Changes in the Microstructure and Components of *Eulaliopsis binata* Treated by Continuous Screw Extrusion Steam Explosion

Yanhong Feng, Bo Lei, Yong Liang, Huiting Zhong, Xiaochun Yin,* Jinping Qu, and Hezhi He

Eulaliopsis binata (EB) was pretreated by continuous screw extrusion steam explosion (SESE), with the aim of converting EB into useful materials on an industrial scale. The three main chemical components were characterized by component analysis using the Van Soest fiber detergent system, ultraviolet (UV) absorption spectrophotometry, gel permeation chromatography (GPC), and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy. Changes in the contents of detergent soluble species in the Van Soest process revealed the partial degradation of hemicellulose and cellulose, and the partial removal of lignin. GPC indicated that the molecular weight of lignin decreased from 4194 to 3710 g/mol over the first three SESE pretreatment cycles, but then increased to 4592 g/mol following the fourth SESE pretreatment cycle. UV absorption and ^{13}C-NMR results indicated the partial removal of lignin and the depolymerization and repolymerization of lignin during SESE pretreatment. Scanning electron microscopy (SEM) showed that the epidermis and parenchyma of EB were almost completely desquamated. X-ray diffraction (XRD) revealed that the crystallinity of EB initially increased and then subsequently decreased slightly, with increasing number of SESE pretreatment cycles. The varying physicochemical properties of EB resulting from different numbers of pretreatment cycles may find use in more diverse applications.

Keywords: Screw extrusion steam explosion; Eulaliopsis binata; Van Soest; Microstructure; Components; Lignin

Contact information: National Engineering Research Center of Novel Equipment for Polymer Processing, The Key Laboratory of Polymer Processing Engineering of the Ministry of Education, South China University of Technology, Guangzhou 510641, P.R. China;
** Corresponding author: xcyin@scut.edu.cn*

INTRODUCTION

Lignocellulosic materials such as agricultural, herbage, hardwood, and softwood products are potentially viable sources for the production of biofuels, value-added biochemicals, and composites, because of their abundance, affordability, and renewability. They consist primarily of plant cell wall materials, and are a complex natural composite of the three main biopolymers: cellulose, hemicellulose, and lignin. However, the recalcitrant structure of lignocellulosic biomass materials makes it difficult to achieve efficient conversion into useful products with high commercial value (Zhang *et al*. 2007). There is increasing interest in developing pretreatment methods to fractionate the structure of lignocelluloses and make it easier to obtain valuable resources from these materials.

Various pretreatment strategies have been examined, including the use of dilute-acid hydrolysis, alkaline hydrolysis, hot-water extraction, and steam explosion processes (Singh *et al*. 2015). The resulting pretreated biomass materials have mainly been applied in biorefining, reinforcing materials, and functional materials, by enhancing their susceptibility to enzymes and chemical reagents (Karimi and Taherzadeh 2016). Steam explosion is the most widely employed physical-chemical pretreatment method for the fractionation of biomass materials. This is because of its low energy requirements, eco-friendliness, and potential to disrupt cellulose, delignification, and the hydrolysis of hemicellulose (Tatsuro Sawada and Nakamura 2001; Singh *et al*. 2015). Most reported steam explosion processes have been carried out in batch reactors, which are characterized by intermittent processes under given conditions for a short period of time. These processes are unsuitable for the industrial scale, because of their relatively low efficiency, chemical recycling problems, and high costs (Bura *et al*. 2002; Kumar and Murthy 2011). Continuous steam explosion processes have recently been reported for pretreating lignocellulose. However, these processes have usually been based on the mechanical pulping refiner principle. This has drawbacks, including difficulties in controlling the moisture contents of materials, severe abrasion of the blow valve, and significant steam loss during discharge (Zimbardi *et al*. 1999; Fang *et al.* 2011a). Some of these processes have also been catalyzed by dilute acids or other chemical reagents. These factors clearly limit a large scale application (Fang *et al*. 2011b; Chen *et al*. 2013).

Developing improved continuous steam explosion processes is important for the effective pretreatment of lignocellulosic materials on the industrial scale. Screw extrusion is widely used in the food and polymer molding industries (Cheng and Friis 2010; He *et al*. 2014). This process can be applied to continuous steam explosion processes. This inspired us to develop a screw extrusion steam explosion (SESE) process for pretreating lignocellulosic materials. The advantages of the SESE process compared with traditional steam explosion processes are its low energy consumption, continuous production, and ease of industrialization (Chen *et al*. 2014). The SESE process can be adapted to pretreat a wide range of biomass materials. It is easily controlled by adjusting the screw speed, die-head gap, moisture content, and feed rate.

