

EFFECT OF PRETREATMENT OF BAGASSE PULP ON PROPERTIES OF ISOLATED NANOFIBERS AND NANOPAPER SHEETS

Mohammad L. Hassan

Research Professor
Cellulose and Paper Department and
Centre of Excellence for Advanced Sciences
Advanced Materials and Nanotechnology Group
National Research Center
Dokki, Cairo, Egypt 12622

Aji P. Mathew

Assistant Professor
Division of Manufacturing and Design of Wood and Bionanocomposites
Luleå University of Technology
Luleå, Sweden

Enas A. Hassan

Researcher
Cellulose and Paper Department
National Research Centre
Dokki, Cairo, Egypt 12622

*Kristiina Oksman**

Professor and Head of Department
Division of Manufacturing and Design of Wood and Bionanocomposites
Luleå University of Technology
Luleå, Sweden

(Received February 2010)

Abstract. Nanofibers were isolated from bagasse pulp pretreated with dilute hydrochloric acid, dilute sodium hydroxide, cellulase, or xylanase enzymes using high-shear ultrafine grinding and high-pressure homogenization. The effect of the different pretreatments on chemical composition and structure of isolated nanofibers was studied using chemical analyses, X-ray diffraction, and Fourier transform infrared. The dimensions and properties of the isolated nanofibers were followed at the different processing stages using optical microscopy, transmission electron microscopy, atomic force microscopy, and tensile properties (wet and dry). The diameter of the microfibrils was in the range of 7-30 nm for untreated and pretreated bagasse pulps while larger microfibrillar bands (to 150 nm wide) were observed for untreated bagasse pulp than the pretreated pulps (to 90 nm wide). Nanopaper sheets made from nanofibers isolated from alkali- and xylanase-treated pulps showed better wet and dry tensile strength than those made from the other pulps.

Keywords: Bagasse, nanofibers, nanopaper, xylanase, cellulase.

INTRODUCTION

Cellulose, the most abundant natural polymer on earth, has been receiving increasing interest

since the emergence of interest in nanomaterials and nanotechnology. There is a growing interest from industry to use the nanocellulosic materials in applications such as packaging, biomedicine, adhesives, electronics, and advanced nanocomposites (Hubbe et al 2008). Three types of

* Corresponding author: kristiina.oksman@ltu.se

nanocellulosic materials have been investigated in recent decades. The first is cellulose nanocrystals, which are prepared by the acid hydrolysis of cellulose fibers followed by mechanical action. Second is electrospun cellulose nanofibers, which are prepared by electrospinning of cellulose after its dissolution in specific solvent systems. Third is microfibrillated cellulose, which is prepared by mechanical processes that involve very high shear forces to defibrillate cellulose fibers.

In the cell wall of lignocellulosic materials, cellulose exists as aggregates of nanosized fibrils. Different terms are used to describe these nanofibrils depending on their width, namely, elementary fibrils (~3-5 nm width), microfibrils (~10-30 nm), and microfibrillar bands (~100 nm); the length of these nanofibrils is on the order of several hundreds of nanometers. Nanofibrils are embedded in a matrix of noncellulosic material (Fengel and Wegener 1989; Klemm et al 2005). Different technologies have been used for isolation of the microfibrils from plant fibers such as high-pressure homogenizers (Zimmermann et al 2009), cryocrushing (Bhatnagar and Sain 2005; Chakraborty et al 2005; Alemdar and Sain 2008), ultrasonics (Zhao et al 2007), and high-shear ultrafine friction grinders (Iwamoto et al 2005, 2008; Subramanian et al 2008; Abe and Yano 2009; Hassan et al 2010). The terms microfibrillated cellulose or cellulose nanofibers have been used to describe the isolated nanosized fibers.

The use of chemical pretreatment of fibers before the mechanical action using some of the aforementioned technologies has been used to facilitate the isolation of the nanofiber from the cell wall, reduce the possible damage to the microfibril structure, and to reduce energy consumption. For example, chemical treatment with an alkaline solution was used to isolate nanofibers from sugar beet (Dufresne et al 1997; Dinand et al 1999), potato tuber cells (Dufresne et al 2000), *Opuntia ficus-indica* (Malainine et al 2003; Habibi et al 2008), lemon and maize (Rondeau-Mouro et al 2003), soybean (Wang and Sain 2007), sisal (Morán

et al 2008), hemp fiber (Wang et al 2007), and banana rachis (Zuluaga et al 2009). The use of 2,2,6,6-tetramethylpiperidine-1-oxyl oxidation was used as a pretreatment before mechanical treatment for nanofiber separation (Saito et al 2006).

The use of enzyme technology in the area of papermaking has been practiced industrially because of the advantage of being environmentally friendly and a lower cost than chemical methods. Enzymes could enhance or restore fiber strength, reduce beating times in refining processes, and increase interfiber bonding through fibrillation (Kenealy and Jeffries 2003). However, in the area of nanofiber isolation, few studies have been carried out on the use of enzymes to facilitate removal of nanofibers, improve the properties of isolated nanofibers, and reduce energy consumption. For example, wood fiber pulp has been treated with cellulase enzymes or acid hydrolysis in combination with mechanical shearing to isolate cellulose nanofibers from the wood fiber cell wall. Treatment with cellulases was superior to the use of acid hydrolysis regarding the properties of isolated nanofibers and the fibers easily passed through a high-pressure homogenizer (Henriksson et al 2007; Pääkkö et al 2007).

In previous research, grinding of bagasse and rice straw pulps using a high-shear ultrafine grinder ("supermass colloidier") was used for isolation of microfibrils and microfibrillar bands. Up to 30 passes through the grinder was necessary to obtain nanofibers with a narrow diameter range. Subsequent passing of the isolated nanofibers through a high-pressure homogenizer resulted in reduction of remaining microfibrillar bands to smaller and more uniform diameter microfibrils (Hassan et al 2010). In the current work, the effect of pretreatment of bagasse pulp with chemical and enzymatic methods on properties of isolated nanofibers using an ultrafine grinder and a high-pressure homogenizer was studied to improve the strength properties of cellulose nanofibers and/or reduce the energy consumed in the grinding and homogenization processes.

EXPERIMENTAL

Materials

Bleached kraft bagasse pulp was supplied by Qena Company for Pulp and Paper, Egypt. Chemical composition of bagasse pulp was 70.6% α -cellulose, 29.7% pentosans, 0.82% ash, and a degree of polymerization (DP) of 1135.

Pretreatments of Bagasse Pulp

Bagasse pulp was treated with 5% hydrochloric acid for 45 min at 50°C. After treatment, the pulp was thoroughly washed with water to a neutral pH. For alkali pretreatment, bagasse pulp was treated with 5% sodium hydroxide for 45 min at 50°C. After treatment, the pulp was washed thoroughly with water, dilute acetic acid (2% solution), and finally with water to a neutral pH.

Trichoderma reesei NRRL 6156 fungus was used for production of crude xylanase enzymes using a PMY liquid medium (Nobuyuki et al 1995). The same fungus was used for production of cellulases using cellulose-yeast nitrogen-base carboxymethyl cellulose culture medium (Ahmed et al 2005). Xylanase and cellulase activities were determined as described earlier (Ghose 1987; Bastawde 1992).

