_5.

Solid contents

The total solid content in the prehydrolysis liquor (PHL) was determined by drying at 105 °C up to constant weight.

Scanning Electron Micrograph (SEM)

SEM (JEOL 6400, Tokyo, Japan) was used for the microstructural analysis of the MCC samples with gold coating. All images were taken at an accelerating voltage of 15kV. TEM

observations were made with JOEL 2011 STEM. For TEM, a 20 µL drop of solution was pipetted onto a carbon coated copper grid.

Thermo Gravimetric Analysis (TGA)

Thermogravimetrical analysis was performed with about 5 mg of air-dried sample, using a Perkin-Elmer set-up (TAQ 500) and a heating rate of 20 °C/min, under nitrogen atmosphere.

Fourier Transform Infrared Spectroscopy (FTIR)

The dried samples were embedded in KBr pellets and analyzed with a Shimadzu FTIR spectrometer (model 8201PC). The spectra were recorded in the absorption band mode, in the 4000-400 cm^{-1} range.

X-ray diffraction

The X-ray powder patterns for α-cellulose, MCC, were scanned and recorded on a Bruker D8 Advance spectrometer equipped with a D-5000 rotating anode X-ray generator from 10-40 of 2-theta (scanning angle), using the Cu Kα radiation generated at 30 mA and 40 kV. The crystalline indices of the samples were calculated from the X-ray diffraction patterns, based on the following equation:[18]

$$Xc = I_{002}\text{-}I_{am}/I_{002}\ X100$$

where Xc is the crystallinity index, I_{002} is the peak intensity from the 002 latice plane (2-theta = 22°) and I_{am} the peak intensity of the amorphous phase.

RESULTS AND DISCUSSION

Chemical composition

The results of the chemical analysis of corn stalks, rice straw, wheat straw and dhaincha are shown in Table 1. The Klason lignin content in these agricultural wastes was of 16-22%, which was similar to hardwood.[19] Dhaincha had the highest lignin content. The α-cellulose content of these wastes was lower than in hardwood.[20] The pentosan contents in dhaincha, rice straw, wheat straw and corn stalks were of 19.7, 23.5, 23.1 and 21.2%, respectively, which agrees with earlier results, such as those of Atchinson.[21]

Fibre fractionation

The results of formic acid/peroxyformic acid/hydroxide peroxide ($FA/PFA/H_2O_2$) fractionation of dhaincha, corn stalks, rice straw and wheat straw are shown in Table 2. After peroxide bleaching, the pulp yield was of 37 to 44% – as depending on the raw materials involved – which is a very high bleach yield for cellulose pulp in comparison with other processes; for example, the unbleached pulp yield[22] in kraft processes from corn stalks was of 33-37%. The unbleached pulp yield[23] from oil palm fibre by prehydrolysed soda process was of 31.4%. The α-cellulose content in the pulps was between 89 and 94%, indicating that hemicelluloses and lignin were effectively removed by the $FA/PFA/H_2O_2$ process. The higher yield in such a case is due to the fact that α-cellulose is highly protected.[24] It has been reported[14,23-25] that acetic and formic acid can effectively remove lignin and hemicelluloses from other lignocellulosics, such as dhaincha, wheat straw, at atmospheric pressure.

Table 1
Chemical characterization of corn stalks, rice straw, wheat straw and dhaincha

	Dhaincha	Rice straw	Wheat straw	Corn stalks
Klason lignin, %	21.9	16.3	17.2	18.2
α-cellulose, %	37.9	38.1	37.8	39.4
Pentosan, %	19.7	23.5	23.1	21.2

Table 2
$FA/PFA/H_2O_2$ fractionation of agricultural wastes

Raw materials	Cellulose fraction		Spent liquor		Total recovered biomass
	Yield (% on original material)	α-cellulose content (%)	Total solid (% on original material)	Lignin (% on original material)	
Dhaincha	42.8	94.1	20.0	19.7	82.5
Rice straw	37.3	91.2	32.9	15.6	85.8
Wheat straw	39.1	89.0	28.6	16.2	83.9
Corn stalks	43.0	89.6	31.2	16.2	90.4

