

Effect of Pretreatment Method on Structural Changes of Coconut Coir Dust

Hanny F. Sangian,^a and Arief Widjaja^{b,*}

The pretreatment of a high lignin substrate, coconut coir dust, was studied by chemical techniques (NaOH solution, ionic liquid, and NaOH followed by ionic liquid) and by a physical method (subcritical water, SCW). Following substrate pretreatment and a washing step, structural analyses were performed by scanning electron microscopy, X-ray diffraction (XRD), and Fourier transform infrared spectroscopy. It was found that all substrates pretreated by chemical methods had more amorphous structures than the untreated substrate. The XRD patterns of the chemically treated substrates shifted toward higher angles by 0.50° to 1.00°. However, the XRD peak symmetry of the SCW-treated substrate did not shift, but its crystallinity index decreased. The results revealed that lignocellulose treated with NaOH followed by ionic liquid at 120 °C for 30 min showed the greatest extent of structural transformation.

Keywords: Sodium hydroxide; Ionic liquid; Pretreatment; Subcritical water

Contact information: a: Department of Physics, Sam Ratulangi University, Manado, North Sulawesi, 95115, Indonesia; b: Department of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, East Java, 60111, Indonesia; *Corresponding author: arief_w@chem-eng.its.ac.id

INTRODUCTION

Lignocellulose from coconut coir is abundantly available in tropical countries, including Indonesia. Coconut coir is primarily composed of cellulose and hemicellulose, which can be converted into more valuable products (Van Dam *et al.* 2006). Ding *et al.* (2012) found that coconut coir consisted of 21.6% cellulose, 17.3% hemicellulose, and 46.4% lignin. Because coconut coir contains both cellulose and hemicellulose, it could be converted into reducing sugars and then fermented into biofuel (hydrogen) as a substitute for fossil-based energy (Widjaja *et al.* 2015; Sangian 2016).

An efficient process is crucial for the conversion of lignocellulose into value-added materials. Lignin, which was once considered waste, has been integrated into the process of converting biomass into biofuels and valuable materials (Hasunuma *et al.* 2013), which fits the definition of a biorefinery. Furfural and levulinic acid have been hydrogenated successfully to produce furfuryl alcohol and the promising biofuel γ -valerolactone (GVL) (Yan and Chen 2013). This new material, GVL, is derived from lignocellulose and is considered a promising substance for production of fuels and green chemicals, as reviewed by Yan *et al.* (2015). Current investigations are focused on new catalysts, called *layered double hydroxides* (LDH) and *derived metal oxides* (DMO), which can be tuned by controlling the acid and alkaline properties around the metal center and which display many good functions for biorefinery (Yan *et al.* 2017).

However, prior to conversion, biomass must first be subjected to a pretreatment process, which is the most important step for various applications (Liu *et al.* 2011; Hong *et al.* 2012). A physical method using steam explosion pretreatment was conducted with

the aim of modifying the characteristics of wood and corn residue (Muzamal *et al.* 2016; Yang *et al.* 2017). Employing acid and alkaline conditions is a very popular and common method for chemical pretreatment on lignocellulose.

For sugarcane bagasse, organic waste, and Napier grass, treatment with such substances has been used to successfully degrade lignin and to alter the morphological structure (Spigno *et al.* 2008; Chang *et al.* 2011; Kumar *et al.* 2014). These studies have shown that acid and alkaline treatments can dissolve lignin components and modify cellulosic structures, increasing the digestibility for enzymatic hydrolysis. The monomer chains of polysaccharides are easily released and hydrolyzed after chemical pretreatment (Araque *et al.* 2008; Zhao *et al.* 2009; Chaudhary *et al.* 2012; Asgher *et al.* 2013; Sun *et al.* 2013 Sangian *et al.* 2015a).

Currently, investigators have been examining the use of pure ionic liquids (ILs) for biomass pretreatment before enzymatic hydrolysis. The application of ILs is referred to as a green technology because used ILs can be recycled, and thus, ideally this technique prevents pollution and does not harm the environment (Feng and Chen 2008). It has been shown that ILs could modify the cellulose structure and improve sugar yields (Su *et al.* 2012; Aver *et al.* 2014; Liu *et al.* 2017). ILs also have the ability to dissolve lignin for pulp preparation (Duan *et al.* 2016).

Water under subcritical conditions (subcritical water, SCW) is directly ionized into H^+ and OH^- , which act as acid and alkaline agents and reactants simultaneously (Peterson *et al.* 2008). Because SCW has many advantages, this method is widely used for biomass pretreatment. SCW hydrolysis has been reported to directly liberate monomers and oligosaccharides from polysaccharides (Kumar *et al.* 2010; Ju *et al.* 2011; Zhao *et al.* 2011).

Previous investigations of SCW treatment of biomass have been carried out under isobaric conditions. The SCW method combined with acid is an alternative approach for biomass pretreatment because it can be conducted at moderate pressures and temperatures (Ahmed *et al.* 2013). Substrates derived from industrial waste, such as coconut husks, grape seeds, sugarcane bagasse, and pressed palm, were pretreated under SCW conditions, in which a pressurized semi-batch reactor was used with steam compression, to successfully liberate reduced sugars (Prado *et al.* 2014a,b).

This investigation focuses on the pretreatment and characterization of lignocellulose in coconut coir dust treated by chemical methods (NaOH, IL, and NaOH + IL) and by SCW, which has not been previously examined. SCW hydrolysis was conducted by varying the pressure in the reactor from 40 to 160 bar for a water volume of 0.120 L. All substrates treated chemically and with SCW were characterized by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

The substrate, coconut coir dust, was obtained from Traditional Copra Industry in the southern part of Minahasa Regency, North Sulawesi, Indonesia. The reactants, trimethyl phosphate (TMP) and 1-methylimidazole (MIM), were obtained from Sigma-Aldrich (St. Louis, MO, USA). The IL, 1,3-dimethyl phosphate [MMIM][DMP], was

synthesized according to a previously reported method (Renken *et al.* 2007; Yang *et al.* 2010; Thomas *et al.* 2011).

Successful synthesis of the IL was confirmed using ^1H NMR (JNE-ECS-400 MHz, Jeol Resonance, Tokyo, Japan), as previously reported (Sangian *et al.* 2015b). The chemical pretreatments employed in this study were adapted from previous reports (Yuan *et al.* 2013).

Pretreatment Methods

Prior to IL pretreatment, the substrate (50 g, 120 mesh) was added to 250 mL of NaOH at 80 °C for 15 h, and then the solid was neutralized by washing with warm water. Subsequently, 6 g of NaOH-treated substrate was mixed with 100 mL of IL at various temperatures (100, 120, and 150 °C) for 10 min to 15 h.

After IL pretreatment, 20 mL ethanol was poured into the mixture to break the IL and cellulose bonds. The precipitated solid was recovered by filtration under reduced pressure (Whatman paper No. 2, GF/B 25 mm). An IL pretreatment was also performed following washing in warm water, without first treating the substrate with NaOH.

