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Relation of lignin and sugar yield from sugarcane bagasse degradation by sodium hydroxide

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Abstract

bagasse as waste is the one biomass that can be considered as lignin and sugar. Sugarcane bagasse contain lignin, hemicellulose and cellulose that can be converted into sugar and lignin through chemical pretreatment. In order to use sugarcane bagasse as a substrate for lignin and sugar production optimum conditions for sodium hydroxide hydrolysis of the bagasse were invested. Lignin and sugar are extracted together. The condition were varied in term of sodium hydroxide concentration (0.0-0.5%, w/v), reaction time (1-5h) and incubation temperature (100°C). The efficiency of sodium hydroxide hydrolysis, were shown on the maximum weight loss percentage of 46.22% under the condition of 0.5% of NaOH at 100 °C for 5h. The hydrolysate were maximum lignin, 69.18%, glucose, 1.24% and xylose, 12.70%. The sugarcane bagasse, hydrolysate and residue obtained were characterized by FT-IR. The results shown that C=C in aromatic skeletal of lignin was degraded from sugarcane bagasse and some peak indicate the presence of cellulose degradation products in liquid sugar. The lignin and lignin degradation were precipitated from solution and then epoxy production, and the sugar solution are then fermented into bioethanol and bio-plastic in the further.

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Introduction

One of the most interesting topics in bio-base material today is to convert biomass-derived waste and feedstock to highly added value-materials. The sugarcane bagasse is a fibrous residue of sugarcane. It is a by-product of the sugar-ethanol industry that represents a large volume about 25% of fresh sugarcane. In Thailand, amount of sugarcane produced in the harvesting period 2016-2017 was 103,533,437 tons (Office of Cane and Sugar Board, Thailand, Annual Report 2018). Most of the bagasse is currently used as fuel. In sugar and ethanol industry, sugarcane bagasse is mainly converted into energy through combustion (Leibbrandt *et al.,* 2011). The present, the burning of lignocellulose materials been used as clean energy alternative to coal in the production of electricity, with sugarcane bagasse being one of the biomasses with highest potential (Mashoko *et al.,* 2013). Environmental gains would be greater if the burning the surplus were avoided and the sugarcane bagasse become feedstock for the sugar, ethanol or bio-plastic production. Sugarcane bagasse, a byproduct of the industry, consists of 50% cellulose, 25% hemicellulose and 25% lignin (Amine *et al.,* 2013). Cellulose is a homopolymer of D-glucose units. Hemicellulose are composed of xylan, mannan, b-glucannand and xyloglucan polysaccharides (Vibe Scheller and Ulvskov., 2010). Lignin is an amorphous, and consists in a complex phenolic polymer composed of three phenylpropane monomeric building unit. The major chemical functional groups present in lignin structure include hydroxyl, methoxyl, carbonyl and carboxy moieties in various amounts depending on its botabic origin (Sarkanen and Hergert., 1971). Hydroxy groups and free positions in the aromatic ring are the most characteristic functions in lignin.

Today, there are a lot of bagasse applications such as fertilizer, fuel to generate electricity, building materials, bio-sorbents and sugar for bioethanol and bio-plastic production. Especially, sugar production to produce ethanol and bio-plastic, which is an important process. Two important steps in extraction sugar from bagasse are pretreatment and hydrolysis. The pretreatment is one method for delignification. The pretreatment method can be employed in various ways. These methods can be categorized into biological, physical, chemical and physicochemical pretreatments. The appropriate pretreatment method can be selected depending on the requirements of hydrolysis and fermentation that have the goals of sugar process, bioethanol process or bio-plastic process such as high yields of sugar, fermentable sugar for ethanol or bio-plastic, low product cost (Pandey *et al.,* 2010), low toxic compounds and recovery of spent chemicals (Kumar *et al.,* 2009).

