EXTRACTION OF LIGNIN FROM SUGARCANE BAGASSE BY USING ALKALINE PROCESS AND ALCOHOLIC ALKALINE PROCESS

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Abstract

The present work was focused on the extraction of lignin from sugarcane bagasse by two extraction processes: alkaline process and alcoholic alkaline process. A Box-Behnken Design of Response Surface Methodology (RSM) was chosen to investigate the optimum conditions of extraction process. Three independent variables in two processes consisting of concentration of solvent, volume of solvent and extraction time were determined based on the yield of lignin. The optimum conditions in alkaline process were found at NaOH concentration of 15%, NaOH volume of 304 mL and extraction time of 2 hr with predicted final lignin yield of 35.62 (%w/w). In alcoholic alkaline process, the optimum conditions were observed at NaOH concentration of 15%, alcoholic NaOH volume of 303 mL and extraction time of 3 hr with predicted lignin yield of 57.19 (%w/w). Predicted values were observed to be in good agreement with experimental values. Then the functional groups present in prepared lignins were identified by using UV-visible spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). The prepared samples were confirmed as lignin due to the presence of phenolic hydroxyl groups, carbonyl groups and methoxy groups.

Keywords: sugarcane bagasse, lignin, alkaline process, alcoholic alkaline process, response surface methodology

Introduction

The lignocellulosic biomasses such as agricultural wastes, crops and wood wastes are renewable resources for making of chemicals and fuels. The main constituents such as cellulose, hemicellulose and lignin are strongly bonded together to form a lignocellulosic matrix. The lignocellulosic biomasses are difficult to break down the components and digest the hemicellulose due to their structural features (Behera et al., 2014). After the cellulose, lignin is the second most plentiful natural bio polymer. Lignin is hard to break down or remove because of its strong lignocellulosic structure (Cesarino et al., 2012). It consists of three major phenylpropanoid monomers mostly the hydroxycinnamyl alcohols: coniferyl alcohol (G), sinapyl alcohol (S) and p-coumaryl alcohol (H). It can be substituted in expensive phenol part because it is bio-renewable, inexpensive and non-toxic natural polymer (Ji et al., 2018). Lignin can be taken out from Sulfur processes (Sulfite process and Kraft process) in pulping industry (Cao et al., 2018). Lignosulfonate lignin and Kraft lignin are sulfur-containing lignins and thus they are not only low in reactivity but also tend to be corrosive to the equipment. Moreover, the presence of sulfur renders them hazardous for the environment. Sulfur free processes such as solvent pulping process and alkaline pulping process can be used to alter the chemical structure of isolated lignin polymers. Lignin can be extracted from numerous biomasses by using various treatment processes such as physical treatment (milling and grinding), chemical treatment (acid, alkali and organic solvents), physicochemical treatment (steam explosion, hydrothermolysis and wet oxidation) and biological treatment (enzymatic hydrolysis) or their combination. Organosolvents have been used to extract high quality and pure lignin but the yield of lignin was very low (D. Li et al., 2020). Alkali pretreatment have been used to separate lignin binding with hemicellulose and also to enhance the digestibility of hemicellulose. However, in the conventional method of NaOH pretreatment, half or more amount of the hemicellulose is dissolved in black liquor (Tsegaye et al., 2019). The present

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study was aimed to extract the lignin from sugarcane bagasse by the alkaline process and alcoholic alkaline process and to analyze the characteristics of prepared lignins.

Materials and Methods

Materials

Sugarcane Bagasses (*Saccharum officinarum*) were collected from Kyun-Lay Village, Kyaiklat Township, Ayeyarwady Region. Ethanol (99%), n-Hexane (95%), Sulphuric acid (98%) and Sodium hydroxide (Analar grade (B.D.H, England)) were purchased from Academy Chemical Shop, Pabedan Township, Yangon Region.

Methods

Analysis of Sugarcane Bagasse

Determination of Lignin Content

Lignin was determined according to ASTM D 1106-56 (1977). The dried sample 1 g was weighed and transferred to a beaker, then 10 mL of 72% sulphuric acid was added carefully and the mixture was stirred with a glass rod. The mixture was diluted with water until the final volume was 300 mL. The solution was refluxed at 100°C for 3 hr and then filtered with vacuum filter. The filter containing lignin residues was dried in an oven at 105°C for 12 hr, cooled in a desiccator for 15 min and then weighed accurately. The cycle of heating, cooling and weighing were repeated until a constant weight was obtained.

$$\text{Lignin content (\%)} \quad = \quad \frac{\text{Weight of lignin}}{\text{Weight of initial sample}} \quad x \quad 100$$

