# Effects of Pectinase Treatment on Pulping Properties and the Morphology and Structure of Bagasse Fiber

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Bagasse was pretreated by pectinase, and both the control and pretreated bagasse were subjected to soda-anthraguinone (AQ) pulping. There were significant improvements in pulp properties after pectinase treatment, such as relative increases of brightness (5.5%), breaking length (17.1%), burst factor (16.5%), and tear factor (7.0%). The samples were analyzed by a fiber analyzer, scanning electron microscope (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). The pectinase treatment changed the material properties, which would improve the efficiency of subsequent pulping, such as increasing the fiber length (20.0%), lowering the fines length (10.6%), and increasing the percentage of flexible fiber. Pectinase treatment removed some non-cellulose components; in particular, the pectin and alcohol-benzene extractives were decreased by 19.4% and 37.3% after enzymatic treatment. The hemicellulose and lignin were decreased by 5.5% and 1.9%, respectively. A bulkier and more collapsed fiber surface was observed in the treated fibers, which suggested greater pore volume and more accessible surface area. Treatment caused a slight incline by 4.8% in the crystallinity index. Some chemical structures in pectin, hemicellulose, and lignin were partly broken, showing the effect of pectinase treatment on the degradation of non-cellulose components. Pectinase treatment prior to pulping is therefore recommended, given its efficiency and eco-friendly nature.

Keywords: Pectinase treatment; Bagasse pulp; Fiber properties; Surface characterizations; Chemical components; Structures

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# INTRODUCTION

Sugarcane bagasse, which is a fibrous residue of cane stalks left over after the juice has been extracted from sugarcane, is one of the largest cellulosic agro-industrial by-products (Pandey *et al.* 2000). However, while part of it is burned in sugar factories for energy purposes (heat and electrical), there is a considerable surplus of bagasse that can be used in pulp and paper production (Singhal *et al.* 2015).

Soda-AQ pulping produces a pulp with better properties, but at the same time it brings about some disadvantages, such as high chemical charge in pulping and bleaching and environmental pollution (Ates *et al.* 2008). Biopulping, which was used in fiber pretreatment prior to chemical pulping, would be a potential solution for some of the soda-AQ pulping problems, by, for instance, decreasing chemical consumption during pulping and bleaching (Liu *et al.* 2017), improving pulp strength properties, and reducing environmental pollution (Kaur *et al.* 2010; Martin-Sampedro *et al.* 2011). Biopulping can

result in better fiber qualities due to the superior flexibility, compressibility, collapsibility, and conformability of fibers produced using this method (Chen *et al.* 2010).

The enzymes used in biopulping include cellulases, hemicellulases, and ligninolytic enzymes such as lignin peroxidases, manganese peroxidases, and pectinases (de Souza-Cruz et al. 2004; Sittidilokratna et al. 2004; Martin-Sampedro et al. 2011). Of the enzymes mentioned above, pectinase has been applied in biopulping due to its selective removal of pectin. Pectic substances are common in the plant kingdom and form a major component of the middle lamella along with lignin, a thin layer of adhesive extracellular material found between the primary cell walls of adjacent young plant cells. Pectin is the protective barrier of the fiber, and the removal of pectin can promote the accessibility of certain chemicals and accelerate subsequent soda-AQ pulping (Pedrolli et al. 2009). In addition, pectinase is a multicomponent enzyme that includes pectin esterase, polygalacturonase, and lyase; it catalyzes the hydrolysis of the glycosidic bonds in pectic polymers and cleaves either at the end (exo-) or in the interior (endo-) of the pectin chain (Hoondal et al. 2002). Moreover, pectinases have been used in the treatment of plants to eliminate pectin, thus modifying the physical network of fibers and further affecting the properties of the fibers as well as separating the fibers (Evans et al. 2002). To date, there has not been a thorough study into the application of pectinase pretreatment prior to chemical pulping, which has implications for the improvement in pulping efficiency and quality.

The properties of fibers, such as fiber length, fines content, and width, greatly affect the quality of pulp and paper. Moreover, the content of lignin, the lignin distribution, and the hemicellulose content in lignocellulosic substrates determine the subsequent pulping efficiency (Kumar and Wyman 2009). The pulping process is also greatly influenced by the exterior and interior characteristics of the substrates, including cellulose crystallinity, pore volume, and accessible surface area (Tozzi *et al.* 2013; Song *et al.* 2016). Therefore, it is important to study the variation in physico-chemical parameters that occur after pectinase pretreatment.

In this study, the effects of pectinase pretreatment on the pulp properties were evaluated, and the chemical components of bagasse and the fiber properties after pectinase pretreatment were measured. Scanning electron microscopy (SEM) was conducted to monitor the morphological changes occurring on the surface of the bagasse. Finally, the functional groups and crystalline structure of bagasse fiber were analyzed using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), respectively.