In many previous research, sodium hydroxide used in pretreatment sugarcane bagasse, (Ju *et al.,* 2011; Moraes Rocha *et al.,* 2011; Amine *et al.,* 2013; Jimmy *et al.,* 2015; Aoul-hrouz *et al.,* 2017; Kyaw *et al.,* 2017; Paulo *et al.,* 2018). After pretreatment, acid or enzyme can be used break down hemicellulose and cellulose to sugar (xylose arabinose and glucose). These sugar can used substrate for ethanol and lactic acid fermentation.

The problem of sugar production from lignocellulose is lignin. It is complex polymer compound contained in plant tissue. In sugar extraction and bioethanol production from lignocellulose materials, it is necessary to remove lignin due to its recalcitrant nature. Lignin is toxic for microorganism during fermentation. After hydrolysis, lignin was removed. The lignin precipitation will be shown in the next research.

The previous research has shown that acid digestion requires acid-resistant and high temperature materials for reactor production. This is expensive and increases production cost. In addition, the use of enzyme for degradation is difficulty condition and high cost. So that, it is possible to reduce the step by using only one step. The aim of this research focus on lignin and sugar degradation from sugarcane bagasse by only one step. The effect of sodium hydroxide concentrations and reaction times were studied. These effect can used correlation to lignin and sugar yield.

Materials and methods

Raw material and pretreatment Sugarcane bagasse (cv. Khonkaen 3, Udon Thani Province, Thailand) was dried at 80°C for 12h. Then, it was cut to 1 cm of particles. The particle was soaked in different concentration of sodium hydroxide solution (1:10 = SCB: solution of 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5%(w/v) NaOH) for 12h at room temperature as pretreatment. At the end of the pretreated sugarcane bagasse was kept for lignin and sugar degradation. All conditions were used 3 g sugarcane bagasse.

Lignin and sugar degradation

Lignin and sugar were degraded from the pretreated sugarcane bagasse, in a solid-to-liquid ratio of 1:10, was placed in a reaction in aqueous 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% NaOH (w/v) solution. The reaction was used to degraded lignin and sugar from sugarcane bagasse (Preeya Kaewnaree, 2015). The reaction was varied at 1, 2 3, 4 and 5h, under agitation in a temperature of 100°C At the end of reaction the delignified and desugar material was filtrated to obtain the black liquor without any fibrous materials. The black liquor, was used lignin precipitation. The fibrous materials was washed to 7.0 pH with distilled water and dried at 90°C for 16h. The weight of fibrous materials was calculated for percentage of weight loss. The characterization of hydrolysate, lignin and fibrous materials was investigated.

Lignin precipitation

The hydrolysate from sodium hydroxide hydrolysis was adjusted pH from 9.0 to 5.0 by hydrochloric acid (Veerasak and Preeya., 2017). The solution was kept in funnel at room temperature for 6h. Two phased of solution were separated. Lower phased of lignin was separated, centrifuged at 10,000 rpm and dried at 90°C for 6h. The dried lignin and supernatant were kept for analysis. And upper phase is sugar solution which was determined glucose, xylose and arabinose.

Analytical methods

High performance liquid chromatography (HPLC)

After centrifugation of hydrolysate precipitation under various sodium hydroxide hydrolysis conditions, the supernatants were determined for sugars (xylose, glucose and arabinose) concentrations using HPLC 20A (Shimadzu, Japan) with an Aminex Bio RAD column and a refractive index detector (Oven temperature, 65 °C; isocratic elution, flow rate of 0.6ml min^{-1} ; mobile phase, 0.005 M H_2SO_4) (Pattana *et al.,* 2009).

Fourier transform infrared spectroscopy (FTIR)

One gram of oven dried sugarcane bagasse, dried lignin or fibrous materials and KBr anhydride were mixed to homogeneous. The mixture was transferred to the FT-IR, analyses were performed using a Spectrum 2, PerkinElmer, USA. The powder samples were supported by KBr pellets the spectra were recorded in the range of 500-4,000 cm^{-1} (Priscila *et al.,* 2012).