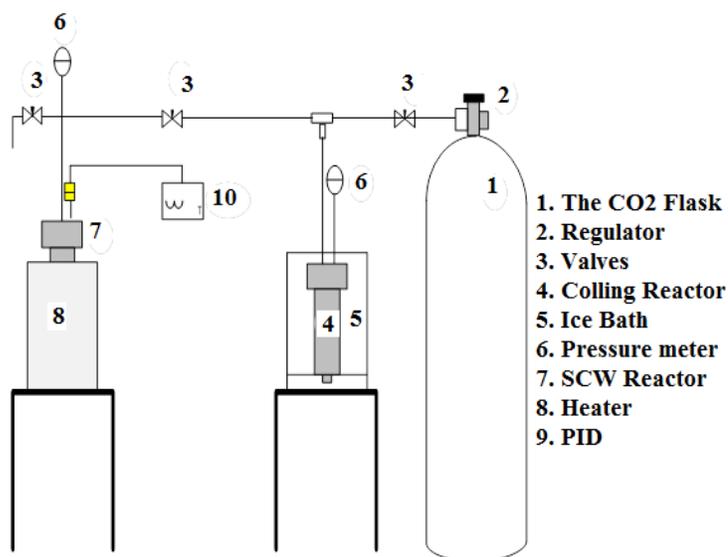


Fig. 1. Design of the subcritical water reactor used in this work equipped with a *proportional–integral–derivative (PID) controller*

The SCW apparatus mainly consisted of a CO₂ cylinder, a regulator, a pressure meter, valves, a SCW reactor, a transit reactor, and a heater, as shown in Fig. 1. Unlike the chemical pretreatment processes, a wash step was not included in the SCW hydrolysis process, and thus not much water was used. Native coconut coir powder (6 g) was mixed with 0.120 L water in the reactor. Prior to SCW hydrolysis, the pressure was generally set 5 to 10 bar lower than required. Carbon dioxide was supplied to the reactor at 40, 80, and 160 bar. When the pressure was maintained at 40 bar, the temperature was 120, 130, 140, or 150 °C.

When the pressure was 80 bar, the temperature was 150, 160, 180, or 200 °C. When the pressure was 160 bar, the temperatures was 200, 220, 260, or 300 °C. SCW hydrolysis was conducted for 1 h under all conditions. After hydrolysis, the reactor was cooled to 30 °C. The solid was separated from the liquid by filtration and then dried for 2 days at 60 °C prior to analysis.

Chemical Composition Determination

The chemical composition of the coconut coir dust was determined by using a previously reported method (Datta 1981). These measurements were conducted in triplicate.

Characterization

XRD (Philips, X'Pert X-ray Diffractometer, Almelo, Netherlands) and FTIR spectroscopy (FT/IR MODEL 4200 JASCO, Tokyo, Japan and Nicolet iS 10 FT-IR Spectrometer, Waltham, MA, USA) were used to examine the structure of the substrate after chemical and SCW pretreatment for comparison with the substrate without treatment. The surface characteristics of the samples were analyzed with SEM (EVO®LS 10; Carl Zeiss Micro Imaging GmbH, Göttingen, Germany).

The crystallinity parameters determined from these analyses were the crystallinity index (CrI) and the lateral order index (LOI). The CrI compares the maximum intensity of the 002 peak (I_{002}) and the minimum intensity (I_{AM}) situated between the 002 peak and the 101 peak in the XRD pattern after subtraction of the background intensity (I_{BG}), as follows (Park *et al.* 2010):

$$CrI (\%) = \frac{I_{002} - I_{AM}}{I_{002}} \times 100 \quad (1)$$

The LOI is calculated from the absorbance ratio of chemical bonds at 1446 and 896 cm^{-1} in the FTIR spectrum. This is an old method, but it has been verified by other authors (Nada *et al.* 2009). The LOI was determined using the following equation:

$$LOI (\text{cm}^{-1}/\text{cm}^{-1}) = \frac{A_{\text{at } 1426/\text{cm}}}{A_{\text{at } 896/\text{cm}}} \quad (2)$$

The FTIR analysis was carried out on limited samples: native substrate and the substrates pretreated with IL (120 °C, 15 h), 1% NaOH (80 °C, 16 h), NaOH+IL (150 °C, 15 h), and SCW (80 bar, 150 °C, 1 h).

RESULTS AND DISCUSSION

Chemical Compositions

Table 1 shows the chemical compositions of the substrates after chemical pretreatment (IL, NaOH, and NaOH+IL) at various temperatures and times. The native substrate consists of 27.1% cellulose, 17.8% hemicellulose, and 40.2% lignin. Following pretreatment with 1% NaOH, the cellulose content increased to 44.0%, whereas the lignin content decreased to 22.7%. This significant decrease was caused by lignin dissolving in the alkaline solution. When the NaOH+IL method was applied at 120 °C, the cellulose content increased to 55.1% (10 min), 49.9% (30 min), 48.0% (5 h), and 44.3% (15 h). However, when only the IL was employed for pretreatment, lignin degradation did not occur.

The SCW pretreatment produced two products, the SCW-treated substrate and sugar liberated directly during pretreatment, which will be published in another paper. Table 2 displays the chemical compositions of the SCW-treated substrates that were not decomposed into sugars. The cellulose contents changed slightly at pressures of 40 to 80

bar, whereas the hemicellulose content decreased significantly compared with that in the native substrate.

Hemicellulose was probably converted into the sugars at lower pressures, as its structure is more amorphous than that of cellulose. The cellulose content started decreasing when the pressure was increased to 80 bar, which indicates that some cellulose was dissolved as water-soluble substances.

Meanwhile, the lignin content increased to 47.4% at 40 bar and 120 °C. The changes in the chemical compositions were also caused by the release of some sugars from the fibers in SCW.

Table 1. Chemical Compositions of Substrates Treated by Chemical Methods

Pretreatment	Chemical contents (%)				
	Cellulose	Hemicellulose	Lignin	Extract	Ash
No Pretreatment	27.1	17.8	40.2	12.2	2.60
1% NaOH (80 °C, 16 h)	44.0	19.2	22.7	13.5	0.63
IL (120 °C, 15 h)	22.1	25.8	47.4	3.9	0.69
1% NaOH + IL (120 °C, 15 h)	44.3	19.9	21.2	14.5	0.17
1% NaOH + IL (120 °C, 5 h)	48.0	20.0	21.1	10.8	0.17
1% NaOH + IL (120 °C, 30 min)	49.9	21.2	19.3	8.8	0.70
1% NaOH + IL (120 °C, 10 min)	55.1	23.0	17.6	3.6	0.72
1% NaOH + IL (100 °C, 30 min)	50.2	18.7	19.4	11.0	0.62
1% NaOH + IL (150 °C, 30 min)	47.5	16.8	22.3	13.0	0.40

Table 2. Chemical Compositions of Substrates Treated by the SCW Method

Variables (bar, °C)	Chemical contents (%)				
	Cellulose	Hemi-	Lignin	Extract	Ash
No Pretreatment	27.1	17.8	40.2	12.2	2.60
(40, 120)	26.2	13.9	47.4	10.8	1.75
(40, 130)	31.5	9.4	39.9	14.8	4.41
(40, 140)	31.9	7.5	47.0	9.3	4.26
(40, 150)	26.5	9.8	51.0	10.2	2.42
(80, 150)	30.0	4.9	53.8	8.9	2.46
(80, 160)	30.4	4.0	55.5	8.1	1.95
(80, 180)	28.9	6.1	55.0	8.2	1.70
(80, 200)	26.1	6.2	54.8	12.4	2.48
(160, 200)	24.3	6.6	47.1	21.5	2.25
(160, 220)	21.4	8.8	46.2	23.1	2.16
(160, 260)	19.5	12.6	49.6	17.8	3.50

Surface Morphology

Figure 2 shows SEM images of the substrates treated with NaOH, IL, and NaOH+IL, as well as the control (without pretreatment). The surface of the original lignocellulose was more textured than that of the treated substrates. Lignin, which has an amorphous structure, was still tightly bound to cellulose and hemicellulose. When NaOH was employed for pretreatment, part of the lignin and hemicellulose was liquefied and removed from the substrate, so that the cellulose was more exposed.