In the current study, a SESE apparatus was assembled and used for pretreating *Eulaliopsis binata* (EB). The SESE conditions were optimized to maintain an appropriate mobility of EB in the charging barrel and die, which is important for achieving the desired pretreatment effect. The effect of different SESE pretreatment intensities on different biomass materials should also be explored, to minimize energy consumption and maximize the pretreatment efficiency. EB is a naturally fibrous material and an excellent candidate for the investigating the SESE apparatus, because its threadlike fibers possess high strength and toughness. There has been little previous investigation on the pretreatment of EB, which may have restricted its application (Tang *et al.* 2011). Developing efficient pretreatment methods to better facilitate the subsequent application of lignocellulosic biomass materials is clearly important.

The current study explores the impact of SESE pretreatment on the structure and chemical components of EB. The intensity of the pretreatment process was altered by increasing the number of SESE pretreatment cycles. The findings provide insight about the impact of SESE pretreatment on the structural and chemical properties of EB, and could also aid our understanding of the effects of SESE pretreatment on non-wood biomass materials. Understanding differences in physicochemical properties following different pretreatment processes may also promote the application of EB in other fields.

EXPERIMENTAL

Materials

EB was purchased from a XianHe paper mill (Henan, P.R. China), and cut into chips approximately 10 mm long. The moisture content of the chipped material was adjusted to approximately 50%. Enzymatic hydrolysis of the chipped material was carried out using cellulase and hemicellulase, which were purchased from Sigma-Aldrich

(Shanghai, P.R. China). All other chemicals used in the study were of analytical grade from Sinopharm Chemical Reagents (Shanghai, P.R. China), and were used as received.

Screw Extrusion Steam Explosion Pretreatment

The continuous steam explosion apparatus consisted of a feeding device, screw, barrel, die, electric motor, and gearbox (Ma *et al*. 2016). The rotation of the screw continuously conveyed the EB chips with 50% moisture content forward. They were squashed, compacted by the screw, and gradually heated because of friction between the chips, screw, and barrel. The pressure and temperature of the compacted EB chips were *ca.* 1.5 MPa and 150 °C, respectively, at the point when they had been conveyed to the die. The compacted EB was then continuously discharged from the die, with a slit width of 1 mm. Pressurized water in the fiber bundles was instantaneously vaporized, which resulted in destruction of the tissue structure of the fiber bundles. This pretreatment process was repeated four times. This pretreatment was continuous because the EB chips were conveyed, compacted, heated, and exploded continuously from the feed inlet to the die. There was no upper limit for the amount of raw material that could be pretreated in a single cycle. This situation differs from conventional batch steam explosion, which pretreats a specific quantity of material in a single cycle. The intensity of conventional batch steam explosion is usually controlled by changing the treatment time, temperature, and pressure (Kang *et al*. 2013). However, the intensity of the current SESE pretreatment process was controlled by simply changing number of pretreatment cycles from 1 to 4. Samples were denoted as SESE-N, where N indicates the number of pretreatment cycles.

Extraction of Enzymatic Mild Acidolysis Lignin (EMAL)

The structure of milled wood lignin (MWL) is similar to that of native lignin, and has been investigated extensively. It was recently reported that EMAL can be generated in higher yields and with higher purity than MWL. EMAL was isolated according to the method proposed by Li *et al*. (2013). Briefly, EB was dewaxed in a Soxhlet extractor for 6 h with a 2:1 (v/v) mixture of toluene and ethanol, before being dried in an oven with circulating air at 60 °C for 24 h. EB (3 g of dried material) was subjected to enzymatic hydrolysis with cellulase (0.24 g) and hemicellulase (0.24 g), which was carried out at 50 °C in 100 mL of deionized water in a round-bottomed flask equipped with magnetic stirring for 48 h under mildly acidic conditions (pH 4.5), using a citric acid buffer solution. After the enzymatic hydrolysis, the suspension was filtered, and the resulting residue was washed twice with 0.01 M HCl.