Determination of Holocellulose Content

Holocellulose content was determined by using ASTM D 1104-56 (1978). Sample 2 g was dissolved into 150 mL of distilled water and the mixture was added into a 250 mL Erlenmeyer flask. $NaClO_2$ 1 g and 3 mL of 10% acetic acid were added while slowly shaking the flask. The flask was placed into a water bath and heated at 70 to 80°C for 30 min. Further addition of 1 g of $NaClO_2$ and 3 mL of 10% acetic acid were conducted until 4 g of $NaClO_2$ had been added into the mixture. Then the flask was further heated for 30 min. After cooling, the sample was filtered and washed with hot water until free of acid. Then the insoluble portion was dried in an oven at 105°C for 4 hr, placed in a desiccator and weighed. The cycle of heating, cooling and weighing were repeated until a constant weight was obtained.

$$\text{Holocellulose content (\%)} \quad = \quad \frac{\text{Weight of holocellulose}}{\text{Weight of initial sample}} \quad x \quad 100$$

Determination of Alpha-cellulose Content

Alpha cellulose content was determined according to ASTM D 1103-60 (1978). Holocellulose 3 g was placed into a 250 mL Erlenmeyer flask. The sample was then treated with 50 mL of 17.5% NaOH for 30 min and then 50 mL of distilled water was added to it. The reaction was continued for 5 min. The contents were filtered by using vacuum filtration. The residue was washed first with 50 mL of 8.3% NaOH, then with 40 mL of 10% acetic acid and finally, with hot water. After that, the cellulose was dried in an oven at $103 \pm 2^{\circ}$C, placed in a desiccator and weighed. The process of heating, cooling and weighing were repeated until a constant weight was obtained.

$$\text{Alpha cellulose content (\%)} = \frac{\text{Weight of alpha cellulose}}{\text{Weight of holocellulose}} \times 100$$

Determination of Hemicellulose Content

The hemicellulose content was obtained by subtracting the weight of alpha-cellulose content from that of holocellulose content (TAPPI 1999b).

Determination of Moisture Content

The moisture content of sugarcane bagasse was determined by AOAC method no. 942.05 (AOAC 2000). Sample 5 g was weighed accurately in a clean, dry and previously weighed dish. The sample was dried at 105ºC for 3 hr in a hot air oven. After drying, the sample was removed from the oven and placed in a desiccator for 30 min and weighed accurately. The cycle of heating, cooling and weighing were repeated until a constant weight was obtained. The percentage moisture content was obtained as follows:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

Determination of Ash Content

The ash content of sugarcane bagasse was determined according to AOAC method no. 942.05 (AOAC 2000). Sample 5 g was weighed in a clean and previously weighed porcelain crucible and ignited in a muffle furnace at 600ºC for 4 hr. The crucible containing the ash was placed in a desiccator for 30 min and weighed. Heating, cooling and weighing were repeated until a constant weight was attained. The following formula was used to calculate the percentage of ash content.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Extraction of Lignin from Sugarcane Bagasse

Bagasse powder (<0.42 mm in size) was pretreated with n-Hexane (95 %) in solid to liquid ratio of 1:16 g/mL at 70°C for 6 hr in a Soxhlet extractor to remove the minor constituents (extractives) such as wax, tannin. Then the solid residues were dried in a hot air oven at $60°C$ until a constant weight was obtained.

Pretreated solid residues 25 g were heated with 300 mL of hot distilled water at $120°C$ for half an hour in a heating mantle and then filtered on filter cloth. In alkaline process, the solid residues were treated with 304 mL of 15% NaOH solution at 100°C for 2 hr in two neck flat-bottomed flask equipped with condenser and magnetic stirrer. In alcoholic alkaline process, the concentration of ethanol was kept constant at 64% to make the alcoholic NaOH solution. In this process, the solid residues were treated with 15% NaOH concentration, 303 mL of alcoholic NaOH at 100°C for 3 hr. After the reaction time, the mixture was filtered about three times under vacuum filtration. The lignin in the black filtrate was precipitated with 50% H_2SO_4 in order to adjust pH 2-3. Then the precipitated lignin was filtered through vacuum filtration and washed with hot distilled water to reach neutral pH (Runcang Sun et al., 1998). Then the lignin cake was dried in hot air oven at 60°C until a constant weight was obtained. In alcoholic alkaline process, ethanol was recovered from the black liquor by distillation.

Process Optimization by Response Surface Methodology (RSM)

Response Surface Methodology (RSM) was used to determine the optimum conditions of lignin extraction from bagasse by using two processes with the process independent variables such as concentration of solvents (%w/v), volume of solvents (mL) and extraction time (hr) as shown in Table (1). Box-Behnken Design was chosen by using the statistical software (Design Expert, version 11, Stat Ease Inc.,) and the 15 experimental runs were conducted as shown in Table (2).