Enzymatic pretreatment of bagasse pulp with cellulase or xylanase enzymes was carried out as follows: 20 g of bleached bagasse pulp was treated with crude xylanase or cellulase enzymes in citrate buffer (pH 5.3) in a 500-mL conical flask at 10% consistency. In preliminary experiments to reach the effective concentrations, the concentrations of cellulase and xylanase enzymes tested were 2.5-10.5 IUg⁻¹ and 15-60 IUg⁻¹, respectively. The reaction mixture was kept under shaking conditions (200 rpm) at 50°C for 4 h. At the end of the reaction period, the pulp was filtered and washed thoroughly with distilled water. The effect of the different pretreatments on chemical composition (α -cellulose, DP, pentosans) of isolated nanofibers was determined according to standard methods (Browning 1956). The nanofibers were isolated by passing the pretreated

pulps through the supermass colloidizer 30 times followed by passing the resultant fibers 2 times through the high-pressure homogenizer.

Preparation of Fibers

Grinding of fibers. The pulps were first disintegrated by a high-shear mixer using pulp suspensions of 2% consistency. The fibers were then ground using a supermass colloidizer (MKCA6-2, Masuko Sanguo, Japan) and passed through the instrument up to 30 times. The gap between the discs was adjusted to 9 μ m.

Homogenization of the fibers. The refined fibers were homogenized using a two-chamber high-pressure homogenizer (APV-2000; Denmark) after being diluted with water to 1% consistency and passed through the instrument up to 10 times. The pressure was kept at 4 MPa in one chamber and 40 MPa in the other.

Nanopaper Preparation

Nanopaper sheets were made by vacuum filtration of 0.5% (by weight) suspension of refined or homogenized fibers using filter paper (Munktell filter paper, grade OOH). The target basis weight was 800 kg/m². The sheets were dried under pressure of 8 kPa at 100°C for 2 h and then at 60°C for 8 h without pressure. The nanopaper sheets were conditioned at 65% RH for 48 h at 25°C before testing.

Experimental Analysis

Nanopaper testing. Tensile testing was carried out according to the TAPPI T494 standard method using an Instron universal testing machine at a constant crosshead speed of 62.5 mm/min. Wet tensile strength testing was carried out using a Lloyd universal testing machine according to the TAPPI T456 standard method. At least six specimens were tested from each material.

Microscopic observations. Optical microscopy (OM) was carried out with an Olympus microscope using one drop of dilute suspension of the fibers to study the effectiveness of the

grinding process for fiber size reduction. To study the diameter of the isolated nanofibers, transmission electron microscopy (TEM) was carried out using a JEOL 1230 transmission electron microscope (Jeol Instruments, Tokyo, Japan) with 100-kV accelerating voltage. One drop of fiber suspension was placed on a copper grid bearing a carbon film. Atomic force microscopy (AFM) of the isolated nanofibers was carried out using a Veeco MultiMode scanning probe microscope equipped with a Nanoscope V controller. One droplet of the aqueous fiber suspension was dried on a mica surface before the AFM examination and images were collected using a tapping-mode etched silicon tip with a nominal spring constant of 5 N/m and a nominal frequency of 270 kHz. Scanning electron microscopy was performed using a Jeol JXA 840A system running at 5–10 keV. Before scanning, samples were coated with gold using a sputter coater system (Edwards Sputter Coater, UK).

Fourier transform infrared. A JASCO 300-E Fourier transform infrared (FTIR) spectrometer was used for obtaining the FTIR spectra using a KBr disc technique.

X-ray diffraction. Diffraction patterns were obtained using a Phillips X-ray diffractometer. The diffraction patterns were recorded using Cu-K α radiation at 40 kV and 25 mA. Crystallinity index was calculated from the X-ray diffraction patterns (Fig 1) according to the following equation (Segal et al 1959):

$$\text{Cr I} = \frac{I_{002} - I_{\text{am}}}{I_{002}}$$

where I_{002} and I_{am} are the intensities of the peaks at 2θ of about 22 and 18, respectively.

RESULTS AND DISCUSSION

Effect of the Different Pretreatments on the Composition and Structure of Isolated Bagasse Nanofibers

The ease of isolation of the nanofibers from the cell wall depends on the degree of bonding between microfibrils that form the fibers. In cel-

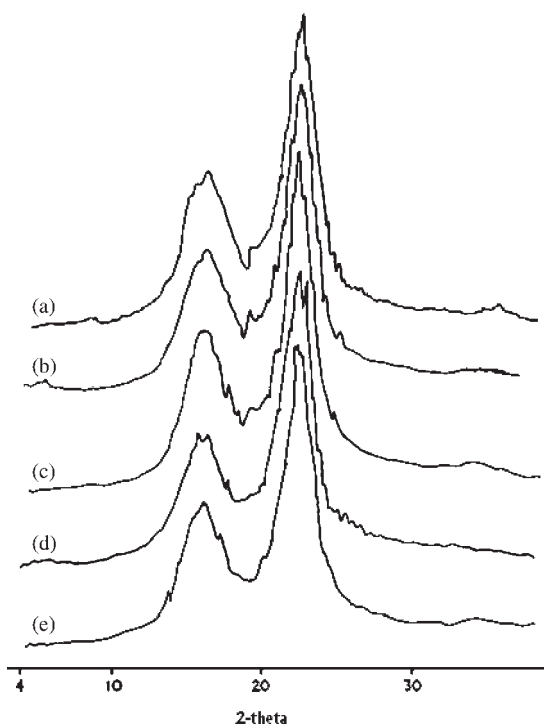


Figure 1. Fourier transform infrared of bagasse nanofibers isolated from (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

lulose bleached pulps, the microfibrils are embedded in a matrix mainly of hemicelluloses, which act as an adhesive between the microfibrils. In this study, bagasse pulp was pretreated by mild hydrolysis using dilute hydrochloric acid, dilute sodium hydroxide, cellulase, or xylanase enzymes with the aim to facilitate the isolation of the nanofibers.

The effect of the different pretreatments on chemical composition of the isolated nanofibers are presented in Table 1. Pretreatment of bagasse pulp with dilute hydrochloric acid (5% at 50°C for 45 min) resulted in removal of most of the hemicelluloses, which consist mainly of xylan in bagasse, and a slight degradation of cellulose chains as noted in the DP value. However, the α -cellulose content of isolated nanofibers was slightly higher than that isolated from untreated pulp. This could be because of partial hydrolysis of amorphous regions of

Table 1. Effect of pretreatment of bagasse pulp on percentage chemical composition of nanofibers isolated by grinding and high-pressure homogenization.

	Untreated	Acid-treated	Alkali-treated	Xylanase-treated	Cellulase-treated
α -Cellulose	71.7	73.3	78.9	73.5	73.3
Pentosans	29.7	3.91	20.7	23.3	27.2
Degree of polymerization	1135	1058	1647	1248	989

cellulose by the dilute acid (Fengel and Wegener 1989) in addition to removal of hemicelluloses.