The resulting residue was suspended in 100 mL of 96:4 (v/v) dioxane/0.01 M HCl, and extracted at 100 °C under a nitrogen atmosphere for 2 h. The resulting mixture was cooled to room temperature, and then filtered to yield a lignin solution. The solid residue was washed with fresh dioxane/water until the filtrate was clear. The combined filtrates were neutralized with sodium bicarbonate under magnetic stirring for 3 h. The mixture was then added dropwise to 1 L of aqueous 0.01 M HCl, and the resulting mixture was allowed to stand at room temperature for 2 h. The precipitated lignin was isolated by centrifugation at 10,000 rpm for 10 min, washed three times with deionized water, and then freeze-dried. Samples were denoted as EMAL_{raw} and EMAL_n (n =1, 2, 3, 4), where n indicates the number of SESE pretreatment cycles.

Characterization

The carbohydrate and lignin contents of the lignocellulosic materials were determined using the Van Soest fiber detergent system (Fibretherm FT12, Gerhard, Germany). The process consisted of steps involving a neutral detergent, acidic detergent, sulfuric acid hydrolysis, and calcination. Losses in weight per process step were noted as neutral detergent soluble (NDS), acid detergent soluble (ADS), sulfuric-acid-hydrolysis-soluble (SHS), and acid detergent lignin (ADL) (Peltre *et al*. 2010), respectively. For the untreated lignocellulosic materials, the NDS group consisted mainly of protein and starch, the ADS group consisted mainly of hemicellulose, and the SHS groups consisted mainly of cellulose. All determinations were performed in triplicate and the results presented as the corresponding mean values.

Morphological and structural characteristics of EB after SESE treatment were investigated by scanning electron microscopy (SEM), using a field-emission scanning electron microscope (LEO1530VP, Zeiss, Germany). Prior to analysis, powder samples were placed on specimen holders using double-sided conductive adhesive tape, and coated with gold to produce a 10-mm-thick film on the sample surface. The X-ray diffraction (XRD) pattern of EB cellulose was recorded on a Bruker D8 Advance X-ray diffractometer (Bruker, Germany) equipped with Ni-filtered Cu $K_{\alpha1}$ radiation (λ = 0.154 nm) at room temperature. The scattering angle range was 5 to 40°, the scanning speed was 8° per min, and the 2θ step interval was 0.02°. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used to investigate the chemical structures of EMAL materials obtained after increasing numbers of SESE pretreatment cycles (Avance III HD 400, Bruker, Germany). Following the enzymatic hydrolysis of EB after SESE pretreatment, ultraviolet (UV) absorption spectrophotometry was used to qualitatively determine the change in the structure of lignin in the filtrate. UV absorption spectra were collected on a UV-VIS spectrophotometer (S3100a, Agilent, Korea).

The average molecular weights of the EMAL materials were determined by gel permeation chromatography (GPC). Samples of EMAL (20 µL) were prepared in tetrahydrofuran (THF) at a concentration of 0.1%, and were injected on to a GPC system (1100, Agilen USA). THF was used as the eluent, with a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Changes in the Chemical Components of EB

Steam explosion is widely used in the physicochemical pretreatment of lignocellulosic biomass, because of its differing effects on cellulose, hemicellulose, and lignin. It is an environmentally-friendly method because of its absence of chemical reagents, and it enhances the fractionation of lignocellulosic biomass (Singh *et al*. 2015). We investigated the effect of SESE pretreatment on the components of EB. Table 1 shows that the relative amounts of the different Van Soest detergent groups varied with increasing number of SESE pretreatment cycles.

A gradual increase in NDS content was observed with increasing number of pretreatment cycles. This indicated that the hydrolysis of hemicellulose by acetic and other organic acids (formed from acetyl and other functional groups) proceeded under the aggressive pretreatment conditions (Jacquet *et al*. 2011). The increase indicated that hydrolyzed products were removed by the neutral detergent (Ma *et al*. 2014).