# EXPERIMENTAL

#### Materials

The bagasse samples were supplied by Guangxi Huajing Group Co., LTD (Nanning, China). The enzymes used in this work contained 3000 IU/mL of pectinase, and a small amount of ligninase, xylanase, and mannase and were purchased from Sukehan Company (Weifang, China).

#### **Enzymatic Pretreatment**

Bagasse was pretreated with the pectinase in a 4 L reactor under the following conditions: temperature, 55 °C; pulp consistency, 10%; initial pH, 9.0 (adjusted with acetate buffer); pectinase dosage, 60 IU/g of oven-dried bagasse; and total time, 60 min (Liu *et al.* 2017). The control samples were subjected to the same treatment conditions

without the addition of the enzyme. After treatment, these pulps were filtered and thoroughly washed with distilled water.

# **Production of Pulps**

All bagasse samples were subjected to soda-AQ pulping in an oil bath rotating digester. The conditions of cooking were as follows: alkali dosage, 13%; anthraquinone dosage, 0.05%; ratio of liquor to bagasse, 5:1 (v/w); maximum temperature, 158 °C; heating time, 100 min; and holding time, 100 min (Liu *et al.* 2017).

# Analysis of Properties of Soda-AQ Pulps

Handsheets were prepared with pulp according to TAPPI T205 sp-02 (2002). The following properties of the handsheets were determined: brightness (TAPPI T217 wd-77 2004); breaking length (TAPPI T494 om-01 2001); burst factor (TAPPI T403 om-10 2010); and tear factor (TAPPI T414 om-04 2004). All experiments were carried out in triplicate, and the results are presented as the average values.

# **Measurement of Fiber Properties**

A Kajaani FiberLab analyzer (Kajaani, Finland) was used to measure the fiber properties, including fiber length, fine, width, curl, and kink index according to the method of TAPPI T271 om-02 (2002) and the procedure of Metso paper company (FiberLab 2001). Values were given as the average of three independent determinations for each sample.

# **Pulp Chemical Components**

The chemical components of the bagasse samples, before and after pectinase pretreatment, were determined as follows: cellulose (TAPPI T17 wd-70 2002), pentosan (TAPPI T223 cm-01 2004), acid-insoluble lignin (TAPPI T222 om-02 2004), ethanolbenzene extractives (TAPPI T204 cm-97 2004), and ash content (TAPPI T211 -02 2004). Values were given as an average of three independent determinations for each sample. Using gas chromatography, an analysis of the pectin degradation was performed to determine acidic sugars. Galacturonic acid was converted to acidic sugars by an acid methanolysis in which the depolymerized carbohydrates were silylated. The contents of the acidic sugars were determined using an Agilent 6890N HPLC (Agilent Technology, Palo Alto, CA, USA) equipped with FID detector and DB-1 (30 m × 0.32 mm × 0.25  $\mu$ m) (Pakarinen *et al.* 2012). The conditions were as follows: oven temperature of 150 °C (5 min), 2 °C min<sup>-1</sup> to 186 °C, followed by 1 °C min<sup>-1</sup> to 200 °C and 20 °C min<sup>-1</sup> to 325 °C; injector temperature of 225 °C; and FID temperature of 280 °C. The injection volume was 2  $\mu$ L with a split ratio of 1:30, and the flow rate of the carrier gas helium was 1 mL min<sup>-1</sup>.

# **SEM Analysis**

The scanning electron microscope (SEM) images of bagasse fibers were obtained using an S-3400N-II microscope (Hitachi, Tokyo, Japan). Gold was sputtered on the samples (one cycle of 120 seconds) before they were examined in secondary electron mode at a beam current of 100 mA and an accelerating voltage of 10 KV.

# **XRD** Analysis

The cellulose crystallinity of bagasse fiber was characterized using powder X-ray diffraction (XRD). The X-ray source was Cu target, and the  $K_{\alpha}$ -ray was generated at 40 KV and 40 mA. The CuK $\alpha$  radiation was filtered with nickel filters, and its radiation

wavelength was 0.15418 nm. Scans were obtained from 5 to 50 degrees  $2\theta$  in 0.02 degrees steps for 0.1 seconds per step. The XRD spectra were further analyzed using Jade 8 software (Livermore, USA), through which crystallinity data were obtained. The crystallinity index (CI) is typically used to illustrate the relative amount of crystalline material in cellulose. It was calculated from the height ratio between the intensity of the crystalline peak ( $I_{002}$ - $I_{am}$ ) and the total intensity ( $I_{002}$ ) according to Segal's method as follows (Segal *et al.* 1959),

$$CI = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$
(1)

where  $I_{002}$  is the maximum intensity of the crystalline peak at  $2\theta$  between 22° and 23° for cellulose I (between 18° and 22° for cellulose II) and  $I_{am}$  is the minimum intensity of the amorphous cellulose at  $2\theta$  between 18° and 19° for cellulose I (between 13° and 5° for cellulose II) (Roncero *et al.* 2005).