Mass balance

The total biomass recovered in the FA/PFA/H_2O_2 process was of 83-90%, which is the sum of cellulose yield, precipitated lignin and solid content in the filtrate after lignin precipitation. The solid content represents mostly sugars. The sum was relatively lower, mainly because: 1) some biomass, such as lignin, hemicelluloses, volatile compounds (*e.g.* methanol), was lost during alkaline peroxide treatment – this has been well supported by literature results, for example, in the alkaline peroxide process, He *et al*.[27-28] reported that methanol is formed with a pulp yield loss of about 4-12%; 2) the formation of 4-*O*-methy glucuronic acid and other degradation products; 3) inorganic compounds dissolved from these nonwoods.

The spent liquors from the FA/PFA treatments were further concentrated, followed by dilution with water, so that lignin could be precipitated. The highest amount of lignin was recovered (20% based on original material) from dhaincha, which represented 90% of the Klason lignin content. After the FA/PFA treatment, the recovered lignin from the other agricultural wastes represented 89-96% of the Klason lignin. The formic acid treatment (in 90% v/v formic acid solution) of rice straw at atmospheric pressure dissolved about 90% of the lignin present in the raw material.[28] Seisto and Poppius[29] found 11.3-13.0% lignin (based on original materials) from different grasses in the FA/PFA process, which accounted for 56-59% of the Klason lignin. As a nontoxic, low-cost and renewable resource, lignin has been considered as a substitute for some petrochemical products, to deal with the petroleum crisis and environment pollution caused by non-biodegradable polymers.[30-31] For instance, as part of the starting material, lignin has been studied to produce different polymers, such as polyurethane[32] and phenol-formaldehyde resin.[33-34] The value-added applications of lignin help boost the economic viability of the biorefinery, make lignin a source of renewable materials.

The solid content in the spent liquor (mostly mono and oligo sugars) is of 20-33%, as shown in Table 2. The solid contents in rice straw, wheat straw and corn stalks were of 32.9, 28.6 and 31.2%, respectively. Pan and Sano[24] reported that 63.5% of xylose/xylan was dissolved in the spent liquor during the acetic acid treatment of wheat straw, while cellulose was highly protected. Lam *et al.*[28] observed that above 80% of pentose sugars were dissolved from rice straw in the formic acid treatment at atmospheric pressure. The sugars in the biorefinery process can be transformed into building-block chemicals, both by fermentation and enzymatic and chemical transformations. The key building-block chemicals will include ethanol, C3 to C6 carboxylic acids (*e.g.*, hydroxypropanoic and glucaric acids) and alcohols, such as glycerol and sorbitol. Xylose/xylan can be converted into xylitol, yeast, furfural and others, by chemical or enzymatic processes. Recently, the U.S. Department of Energy has published a comparative study on the top 12 chemicals from carbohydrate biomass, identifying several particularly promising compounds – including sorbitol, levulinic acid and glycerol.[35]

Preparation of microfibrils

Microfibrils can be prepared through acid hydrolysis,[17] known as effective in dissolving the amorphous cellulose, leaving behind the microcellulose crystalline. The yields of microfibrils from these raw materials were of 20-31%.

Acid hydrolysis of cellulose produced mainly glucose from the amorphous part.[8] The glucose present in the hydrolysate can be further utilized for the production of cellulosic ethanol following the biorefinery concept, as reported by many recent studies.[36-38]

It is well accepted that the dimensions of the microfibrils are vital in yielding good mechanical properties.[39] The size characteristics of the microfibril samples prepared from the cellulose of different agricultural wastes, based on acid hydrolysis, are shown in Table 3 and Figure 1. The diameters were calculated based on the image processing software ImageJ, using SEM images. 200 measurements were carried out from different images. The average diameters obtained are of 6.8, 8.7, 9.3 and 6.6 μm for dhaincha, rice straw, wheat straw and corn stalks, respectively, *i.e.* similar or lower than those reported by others; Alemdar and Sain[40] produced MCC of 10-15 μm from rice straw and soy hull.