Table 1. Changes in Components of EB after Increasing Number of SESE Pretreatment Cycles

Composition (% w/w)	NDS	ADS	SHS	ADL
Raw materials	9.07±0.23	33.04±0.83	48.02±1.23	9.73±0.31
SESE-1	14.60±0.41	32.61±0.97	47.68±1.14	4.77±0.26
SESE-2	14.46±0.35	33.47±0.86	44.91±0.95	4.42±0.19
SESE-3	19.54±0.61	33.99±1.13	40.95±1.27	4.45±0.29
SESE-4	18.78±0.57	33.59±1.05	41.15±0.86	5.41±0.22

The partial degradation of lignin-carbohydrate complexes would lead to the formation of hydrophilic products, which would be removed by neutral detergent (Aimi *et al*. 2005). The ADL content initially decreased with SESE pretreatment. This indicated that products resulting from the partial degradation of lignin and lignin fragments were removed by the neutral and acidic detergents with hemicelluloses. A slight increase in ADL content was observed after the fourth pretreatment cycle, indicating a gradually increasing degree of repolymerization of lignin (Pielhop *et al*. 2015). The decrease in SHS content was consistent with the degradation of cellulose, and subsequent removal of degradation products by the acidic detergent. The small changes in ADS content were attributed to two reasons. The partial degradation production of cellulose was removed by the acid detergent, which increased the ADS content. The partial degradation production of hemicellulose was also removed by the neutral detergent, which decreased the ADS content. The changes in the soluble substance contents provided indirect information about the effect of SESE pretreatment on the fractionation of the three components of lignocellulose.

Characterization of Lignin in Filtrates Obtained from EB

UV absorption spectroscopy can be used to study the structure of lignin, because its aromatic rings and conjugated carbonyl structure absorb in the UV. Figure 1 shows UV absorption spectra of lignin in the different filtrates.

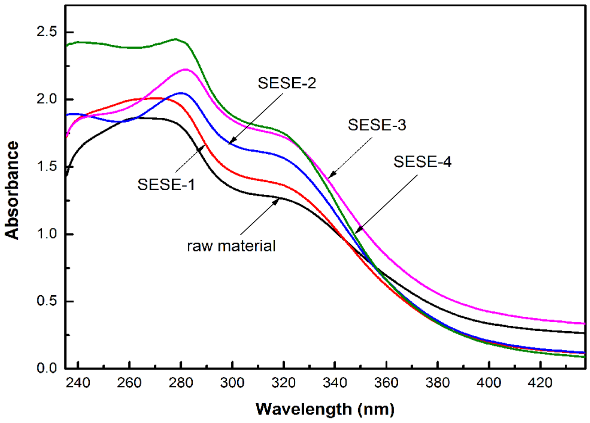

Fig. 1. UV spectra of EB enzymolysis filtrates with increasing number of SESE pretreatment cycles.

The spectra all contained a typical absorption peak at around 280 nm, which was attributed to non-conjugated phenolic groups (*i.e.* aromatic rings) of lignin. These groups are generally derived from the polylignol copolymers of sinapyl, coniferyl, and *p*-coumaryl alcohols (Wang and Chen 2013). The peak at 275 nm in the spectra of the raw material and SESE-1 indicated that the lignin in EB mainly consisted of syringyl units. The slight shift in the maximum of this peak from 275 (raw materials, SESE-1) to 280 (SESE-2, SESE-3, SESE-4) nm was attributed to a decrease in the number of syringyl units resulting from the cleavage of ether linkages and removal of methoxyl groups at the C-3/C-5 position (Wang *et al*. 2012). There was also a gradual increase in the intensity of the lignin peak at around 280 nm. This may have been related to an increase in the amount of lignin degradation fragments, and degradation of the carbohydrate.

The shoulder peak at around 320 nm was attributed to the high content of phenolic acid groups (Mitsuhashi *et al*. 2008). This indicated a low molecular weight and high chemical activity of lignin in the filtrate. The spectra exhibited a gradual increase in the intensity of the shoulder peak at around 240 nm. This may have been related to an increase in furfural in the filtrates, because of the degradation of hemicellulose upon SESE pretreatment (Li *et al*. 2015).

Molecular Weight and Polydispersity Characteristics of EMAL

GPC is a fast and effective method for analyzing polymers according to their molecular size. The weight-average molecular weights (M_w), and number mean molecular weight (M_n) and polydispersity values (M_w/M_n) of the EMAL materials were calculated from their GPC curves, and are shown in Table 2.