# **FTIR Spectra Analysis**

The analysis of the samples was performed using a Perkin-Elmer 2000 spectrometer (Phoenix, USA). A total of 100 scans, at a resolution of  $2 \text{ cm}^{-1}$ , were taken for each sample. The fibers were cut in an IKA MF10 cutting mill and then sieved to produce fibers in a size range from 106 to 212 mm. A mixture of 5.0 mg dried sample and 200 mg KBr was pressed into a wafer for the FTIR measurement.

# **RESULTS AND DISCUSSION**

# Effects of Pectinase Pretreatment on Soda-AQ Pulps Properties

The enzyme-treated and control bagasse samples were subjected to soda-AQ pulping under the same conditions. The brightness and physical strength properties of the handsheets prepared with the soda-AQ pulps are shown in Table 1.

	Brightness (% ISO)	Breaking Length (km)	Burst Factor (kPa⋅m²/g)	Tear Factor (mN·m²/g)
Control	35.00 ± 0.05	1.87 ± 0.04	3.22 ± 0.02	6.25 ± 0.03
Pretreatment	36.91 ± 0.07	2.19 ± 0.03	3.75 ± 0.04	6.69 ± 0.05

Table 1. Effects of Pectinase Pretreatment on Pulps Properties

Compared with the control, the pulps from bagasse pretreated with pectinase had higher ISO brightness (about 2% ISO higher). After pectinase pretreatment, some properties of physical strength of the pulps were improved, such as breaking length by 17.1%, burst factor by 16.5%, and tear factor by 7.0%. These results revealed that pretreatment with pectinase before pulping could yield further improvements in the paper quality. The results were similar to findings form other researchers (Pulkkinen *et al.* 2006; Garmaroody *et al.* 2011). The pectinase pretreatment may facilitate an increase in fiber average length and variations in the level of fiber bonding (Bajpai 1999). A longer average fiber length could lead to a higher tear index for pulps. Moreover, the higher level of fiber bonding could lead to a higher breaking length and better burst factor. Additionally, the pulp brightness was slightly improved by pectinase pretreatment. This result might reflect the decrease in residual lignin content in the pulps. A thorough study into the properties

and morphology of bagasse fiber after pectinase treatment would promote a deeper understanding about the effects of pectinase pretreatment on soda-AQ pulping.

#### Effects of Pectinase Pretreatment on Fiber Properties of Soda-AQ Pulps

Table 2 shows the effects of pectinase pretreatment on the soda-AQ pulp fiber properties, such as average fiber length, average fines length, average fiber width, fiber curl, and fiber kink index. The pectinase-pretreated samples had greater fiber lengths than did the controls. This result could partly be explained by a reduction in the fines fraction due to the decrease in F(1) and F(n). Compared with the control, slight increases in the fiber curl and kink indices were observed after pretreatment. Also, an increase in the fiber flexibility would be beneficial to the improvement of fiber length due to the increase in the curl and kink index. These findings were in agreement with other results (Evans *et al.* 2002).

	Fib	ber Length (mm)			Fines Length (%)		Fines Length (%)		Fines Length (%)		Width (µm)	Curl (%)	Kink Index
	L(I)	L(w)	L(n)		F(l)	F(n)							
Control	0.92 ±	1.57 ±	0.45 ±		7.10 ±	28.28 ±	19.09 ±	11.40 ±	0.72 ±				
	0.03	0.03	0.02		0.06	0.04	0.02	0.04	0.02				
Pretrea	1.03 ±	1.68 ±	0.54 ±		6.19 ±	25.27 ±	18.90 ±	11.70 ±	0.80 ±				
tment	0.02	0.04	0.01		0.05	0.06	0.04	0.03	0.03				

 Table 2. Effects of Pectinase Pretreatment on Fiber Properties of Soda-AQ Pulps

L(I), length-weight fiber length; L(w), weight-weight fiber length; L(n), arithmetic fiber length; F(I), length-weight fiber fines; F(n), arithmetic fiber fines

However, both the length-weight fiber fines (Fine (1)) and the arithmetic fiber fines (Fine (n)) of the bagasse soda-AQ pulp were reduced. In this respect, after pretreatment, the lower content of fine length and the higher percentage of flexible fiber with more collapsibility in the soda-AQ pulp could produce a paper with improved qualities (Hoondal *et al.* 2002), which agreed with the results of the superior pulp physical properties displayed in Table 1. The higher fiber curl and kink indices in the pulp could be explained by their higher fiber coarseness and higher cell wall flexibility, and that the effect of pectinase treatment on the chemical components of fibers was also responsible for this result.