Dilute alkali treatment resulted in partial removal of hemicelluloses; however, it could not remove significant amounts of hemicelluloses like in acid treatment. This could be because of the low concentration of the alkali used, which has a limited swelling effect on the microfibrils (Hayashi et al 1987). In addition, xylan probably binds not only to the surface of cellulose microfibrils in the cell wall, but it can also be entrapped within the microfibrils (Kacuráková et al 2000; Liu et al 2006; Habibi et al 2008). Dilute alkali can also dissolve short-chain cellulose (beta and gamma cellulose) and consequently α -cellulose content and DP of the isolated nanofibers were higher than that isolated from untreated pulp.

Xylanase treatment of bagasse pulp resulted in partial removal of hemicelluloses. This is because of the removal of the xylan fraction by xylanase enzymes, which are specific in their action and work mainly to degrade hemicelluloses to soluble oligosaccharides (Suurnakki et al 1994). As a result of removal of hemicelluloses, increases in α -cellulose content and DP of the isolated nanofibers occurred. Treatment of bagasse pulp with cellulases resulted in a decrease in DP from hydrolysis of cellulose by the enzymes (Oksanen et al 1997). A slight decrease in hemicelluloses was also noted. The effect of the pretreatments on structure of cellulose and its crystallinity was studied using XRD and FTIR. Figures 1 and 2 show the XRD patterns and FTIR spectra of isolated nanofibers, respectively.

The nanofibers isolated from untreated and pretreated bagasse fiber samples showed the

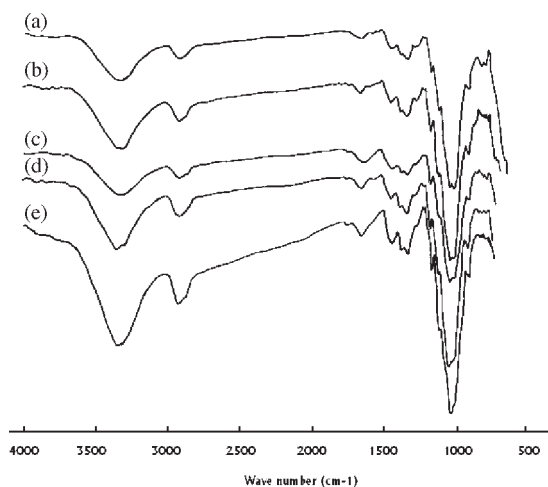


Figure 2. XRD patterns of bagasse nanofibers isolated from (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

characteristic peaks of cellulose at 2θ angles 16, 22, and 34 from reflections from $1\bar{1}0$ and 110 , 020 , and 004 lattice planes, respectively (Sassi and Chanzy 1995). The overlapping of $1\bar{1}0$ and 110 peaks suggest that the structure of the cellulose in both untreated and pretreated bagasse pulp corresponds to cellulose I (Lai-Kee-Him et al 2002; Wada et al 2004). The crystallinity index values were 74.2, 76.1, 76, 76.4, and 73.9 for untreated, alkali-, acid-, cellulase-, and xylanase-treated pulps, respectively. The higher crystallinity values of cellulose for acid-treated fibers than the untreated sample could be because of hydrolysis of some of amorphous cellulose by the action of the dilute acid in addition to removal of the amorphous hemicelluloses. In alkali treatment, the higher crystallinity index than that of the untreated sample could result from dissolution of short-chain amorphous cellulose in addition to partial removal of hemicelluloses. Because xylanase enzymes are specific in action, they partially remove xyans without significant effects on cellulose and therefore the crystallinity of cellulose for xylanase-treated fibers was similar to that of untreated fibers. For cellulase-treated pulp, the greater crystallinity than that of untreated sample could be from

removal of amorphous cellulose fraction by the action of the enzymes.

FTIR spectroscopy was used to investigate the structure of the nanofibers isolated from the pretreated fibers. The change in crystallinity of cellulose was followed according to O'Connor et al who used the ratio of the relative intensities of bands at 1429 cm^{-1} (CH_2 scissoring vibration band) and 893 cm^{-1} (C1-H group vibration) for calculation of empirical crystallinity index (O'Connor et al 1958). The relative intensity of these bands (A_{1429}/A_{894}) for untreated, acid-, alkali-, cellulase-, and xylanase-treated pulps was 0.75, 0.86, 1.12, 0.79, and 0.75, respectively. These results are in accordance with the crystallinity index values calculated from the XRD patterns. The FTIR spectra of untreated and pretreated samples were very similar indicating no drastic change in the structure of the fibers as a result of the pretreatments.

Effect of Pretreatments on Size and Strength of Isolated Nanofiber After Grinding

Untreated and pretreated bagasse pulps suspensions (2% consistency) were passed through the supermass colloidizer and the change in morphology of fibers after different numbers of passes through the grinder was followed by OM. OM images showed that 20-30 passes were necessary for effective fibrillation of the fibers. As shown in Fig 3, acid-treated pulp was easily ground after 20 passes, whereas for other pulps, more coarse fibers were present. This could be because of greater brittleness of the acid-treated fibers than the other pretreated fibers. After 30 passes, almost no coarse fibers were seen in any pulps. The dimensions of the ground fibers after 30 passes through the supermass colloidizer were studied using both TEM and AFM and the results are shown in Figs 4 and 5, respectively. As shown in the TEM micrographs, the diameters of the ground fibers from untreated and pretreated pulps were in the nanosized range. In addition to appearance of the microfibrils in the images of the different samples, microfibrillar bands of larger diameter were obvious.

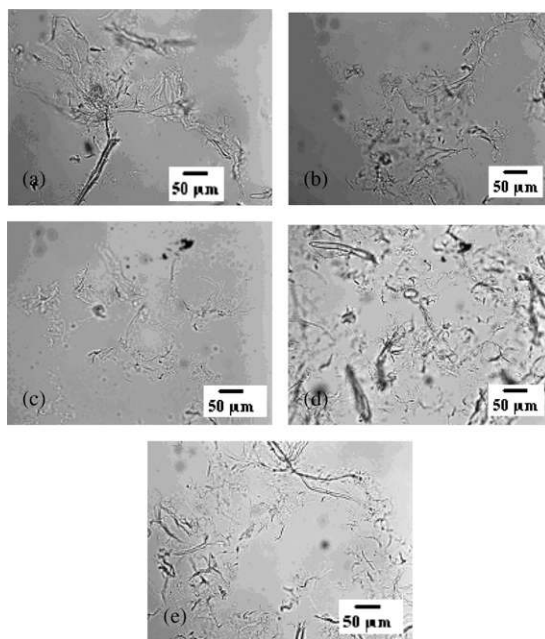


Figure 3. Optical microscopy images of bagasse fibers obtained by grinding different pretreated pulps by the supermass colloidizer for 20 passes: (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

No statistical analysis was carried out on the dimensions of the isolated nanofibers, but the range representing the highest and lowest width observed in the images was measured (Table 2). Although the diameters of microfibrils isolated from the different pulps were similar, the microfibrillar bands had larger diameters for untreated pulp than the other pulps. For the acid-pretreated fibers, broken nanofibers were obvious (Fig 1C). The AFM images also confirmed isolation of the nanofibers by the action of the supermass colloidizer, and the range of the diameters of the isolated microfibrils and microfibrillar bands was in a good agreement with TEM observations.