UV spectrophotometer

Aspectrophotometer Shimadzu UV-vis 2000 was used analyze the lignin. The black liquor were diluted for 250 500 750 1,000 and 1,500 dilutions and their absorbance was measured in the wavelength range from 200 to 800 nm (Priscila *et al.,* 2012). The 100 ml of dilutions were dried (in a temperature of 90°C for 16h) for calculation of weight percentage. The absorbance of samples was measured at 283 nm. Calibration curves of the lignins (absorbance vs concentration) were carried out to determine the Beer-Lambert parameters. Due to the presence of aromatics rings in lignin (Fergus *et al.,* 1969), analysis of the slope in a graphic of absorbance vs concentration measured at the wavelength of 283 nm.

Results and discussion

Previous work was emphasize on the hydrolysis of bagasse which involves 2 important steps and procedures 1) the application of bagasse was soaked in sodium hydroxide to extract lignin from bagasse 2) hydrochloric acid solution and potassium sulpaete as catalyst was then applied to the remaining bagasse applying a temperature of 121°C for 5 h achieving a maximum yield on total sugar of 87.86 g/L and reducing sugar of 43.84 g/L were observed (Preeya, 2015). But with this experiment, 2 steps of sugarcane bagasse hydrolysis were reduced from 2 steps down to 1 step. Only Sodium hydroxide was used for hydrolysis. Both sugar and lignin were degrated under the same time. The lignin was separated from solution by sedimentation resulting in sugar solution. After that sugar and type of sugar were determined. This process reduces hydrochloric acid and catalyst to achieve safer process and would significantly lower chemical use.

Table 1. Effect of sodium hydroxide concentration and reaction time to degradation efficiency of sugarcane bagasse.

Reaction time (h)	Initial weight of SCB (g)	Sodium hydroxide concentration (%)	Final weight of SCB (g)	Weight loss percentage (%)
1	3	Control	2.05±0.03	31.35±0.67
		0.1	1.86±0.10	37.96±0.67
		0.2	1.88±0.12	37.02±0.67
		0.3	1.86±0.23	37.82±0.33
		0.4	1.85±0.30	38.16±0.67
		0.5	1.75±0.03	41.65±0.67

Continued

Reaction time (h)	Initial weight of SCB (g)	Sodium hydroxide concentration (%)	Final weight of SCB (g)	Weight loss percentage (%)
2	3	Control	1.80±0.18	32.41±0.83
		0.1	1.86±0.16	37.68±0.00
		0.2	1.85±0.02	38.09±0.33
		0.3	1.77±0.16	40.94±0.67
		0.4	1.68±0.9	43.93±0.67
		0.5	1.74±0.15	43.93±0.33
3	3	Control	1.71±0.17	34.93±0.33
		0.1	1.90±0.13	36.45±0.67
		0.2	1.75±0.28	41.37±0.33
		0.3	1.67±0.19	44.27±0.00
		0.4	1.64±0.19	45.03±0.67
		0.5	1.64±0.36	45.21±0.33
4	3	Control	1.66±0.04	34.66±0.17
		0.1	1.72±0.21	42.49±0.67
		0.2	1.77±0.27	40.81±0.00
		0.3	1.69±0.14	43.42±0.00
		0.4	1.71±0.26	42.68±0.00
		0.5	1.80±0.15	39.71±0.67
5	3	Control	1.76±0.41	37.91±0.83
		0.1	1.73±0.39	42.20±0.33
		0.2	1.71±0.02	42.99±0.33
		0.3	1.62±0.21	46.16±0.33
		0.4	1.65±0.22	46.09±0.00
		0.5	1.61±0.34	46.22±0.00

Effect of sodium hydroxide concentrations and reaction times on weight loss percentage of sugarcane bagasse

The sodium hydroxide degradation had an exhibited effect on lignin and sugar degradation from the sugarcane bagasse. The lignin and sugar yield in the solution after degradation by 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) of sodium hydroxide were determined on weight loss of sugarcane bagasse. The Table 1 shown the percentage of weight loss and the relative extraction yield by the process, which exhibited sugarcane bagasse degradation. This indicates that sodium hydroxide could break the lignin, hemicellulose or cellulose.