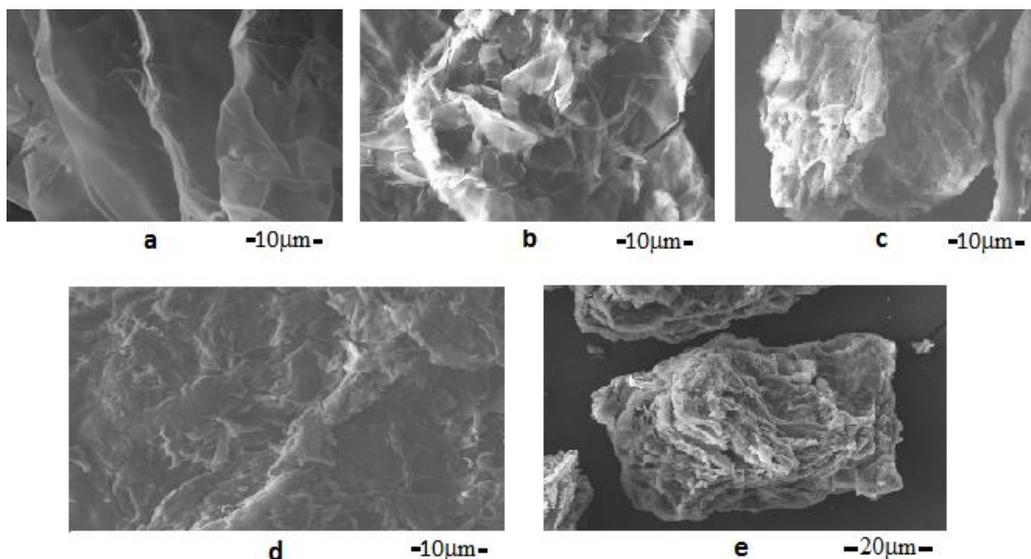


Fig. 2. SEM images of the native substrate (a) and substrates treated with NaOH, 80 °C, 16 h (b), IL, 120 °C, 15 h (c), NaOH+IL, 120 °C, 15 h (d), and NaOH+IL, 120 °C, 30 min (e)

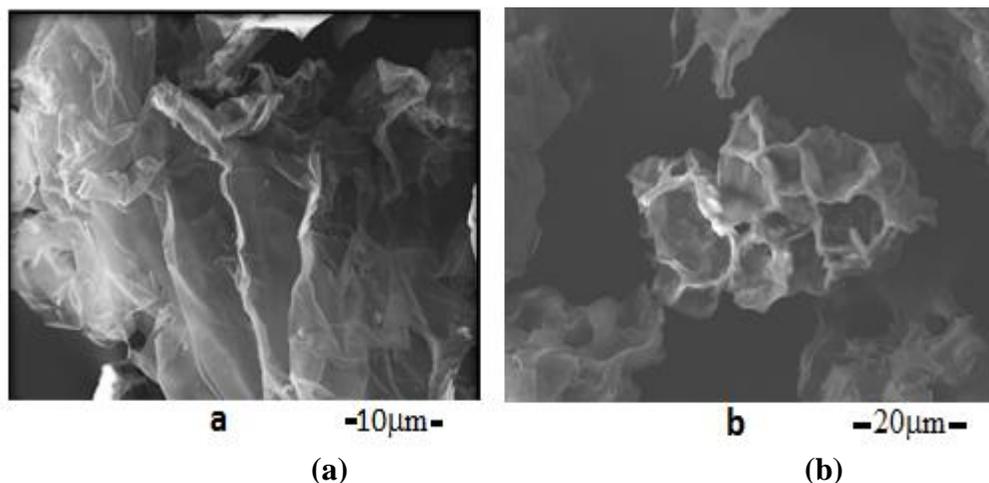


Fig. 3. SEM images of native substrate (a) and a SCW-treated substrate (80 bar, 150 °C, 1 h) (b)

Meanwhile, in the NaOH+IL-treated substrate, the cellulose was very porous and textured. After alkaline pretreatment (delignification), the IL interacted freely with cellulose through an electrical attraction between the ions (cation, anion) of the IL and electrical dipoles of the substrate. The SEM image shows that the substrate treated with NaOH+IL at 120 °C for 30 min was more textured than that treated at 120 °C for 15 h. Thus, it was concluded that NaOH+IL pretreatment increased the surface area of cellulose

relative to that in the control. These conditions can improve the digestibility for enzymatic hydrolysis in sugar preparation (Li *et al.* 2009; Silva *et al.* 2011; Qiu *et al.* 2012).

The surface structure of the substrate treated by SCW at 80 bar and 150 °C was also investigated in comparison with that of the native substrate, as shown in Fig. 3. The surface morphology of the substrate was remarkably altered following SCW pretreatment, as some lignin and hemicellulose, as well as monomers, were dissolved in water (Oztruk *et al.* 2010).

Crystalline Characteristics

The crystallinity of the substrates was analyzed using XRD (Fig. 4) and the CrI values were calculated using Eq. 1 (Park *et al.* 2010). Distinct peaks were located at 22° (002), 16° (101), and 35° (040) whereas contributions from amorphous regions of the substrate were determined at 18, which are similar to the original cellulose characteristics (Nazri *et al.* 2016).

Table 3 shows the CrI values of the substrates after chemical pretreatment and that of the substrate without pretreatment. The CrI value of the native substrate (44.5%) was much lower than that of the NaOH-treated substrate owing to the presence of lignin, which was still connected to cellulose and had an irregular structure. As shown in Fig. 4, the CrI values of the substrate improved slightly to 47.5% following IL pretreatment at 120 °C for 15 h.

Table 3. CrI Values of Substrates Treated by Chemical Methods

Pretreatment	I_{002}			I_{AM}			CrI (%)
	2θ	BG*	Int	2θ	BG	Int	
No pretreatment	23.006	100.152	337	18.644	112.234	249	44.5
NaOH + IL (120 °C, 15 h)	23.006	119.800	446	18.978	126.494	307	45.6
NaOH + IL (120 °C, 5 h)	23.524	97.804	380	18.594	101.057	220	58.5
NaOH + IL (120 °C, 30 min)	23.023	95.081	530	18.026	103.376	296	56.8
NaOH + IL (120 °C, 10 min)	23.056	169.629	517	18.026	191.963	408	40.4
NaOH + IL (100 °C, 30 min)	23.023	140.024	418	18.327	155.520	351	31.4
NaOH + IL (150 °C, 30 min)	23.073	113.858	424	18.895	127.640	313	42.1
NaOH (80 °C, 16 h)	22.240	18.964	207	18.080	14.388	83	62.0
IL (120 °C, 15 h)	23.480	20.328	112	17.440	13.684	59	47.2

*BG: Background

When alkaline solution (NaOH) was employed in the pretreatment, the CrI value noticeably increased to 62.0% owing to delignification and dissolution of hemicellulose, which exposed cellulose (Koullas *et al.* 1993). When the substrate was subjected to the NaOH+IL method at 120 °C (10 min) and 150 °C (30 min), the CrI values decreased remarkably to 40.4% and 42.1%, respectively. These results showed that treatment of coconut coir dust with NaOH followed by an IL greatly transformed the original substrate to a more amorphous substrate, which could increase the digestibility for enzymatic hydrolysis (Liu *et al.* 2012). The symmetry of the XRD patterns also changed, with an

approximately 0.50° to 1.00° shift toward higher angles, which is indicative of crystals dilation after the NaOH and NaOH+IL pretreatments. This change is comparable with previous observations (Zhu *et al.* 2012).