Tensile strength depends on the extent of the hydrogen bonding between the fibrils, the degree of fibrillation as well as DP of the cellulose chains. In addition, the presence of hemicelluloses on the surface of the fibrils can also affect tensile strength. The effect of the different

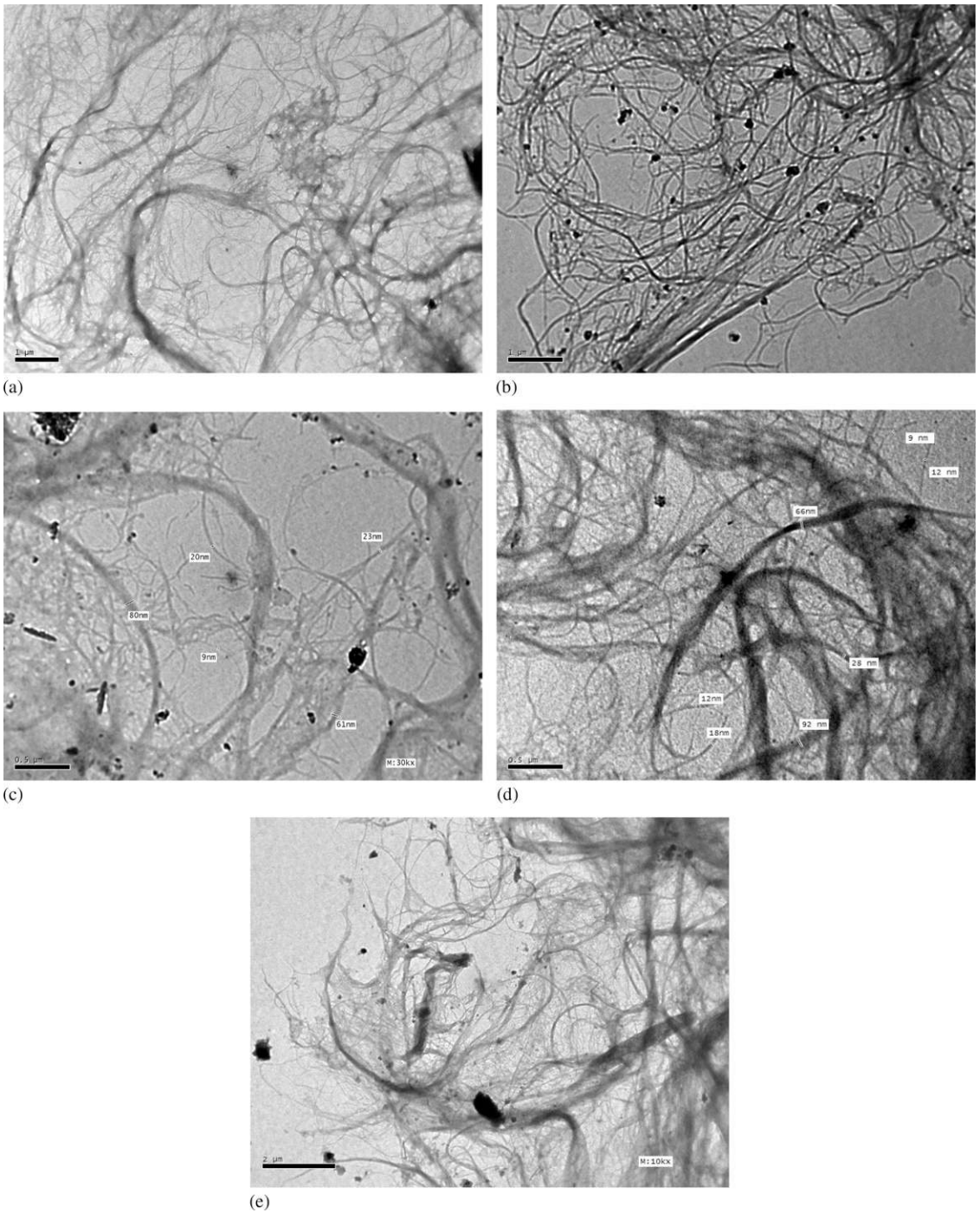


Figure 4. Transmission electron microscopy images of nanofibers obtained by grinding different pretreated pulps by the supermass collioder for 30 passes: (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

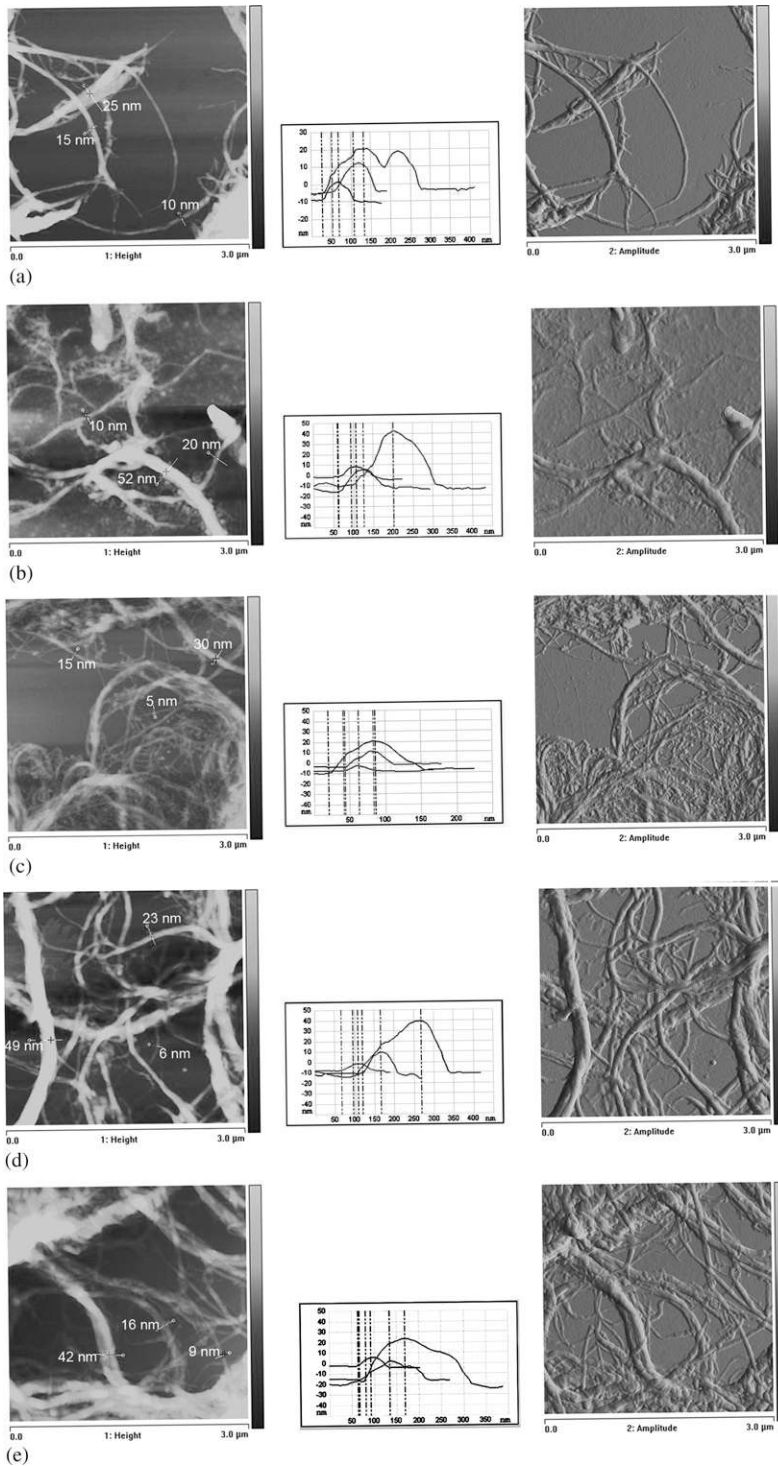


Figure 5. Atomic force microscopy images of nanofibers obtained by grinding different pretreated pulps by supermass collioder for 30 passes: (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