Figure 2 presents the SEM micrographs of microfibrils obtained from different agricultural wastes. It is clearly seen that they are fibrillated to nano-size. The ultrasonic homogenization will certainly separate these fibrils, which was out of our scope.

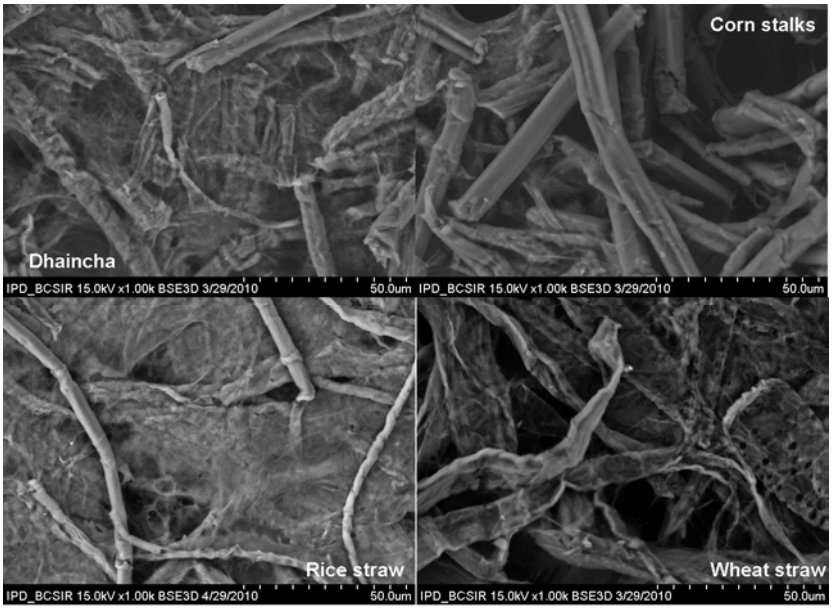
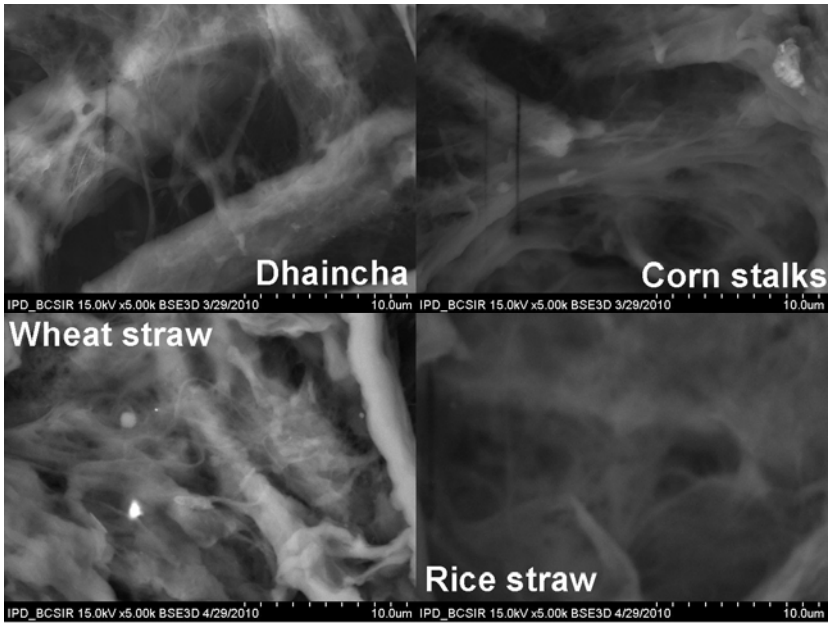


Figure 1: Extracted microfibrils from agricultural wastes (Magnification x1000)



.Figure 2: Extracted microfibrils from agricultural wastes (Magnification x5000)

Table 3
Dimension characteristics of microfibrils isolated from different agricultural wastes