	Cellulose (%)	Pentosan (%)	Acid- insoluble Lignin (%)	Alcohol- benzene Extractive (%)	Ash Content (%)	Galacturonic Acid (%)
Control	46.85 ± 0.05	26.48 ± 0.05	20.84 ± 0.05	2.71 ± 0.03	3.33 ± 0.06	0.98 ± 0.02
Pretreatment	47.98 ± 0.04	25.02 ± 0.06	20.44 ± 0.04	1.70 ± 0.05	2.98 ± 0.03	0.79 ± 0.03

Table 3. Effects of Pectinase Pretreatment on Chemical Components of Bagasse

#### Effects of Pectinase Pretreatments on Chemical Components of Bagasse

The main chemical components of the enzymatic pretreated and control bagasse samples are shown in Table 3. The galacturonic acid and pentosan contents after enzyme treatment were decreased by 19.4% and 5.5%, respectively, compared with the control.

These results indicated that the pectin bound to the hemicellulose was partly reduced, which led to the disintegration and rupture of the fiber network and the dissolution of part of the hemicellulose. George *et al.* (2014) and Zhang *et al.* (2013) found similar results that hemicellulose was partly removed after pectinase pretreatment by XPS and HPLC analysis.

Similarly, the ethanol-benzene extractives and ash were decreased by 37.3% and 10.5%, respectively. The main reason for this was that pectinase degraded some of the pectin in the bagasse, which made the loosened waxes on the surface fibers more easily removable and decreased the ethanol-benzene extractives (Abdel-Halim *et al.* 2008). Additionally, the pretreatment with the crude pectinase enzyme had degraded some resin and fatty compounds of the bagasse, leading to a decrease in the ethanol-benzene extractive content and ash content.

The lignin linked with ethanol-benzene extractives, ash, and pentosans in bagasse was also induced to fracture, resulting in a slight removal of acid-insoluble lignin. Importantly, harmful pectin in the aqueous phase of pulp would be depolymerized by the pectinase pretreatment, and consequently rendered harmless to the papermaking process by lowering the cationic demand (Ricard and Reid 2004). Simultaneously, the results may partially explain the differences in the pulping results between pectinase-pretreated bagasse and the control.

Although the effect of pectinase treatment on the removal of non-cellulose components was not obvious, the treatment was still beneficial to the subsequent pulping (as shown in Table 1). Therefore, the pectinase pretreatment made the pulping process not only economically feasible but also eco-friendly.

# Effects of Pectinase Pretreatments on Fiber Surface Morphologies of Bagasse

SEM images of bagasse fibers at magnifications between 500 and 1000 were obtained. The fiber sections were randomly selected. The SEM observation in Fig. 1 revealed that the surface of the controls were relatively smooth and rigid, and fewer damages and pores were observed on the exterior epidermis due to its wax-like hydrophobic covering on the fiber surface (Fig. 1a). However, the fiber surfaces pretreated by pectinase were decomposed, collapsed, and uneven (Fig. 1b).

Serious exfoliations emerged on the exterior area of the epidermis after the pectinase treatment, and some fragments were still present on the surface of the fibers, which indicated the incomplete activity of the pectinase to break down or depolymerize the pectin molecules in the partial areas of the fibers.

Additionally, these looser and bulkier surface characters in Fig. 1b might also have been caused by the removal of some characteristic structures by the pectinase treatment, such as the releasing of silica, the breaking of the intra-molecular hydrogen bonding of microfibril, and the dissolution of hemicellulose and lignin (Table 3). The changes in the fiber morphologies after pectinase treatment were conducive to improving pore volume and accessible surface area, and then promoting the entry of chemicals into the interior fiber during the pulping process.

Moreover, a more efficient lignin removal process with milder pulping conditions, less degradation of hemicelluloses and celluloses, higher pulp yield, and inexpensive and environmentally attractive option, were expected to be realized by pectinase treatment prior to pulping.

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Fig. 1. SEM images of bagasse fibers: (a) Control, (b) Pectinase-pretreated

#### Effects of Pectinase Pretreatments on Fiber Crystallinity of Bagasse

Changes in the crystallinity of undissolved cellulose due to chemical/mechanical/ biological treatment would greatly influence the cellulose dissolution, accessibility, and elastic state (Park *et al.* 2010). X-ray diffraction is an effective way to analyze cellulose crystallinity. The crystallinity changes in the samples, corresponding to both pectinasepretreated and control test samples, are shown in Table 4.

Sample	I <sub>am</sub> , a.u.	<i>I</i> <sub>002</sub> , a.u.	CI
Control	1643	2413	31.92
Pretreatment	2870	4312	33.45

Table 4. XRD Parameters for Control and Pretreated Samples

The crystallographic planes were labeled to indicate their crystal lattice assignments based on the observations of Ouajai and Shanks (2005). Reflections from the 16.0 °  $2\theta$  (assigned to the 101 crystallographic plane), 19.0 °  $2\theta$  (assigned to amorphous phase), and 22 °  $2\theta$  (assigned to the 002 crystallographic plane of cellulose I) were obtained in both the control test and pectinase-pretreated sample. Hence, there was no crystalline transformation of the crystalline structure, as the typical crystallographic pattern of cellulose I was acquired in the cellulose of bagasse fiber treated by pectinase.