Varying the time of the pretreatment using the NaOH+IL method at 120°C resulted in changes in crystallinity. At 10 min, the CrI value was relatively low (40.4%). As the time was increased to 30 min and 5 h, the CrI value increased significantly to 56.8% and 58.6%, respectively owing to dissolution of lignin and hemicellulose. Further increasing the pretreatment duration to 15 h, however, decreased the CrI value to 45.6%, which may be caused by degradation of cellulose.

The XRD patterns of coconut coir dust before and after SCW treatment are shown in Fig. 5. The CrI value of the untreated substrate was relatively high (45.8%), whereas hydrolysis by SCW (80 bar, 150°C) decreased the CrI value of the substrate to 42.5%. This decrease in CrI was due to the degradation of cellulose and hemicellulose into reduced sugars and the transformation of the substrate structure into an irregular structure (Cheng *et al.* 2011). A significant difference in the symmetry of the XRD patterns was observed between SCW-treated solids and chemically treated solids. As discussed previously, a peak shift of 0.50° to 1.00° was observed for the substrates treated by chemical methods. However, Fig. 5 reveals that the symmetry of the XRD patterns did not change following SCW treatment. These results indicate that the crystalline structure of the SCW-treated substrate was not diluted, but the CrI was changed.

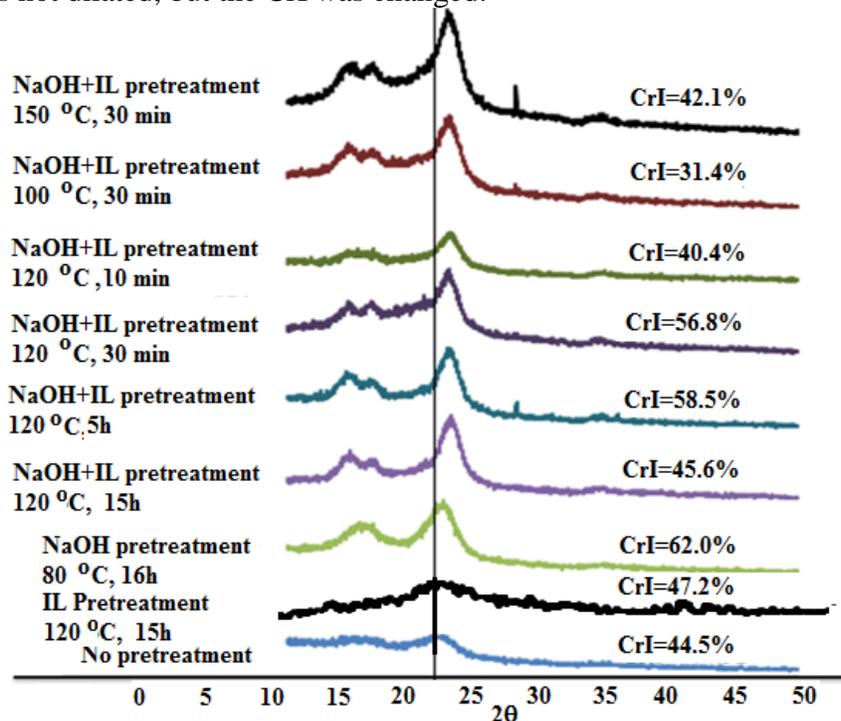


Fig. 4. X-ray diffraction patterns of coconut coir dust before and after various chemical pretreatments

In SCW and supercritical conditions, water is ionized to H^+ (proton) + OH^- , which act as reactants and catalysts (acid and alkaline) to break the H and β -(1,4)-glycosidic bonds connecting fibers. The reaction can be written as follows: $\text{Fiber---OH---OH---fiber} + \text{H}^+ + \text{OH}^- \rightarrow \text{Fiber---OH---OH}^-$ and $\text{H}^+---\text{OH---fiber}$. The length of the polysaccharide chains continues to be shortened until simple monomers are obtained: $\text{---fiber---O---fiber---} + \text{H}^+$

+ OH⁻ → sugars. The concentration of protons is much higher than those of the other reactants, and these reactions proceed following collisions with H⁺.

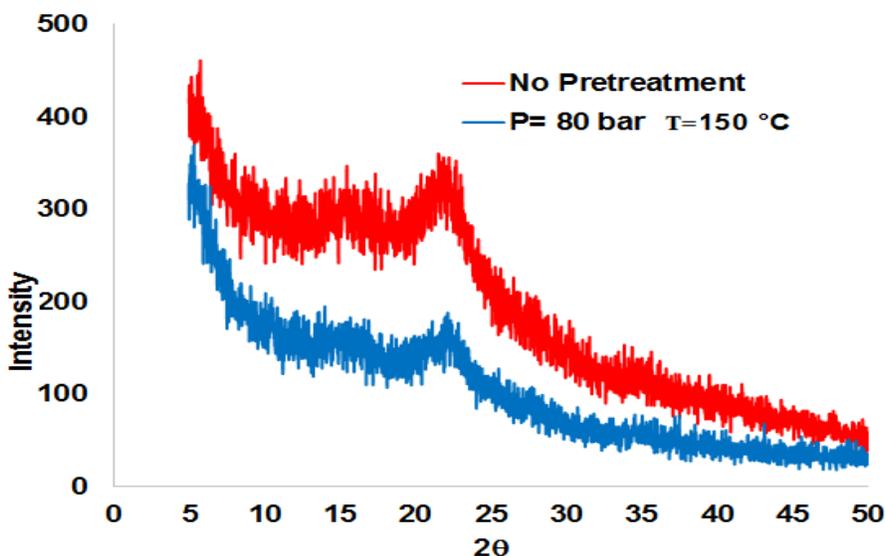


Fig. 5. X-ray diffraction patterns of coconut coir dust before and after SCW treatment

FTIR Analysis of Substrates

FTIR spectroscopy in the range from 400 to 4000 cm⁻¹ was also used to analyze the structure of the substrates treated by chemical methods.

Table 4. Wavenumbers of FTIR Absorption Bands for Native and Treated Lignocelluloses

Wavenumbers (cm ⁻¹)					Bond absorptions
Native	IL	NaOH	NaOH+IL	SCW*	
3434	3432	3434	3429	3324	O–H stretching and intramolecular vibrations
2926	2924	2923	2923	2905	C–H and alkane stretching (on cellulose)
1627	1630	1629	1653	1605	OH bending of absorbed water
1516	1508	1509	1506	1505	C=C aromatic stretching (dominated by lignin)
1446	1449	1452	1424	1451	CH ₂ and OCH in plane bending (on cellulose)
1375	1383	1373	1372	1357	CH ₃ bending (on cellulose)
1257	1260	1268	1268	1232	G ring stretching (C–C, and C–O, and stretching and COH bending at C6 in cellulose)
1033	1035	1031	1032	1031	C–C, C–OH, C–H ring, and side group vibrations
896	895	895	897	894	COC, CCO (glycosidic connections on cellulose), and CCH stretching
672	670	671	672	659	C–OH out of plane bending
609	607	578	609	558	-

* Conducted at 150 °C and 150 bar for 1 h.