Diameter, µm	Dhaincha	Rice straw	Wheat straw	Corn stalks
Maximum	7.8	10.8	12.1	7.5.
Minimum	5.9	6.6	8.6	5.8
Average	6.8	8.7	9.3	6.6
Standard deviation	1.31	1.72	1.38	1.31

\

Thermogravimetric analysis

The thermal properties are critical for many applications, including the use of microfibrils for the production of biocomposites. In such applications, the processing temperature may rise above 200 ºC. The TG curves of microfibrils obtained from different agricultural wastes are shown in Figure 3. For all samples, initial weight loss was observed from 50 to 150 ºC, due to moisture evaporation. The degradation behaviour of all microfibrils was almost similar, starting above 300 ºC. The degradation temperature for the micrifibrils extracted from rice straw started at 332 ºC and continued up to 370 ºC, where all cellulose was pyrolyzed, the solid residues being of about 20%. The high residue was due to the high silica content of rice straw. The microfibrils from wheat straw showed an almost similar degradation temperature, but lower residues (12%). The degradation peaks and residues for microfibrils from dhaincha and corn stalks were of 368 and 353 ºC and 10%, respectively. Yang *et al.*[41] showed that cellulose decomposition started at 315 ºC and continued until 400 ºC, the maximum weight loss rate being reached at 355 ºC. It was reported[40] that the degradation temperatures of nanofibrils from wheat straw and soy hulls were of 296 and 290 ºC, respectively. The solid residue was of 12-14% at 600 ºC. Based on the above results, one can conclude that the microfibril samples from such agricultural wastes have good thermal stability at 300 ºC and will be suitable for the production of biocomposites.

FTIR

Figure 4 shows the FTIR peak assignments of cellulose and microfibrils extracted from rice straw, wheat straw, corn stalks and dhaincha. The absence of a prominent peak at 1737 cm^{-1} in the extracted cellulose indicated that the FA/PFA/H_2O_2 treatment effectively removed lignin and hemicelluloses from the raw materials. This peak is explained by either the acetyl and uronic ester groups of hemicelluloses or the ester linkage of the carboxylic group of ferulic and *p*-coumeric acids of lignin and/or hemicelluloses.[42-43] No peak was found at 1513 cm^{-1}, which was attributed to the effective removal of lignin during the treatment.

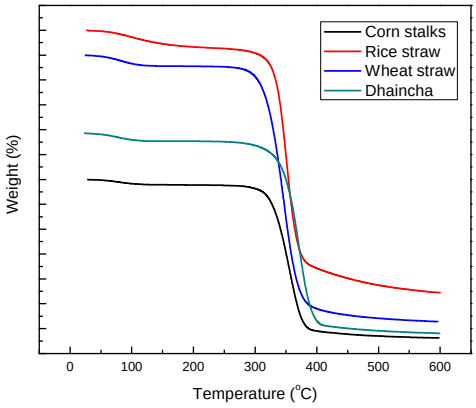


Figure 3: Thermal degradation behaviour of microfibrils isolated from agricultural wastes