The percentage of cellulose crystallinity was calculated by the area method. The degree of crystallinity (CI) was slightly higher for the cellulose of bagasse fiber with pectinase treatment (33.4%) than for that of the control sample (31.9%). This may have been due to the partial removal of pectin, amorphous hemicellulose, and lignin from bagasse as observed in Table 3, which brought about a relative increase in the degree of crystallinity. Additionally, the ordered crystalline and less ordered amorphous regions were

two-phase of cellulose models. The amorphous regions were more susceptible to the enzymes or chemical reagents than were the crystalline regions (Marcovich *et al.* 2001). To some extent, the increased degree of crystallinity (CI) would decrease the accessibility of subsequent chemical reagents to internal fiber. However, in addition to the CI, there were some other factors that also affected the accessibility of the chemicals to the fiber. The other characteristics of the substrate such as available particle size, surface area, and degree of polymerization could all have affected the accessibility of substrate (George *et al.* 2014). However, larger scale structures in celluloses may have drastically impacted the accessibility of enzymes and chemicals to the fiber (Dong *et al.* 1998). For instance, chemicals could barely attack the amorphous component packed sufficiently by the adjoining crystallites. Moreover, since the transition region of paracrystalline cellulose existed in between the crystalline and amorphous zones, assessment of the accessibility of the substrate by CI amount was even more difficult (Larsson *et al.* 1997). Therefore, the effects of pectinase treatment on bagasse fiber must be considered compositely.

#### FTIR Analysis of Bagasse Fiber

Infrared spectra of the bagasse fiber are shown in Fig. 2. The 1500 cm<sup>-1</sup> band corresponding to the stretching vibration of benzene rings, which had a stable intensity in the infrared spectra, was employed as the base band to identify the intensity of the other bands. The relative intensities of characteristic peaks are shown in Table 5.



Fig. 2. FTIR spectra of bagasse fibers: (A) Control, (B) Pectinase-pretreated

In Fig. 2, the spectrum of the treated bagasse fiber is similar to that of the control fiber, which indicated that pectinase treatment did not obviously change the chemical structures of the bagasse fibers. However, some peak intensities such as those at 895 cm<sup>-1</sup>, 830 cm<sup>-1</sup>, 1037 cm<sup>-1</sup>, and 3340 cm<sup>-1</sup> were remarkably reduced, by 45.3%, 27.2%, 26.0%, and 24.0%, respectively, which was attributed to the OH and C=O stretching vibration of

the celluloses and hemicelluloses (Rocha 2001; Nie *et al.* 2015) and the anomeric carbon (C1) vibration of the hemicelluloses (Peng 2010), respectively. In consideration of the decrease in hemicellulose content after pectinase treatment (Table 3), these weakened bands in FTIR spectra further suggested that some hemicelluloses were degraded during pectinase treatment, due to the less ordered amorphous regions and easily hydrolyzed properties of the hemicelluloses (Park *et al.* 2010).

Wavenumber (cm <sup>-1</sup> )	Relative Intensity (control) <i>A</i> i/A <sub>1500</sub>	Relative Intensity (treated) <i>A</i> i/A <sub>1500</sub>	Reduction Rate (%)	Corresponding Structures
3338-3348	1.7576	1.3364	23.96	OH stretching vibration
2916-2922	1.0379	0.8864	14.60	CH stretching vibration
1724-1730	1.2348	1.1000	10.92	C=O stretching vibration
1640-1642	0.2841	0.2722	4.19	antisymmetric COO- stretching
1602-1603	1.5833	1.3272	16.18	Benzene ring stretching vibration
1509-1511	1.2045	1.1955	0.75	Benzene ring stretching vibration
1418	1.5833	1.3364	15.60	Benzene ring stretching vibration
1370-1371	1.8636	1.5227	18.30	CH stretching vibration
1314-1315	1.9621	1.5727	19.85	C=O stretching vibration of phenolic ether; syringyl and guaiacyl lignin
1240-1243	3.1742	2.4363	23.25	C-O-C stretching vibration
1158-1161	3.0303	2.4091	20.50	C-O-C symmetric bending
1035-1037	7.2576	5.3727	25.97	C=O stretching vibration
895-898	2.7424	1.5000	45.30	C=C stretching vibration; the anomeric carbon (C1) vibration of hemicelluloses
800-850	2.5833	1.8818	27.16	CH bending vibration of aromatic ring

Table 5. Relative Intensity of Characteristic Peaks

Compared with the control sample, the band intensity at 1730 cm<sup>-1</sup> that was attributed to the C=O stretching of methyl ester and carboxylic in pectin (Ouajai and Shanks 2005) decreased by 10.9% after treatment, indicating that the pectin had been removed after the treatment. This result was also consistent with the observation in Table 3.