Table 2. Effect of sodium hydroxide concentration to relation of sugar and lignin in sugarcane bagasse degradation**.**

Reaction time (h)	Sodium hydroxide concentration (%)	Total sugar (%)	Percentage of lignin (%)		Component of sugarcane bagasse solutions (%w/w)			
			Dried weight	UV-Vis	Glucose	Xylose	Arabinose	Total
1	Control	0.76±0.25	8.59±1.41	10.48±0.91	0.73±0.15	2.03±0.01	-	2.76±0.08
	0.1	1.92±0.16	18.04±1.00	15.07±0.39	0.60±0.03	1.31±0.24	-	1.93±0.14
	0.2	2.18±0.18	17.84±0.48	22.98±0.91	0.73±0.02	1.44±0.06	-	2.18±0.04
	0.3	3.23±0.05	17.59±0.28	15.49±1.13	0.96±0.05	2.26±0.03	-	3.23±0.04
	0.4	1.10±0.39	18.56±0.28	20.28±1.26	0.09±0.02	1.00±0.07	-	1.10±0.05
	0.5	1.55±0.14	20.59±0.73	21.60±1.87	0.00±0.00	0.55±0.14	-	0.56±0.07
2	Control	1.87±0.09	8.54±1.04	9.13±0.15	0.63±0.09	3.24±0.13	-	3.88±0.11
	0.1	5.86±0.03	15.81±1.97	15.75±2.00	1.09±0.11	4.76±0.13	-	5.86±0.12
	0.2	6.36±0.26	15.72±1.67	18.00±0.87	0.45±0.08	5.90±0.08	-	6.37±0.08
	0.3	5.12±0.63	17.82±1.03	19.80±1.43	1.00±0.01	4.03±0.03	-	5.13±0.02
	0.4	3.44±0.04	20.59±1.63	21.29±2.35	0.90±0.07	2.53±0.34	-	3.44±0.21
	0.5	1.09±0.06	20.58±0.98	22.40±1.22	0.50±0.04	0.58±0.06	-	1.09±0.05

Continued

Reaction time (h)	Sodium hydroxide concentration (%)	Total sugar (%)	Percentage of lignin (%)		Component of sugarcane bagasse solutions (%w/w)			
			Dried weight	UV-Vis	Glucose	Xylose	Arabinose	Total
3	Control	1.14±0.33	10.79±1.00	11.21±0.74	0.82±0.02	3.31±0.31	-	4.14±0.17
	0.1	10.13±0.64	13.32±1.03	8.30±0.43	1.24±0.06	8.89±0.08	-	10.13±0.06
	0.2	1.59±0.20	19.77±0.83	11.65±1.22	0.43±0.04	1.16±0.16	-	1.60±0.10
	0.3	1.52±0.17	18.74±1.23	10.38±1.04	0.41±0.01	1.11±0.25	-	1.53±0.13
	0.4	1.50±0.22	19.53±1.45	19.53±1.26	0.39±0.07	1.10±0.05	-	1.50±0.06
	0.5	2.94±0.39	21.26±0.95	23.34±1.78	0.84±0.02	2.09±0.06	-	2.94±0.04
4	Control	2.13±0.04	11.53±1.13	12.85±1.87	1.09±0.07	6.03±0.07	-	7.13±0.07
	0.1	8.18±0.76	17.30±0.91	18.05±1.43	1.06±0.05	7.12±0.11	-	8.19±0.08
	0.2	2.45±0.16	19.35±1.04	18.14±1.13	0.80±0.04	1.65±0.12	-	2.46±0.08
	0.3	2.83±0.08	20.58±0.92	21.92±2.39	0.84±0.07	1.98±0.31	-	2.83±0.19
	0.4	2.65±0.05	21.02±1.35	22.28±2.06	0.00±0.00	0.65±0.05	-	0.66±0.03
	0.5	3.28±0.36	21.43±1.31	23.36±0.96	0.90±0.03	2.37±0.13	-	3.28±0.08