In this section, the FTIR results are discussed only for selected samples (native substrate and the substrates treated with IL (120 °C, 15 h), 1% NaOH (80 °C, 16 h), and NaOH+IL (150 °C, 15 h), as the XRD results sufficiently described the changes in the substrate crystallinity. In general, the observed FTIR absorption bands varied considerably depending on the pretreatment method, as shown in Table 4, Fig. 6, and Fig. 7. It was found that the spectra of substrates treated with IL and without pretreatment were relatively similar, which is reasonably consistent with the XRD analysis.

The wavenumbers of the main bands for native and treated lignocelluloses, which describe the bond absorptions, are shown in Table 4, and these results are relatively comparable to other findings (Spiridon *et al.* 2010; Poletto *et al.* 2014). To determine the extent of damage to the original cellulose, the bands at 2923 to 2926 cm^{-1} (C–H stretching), 1424 to 1429 cm^{-1} (CH_2 and OCH bending), 1372 to 1383 cm^{-1} (CH_3 bending), and 895 to 897 cm^{-1} (glycosidic connection motions) were analyzed. The NaOH-treated substrate exhibited absorption corresponding to OH stretching at 3434 cm^{-1} , which was similar to that of the native substrate; C–H symmetrical stretching at 2923 cm^{-1} , which was close to that following NaOH+IL pretreatment; OH bending of absorbed water at 1629 cm^{-1} ; and C–C, C–OH, C–H ring, and side groups at 1031 cm^{-1} . After NaOH+IL pretreatment, the absorption bands were observed at 3429, 2923, 1653, 1424, 1372, 1032, and 897 cm^{-1} , and the C=C aromatic stretching exhibited a drastic shift to lower wavenumbers (1506 cm^{-1}), which is related to delignification of the substrate.

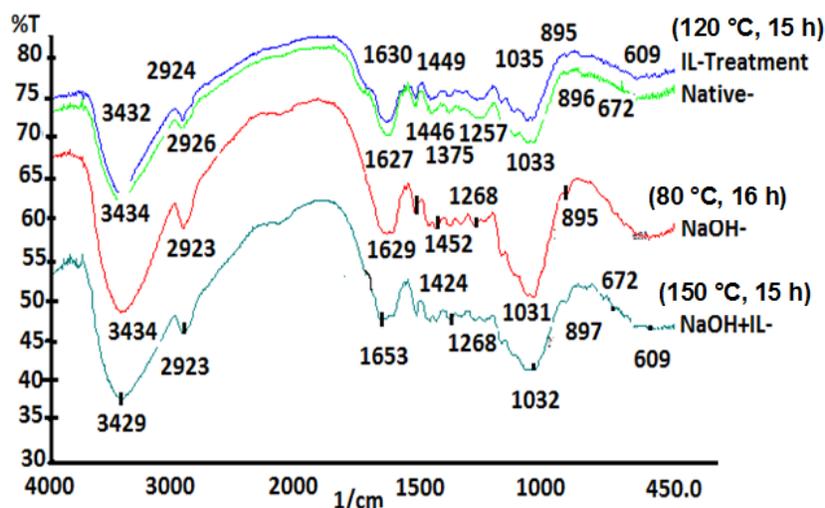


Fig. 6. FTIR spectra (transmittance vs. wavenumber) of the native substrate and the substrates treated with ionic liquid (120 °C, 15 h), 1% NaOH (80 °C, 16 h), and NaOH+IL (150 °C, 15 h)

Meanwhile, the SCW-treated substrate exhibited absorption bands at 3324 (O–H stretching), 2905, 1505 (C=C aromatic vibration), and 894 cm^{-1} (C–H and glycosidic stretching). The spectra of the IL-treated substrate showed a slight shift toward lower wavenumbers, with the exception of the CH_3 bonds, which shifted to 1383 cm^{-1} from 1375 cm^{-1} for the native substrate. The increase in OH bending was influenced by water absorbed by substrate during the wash step, similar to the trend observed for the NaOH- and NaOH+IL-treated substrates. The CH_2 bending motions in the IL- and NaOH-treated substrates shifted toward longer wavenumbers (1449 cm^{-1} and 1452 cm^{-1}), whereas that of the NaOH+IL-treated substrate shifted noticeably toward shorter wavenumbers (such as 1424 cm^{-1}).

Generally, for all treated substrates, the C=C stretching vibrations on the aromatic plane in the lignin chains showed a noticeable shift to lower wavenumbers (1508, 1509, and 1506 cm^{-1}) relative to native lignocellulose (1516 cm^{-1}). This change was attributed to the decrease in lignin chains after pretreatment. The vibrations of C–C, and C–O, and C–O–H bonds located at C6 in cellulose shifted to 1260 cm^{-1} (IL), 1268 cm^{-1} (NaOH), and 1268 cm^{-1} (NaOH+IL) from that 1257 cm^{-1} for the native substrate. This change was caused by the breakage of many H-bonds between fibers in the substrates.

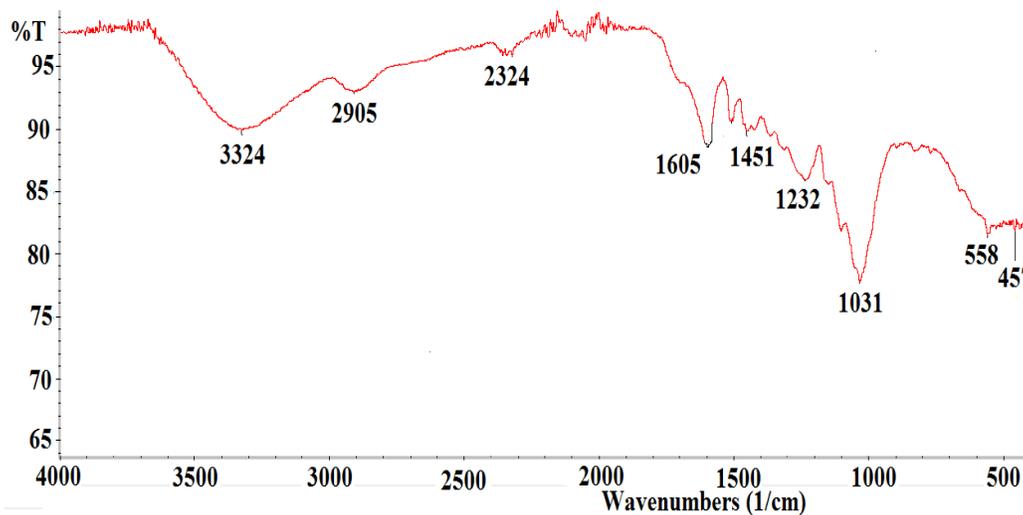


Fig. 7. FTIR spectrum (transmittance vs. wavenumber) of the SCW-treated substrate (80 bar, 150 °C, for 1 h)

Meanwhile, little change was observed in the bands corresponding to the C–O–C, C–C–O, and C–C–H vibrations of the glycosidic connections, which means that the IL, NaOH, and NaOH+IL pretreatments did not damage the glycosidic connections significantly. The shift in wavenumbers was attributed to degradation of the substrate after pretreatment and transformation of the substrate from a high crystallinity structure to a low crystallinity or amorphous structure (Sun *et al.* 2008). In FTIR, another method used to analyze the crystallinity is the LOI, which is defined as the ratio of the absorbances at around 1426 and 896 cm^{-1} (O'Connor *et al.* 1958; Nelson *et al.* 1964a; Nelson *et al.* 1964b).