Additionally, the degree of methylation in pectin was clearly indicated (Bociek and Welti 1975). Generally, both esterified and carboxylic groups exist in the structure of pectin. However, the removal of the carboxylic section in pectin could not be explained by the information on the C=O band alone due to the close original position between carboxylic acid and esterified pectin bands. Moreover, the acetyl group of the hemicelluloses also causes disturbances because of the absorbance in the same region (Himmelsbach et al. 2002). Fortunately, the band at 1642 cm<sup>-1</sup> assigned to the antisymmetric COO- stretching is a characteristic band for the carboxylate ion in the water-extracted pectin (Synytsya et al. 2003), leading to a good separation of the carboxylate and ester bands. As shown in the spectra, an increase in the 1642 to 1730 cm<sup>-1</sup> ratio was obtained, suggesting a high degree of methyl ester content after pectinase treatment. The specific action of the enzyme on the nonesterified fraction might be responsible for this consequence. Also, the decreased intensity of the OH stretching band was caused by the higher content of methyl ester. The peak intensities at 1602 cm<sup>-1</sup>, 1418 cm<sup>-1</sup>, and 1314 cm<sup>-1</sup> assigned to the stretching vibration of the lignin benzene rings and to the syringyl and guaiacyl lignin were reduced by 16.2%, 15.6%, and 19.8%, respectively. There was also reduced band intensity at 1240 and 1158 cm<sup>-1</sup>, which was attributed to C-O-C stretching vibration and C-O-C symmetric bending. As shown in Fig. 2, the pectinase treatment caused an 18.3% reduction in the peak intensity at approximately 1370 cm<sup>-1</sup>, which was attributed to the C-H bending vibration of the celluloses and hemicelluloses. These results confirmed that part of lignins and hemicelluloses were degraded or removed after pectinase treatment.

# CONCLUSIONS

- 1. Pectinase pretreatment has potential for improving the efficiency and environmentalfriendliness of bagasse soda-AQ pulping. The brightness and physical strength properties of the pulp were noticeably improved by the pectinase pretreatment.
- 2. The properties of the pulp fibers after pretreatment, such as higher fiber length, lower fine length, and higher percent of flexible fiber, would be beneficial to subsequent pulping. The ash, organic solvent extractives, and pectin were partly removed, and the hemicellulose and lignin were slightly degraded by the pretreatment, leaving the fibers with a relatively high cellulose content as measured from Fourier transform infrared spectroscopy (FTIR). The scanning electron microscopy (SEM) study exhibited that the fiber surfaces became looser and collapsed after pretreatment, and X-ray diffraction (XRD) demonstrated that the pectinase treatment was conductive to a slightly higher crystallinity degree of the celluloses. Some structural changes in pectin, hemicellulose, and lignin occurred after pretreatment.