Table 2. Weight-average Molecular Weights (M_w, g/mol) and Polydispersity Values of EMAL Obtained from EB

	$EMAL_{raw}$	$EMAL_1$	$EMAL_2$	$EMAL_3$	$EMAL_4$
M_w	4194	4127	3985	3710	4592
M_n	2659	2857	2798	2622	3079
M_w/M_n	1.577	1.475	1.424	1.415	1.491

The M_w of EMAL underwent two substantial changes, because of variations in the molecular structure of lignin during SESE pretreatment. The M_w of EMAL gradually decreased from 4194 to 3710 g/mol with increasing number of SESE cycles. This may have been related to the depolymerization of lignin, because of the cleavage of ether linkages under acidic conditions during the two previous pretreatment cycles (Li *et al.* 2007). However, the M_w of EMAL subsequently increased to 4592 g/mol following the fourth SESE pretreatment cycle. This indicated that repolymerization gradually became more significant, resulting in an increase in the heterogeneous structure of lignin. Carbonium ions formed under acidic conditions were an intermediate in the depolymerization and repolymerization. Repolymerization became easier under the intense acidic conditions after the fourth SESE pretreatment cycle. Repolymerization is sometimes suppressed by changing the pH or adding a carbonium ion scavenger during steam explosion to increase the delignification efficiently (Toledano *et al.* 2014). The polydispersity of the EMAL initially decreased and subsequently increased as the structure of lignin changed during SESE pretreatment.

Solid-State ^{13}C-NMR Spectroscopy of EMAL

Figure 2 shows solid-state ^{13}C NMR spectra of EMAL materials obtained following increasing number of SESE pretreatment cycles of EB. Figure 2 provides qualitative information about each sample, and the effects of SESE pretreatment on lignin. Table 3 shows assignments of ^{13}C NMR peaks to specific functional groups. The absence of signals in the range 90 to 102 ppm indicated a low residual sugar concentration in the lignin (Sun *et al.* 2000).

The aromatic region of the ^{13}C NMR spectra (103 to 153 ppm) contained several signals. The signal at 152.5 ppm was attributed to C-3/C-5 carbons in etherified syringyl units. The signal at 147.0 ppm was attributed to C-3/C-5 carbons in non-etherified syringyl units, and to C-4 carbons in etherified guaiacyl units of lignin. The relative intensity ratio of the NMR signals at 152.5 and 147 ppm gradually decreased with increasing number of SESE pretreatment cycles. This indicated a decrease in the number of lignin ether bonds during SESE pretreatment. The signals at 114.9 and 132.6 ppm were assigned to C-5 carbons of non-etherified guaiacyl units, and C-1 carbons of non-etherified syringyl units, respectively. The weak peak at 103.6 ppm was attributed to C-2/C-6 carbons of syringyl units, and the signal at 127.3 ppm to C-2/C-6 carbons in hydroxyphenyl units (El Hage *et al.* 2009). Figure 2 shows that EMAL derived from EB contained a high proportion of syringyl moieties.

Table 3. Assignment of Peaks in Solid-state ^{13}C NMR Spectra of EMAL Obtained from EB

δ (ppm)	Assignment
182.8	C in carbonyl groups
167.3	Phenolic hydroxyl groups
152.5	C-3/C-5 S^a etherified
147.0	C-3/C-5 S^a non-etherified, C-4 G^b etherified
132.6	C-1 S non-etherified
127.3	C-2/C-6 H^c
114.9	C-5 G non-etherified
103,6	C-2/C-6 S
81.1	C_β in β-O-4
73.3	C_α in β-O-4
61.0	C_γ in β-O-4
56.0	OCH_3 G S
34-11	Saturated hydrocarbon side-chains

^{a} S=syringyl; ^{b} G=guaiacyl; ^{c} H=hydroxypropyl.