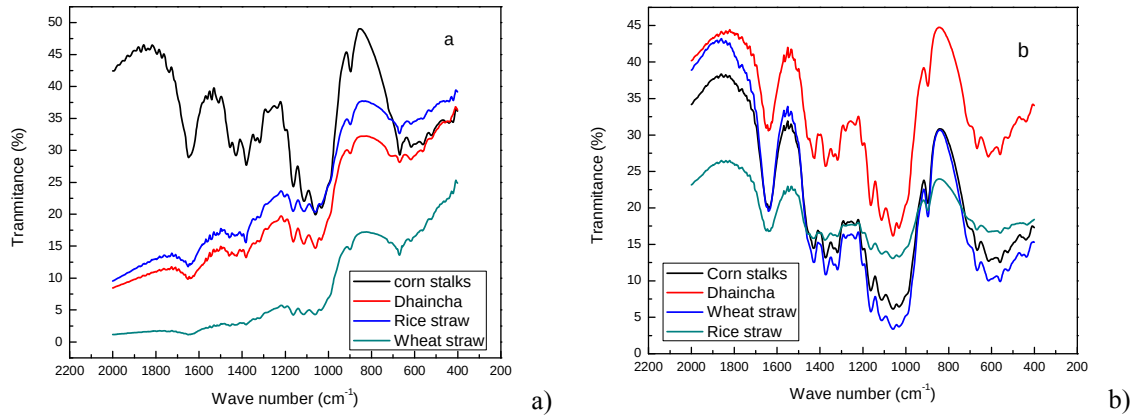


Figure 4: FT-IR spectra of a) cellulose and b) microfibrils

Acid hydrolysis removed the amorphous cellulose on the surface, therefore, more C-OH, C-O-C and C-C bonds were exposed, resulting in increased stretching absorbency.[43] There is an obvious absorption at 710 cm^{-1} and a weak shoulder at 750cm^{-1}, both assigned to I_α (triclinic) and I_β (monoclinic) cellulose.[44-45] These results indicate that the samples are rich in I_β.

X-Ray diffraction

The X-ray diffraction patterns showed a peak at 2-Theta 22º, indicating that all microfibrils were made up of cellulose I (Fig. 5). The crystallinity index of microfibrils was of 52.8, 53.3, 58.3 and 55.3% for dhaincha, rice straw, wheat straw and corn stalks, respectively, lower than the original cellulose from the corresponding raw materials. Fahma *et al.*[46] reported that the crystallinity index of nanofibres tended to decrease with increasing the sulphuric acid treatment time. The sulphuric acid penetrates the amorphous region of cellulose, causing the hydrolytic cleavage of the glucosidic bonds and releasing individual crystallites.[12] The crystallinity index of flax and rutabaga nanofibrils was estimated to be of 59 and 64%, respectively.[40] The higher crystallinity is associated with the higher tensile strength of MCC.

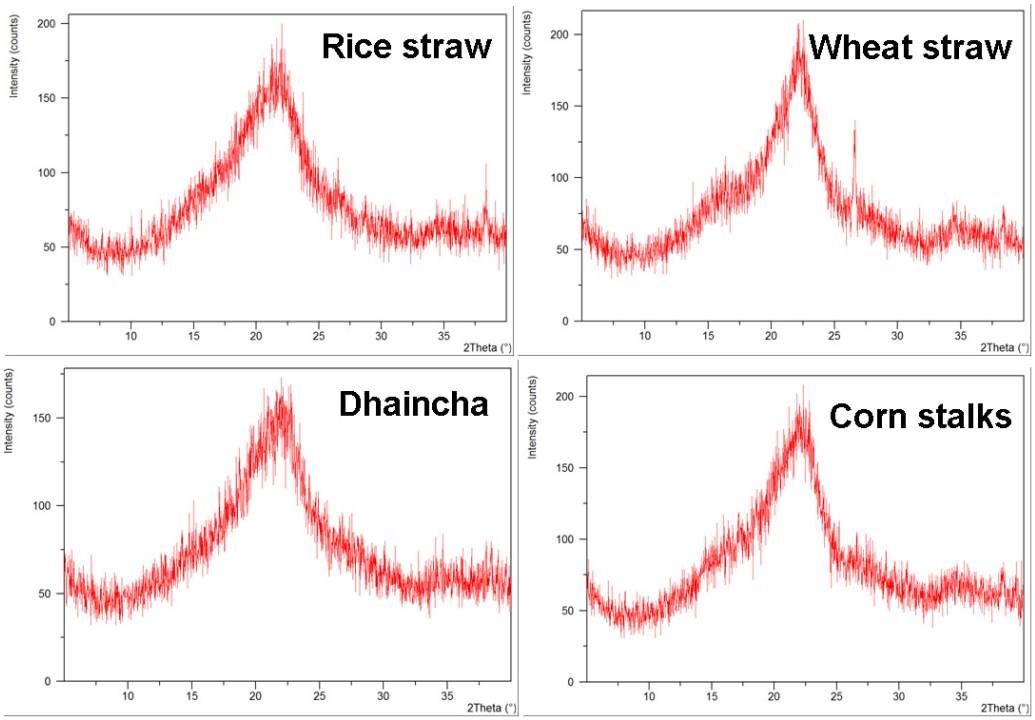


Figure 5: X-ray diffraction patterns of microfibrils isolated from agricultural wastes