Table 2. Range of diameter of microfibrils and microfibrillar bands of nanofibers isolated from untreated and pretreated bagasse pulps by grinding using the supermass colloid.

Pulp treatment	Range of diameter of microfibrils (nm)	Range of diameter of microfibrillar bands (nm)
Untreated	7-24	Up to 150
Acid-treated	9-23	Up to 80
Alkali-treated	11-30	Up to 75
Xylanase-treated	9-25	Up to 90
Cellulase-treated	9-39	Up to 92

pretreatments carried out in this work on surface properties of the isolated nanofibers should be also taken into consideration when addressing the tensile strength properties of the nanopaper sheets. The effect of the grinding process of pretreated bagasse pulps on the tensile strength properties (tensile strength and tensile modulus) of nanopaper sheets made from the isolated nanofibers are shown in Table 3. Consequently, passing both untreated and treated bagasse pulps for only 10 times through supermass colloid resulted in significant increases in tensile strength and tensile modulus compared with paper sheets made from the original pulp. After 30 passes through the supermass colloid, both alkali and xylanase treatments of bagasse pulp resulted in higher tensile strength than the other samples. These results may indicate better fibrillation in case of alkali and xylanase treatments, although the TEM and AFM images showed similar average diameters for different pretreatments. The higher tensile strength for alkali and xylanase pretreatments could also be partially attributed to the higher α -cellulose content, crystallinity, and DP of the nanofibers isolated from the alkali- and xylanase-treated pulps than the other samples. Nanofibers isolated from both alkali- and xylanase-treated pulps had higher tensile strength than those isolated from untreated fibers, although the former have less hemicelluloses, which are known to improve the interfibrillar bonding and have a favorable effect on mechanical properties of paper sheets (Clark 1981). Also, TEM images show the presence of microfibrillar bands of larger diameter in case of nanofibers isolated from untreated bagasse pulp

Table 3. Tensile strength properties of paper sheets made from refined bagasse nanofibers isolated from untreated and pretreated bagasse pulps.^a

Number of passes through supermass colloid	Untreated bagasse		Alkali-treated		Acid-treated		Cellulase-treated		Xylanase-treated	
	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)
10	104.5 (15.6)	5.9 (0.35)	101.4 (6.47)	5.77 (0.37)	80.7 (13.85)	5.38 (0.18)	95.9 (4.3)	5.92 (0.19)	111.3 (15.2)	6.95 (0.59)
20	99.9 (19.5)	5.6 (0.44)	121.4 (10.8)	5.68 (0.41)	101.8 (8.33)	5.35 (0.25)	111.8 (13.15)	6.49 (0.23)	136.7 (13.6)	6.80 (0.32)
30	110.2 (13.0)	6.1 (0.61)	138.2 (9.71)	6.18 (0.30)	100.7 (7.98)	5.08 (0.28)	107.1 (18.11)	5.86 (0.54)	138.3 (10.9)	6.17 (0.25)

^a Original bagasse paper sheets had tensile strength of 32.8 MPa and tensile modulus of 3.2 GPa. Values between parentheses are the standard deviations.

than those observed for other pretreated pulps. In a previous study on treatment of wood pulp by xylanase enzymes, it was found that the decrease in strength properties was recovered after refining (Wong et al 1996; Roncero et al 2005). This was explained as a possible result of greater external fibrillation of treated pulp after enzymatic removal of xylan from the surface of the fibers (Roncero et al 2005). The improvement in tensile strength as a result of increasing the number of passes through the supermass colloidizer was more rapid for xylanase-treated pulp than that of alkali-treated pulp. The highest tensile strength was recorded for xylanase-treated pulp after passing through supermass colloidizer 20 times, while the highest strength in case of alkali-treated pulp was recorded after 30 passes. The tensile modulus of nanopaper sheets made from the xylanase-treated pulp was generally higher than the other samples.

For cellulase-treated pulp, tensile strength was close to that of untreated pulp, but the maximum tensile strength was achieved only after 20 passes through the supermass colloidizer. Previous studies on the action of cellulases on cellulose pulp showed that despite the hydrolysis action of cellulases on cellulose pulp, they can also have positive effects on strength properties when a limited hydrolysis of fibers is carried out because of improved beatability of the pulp (Stork and Puls 1996).

The lower strength properties of acid-pretreated pulp could be because of its lower DP and presence of broken nanofibers as seen from the TEM images. In addition, removal of most of hemicelluloses by the action of the acid is expected to lower the strength.

Effect of High-Pressure Homogenization on Fiber Size and Strength

The nanofibers isolated by grinding using the supermass colloidizer were diluted to 1% consistency and passed through the high-pressure homogenizer up to 10 times. The change in dimensions as a result of homogenization was followed using TEM and AFM (Figs 6 and 7,

respectively). TEM and AFM images did not show reduction in the size of the microfibrils obtained by the grinding process, but the existence of the microfibrillar bands was much less for all samples from high-pressure homogenization. No significant change in diameters of the obtained bagasse nanofibers took place in increasing the number of passes from one to 10.

Table 4 shows the effect of high-pressure homogenization of the nanofibers isolated by grinding on tensile strength of nanopaper sheets. As shown, passing the ground fibers through the high-pressure homogenizer only one or two times resulted in an improvement in tensile strength and tensile modulus. The improvement could be because of better microfibril separation from the larger diameter microfibrils bands as observed in the TEM images. Passing the ground fibers through the high-pressure homogenizer more than two times had insignificant or detrimental effects on the tensile strength properties of nanopaper sheets.

Nanopaper sheets prepared from nanofibers isolated from alkali- and xylanase-treated bagasse pulp had higher tensile strength properties than those made from nanofibers isolated from untreated, cellulase-treated, and acid-treated pulps. The highest tensile strength was recorded for the alkali-treated sample after passing through the homogenizer two times (tensile strength ~150 MPa and tensile modulus 7.64 GPa). For the xylanase-treated sample, the highest tensile strength was ~142 MPa and tensile modulus 6.8 GPa, which were obtained by passing it through the homogenizer an additional time.