The LOI value describes the structural order of cellulose before and after pretreatment. Table 5 shows the LOI values for the substrates before and after pretreatments, as obtained from duplicate FTIR measurements. A general decrease in the LOI values was observed following pretreatment, except for alkaline pretreatment, which had a slightly increased LOI owing to lignin dissolution (Mirahmadi *et al.* 2010). The trend observed for the average LOI values for all substrates was comparable to that observed for the CrI values determined by XRD.

The average LOI value of the native substrate was 1.25, and a slight decrease to 1.23 was observed for the IL-treated substrate. When the substrate was treated with NaOH, the LOI value increased considerably to 1.27, which was attributed to increased absorbance corresponding to CH₂ scissoring vibrations at 1452 cm^{-1} owing to decreased lignin bonding to cellulose after alkaline treatment. It was confirmed that the NaOH-treated substrate had the most regular structure, with the highest CrI and LOI values. The LOI value of the NaOH+IL-treated substrate decreased greatly to 1.07, which was influenced primarily by a weakening of CH₂ bending motions. When the substrate was subjected to SCW treatment

at 150 °C and 80 bar for 1 h, the LOI value decreased significantly to 0.89. The large decreases in the LOI values for the NaOH+IL- and SCW-treated lignocelluloses indicate that the substrates transformed into amorphous structures (Spiridon *et al.* 2010).

Table 5. Lateral Order Index (LOI) Determined from Duplicate FTIR Measurements

Substrate	Transmittance		Absorbance		LOI
	At 1426 cm ⁻¹	At 896 cm ⁻¹	At 1426 cm ⁻¹	At 896 cm ⁻¹	
Native	62.50	70.25	37.50	29.75	1.25
IL	72.00	77.25	28.00	22.75	1.23
NaOH	54.75	64.25	45.25	35.75	1.27
NaOH+IL	41.50	45.50	58.50	54.50	1.07
SCW (150 °C, 80 bar)	89.74	88.44	10.26	11.56	0.89

The trend observed for the LOI values was found to be relatively close to that observed using XRD to describe the structural changes of the substrates. Of all the treated substrates, the NaOH-treated substrate had the most crystalline structure, based on the LOI values. High transmittance through this substrate occurred owing to the crystalline structure of cellulose that was exposed by delignification. The NaOH+IL-treated substrate exhibited the lowest transmittance, attributed to a transformation from genuine cellulose to amorphous cellulose, which would be easily hydrolyzed enzymatically into reduced sugars.

Structural parameters, CrI and LOI values, and SEM images can be used to infer the cellulose (high crystallinity), hemicellulose, and lignin (low crystallinity) contents after pretreatment as reviewed by Zhao *et al.* (2012). As described previously, the CrI of the NaOH-treated substrate was the highest among the substrates, mainly owing to the removal of lignin, which has an amorphous structure, resulting in an increased cellulose content. Meanwhile, the CrI of the SCW-treated substrate (80 bar and 150 °C) decreased, as the lignin composition content increased.

This observation indicates that the treated substrate became more amorphous owing to the presence of lignin. As change was observed in the symmetry of the XRD pattern, pressure had no effect on the cellulose structure. The chemical composition of a substrate contributes enormously to the biomass structure before and after pretreatment. Generally, for substrates subjected to chemical treatment, cellulose I crystals were transformed into cellulose II, as indicated by shifting of the XRD symmetries.

CONCLUSIONS

1. Lignocellulose, which has a high lignin content, was successfully pretreated using NaOH solution, IL, and NaOH followed by IL treatments at various temperatures and times. The substrates obtained using chemical methods were compared with that obtained using a physical technique (SCW).
2. Shifted XRD patterns were observed for all substrates after chemical pretreatment, but no shift was observed for the substrate subjected to the SCW method.

- SEM, XRD, and FTIR analyses revealed that the NaOH+IL pretreatment achieved the greatest structural alteration of the substrate into a more amorphous form. The surface morphology of the SCW-treated substrate (80 bar, 150 °C, 1 h) was transformed relative to that of the control. Moreover, the CrI and LOI values of the SCW-treated substrate decreased significantly relative to those of the control, indicating that the substrate was transformed into a more amorphous structure.
- The CrI value and surface morphology of the SCW-treated substrate (80 bar, 1 h) changed considerably, but the XRD symmetry was constant.
- The lignin content contributed substantially to the structure of lignocellulose before and after pretreatment.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Higher Education Department of the Indonesian Government for financial support and the Biochemical Engineering Lab of ITS Surabaya that provided the authors with wide access to conduct many experiments.

REFERENCES CITED

- Ahmed, I. N., Sylviana, S., Lien, H. H., Suryadi, I., and Yi-Hsu, J. (2013). "Subcritical water and dilute acid pretreatments for bioethanol production from *Melaleuca leucadendron* shedding bark," *Biochem. Eng. J.* 78, 44-52. DOI: 10.1016/j.bej.2013.03.008
- Asgher, M., Ahmad, Z., and Iqbal, H. M. N. (2013). "Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production," *Ind. Crop. Prod.* 44, 488-495. DOI: 10.1016/j.indcrop.2012.10.005
- Aver, K. R., Scortegagna, A. Z., Fontana, R. C., and Camassola, M. (2014). "Saccharification of ionic liquid-pretreated sugar cane bagasse using *Penicillium echinulatum* enzymes," *J. Taiwan Inst. Chem. Eng.* 45(5), 2060-2067. DOI: 10.1016/j.jtice.2014.04.017
- Chang, C. W., Chin, W., Yu, W. C., Chen, W. J., Chang, R. F., and Kao, W. S. (2011). "A study on the enzymatic hydrolysis of steam exploded Napier grass with alkaline treatment using artificial neural networks and regression analysis," *J. Taiwan Inst. Chem. Eng.* 42(6), 889-894. DOI: 10.1016/j.jtice.2011.04.002
- Chaudhary, G., Singh, L. K., and Ghosh, S. (2012). "Alkaline pretreatment methods followed by acid hydrolysis of *Saccharum spontaneum* for bioethanol production," *Bioresource Technol.* 124, 111-118. DOI: 10.1016/j.biortech.2012.08.067
- Cheng, G., Varanasi, P., Li, C., Liu, H., Melnichenko, Y. B., and Simmons, B. A. (2011). "Transition of cellulose crystalline structure and surface morphology of biomass as a function of ionic liquid pretreatment and its relation to cellulase hydrolysis," *Biomacromolecules* 12(4), 933-941. DOI: 10.1021/bm101240z
- Datta, R. (1981). "Acidogenic fermentation of lignocellulose-acid yield and conversion of components," *Biotechnology and Bioengineering* 23(9), 2167-2170. DOI: 10.1002/bit.260230921