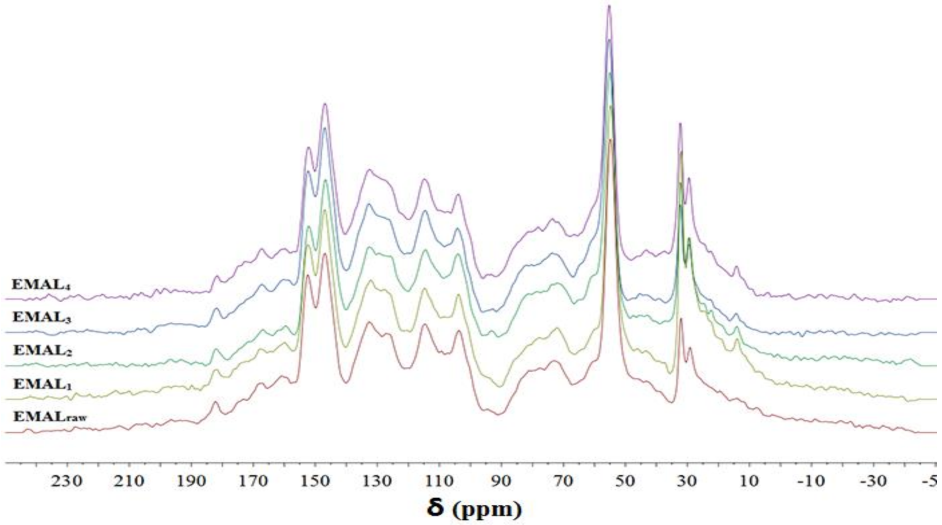

Fig. 2. Solid-state ^{13}C NMR spectra of EMAL obtained from EB after increasing number of SESE pretreatment cycles

Signals observed in the range 50 to 86 ppm were attributed to the oxygenated and non-oxygenated interunit linkages in lignin. The β-O-4 linkages responsible for the main intermonomeric linkages in lignin were observed at 81.1, 73.3, and 61 ppm, which corresponded to C_β, C_α, and C_γ, respectively (Yan *et al*. 2009). The intensity of the signals at 81.1 and 61 ppm decreased with increasing number of SESE pretreatment cycles. This provided evidence for cleavage of the β-O-4 structure, and the process involved in the depolymerization of lignin.

Signals in the range 11.1 to 34.4 ppm were assigned to the saturated hydrocarbon moieties of aliphatic side chains (Mao *et al.* 2006), and their intensities increased following SESE pretreatment. This result was consistent with the formation of C–C bonds, and the repolymerization of lignin. The gradual increase in the intensity of the signal at 167.3 ppm (phenolic hydroxyl groups) was also consistent with a decrease in the number of β-O-4 structures following SESE pretreatment (Wen *et al*. 2013). The large number of phenolic hydroxyl groups in lignin would promote its chemical modification.

Fig. 3. Appearance of (a) raw material, (b) SESE-1, (c) SESE-2, (d) SESE-3, and (e) SESE-4

Changes in the Structure and Morphology of EB

Changes in the color and physical appearance of EB following SESE pretreatment are shown in Fig. 3. EB changed from green-yellow to dark brown, as a consequence of the SESE pretreatment. This was most likely because of chemical reactions of lignin in the cell walls (Zhang and Cai 2005). Figure 3 also shows that the macroscopic size of the EB fiber bundles decreased with increasing number of SESE cycles.

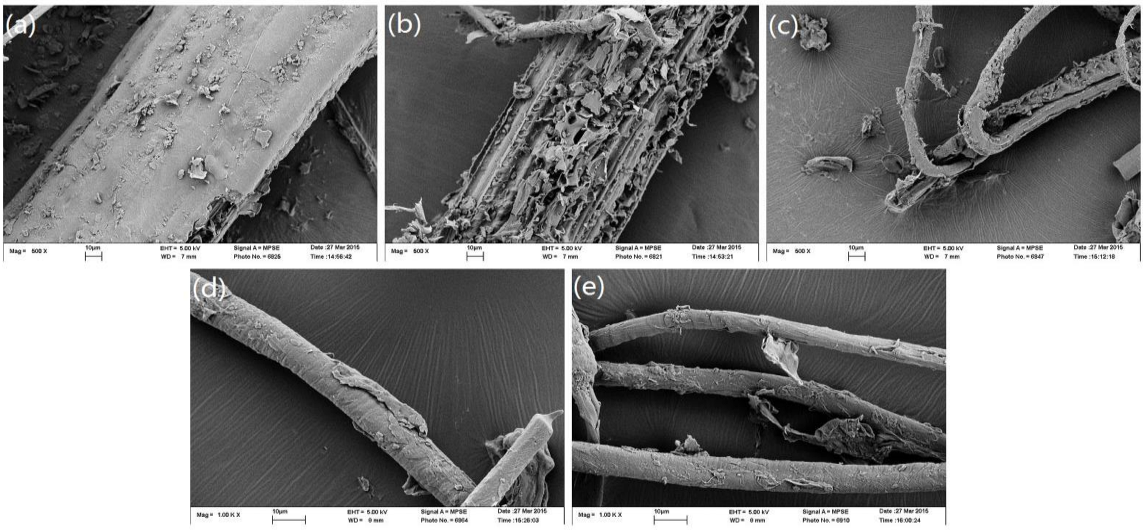

Fig. 4. SEM images of (a) raw material, (b) SESE-1, (c) SESE-2, (d) SESE-3, and (e) SESE-4