Continued

Reaction time (h)	Sodium hydroxide concentration (%)	Total sugar (%)	Percentage of lignin (%)		Component of sugarcane bagasse solutions (%w/w)			
			Dried weight	UV-Vis	Glucose	Xylose	Arabinose	Total
5	Control	2.70±0.35	12.71±0.48	12.64±0.67	0.00±0.00	12.70±0.35	-	12.70±0.18
	0.1	3.32±0.30	19.88±1.03	8.67±0.61	0.91±0.08	2.40±0.02	-	3.32±0.05
	0.2	3.10±0.32	20.38±0.51	22.89±1.13	0.83±0.02	2.27±0.08	-	3.11±0.05
	0.3	3.16±0.36	21.59±0.98	24.60±2.87	0.77±0.06	2.39±0.09	-	3.16±0.08
	0.4	2.79±0.42	21.96±0.58	25.98±2.91	0.76±0.06	2.03±0.08	-	2.79±0.07
	0.5	2.83±0.03	22.68±0.97	27.18±2.48	0.81±0.08	2.01±0.11	-	2.83±0.10

The highest percentage of weight loss was achieved when applying 0.5% of sodium hydroxide for 5h. The sodium hydroxide concentrations and reaction times of high degradation were 0.5% for 5h, 0.4% for 2h, 0.4% for 3h, 0.3% for 4h and 0.3% for 5h. The percentages of weight loss were 41.65±0.67, 43.93±0.67, 45.03±0.67, 43.42±0.00 and 46.16±0.33, respectively. The condition can be selected depending on the requirements of lignin and sugar yield in degraded solution. Amine *et al.* (2013) report, sugarcane bagasse consists of 50% cellulose, 25% hemicellulose and 25% lignin.

Table 3. Position and attribution of the IR absorption bands.

Functional groups	Wave number (cm^{-1})		
	Untreated sugarcane bagasse	Lignin solution	Pretreated sugarcane bagasse
OH	3344.44	3347.23	3339.88
C-H a symmetrical stretching	2898.00	2932.00	2895.00
C=C stretching vibration	1603.61	1569.19	1603.46
C-H bending vibration	-	1411.60	-
CH_2 cocking and	-	-	1319.97
O-H deformation	-	-	-
C-O vibration	1241.03	1243.00	1243.66
			1162.20
C-O symmetrical vibration	1036.44	1051.52	1034.07
C-H deformation			897.17

The results exhibited that under all conditions can degraded other than lignin. The results of the experiments in Table 2 described the composition of the solution from the process. The result was found that the total highest sugar and lignin yield.

The effects of sodium hydroxide concentration and reaction time of sugarcane bagasse degradation are shown in Table 2. The total sugar and lignin was the main product. Sodium hydroxide concentration affects the ability to digest lignin rather than sugar. All condition shown total sugar and lignin yield in the range of 10.13±0.64-10.13±0.64% and 13.32±1.03-22.68±0.97%, respectively. But the sodium hydroxide concentration and reaction time affects the optimum condition for sugar yield.