Table 4
Crystallinity index of microfibrils isolated from corn stalks, rice straw, wheat straw and dhaincha

	Dhaincha	Rice straw	Wheat straw	Corn stalks
Crystallinity (%)	52.8	53.3	58.3	55.3

CONCLUSIONS

Corn stalks, rice straw, wheat straw and dhaincha were effectively fractionated to cellulose, hemicelluloses and lignin by a FA/PFA/H_2O_2 process. The purity of extracted cellulose was of 89-94%, depending on the raw materials. The total biomass recovered in the FA/PFA/H_2O_2 process was of 90-83%. The recovered lignin was of 16-20%, based on the original raw materials. FTIR results indicate that the isolated microfibrils from such agricultural wastes are rich in I_β. The crystallinity index of microfibrils was of 53-55%, and the degradation temperature was above 300 ºC. It may be therefore concluded that these agricultural wastes can be fractionated, by a FA/PFA/H_2O_2 process, in an Integrated

Forest Biorefinery Concept and also that all fractions can be used in producing biomaterials, chemicals and fuels.

REFERENCES

[1] M. S. Jahan, A. S. Y. Ni and Z. He, *J. Biobased Mater. Bio.*, **3**, 380 (2009).
[2] A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak, C. L. Liotta, J. R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, **311**, 484 (2006).
[3] T. Teramoto, S. H. Lee and T. Endo, *Bioresource Technol.*, **99**, 8856 (2008).
[4] FAO STAT Database at www.fao.org
[5] K. Abe and H. Yano, *Cellulose*, **16**, 1017 (2009).
[6] M. Gáspár, G. Kálmán and K. Réczey, *Process Biochem.*, **42**, 1135 (2007).
[7] A. Alemdar and M. Sain, *Bioresource Technol.*, **99**, 1664 (2008).
[8] M. S. Jahan, A. S. Zhibin and H. Y. Ni, *Cellulose*, DOI 10.1007/s10570-010-9481-z (2011).
[9] Y. U. Nelson, A. G. Edgardo and A. W. Ana, *Int. J. Pharm.*, **206**, 85 (2000).
[10] A. M. Bochek, I. L. Shevchuk and V. N. Lavrentev, *Russ. J. Appl. Chem.*, **76**, 1679 (2003).
[11] D. Bhattacharya, L. T. Grminario and W. T. Winter, *Carbohyd. Polym.*, **73**, 371 (2008).
[12] R. Li, J. Fei, Y. Cai, Y. Li, J. Feng and J. Yao, *J. Carbohyd. Polym.*, **76**, 94 (2009).
[13] Y. Habibi, M. Mahrouz and M. R. Vignon, *Food Chem.*, **115**, 423 (2009).
[14] M. S. Jahan, D. A. Nasima Chowdhury and I. M. Khalidul, *Ind. Crop. Prod.*, **26**, 324 (2007).
[15] M. Zhang, W. Qi, R. Liu, R. Su, S. Wu and Z. He, *Biomass Bioenerg.*, **34**, 525 (2010).
[16] M. S. Jahan, D. A. Nasima Chowdhury, M. Khalidul Islam and M. Shahidul Islam, *Cellulose Chem. Technol.*, **41**, 137 (2007)
[17] X. M. Dong, J.-F. Revol and D. G. Gray, *Cellulose*, **5**, 19 (1998).
[18] L. E. Alexander, in "X-ray Diffraction Methods in Polymer Science", edited by R. E. Krieger, Krieger, New York, 1979, pp. 423-424.