Wet Strength of Nanopaper

Wet tensile strength of paper sheets is important in some specialty paper products. It was noticed in the first part of this work that the nanopaper made from nanofibers isolated from untreated bagasse pulp had higher wet tensile strength than paper sheets made from the original pulp fibers. The high wet tensile strength was attributed to greater hydrogen bonding between the nanofibers because of their very high surface

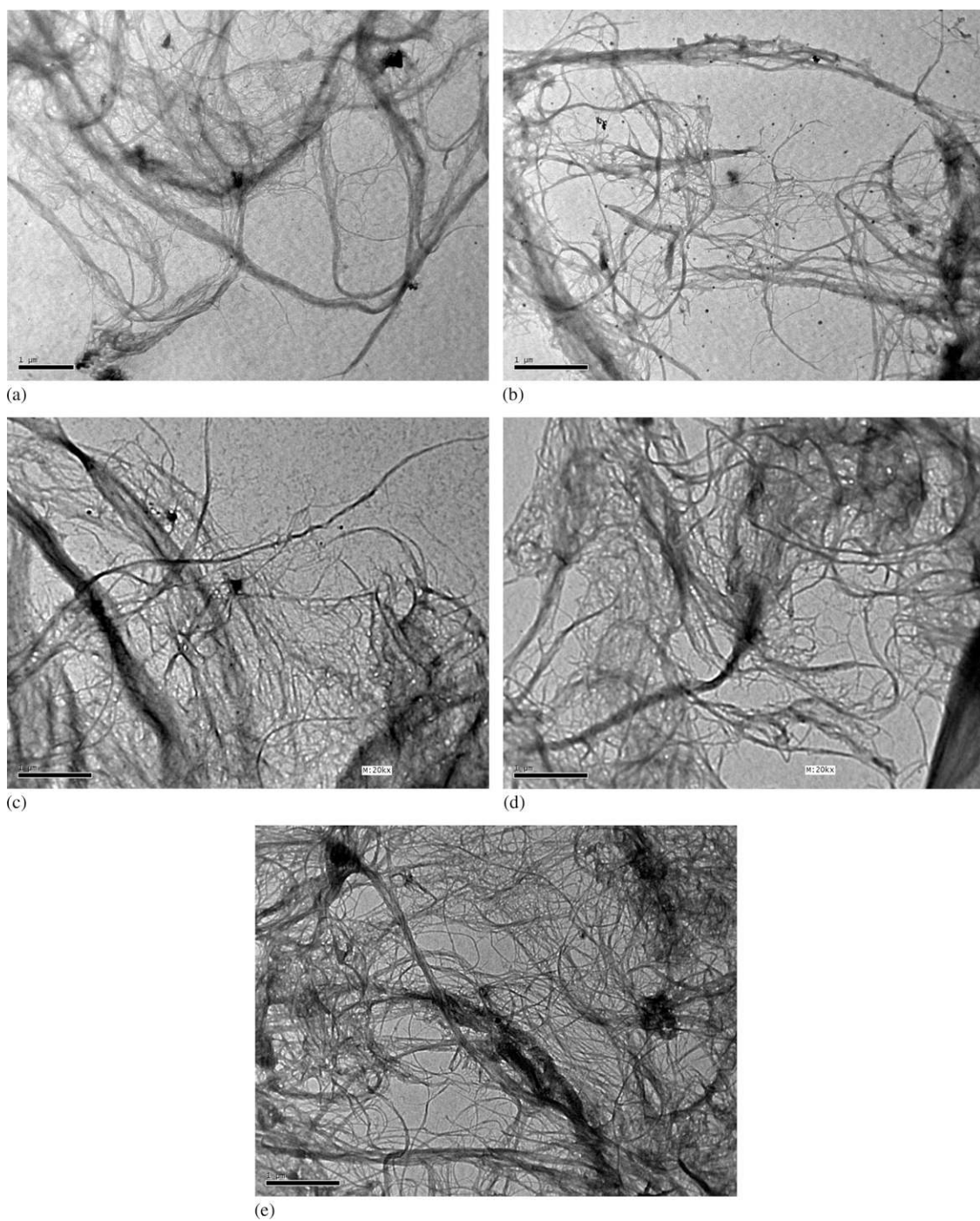


Figure 6. Transmission electron microscopy images of nanofibers obtained by high-pressure homogenization of ground fibers of different pretreated pulps in the high-pressure homogenizer for one pass: (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

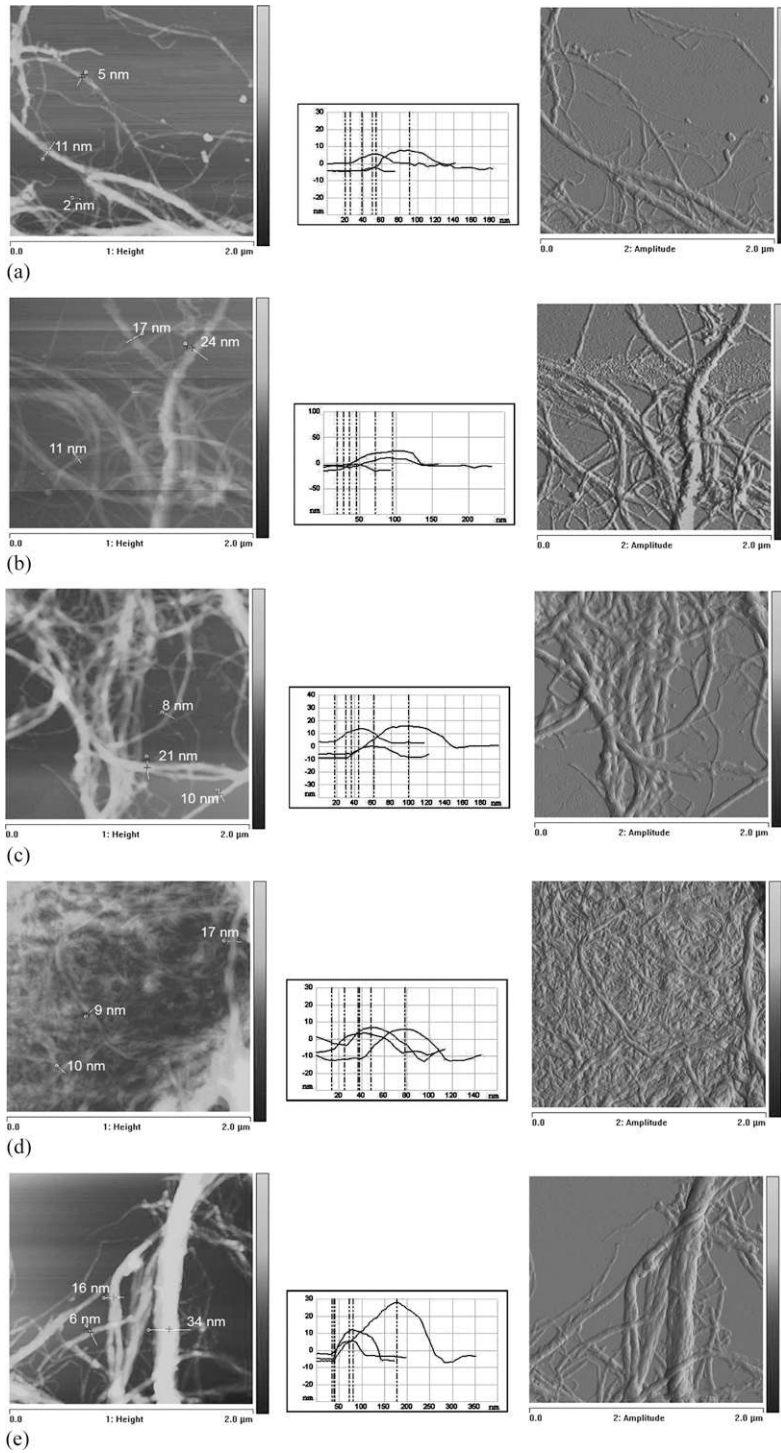


Figure 7. Atomic force microscopy images of nanofibers obtained by high-pressure homogenization of the refined fibers after one pass: (a) untreated, (b) alkali-treated, (c) acid-treated, (d) xylanase-treated, and (e) cellulase-treated pulp.