- Ding, T. Y., Hii, S. L., and Ong, L. G. A. (2012). "Comparison of pretreatment strategies for conversion of coconut husk fiber to fermentable sugars," *BioResources* 7(2), 1540-1547
- Duan, R., Westerlind, B. S., Norgren, M., Anungwom, I., Virtanen, P., and Mikkola, J. P. (2016). "Fibre stress-strain response of high-temperature chemi-thermomechanical pulp treated with switchable ionic liquid," *BioResources* 11(4), 8570-8588. DOI: 10.15376/biores.11.4.8570-8588
- Feng, L., and Chen, Z. (2008). "Research progress on dissolution and functional modification of cellulose in ionic liquids," *J. Mol. Liq.* 142(1-3), 1-5. DOI: 10.1016/j.molliq.2008.06.007
- Hasunuma, T., Okazaki, F., Okai, N., Hara, K. Y., Ishii, J., and Kondo, A. (2013). "A review of enzymes and microbes for lignocellulosic biorefinery and the possibility of their application to consolidated bioprocessing technology," *Bioresource Technol.* 135, 513-522. DOI: 10.1016/j.biortech.2012.10.047
- Hong, B., Xue, G., Weng, L., and Guo, X. (2012). "Pretreatment of maso bamboo with dilute phosphoric acid," *BioResources* 7(4), 4902-4913. DOI: 10.15376/biores.7.4.4902-4913
- Ju, Y. H., Lien-Huong, H., Novy, S. K., Ting-Jiang, G., and Jen-Huo, W. (2011). "Analysis of soluble and insoluble fraction of alkali and subcritical water treated sugarcane bagasse," *Carbohydr. Polym.* 83, 591-599. DOI: 10.1016/j.carbpol.2010.08.022
- Koullas, D. P., Christakofoulo, P. F., Ekos, S. K., Koukios, E. G., and Macius, B. J. (1993). "Effect of alkali delignification on wheat straw saccharification by *Fusarium oxysporum* cellulases," *Biomass Bioenerg.* 4(1), 9-13. DOI: 10.1016/0961-9534(93)90022-v
- Kumar, A., Negi, Y. S., Choudhary, V., and Bhardwaj, N. K. (2014). "Characterization of cellulose nanocrystals produced by acid-hydrolysis from sugarcane bagasse as agro-waste," *J. Mater. Phys. Chem.* 2(1), 1-8. DOI: 10.12691/jmpc-2-1-1
- Kumar, S., Gupta, R., Lee, Y. Y., and Gupta, R. B. (2010). "Cellulose pretreatment in subcritical water: Effect of temperature on molecular structure and enzymatic reactivity," *Bioresource Technol.* 101(4), 1337-1347. DOI: 10.1016/j.biortech.2009.09.035
- Li, Q., He, Y. C., Xian, M., Jun, G., Xu, X., Yang, J. M., and Li, L. Z. (2009). "Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment," *Bioresource Technol.* 100 (14), 3570-3575. DOI: 10.1016/j.biortech.2009.02.040
- Liu, S., Fang, G., Wang, Q., Deng, Y., and Han, S. (2011). "Kinetic modeling of enzymatic hydrolysis of poplar waste oxidation pretreatment," *BioResources* 6(4), 4229-4237. DOI: 10.15376/biores.6.4.4229-4237
- Liu, W., Hou, Y., Wu, W., Ren, S., and Wang, W. (2012). "Complete conversion of cellulose to water soluble substances by pretreatment with ionic liquids," *Korean J. Chem. Eng.* 29(10), 1403-1408. DOI: 10.1007/s11814-012-0023-2
- Liu, W., Qi, W., Zhou, W., Liu, S., Zhang, X., Zhou, J., Yuan, Z., and Zhuang, X. (2017). "Analysis of hydrolyzates produced from cellulose catalyzed by carbonaceous solid acid in an ionic liquid," *BioResources* 12(1), 316-325. DOI: 10.15376/biores.12.1.316-325

- Mirahmadi, K., Kabir, M. M., Jeihanipour, A., Karimi, K., and Taherzadeh, M. J. (2010). "Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production," *BioResources* 5(2), 928-938.
- Muzamal, M., Bååth, J. A., Olsson, L., and Rasmuson, A. (2016). "Contribution of structural modification to enhanced enzymatic hydrolysis and 3-d structural analysis of steam-exploded wood using x-ray tomography," *BioResources* 11(4), 8509-8521. DOI: 10.15376/biores.11.4.8509-8521
- Nada, A. M. A., El-Kady, M. Y., El-Sayed, E. S. A., and Amine, F. M. (2009). "Preparation and characterization of microcrystalline cellulose (MCC)," *BioResources* 4(4), 1359-1371.
- Nazri, Z., Roesyadi, A., and Sumarno, F. I. (2016). "Effects of hydrolysis conditions on the crystallinity, chemical structure, morphology, and thermal stability of cellulose nanocrystals extracted from oil palm biomass residue," *Int. J. ChemTech Res.* 9(9), 456-464.
- Nelson, M. L., and O'Connor, R. T. (1964a). "Relation of certain infrared bands to cellulose crystallinity and crystal latticed type. Part I: Spectra of lattice types I, II, III and of amorphous cellulose," *J. Appl. Polym. Sci.* 8(3), 1311-1324. DOI: 10.1002/app.1964.070080322
- Nelson, M. L., and O'Connor, R. T. (1964b). "Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II: A new infrared ratio for estimation of crystallinity in celluloses I and II," *J. Appl. Polym. Sci.* 8(3), 1325-1341. DOI: 10.1002/app.1964.070080323
- O'Connor, R. T., DuPré, E. F., and Mitcham, D. (1958). "Applications of infrared absorption spectroscopy to investigations of cotton and modified cottons," *Text. Res. J.* 28(5), 382-392. DOI: 10.1177/004051755802800503
- Ozturk, I., Sibel, I., Arif, H., and Oktay, E. (2010). "Hydrolysis of kenaf (*Hibiscus cannabinus* L.) stems by catalytical thermal treatment in subcritical water," *Biomass Bioenerg.* 34(11), 1578-1585. DOI: 10.1016/j.biombioe.2010.06.005
- Park, S., John, B., Michael, E. H., Philip, A. P., and David, K. J. (2010). "Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulase performance," *Biotechnol. Biofuel.* 3(10), 1-10. DOI: 10.1186/1754-6834-3-10
- Peterson, A. A., Vogel, F., Lachance, R. P., Fröling, M., Antal, M. J., and Tester, J. W. (2008). "Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies," *Energ. Environ. Sci.* 1(1), 32-65. DOI: 10.1039/B810100K
- Poletto, M., Ornaghi Jr, H. L., and Zattera, A. J. (2014). "Native cellulose: Structure, characterization and thermal properties," *Materials* 7(9), 6105-6119. DOI: 10.3390/ma7096105
- Prado, J. M., Luis, A. F. R., Tania, C., Mauricio, A. R., Fransisco, M. F., and Angela, A. M. (2014a). "Hydrolysis of sugarcane bagasse in subcritical water," *J. Supercrit. Fluid* 86, 15-22. DOI: 10.1016/j.supflu.2013.11.018
- Prado, J. M., Tania, F. C., Mauricio, A. R., Luis, A. F. R., Fransisco, M. F., and Angela, A. M. (2014b). "Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water," *J. Supercrit. Fluid* 89, 89-98. DOI: 10.1016/j.supflu.2014.02.017
- Qiu, Z., Aita, G. M., and Walker, M. S. (2012). "Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse," *Bioresource Technol.* 117, 251-256. DOI: 10.1016/j.biortech.2012.04.070