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# **REFERENCES CITED**

- Abdel-Halim, E. S., Fahmy, H. M., and Fouda, M. M. G. (2008). "Bioscouring of linen fabric in comparison with conventional chemical treatment," *Carbohydrate Polymers* 74, 707-711. DOI: 10.1016/j.carbpol.2008.04.044
- Ates, S., Atik, C., Ni, Y.H., and Gumuskaya, E. (2008). "Comparison of different chemical pulps from wheat straw and bleaching with xylanase pre-treated ECF method," *Turkish Journal of Agriculture and Forestry* 32, 561-570.
- Bajpai, P. (1999). "Application of enzymes in the pulp and paper industry," *Biotechnology Progress* 15, 147-157. DOI: 10.1021/bp990013k
- Bociek, S. M., and Welti, D. (1975). "Quantitative-analysis of uronic acid polymers by infrared spectroscopy," *Carbohydrate Research* 42, 217-226. DOI:10.1016/S0008-6215(00)84263-9
- Chen, Y., Wan, J., Ma, Y., and Lv, H. (2010). "Modification of properties of old newspaper pulp with biological method," *Bioresource Technology* 101, 7052-7056. DOI: 10.1016/j.biortech.2010.04.015
- de Souza-Cruz, P. B., Freer, J., Siika-Aho, M., and Ferraz, A. (2004). "Extraction and determination of enzymes produced by *Ceriporiopsis subvermispora* during biopulping of *Pinus taeda* wood chips," *Enzyme and Microbial Technology* 34, 228-234. DOI: 10.1016/j.enzmictec.2003.10.005
- Dong, X. M., Revol, J. F., and Gray, D. G. (1998). "Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose," *Cellulose* 5, 19-32. DOI: 10.1023/A:1009260511939
- Evans, J. D., Akin, D. E., and Foulk, J. A. (2002). "Flax-retting by polygalacturonasecontaining enzyme mixtures and effects on fiber properties," *Journal of Biotechnology* 97, 223-231. DOI: 10.1016/S0168-1656(02)00066-4
- FiberLab, M. A. (2001). "Research-quality fiber measurement for mill process management," <www.metso.com>, accessed 11 July 2017.
- Garmaroody, E. R., Resalati, H., Fardim, P., Hosseini, S. Z., Rahnama, K., Saraeeyan, A. R., and Mirshokraee, S. A. (2011). "The effects of fungi pre-treatment of poplar chips on the kraft fiber properties," *Bioresource Technology* 102, 4165-4170. DOI: 10.1016/j.biortech.2010.12.054
- George, M., Mussone, P. G., and Bressler, D. C. (2014). "Surface and thermal characterization of natural fibres treated with enzymes," *Industrial Crops and Products* 53, 365-373. DOI: 10.1016/j.indcrop.2013.12.037
- Himmelsbach, D. S., Khalili, S., and Akin, D. E. (2002). "The use of FT-IR microspectroscopic mapping to study the effects of enzymatic retting of flax (*Linum usitatissimum* L.) stems," *Journal of the Science of Food and Agriculture* 82, 685-696. DOI: 10.1002/jsfa.1090
- Hoondal, G. S., Tiwari, R. P., Tewari, R., Dahiya, N., and Beg, Q. K. (2002). "Microbial alkaline pectinases and their industrial applications: A review," *Applied Microbiology Biotechnology* 59, 409-418. DOI: 10.1007/s00253-002-1061-1
- Kaur, A., Mahajan, R., Singh, A., Garg, G., and Sharma, J. (2010). "Application of cellulase-free xylano-pectinolytic enzymes from the same bacterial isolate in biobleaching of kraft pulp," *Bioresource Technology* 101, 9150-9155. DOI: 10.1016/j.biortech.2010.07.020
- Kumar, R., and Wyman, C. E. (2009). "Does change in accessibility with conversion depend on both the substrate and pretreatment technology?" *Bioresource Technology*

100, 4193-4202. DOI: 10.1016/j.biortech.2008.11.058

- Larsson, P. T., Wickholm, K., and Iversen, T. (1997). "A CP/MAS C-13 NMR investigation of molecular ordering in celluloses," *Carbohydrate Research* 302, 19-25. DOI: 10.1016/S0008-6215(97)00130-4
- Liu, X.Y., Jiang, Y., Xie, Q.X., Nie, S.X., and Song, X.P. (2017). "Effect of alkali pectinase pretreatment on bagasse soda-anthraquinone pulp," *BioResources* 12, 5045-5056.

Marcovich, N. E., Reboredo, M. M., and Aranguren, M. I. (2001). "Modified woodflour as thermoset fillers. II. Thermal degradation of woodflours and composites," *Thermochimica Acta* 372, 45-57. DOI: 10.1016/S0040-6031(01)00425-7

Martin-Sampedro, R., Eugenio, M. E., Carbajo, J. M., and Villar, J. C. (2011).
"Combination of steam explosion and laccase-mediator treatments prior to *Eucalyptus* globulus kraft pulping," *Bioresource Technology* 102, 7183-7189. DOI: 10.1016/j.biortech.2011.04.053

Nie, S. X., Wang, S. F., Qin, C. R., Yao, S. Q., Ebonka, J. F., Song, X. P., and Li, K. C. (2015). "Removal of hexenuronic acid by xylanase to reduce adsorbable organic halides formation in chlorine dioxide bleaching of bagasse pulp," *Bioresource Technology* 196, 413-417. DOI: 10.1016/j.biortech.2015.07.115

Ouajai, S., and Shanks, R. A. (2005). "Morphology and structure of hemp fibre after bioscouring," *Macromolecular Bioscience* 5, 124-134. DOI: 10.1002/mabi.200400151