Table 4. Tensile strength properties of paper sheets made from high-pressure homogenized bagasse nanofibers isolated from untreated and pretreated bagasse pulps.

Number of passes through high-pressure homogenizer	Untreated		Alkali-treated		Acid-treated		Cellulase-treated		Xylanase-treated	
	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)	Tensile strength (MPa)	Tensile modulus (GPa)
0	110.2 (13.0)	6.1 (0.61)	138.2 (9.71)	6.1 (0.30)	100.7 (8.33)	5.08 (0.25)	107.1 (18.11)	5.86 (0.54)	138.3 (13.6)	6.17 (0.32)
1	130.5 (16.6)	8.45 (0.91)	143.7 (11.4)	6.87 (0.34)	104.9 (12.0)	6.08 (0.65)	128.6 (18.6)	6.985 (0.29)	142.4 (16.7)	6.81 (0.4)
2	93.6 (19.7)	6.99 (0.98)	149.6 (12.7)	7.64 (0.58)	112.9 (12.9)	7.06 (0.84)	127.0 (15.4)	7.44 (0.23)	138.5 (15.5)	6.39 (0.61)
5	103.9 (19.5)	7.37 (0.56)	140.9 (12.9)	8.13 (0.67)	108.7 (18.0)	6.75 (0.69)	126.6 (8.3)	6.25 (0.21)	135.5 (15.2)	6.6 (0.53)
10	102.1 (12.9)	6.90 (0.62)	135.7 (16.7)	6.88 (0.64)	112.8 (8.2)	6.07 (0.45)	115.2 (12.6)	7.71 (0.45)	129.5 (19.4)	6.57 (0.69)

Values between parentheses are the standard deviations.

Table 5. Wet tensile strength of paper sheets made from refined and high-pressure homogenized bagasse nanofibers isolated from untreated and pretreated bagasse pulps.

Treatment	Wet tensile strength ^a (MPa)	
	Refined nanofibers ^b	Homogenized nanofibers ^b
None	18.84 (1.95)	19.41 (4.1)
Dilute acid	20.0 (2.2)	21.4 (3.4)
Dilute alkali	26.9 (2.8)	28.1 (4.2)
Cellulase	20.9 (2.2)	23.3 (2.8)
Xylanase	26.2 (3.9)	30.6 (2.7)

Values between parentheses are the standard deviations.

^aWet strength of paper sheets made from original bagasse pulp was 1.5 MPa.

^bRefined fibers were passed through a supermass colloidizer 30 times; homogenized fibers were passed through the high-pressure homogenizer once.

area compared with the original fibers. Table 5 shows the wet tensile strength of nanopaper sheets made from pretreated bagasse nanofibers after grinding by the ultrafine grinder and after high-pressure homogenization. As shown, all nanopaper sheets have wet tensile strengths up to about 12 times greater than those made from the original pulp fibers. Nanofibers isolated from the pretreated pulps have better wet tensile strength than those isolated from untreated bagasse pulp. In addition, nanofibers isolated from both alkali- and xylanase-treated pulp showed significantly higher wet tensile strength than those isolated from cellulase-treated and acid-pretreated pulp. The higher wet tensile strength for nanofibers isolated from the different pretreated pulps than that of untreated nanofibers could be attributed to one or more of the following reasons: better defibrillation, lower hemicelluloses content, higher crystallinity, higher α -cellulose content, or higher DP. The high wet strength of nanopaper sheets, without adding wet strength additives, is of significant importance in applications that require high wet strength such as in food packaging and in composites used in humid conditions.

CONCLUSIONS

Cellulose microfibrils having diameters of a few nanometers (about 5-30 nm) and microfibrillar bands having diameters of about 60-150 nm could be easily obtained through high-shear

refining using a supermass colloidizer without the need for high-pressure homogenization. However, high-pressure homogenization reduced the size of the microfibrillar bands and resulted in more of the smaller sized microfibrils. Pretreatment of bagasse pulp with dilute alkali, dilute acid, xylanase, and cellulose enzymes resulted in smaller diameter microfibrils bands than those isolated from the untreated bagasse pulp. Environmentally friendly treatment of bagasse pulp with xylanase enzymes could be used to enhance the wet and dry strength properties of isolated nanofibers and to reduce the energy needed for isolation of nanofibers (microfibrils and microfibrillar bands) from the fibers.

ACKNOWLEDGMENTS

We thank SIDA for the financial support of the collaboration between Luleå University of Technology, Luleå, Sweden, and the National Research Center, Dokki, Cairo, Egypt.