[19] M. S. Jahan, R. Sabina and A. Rubaiyat, *Turk. J. Agric. For.*, **32**, 339 (2008).
[20] M. S. Jahan, D. A. N. Chowdhury and Y. Ni, *Bioresource Technol.*, **101**, 1892 (2010).
[21] J. E. Atchison, F. Hamilton and B. Leopold, in "Pulp and Paper Manufacture", Vol. 3, Third Edition, Tappi Press, 1987, pp. 22-70.
[22] M. A. Abu-State, F. F. El-Megeid, and R. I. Nesseem, *Ind. Eng. Chem. Prod. Dev.*, **22**, 506 (1983).
[23] W. D. W. Rosli, C. P. Leh, Z. Zaiuddin and R. Tanaka, *Holzforschung*, **57**, 106 (2003).
[24] X. Pan and P. Sano, *Bioresource Technol.*, **96**, 1256 (2005).
[25] M. S. Jahan, Z. Z. Lee and Y. Jin, *Turk. J. Agric. For.*, **30**, 231 (2006).
[26] Z. He, Y. Ni and E. Zhang, *J. Wood Chem. Technol.*, **24**, 1 (2005).
[27] Z. He, Y. Ni and E. Zhang, *Appita J.*, **58**, 72 (2005).
[28] H. Q. Lam, Y. L. Bigot, M. Delmas and G. Avignon, *Ind. Crop. Prod.*, **14**, 65 (2001).
[29] A. Seisto and K. L. Poppius, *Tappi J.*, **80**, 215 (1997).
[30] J. H. Lora and W. G. Glasser, *J. Polym. Environ.*, **10**, 39 (2002).
[31] L. N Zhang and J. Huang, *J. Appl. Polym. Sci.*, **80**, 1213 (2001).
[32] T. Hatakeyama, Y. Izuta, S. Hirose and H. Hatakeyama, *Polymer*, **43**, 1177 (2002).
[33] M. N. M. Ibrahim, A. Md. Ghani, N. Zakaria, S. Shuib and C. S. Sipaut, *Macromol. Symp.*, **274**, 37 (2008).
[34] T. Michinobu, M. Hishida, M. Sato, Y. Katayama, E. Masai, M. Nakamura *et al*., *Polym. J.*, **40**, 68 (2008).
[35] T. Werpy *et al*., "Top Value Added Chemicals From Biomass", Vol. 1, U.S. Dept. Energy, Oak Ridge, TN, August 2004; available at www.eere.energy.gov/biomass/pdfs/35523.pdf
[36] R. J. Bothast, N. N. Nichols and B. S. Dien, *Biotechnol. Prog.*, **15**, 867 (1999).
[37] P. Chandrakant and V. S. Bisaria, *Crit. Rev. Biotechnol.*, **18**, 295 (1998).
[38] M. Sedlak and Y. W. N. Ho, *Appl. Biochem. Biotechnol.*, **114**, 403 (2004).
[39] I. Sakurada, Y. Nukushina and T. J. Ito, *Polym. Sci.*, **57**, 651 (1962).
[40] A. Bhatnagar and M. Sain, *J. Reinf. Plast. Comp.*, **24**, 1259 (2005).
[41] H. Yang, R. Yan, H. Chen, H. L. Dong and C. Zheng, *Fuel*, **86**, 1781 (2007).
[42] A. S. A. Sain and S. Panthapulakkal, *Ind. Crop. Prod.*, **23**, 1 (2006).
[43] X. F. Sun, F. Xu, R. C. Sun, P. Fowler and M. S. Baird, *Carbohyd. Res.*, **340**, 97 (2005).
[44] J. Sugiama, J. Persson and H. Chanzy, *Macromolecules*, **24**, 2461 (1991).
[45] S. Y. Oh, Y. I. Dong, S. Younsook and S. Gon, *Carbohyd. Res.*, **340**, 417 (2005).
[46] F. Fahma, S. Iwamoto, N. Hori, T. Twata and A. Takemura, *Cellulose*, **17**, 977 (2010).