The optimum condition of high sugar yield were 0.1% NaOH for 3 h of reaction time (10.13±0.64%), 0.1% NaOH for 4h of reaction time (8.18±0.76%) and 0.1% NaOH for 4h of reaction time (6.36±0.26%). The highest total sugar and lignin yield were 10.13±0.64% (under 0.3% sodium hydroxide for 1h) and 43.68±0.97% (under 0.5% sodium hydroxide for 5h), respectively. However, at the highest sodium hydroxide concentration (0.5%) and reaction time (5h) were exhibited the maximum lignin concentration. This degradation can also promote the internal surface and solubilize the binding wax between lignin and hemicellulose and cellulose. Remove of lignin is dependent on the sodium hydroxide concentration and content of lignin in lignocellulosic material (Sun and Cheng., 2002). However, if conditions of the oxidation processes are serve, the oxidizing agent , heated under alkaline conditions reacts with lignin and promotes the opening of the phenolic rings. This process leads to addition of carboxylic groups to the macromolecular structure of lignin. The modifications increase the water solubility by the insertion of polar groups into the molecule (Kadla and Chang, 2001; Mancera *et al.,* 2010). Solubilization of lignin with rather carbohydrate removal occurs during the delignification process. These results show that sugar and lignin can be extracted at the same time (Table 2). The component of sugarcane bagasse solutions are shown glucose and xylose yield in Table 2. The results showed that xylose was the main product. The highest xylose, glucose and total sugar were exhibited at 0.1% NaOH for 3 h of reaction time of 8.89±0.08, 1.24±0.06 and 10.13±0.06 %w/w, respectively. Accompanying these monosaccharides, some soluble materials such as lignin, acetic acid and furfural are also produced with can inhibit both growth and sugar utilization of microorganisms during the fermentation process (Aguilar *et al.,* 2002; Lavarack *et al.,* 2002; Rodrguez-Chong *et al.,* 2004; Cheng *et al.,* 2008; Pattana *et al.,* 2009).

The lignin was removed from alkali solution by precipitation process. The percentage of lignin removal was 60-100%. After that lignin was centrifuged and dried. The highest lignin of sugarcane bagasse was 20%(w/v) (the data not show). After lignin precipitation, the solution was detected sugar content by HPLC

FT-IR spectroscopy of sugarcane bagasse, hydrolysate and residue The FTIR spectra corresponding to sodium hydroxide lignin oxidized are given in Fig. 1 and Table 3. As usual, the fingerprint region lies between wavenumbers ranging from 500-4000 cm-1, whereas simple stretching of chemical groups is observed at higher wave number (Mancera *et al.,* 2010). Fig. 1 explains simple comparison between FTIR spectra of untreated sugarcane bagasse (black), pretreated sugarcane bagasse (red) and lignin solution (blue).