- Pakarinen, A., Zhang, J., Brock, T., Maijala, P., and Viikari, L. (2012). "Enzymatic accessibility of fiber hemp is enhanced by enzymatic or chemical removal of pectin," *Bioresource Technology* 107, 275-281. DOI: 10.1016/j.biortech.2011.12.101
- Pandey, A., Soccol, C. R., Nigam, P., and Soccol, V. T. (2000). "Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse," *Bioresource Technology* 74, 69-80. DOI: 10.1016/S0960-8524(99)00142-X
- Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., and Johnson, D. K. (2010).
  "Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulase performance," *Biotechnology for Biofuels* 3, 1-10. DOI: 10.1186/1754-6834-3-10
- Pedrolli, D. B., Monteiro, A. C., Gomes, E., and Carmona, E. C. (2009). "Pectin and pectinases: Production, characterization and industrial application of microbial pectinolytic enzymes," *The Open Biotechnology Journal* 3, 9-18. DOI: 10.2174/1874070700903010009
- Peng, F., Ren, J. L., and Xu, F. (2010). "Comparative studies on the physico-chemical properties of hemicelluloses obtained by DEAE-cellulose-52 chromatography from sugarcane bagasse," *Food Research International* 43, 683-693. DOI: 10.1016/j.foodres.2009.10.020
- Pulkkinen, I., Ala-Kaila, K., and Aittamaa, J. (2006). "Characterization of wood fibers using fiber property distributions," *Chemical Engineering and Processing: Process Intensification* 45, 546-554. DOI: 10.1016/j.cep.2005.12.003
- Ricard, M., and Reid, I. D. (2004). "Purified pectinase lowers cationic demand in peroxide-bleached mechanical pulp," *Enzyme and Microbial Technology* 34, 499-504. DOI: 10.1016/j.enzmictec.2003.12.005
- Rocha, S. M., Goodfellow, B. J., and Delgadillo, I. (2001). "Enzymatic isolation and structural characterisation of polymeric suberin of cork from *Quercus suber* L.," *International Journal of Biological Macromolecules* 28, 107-119. DOI: 10.1016/S0141-8130(00)00163-X

- Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2005). "The effect of xylanase on lignocellulosic components during the bleaching of wood pulps," *Bioresource Technology* 96, 21-30. DOI: 10.1016/j.biortech.2004.03.003
- Segal, L., Creely, J. J., Martin, A. E., and Conrad, C. M. (1959). "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer," *Textile Res. J.* 29, 786-794. DOI: 10.1177/004051755902901003

Singhal, A., Jaiswal, P. K., and Thakur, I. S. (2015). "Biopulping of bagasse by *Cryptococcus albidus* under partially sterilized conditions," *International Biodeterioration & Biodegradation* 97, 143-150. DOI: 10.1016/j.ibiod.2014.10.011

Sittidilokratna, C., Suthirawut, S., Chitradon, L., Punsuvon, V., Siriacha, P., and Kasetsart, U. (2004). "Screening of pectinase producing bacteria and their efficiency in biopulping of paper mulberry bark," in: *Proceedings of 42<sup>nd</sup> Kasetsart University Annual Conference*, Bangkok, Thailand, pp. 305-313.

Song, X., Jiang, Y., Rong, X., Wei, W., Wang, S., and Nie, S. (2016). "Surface characterization and chemical analysis of bamboo substrates pretreated by alkali hydrogen peroxide," *Bioresource Technology* 216, 1098-1101. DOI: 10.1016/j.biortech.2016.06.026

Synytsya, A., Copikova, J., Matejka, P., and Machovic, V. (2003). "Fourier transform Raman and infrared spectroscopy of pectins," *Carbohydrate Polymers* 54, 97-106. DOI: 10.1016/S0144-8617(03)00158-9

TAPPI T17 wd-70 (2002). "Cellulose in wood," TAPPI Press, Atlanta, GA.

- TAPPI T204 cm-97 (2004). "Solvent extractives of wood and pulp," TAPPI Press, Atlanta, GA.
- TAPPI T205 sp-02 (2002). "Forming handsheets for physical tests of pulp," TAPPI Press, Atlanta, GA.
- TAPPI T211 om-02 (2004). "Ash in wood, pulp, paper, and paperboard: Combustion at 525 °C," TAPPI Press, Atlanta, GA.
- TAPPI T217 wd-77 (2004). "Brightness of pulp," TAPPI Press, Atlanta, GA.
- TAPPI T222 om-02 (2004). "Acid-insoluble lignin in wood and pulp," TAPPI Press, Atlanta, GA.
- TAPPI T223 cm-01 (2004). "Pentosans in wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T271 om-02 (2002). "Fiber length of pulp and paper by automated optical analyzer using polarized light," TAPPI Press, Atlanta, GA.

TAPPI T403 om-10 (2010). "Burst strength of paper," TAPPI Press, Atlanta, GA.

TAPPI T414 om-04 (2004). "Internal tearing resistance of paper (Elmendorf-type method)," TAPPI Press, Atlanta, GA.

- TAPPI T494 om-01 (2001). "Tensile breaking properties of paper and paperboard," TAPPI Press, Atlanta, GA.
- Tozzi, E. J., Lavenson, D. M., McCarthy, M. J., and Powell, R. L. (2013). "Effect of fiber length, flow rate, and concentration on velocity profiles of cellulosic fiber suspensions," *Acta Mechanica* 224, 2301-2310. DOI: 10.1007/s00707-013-0922-2
- Zhang, J., Pakarinen, A., and Viikari, L. (2013). "Synergy between cellulases and pectinases in the hydrolysis of hemp," *Bioresource Technology* 129, 302-307. DOI: 10.1016/j.biortech.2012.11.064

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