REFERENCES

- Abe K, Yano H (2009) Comparison of the characteristics of cellulose microfibrils aggregates of wood, rice straw and potato tuber. *Cellulose* 16(6):1017-1023.
- Ahmed A, Singh A, Ward OP (2005) Culture-based strategies for reduction of protease activity in filtrates from *Aspergillus niger* NRRL3. *World J Microb Biot* 21: 1557-1583.
- Alemdar A, Sain M (2008) Isolation and characterization of nanofibers from agricultural residues—Wheat straw and soy hulls. *Biores Technol* 99(6):1664-1671.
- Bastawde KB (1992) Cellulolytic enzymes of a thermotolerant *Aspergillus terreus* strain and their action on cellulosic substrates. *World J Microb Biot* 8:45-49.
- Bhatnagar A, Sain M (2005) Processing of cellulose nanofibers reinforced composites. *J Reinf Plast Comp* 24 (12):1259-1268.
- Browning B (1956) *Methods of wood chemistry*. Volume 1. Interscience, New York, NY. 377 pp.
- Chakraborty A, Sain M, Kortschot M (2005) Cellulose microfibrils: A novel method of preparation using high shear refining and cryocrushing. *Holzforchung* 59(1): 102-107.
- Clark JA (1981) *Pulp technology and treatment for paper*. Miller Freeman Publications, San Francisco, CA.
- Dinand E, Chanzy H, Vignon MR (1999) Suspensions of cellulose microfibrils from sugar beet pulp. *Food Hydrocoll* 13(3):275-283.
- Dufresne A, Cavallé J-Y, Vignon MR (1997) Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils. *J Appl Polym Sci* 64(6):1185-1194.
- Dufresne A, Dupeyre D, Vignon MR (2000) Cellulose microfibrils from potato tuber cells: Processing and characterization of starch-cellulose microfibril composites. *J Appl Polym Sci* 76(14):2080-2092.
- Fengel D, Wegener G (1989) *Wood: Chemistry, ultrastructure, reactions*. Walter de Gruyter, Berlin, Germany. 613 pp.
- Ghose TK (1987) Measurement of cellulase activities. *Pure Appl Chem* 59(2):257-268.
- Habibi Y, Heux L, Mahrouz M, Vignon MR (2008) Morphological and structural study of seed pericarp of *Opuntia ficus-indica* prickly pear fruits. *Carbohydr Polym* 72(1):102-112.
- Hassan M L, Mathew AP, Hassan EA, El-Wakeel NA, Oksman K (2010) Nanofibers from bagasse and rice straw: Process optimization and properties. Submitted for publication to *Wood Sci Technol*.
- Hayashi T, Marsden MPF, Delmer DP (1987) Pea xyloglucan and cellulose. *Plant Physiol* 83(2):384-389.
- Henriksson M, Henriksson G, Berglund LA, Lindström T (2007) An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. *Eur Polym J* 43(8):3434-3441.
- Hubbe MA, Rojas OJ, Lucia LA, Sain M (2008) Cellulosic nanocomposites: A review. *Bioresources* 3(3):929-980.
- Iwamoto S, Abe K, Yano H (2008) The effect of hemicelluloses on wood pulp nanofibrillation and nanofiber network characteristics. *Biomacromolecules* 9(3):1022-1026.
- Iwamoto S, Nakagaito AN, Yano H, Nogi M (2005) Optically transparent composites reinforced with plant fibers-based nanofibers. *Appl Phys (Berl)* 81:1109-1112.
- Kenealy WR, Jeffries TW (2003) Enzyme processes for pulp and paper: A review of recent developments. Pages 210-239 in B Goodell, DD Nicholas, and TP Schultz, eds. *Wood deterioration and preservation: Advances in our changing world*. American Chemical Society, Washington, DC.
- Kacuráková M, Capek P, Sasinková V, Wellner N, Ebringerová A (2000) FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydr Polym* 43(2):195-203.
- Klemm D, Heublein B, Fink H-P, Bohn A (2005) Cellulose: Fascinating biopolymer and sustainable raw material. *Angew Chem Int Ed* 44(22):3358-3393.
- Lai-Kee-Him H, Chanzy H, Müller M, Putaux J-L, Imai T, Bulone V (2002) In vitro versus in vivo cellulose microfibrils from plant primary wall synthases: Structural differences. *J Biol Chem* 277(40):36931-36939.
- Liu CF, Xu F, Sun JX, Ren JL, Sun RC, Curling S (2006) Physicochemical characterization of cellulose from perennial ryegrass leaves (*Lolium perenne*). *Carbohydr Res* 341(16):2677-2687.

- Malainine ME, Dufresne A, Dupeyre D, Mahrouz M, Vuong R, Vignon MR (2003) Structure and morphology of cladodes and spines of *Opuntia ficus-indica*. Cellulose extraction and characterization. Carbohydr Polym 51(1):77-83.
- Morán JI, Alvarez VA, Cyras VP, Vázquez A (2008) Extraction of cellulose and preparation of nanocellulose from sisal fibers. Cellulose 15(1):149-159.
- Nobuyuki K, Tsutsumi Y, Nishida T (1995) Correlation of brightening with cumulative enzyme activity related to lignin biodegradation during biobleaching of kraft pulp by white rot fungi in the solid-state fermentation system. Appl Environ Microbiol 61(2):617-622.
- O'Connor RT, Dupre EF, Mitcham D (1958) Application of infrared absorption spectroscopy to investigation of cotton and modified cotton. Textile Res J 28(5):382-392.
- Oksanen T, Pere J, Buchert J, Viikari L (1997) The effect of *Trichoderma reesei* cellulases and hemicellulases on the paper technical properties of never-dried bleached kraft pulp. Cellulose 4(4):329-339.
- Pääkkö M, Ankerfors M, Kosonen H, Nykänen A, Ahola S, Österberg M, Ruokolainen J, Laine J, Larsson PT, Ikkala O, Lindström T (2007) Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels. Biomacromolecules 8(6):1934-1941.
- Roncero MB, Torres AL, Colom JF, Vidal T (2005) The effect of xylanase on lignocellulosic components during the bleaching of wood pulps. J Biotechnol 96:21-30.
- Rondeau-Mouro C, Bouchet B, Pontoire B, Robert P, Mazoyer J, Buléon A (2003) Structural features and potential texturing properties of lemon and maize cellulose microfibrils. Carbohydr Polym 53(3):241-252.
- Saito T, Nishiyama T, Putaux JL, Vignon M, Isogai A (2006) Homogeneous suspensions of individualized microfibrils from TEMPO-catalyzed oxidation of native cellulose. Biomacromolecules 7:1687-1691.
- Sassi JF, Chanzy H (1995) Ultrastructural aspects of the acetylation of cellulose. Cellulose 2:111-127.
- Segal L, Creely JJ, Martin AE, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Res J 29:786-794.
- Stork G, Puls J (1996) Change in properties of different recycled pulps by endoglucanase treatment. Pages 145-150 in E Srebotnik and K Messner, eds. Biotechnology in the pulp and paper industry. Facultas Universitätsverlag, Vienna, Austria.
- Subramanian R, Knononov A, Kang T, Paltakari J, Paulapura H (2008) Structure and properties of some natural cellulosic fibrils. Bioresources 3:192-203.
- Suurnakki A, Kantelinen A, Buchert J, Viikari L (1994) Enzyme aided bleaching of industrial softwood kraft pulps. Tappi J 77(11):111-116.
- Wada M, Heux L, Sugiyama J (2004) Polymorphism of cellulose I family: Reinvestigation of cellulose IV. Biomacromolecules 5(4):1385-1391.
- Wang B, Sain M (2007) Isolation of nanofibers from soybean source and their reinforcing capability on synthetic polymers. Compos Sci Technol 67(11-12):2517-2521.
- Wang B, Sain M, Oksman K (2007) Study of structural morphology of hemp fiber from the micro to the nanoscale. Appl Compos Mater 14(2):89-103.
- Wong KK, Nelson SL, Saddler JN (1996) Xylanase treatment for the peroxide bleaching of oxygen delignified kraft pulps derived from three softwood species. J Biotechnol 48:137-145.
- Zhao H-P, Feng X-Q, Gao H (2007) Ultrasonic technique for extracting nanofibers from natural materials. Appl Phys Lett 90:073112. 2 pp
- Zimmermann T, Bordeanu N, Strub E (2010) Properties of nanofibrillated cellulose from different raw materials and its reinforcement potential. Carbohydr Polym 79(4):1086-1093.
- Zuluaga R, Putaux JL, Cruz J, Vélez J, Mondragon I, Gañán P (2009) Cellulose microfibrils from banana rachis: Effect of alkaline treatments on structural and morphological features. Carbohydr Polym 76(1):51-59.