SEM images in Fig. 4 show the structural changes occurring in EB following SESE pretreatment. The EB fibers of the untreated material were surrounded by an epidermis (Fig. 4a). Most of the epidermis was lost after the first SESE pretreatment cycle (Fig. 4b). The second pretreatment cycle resulted in squeezing of the parenchyma (Fig. 4c). The parenchyma was further split following the third and fourth pretreatment cycles (Fig. 4d and e, respectively) (Kristensen *et al*. 2008). Individual fibers with relatively smooth surfaces and about 10 µm in width were eventually obtained. The partial defibration of EB was also observed following the fourth SESE pretreatment cycle. Changes in the structure of EB could significantly impact on the application of modified EB materials.

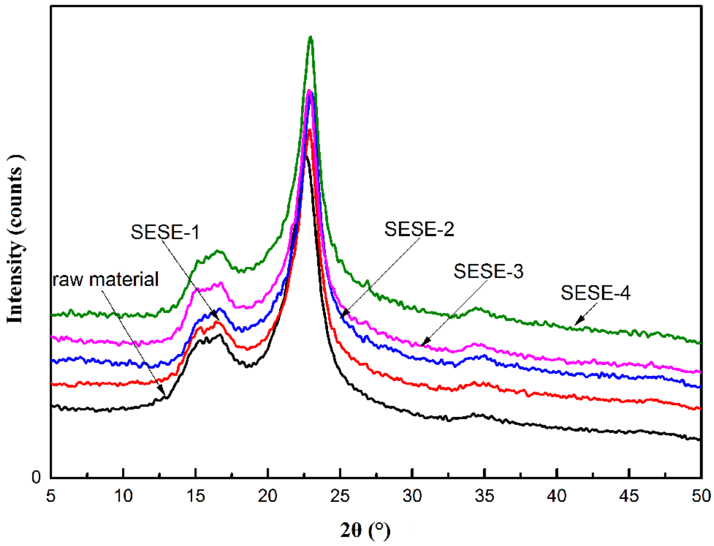

Fig. 5. X-ray diffraction patterns of EB after increasing number of SESE pretreatment cycles

Effect of SESE Pretreatment on Cellulose Crystallinity

The crystallinity of cellulose is important, as it affects the feasibility of different applications of materials containing cellulose. For example, highly crystalline cellulose is suitable as a reinforcing agent, because of its excellent mechanical properties, but is not significantly susceptible to enzymolysis. The possibility of changing the crystallinity of cellulose by SESE pretreatment should therefore be explored.

Table 4. Crystallinity of EB after Increasing Numbers of SESE Pretreatment Cycles

Samples	Crystallinity(%)
Raw material	42.62%
SESE-1	43.01%
SESE-2	47.82%
SESE-3	45.63%
SESE-4	44.97%

XRD patterns of EB materials subjected to increasing number of SESE cycles are shown in Fig. 5. The crystallinities of EB are shown in Table 4. The crystal structure of cellulose-I remained unchanged following the SESE pretreatment cycle. The crystallinity gradually increased until the third SESE pretreatment cycle. This was attributed to the partial disruption and degradation of hemicellulose and lignin. The crystal structure became further disrupted with further pretreatment, causing a slight decrease in crystallinity. The small changes in crystallinity indicated that SESE treatment had a limited impact on cellulose. The crystallinity of the cellulose region remained largely intact, ensuring favorable mechanical properties which are important in reinforcing materials and papermaking.

CONCLUSIONS

1. Changes in the components of EB during SESE pretreatment were investigated using the Van Soest method. The results indicated the partial degradation of hemicellulose and cellulose, and the partial removal of lignin.

2. Depolymerization and repolymerization of lignin were apparent from changes in the weight-average molecular weight and structure. The repolymerization of lignin began to dominate the depolymerization after the fourth pretreatment cycle.
3. The color of EB gradually changed from green-yellow to dark brown, indicating reactions between chemical constituents in the cell walls. The epidermis and parenchyma were almost completely desquamated by the SESE pretreatment. This resulted in lignocellulose fibers of high aspect ratio. Minor disruption in the crystalline region of cellulose was observed after SESE pretreatment.
4. SESE pretreatment impacted on the structure and components of EB, especially pretreatment involving three consecutive SESE cycles. It is important to now optimize this pretreatment process, and investigate its application to other biomass materials.

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