- Renken, A., Hessel, V., Löb, P., Miszczuk, R., Uerdingen, M., and Kiwi-Minsker, L. (2007). "Ionic liquid synthesis in a microstructured reactor for process intensification," *Chem. Eng. Proc.* 46, 840-845. DOI:10.1016/j.cep.2007.05.020
- Sangian, H. F., Kristian, J., Rahma, S., Agnesty, S. Y., Gunawan, S., and Widjaja, A. (2015a). "Comparative study of the preparation of reducing sugars hydrolyzed from high-lignin lignocellulose pretreated with ionic liquid, alkaline solution and their combination," *J. Eng. Technol. Sci.* 47 (2), 137-148 DOI: 10.5614/2Fj.eng.technol.sci.2015.47.2.3
- Sangian, H. F., Kristian, J., Rahma, S., Dewi, H. K., Puspasari, D. A., Agnesty, S. Y., Gunawan, S., and Widjaja, A. (2015b). "Preparation of reducing sugar hydrolyzed from high-lignin coconut coir dust pretreated by the recycled ionic liquid [mmim][dmp] and combination with alkaline," *Bull. Chem. React. Eng. Catal.* 10(1), 8-22. DOI: 10.9767/brec.10.1.7058.8-22
- Sangian, H. F. (2016). "Analysis of retention time and substances released enzymatically from lignocellulose, coconut coir treated by alkaline, ionic liquid [mmim][dmp] and combined method by observing the HPLC-RI spectra," *Int. J. ChemTech Res.* 9(12), 715-724.
- Silva, A. S. D., Lee, S. H., Endo, T., and Bon, E. P. B. (2011). "Major improvement in the rate and yield of enzymatic saccharification of sugarcane bagasse via pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim] [Ac])," *Bioresource Technol.* 102 (22), 10505-10509. DOI: 10.1016/j.biortech.2011.08.085
- Spigno, G., Pizzorno, T., and De Faveri, D. M. (2008). "Cellulose and hemicelluloses recovery from grape stalks," *Bioresource Technol.* 99(10), 4329-4337. DOI: 10.1016/j.biortech.2007.08.044
- Spiridon, I., Teaca, C. A., and Bodirlau, R. (2010). "Structural changes evidenced by FTIR spectroscopy in cellulosic materials after pretreatment with ionic liquid and enzymatic hydrolysis," *BioResources* 6(1), 400-413.
- Su, C. H., Chung, M. M., Hsieh, H. J., Chang, Y. K., Ding, J. C., and Wu, H. M. (2012). "Enzymatic hydrolysis of lignocellulosic biomass in ionic liquid media for fermentable sugar production," *J. Taiwan Inst. Chem. E.* 43(4), 573-577. DOI: 10.1016/j.jtice.2012.02.001
- Sun, Y. C., Xu, J. K., Xu, F., and Sun, R. C. (2013). "Structural comparison and enhanced cellulose hydrolysis of eucalyptus cellulose via pretreatment with different ionic liquids and catalysts," *Process. Biochem.* 48(5-6), 844-852. DOI: 10.1016/j.procbio.2013.03.023
- Sun, Y., Lin, L., Deng, H., Li, J., He, B., Sun, R., and Ouyang, P. (2008). "Structural changes of bamboo cellulose in formic acid," *BioResources* 3(2), 297-315.
- Thomas, M. F., Li, L. L., Handley-Pendleton, J. M., Lelie, D. V. D., Dunn, J. J., and Wishart, J. F. (2011). "Enzyme activity in dialkyl phosphate ionic liquids," *Bioresource Technol.* 102(24), 11200-11203. DOI: 10.1016/j.biortech.2011.09.069
- Van Dam, J. E. G., Van den Oever, M. J. A., Keijsers, E. R. P., Van der Putten, J. C. Anayron, C., Josol, F., and Peralta, A. (2006). "Process for production of high density/high performance binderless boards from whole coconut husk Part 2: Coconut husk morphology composition and properties," *Ind. Crops Prod.* 24(2), 96-104. DOI: 10.1016/j.indcrop.2005.03.003
- Widjaja, A., Agnesty, S. Y., Sangian, H. F., and Gunawan, S. (2015). "Application of ionic liquid [dmim]dmp pretreatment in the hydrolysis of sugarcane bagasse for

- biofuel production,” *Bull. Chem. React. Eng. Catal.* 10(1), 70-77. DOI: 10.9767/bcrec.10.1.7143.70-77
- Yang, F., Li, L., Li, Q., Tan, W., Liu, W., and Xian, M. (2010). “Enhancement of enzymatic *in situ* saccharification of cellulose in aqueous-ionic liquid media by ultrasonic intensification,” *Carbohydr. Polym.* 81(2), 311-316. DOI: 10.1016/j.carbpol.2010.02.031
- Yan, K., Liu, Y., Lu, Y., Chai, J., and Sun, L. (2017). “Catalytic application of layered double hydroxides-derived catalysts for the conversion of biomass-derived molecules,” *Catal. Sci. Technol.* 7, 1622-1645. DOI: 10.1039/C7CY00274B
- Yan, K., and Chen, A. (2013). “Efficient hydrogenation of biomass-derived furfural and levulinic acid on the facilely synthesized noble-metal-free Cu and Cr catalyst,” *Energy*. 58, 357-363. DOI: 10.1016/j.energy.2013.05.035
- Yan, K., Yang, Y., Chai, J., and Lu, Y. (2015). “Catalytic reactions of gamma-valerolactone: A platform to fuels and value-added chemicals,” *Appl. Catal., B*. 179, 292-304. DOI: 10.1016/j.apcatb.2015.04.030
- Yang, H., Li, J., Xu, J., and Mo, L. (2017). “Analysis of catalytic steam explosion pretreatment of corn stalk, lignin degradation, recovery, and characteristic variations,” *BioResources* 12(1), 344-361. DOI: 10.15376/biores.12.1.344-361
- Yuan, T. Q., Wang, W., Xu, F., and Sun, R. C. (2013). “Synergistic benefits of ionic liquid and alkaline pretreatments of poplar wood. Part 1: Effect of integrated pretreatment on enzymatic hydrolysis,” *Bioresource Technol.* 144, 429-434. DOI: 10.1016/j.biortech.2012.12.034
- Zhao, X., Zhang, L., and Liu, D. (2012). “Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose,” *Biofuels Bioprod Bioref.* 6, 465-482. DOI: 10.1002/bbb.1331
- Zhao, H., Jones, C. L., Baker, G. A., Xia, S., Olubajo, O., and Person, V. N. (2009). “Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis,” *J. Biotechnol.* 139(1), 47-54. DOI: 10.1016/j.jbiotec.2008.08.009
- Zhao, Y., Wang, H. T., Lu, W. J., and Wang, H. (2011). “Combined supercritical and subcritical conversion of cellulose for fermentable hexose production in a flow reaction system,” *Chem. Eng. J.* 166 (3), 868-872. DOI: 10.1016/j.cej.2010.11.058
- Zhu, Z., Zhu, M., and Wu, Z. (2012). “Pretreatment of sugarcane bagasse with NH₄OH-H₂O₂ and ionic liquid for efficient hydrolysis and bioethanol production,” *Bioresource Technol.* 119, 199-207. DOI: 10.1016/j.biortech.2012.05.111

Article submitted: January 28, 2017; Peer review completed: May 22, 2017; Revised version received and accepted: September 6, 2017; Published: September 13, 2017.
DOI: 10.15376/biores.12.4.8030-8046