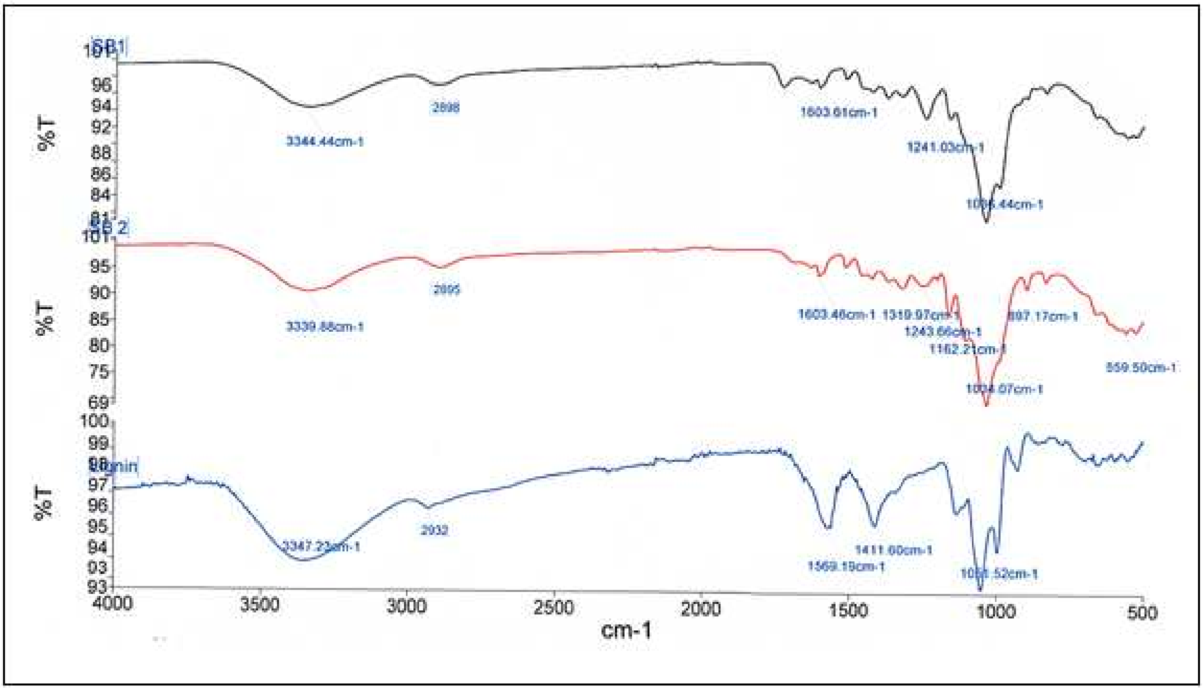

Fig. 1. FTIR spectra of untreated sugarcane bagasse (black), pretreated sugarcane bagasse (red) and lignin solution (blue).

By comparison spectra of untreated sugarcane bagasse, pretreated sugarcane bagasse and lignin solution, a broad peak at around 3,339-3,347 cm^{-1} represents OH groups either from cellulose or lignin. The peak at around 2,895-2,932 cm^{-1} represents the C-H a symmetric stretching in aliphatic methyl. A band at approximately 1,569-1,603 cm^{-1} is characteristic to C=C stretching vibration in aromatic skeletal of lignin. These peak occurred in three samples (Mona *et al.,* 2010). A band appears at approximately at 1411 cm^{-1} is due to bending vibration of C-H of methylene group (scissoring vibration). This peak occurred in only lignin solution. Peak around 1411cm^{-1} indicate the presence of cellulose degradation products in solution. A band appears at approximately at 1319 cm^{-1} is due to cocking of CH_2 and deformation of O-H (Mancera *et al.,* 2010). A band appears at approximately at 1241-1243 cm^{-1} is due to vibration of C-O. These peak occurred in untreated and pretreated sugarcane bagasse. Three sample showed a band at around 1036, 1034, 1051 cm^{-1} which dominates the spectrum of cellulose linkages (Mona *et al.,* 2010)**.** Peak is at around 897 cm^{-1} which is due β-glycosidic linkage (Mona *et al.,* 2010). represents the C-H a deformation out of aromatic rings and C-C in rings.

The many types of lignin was extracted from lignocellulose by chemical method. Chemical modifications in lignin types may result in products with particular characteristics, which can be utilized in different industrial segments (Prisila *et al.,* 2012). Oxidized lignins have properties which are similar to lignosulfonates and can be used in a number of industrial applications, mainly as dispersants and binders (Ferdheim *et al.,* 2002; Priscila *et al.,* 2012).

Conclusion

In this work, the development of pretreatment were used for lignin and sugar degradation under only one step. The lignin and sugar degradation showed highest yield under condition of 0.5% sodium hydroxide, for 5h at 100 °C for three grams of sugarcane bagasse. A hydrolysate solution was detected resulting in maximum lignin, 69.18%, glucose, 1.24% and xylose, 12.70%. The highest lignin of sugarcane bagasse was 20%(w/v). The characterization of untreated sugarcane bagasse, pretreated sugarcane bagasse and lignin solution were detected the correlation on functional group. The three component of sugarcane bagasse was hydrolyzed. The cellulose chain was brake down (A band appears at approximately at 1411 cm^{-1} and a band at around 1036, 1034, 1051 cm^{-1}) resulting in glucose yield. Lignin skeletal was degraded (A band at approximately 1,569-1,603 cm^{-1}) resulting in lignin derivative.

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