Table 1   Variables in Experimental Design for Extraction of Lignin from Sugarcane Bagasse with Alkaline Process and Alcoholic Alkaline Process

Variables	Symbols	Levels			
		Alkaline Process		Alcoholic Alkaline Process	
		Lower	Upper	Lower	Upper
Concentration of solvent (w/v %)	X_1	10	20	10	20
Volume of solvent (mL)	X_2	200	400	200	400
Extraction Time (hr)	X_3	1	3	2	4

Table 2 Experimental Runs According to Box-Behnken Design for Lignin Extraction from Sugarcane Bagasse

Run Order	Alkaline Process			Alcoholic Alkaline Process		
	Concentration of NaOH (%w/v)	Volume of NaOH (mL)	Extraction Time (hr)	Concentration of NaOH (%w/v)	Volume of Alcoholic NaOH (mL)	Extraction Time (hr)
1	10	400	2	15	200	4
2	15	300	2	15	300	3
3	15	200	3	20	200	3
4	15	300	2	20	400	3
5	20	400	2	10	400	3
6	20	300	1	20	300	4
7	15	400	3	15	400	4
8	15	200	1	15	400	2
9	10	300	3	15	300	3
10	10	200	2	15	200	2
11	15	300	2	10	300	4
12	20	300	3	10	300	2
13	10	300	1	15	300	3
14	20	200	2	10	200	3
15	15	400	1	20	300	2

Characterization of Extracted Lignin

Physico-chemical properties (moisture content, ash content and melting point) of lignin were investigated. Moisture content and ash content were determined by using AOAC method no. 934.06 and 942.05 (AOAC 2000).

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Determination of Melting Point

The melting points of isolated lignins were measured in open capillary tube by using Gallenkamp melting point apparatus. The melting point of lignin was determined at the Chemistry Department, West Yangon University.

UV-visible Analysis

The resultant lignin was identified in Shimadzu UV-visible Spectrophotometer (UV-1800) at the Customer Support and Laboratory, Amtt, Mayangone Township, Yangon Region.

Fourier-Transform Infrared (FTIR) Analysis

FTIR analysis was carried out to determine the functional groups of extracted lignin using IR-Tracer 100 S (SHIMADZU, Japan) at the Universities' Research Center, University of Yangon.

Results and Discussion

The chemical compositions such as lignin, alpha-cellulose, hemicellulose, moisture and ash of sugarcane bagasse are shown in Table (3). Lignin is the second most abundant constituent after cellulose in sugarcane bagasse. The compositions of bagasse can be different depending on its location, variety and agricultural practices (X. Li, 2004).

Table 3   Chemical Composition of Sugarcane Bagasse

| Sr No.	Components	Composition (% w/w)
1.	Lignin	38.1 ± 0.2
2.	Alpha-cellulose	47.41 ± 0.67
3.	Hemicellulose	32.35 ± 0.68
4.	Moisture	2.39 ± 0.79
5.	Ash	2.33 ± 0.58

Table (4) shows experimental and predicted yield of lignin from bagasse by alkaline extraction process. It was found that the predicted values by statistical design software were in good agreement with the experimental values. Alkali can be used to enhance the digestibility of hemicellulose and to disrupt lignin binding with hemicellulose. The model regression equation giving lignin content as a function of alkali concentration, alkali volume and extraction time in alkaline process is shown in equation (1). This equation was solved by using MATLAB.

$$\text{Lignin Content (\% w/w)} = 35.24 + 5.84X_1 + 7.11X_2 + 6.39X_3 - 0.600X_1X_2 + 1.95X_1X_3 + 0.4475X_2X_3 - 6.69X_1^2 - 5.29X_2^2 - 6.39X_3^2 \quad \text{---------- eq (1)}$$

Table 4 Experimental and Predicted Yield of Lignin by Alkaline Process

Run Order	Experimental Factors			Response (lignin %)	
	X_1 (%w/v)	X_2 (mL)	X_3 (hr)	Experimental Values (Y) (% w/w)	Predicted Values (% w/w)
1	10	400	2	25.23	25.12
2	15	300	2	35.3	35.24
3	15	200	3	22.43	22.39
4	15	300	2	35.28	35.24
5	20	400	2	35.53	35.60
6	20	300	1	19.76	19.65
7	15	400	3	37.50	37.50
8	15	200	1	10.50	10.50
9	10	300	3	20.65	20.76
10	10	200	2	9.78	9.71
11	15	300	2	35.13	35.24
12	20	300	3	36.4	36.33
13	10	300	1	11.8	11.87
14	20	200	2	22.48	22.59
15	15	400	1	23.78	23.82

The effect of alkali concentration and volume on lignin content at a constant extraction time of 2 hr is presented in Figure (1, a). It was observed that increasing the concentration and volume of NaOH increased the lignin yield. The effect of alkali concentration and extraction time at constant alkali volume of 304 mL is shown in Figure (1, b). It can be seen that increasing the reaction time did not significantly increase the lignin yield; however, increasing the concentration of NaOH increased the lignin content. The interactive effect of volume of NaOH and extraction time on lignin content at constant alkali concentration of 15% is pointed out in Figure (1, c). This figure shows that the lignin content rises with an increase in volume of NaOH and extraction time.

(a)   (b)   (c)
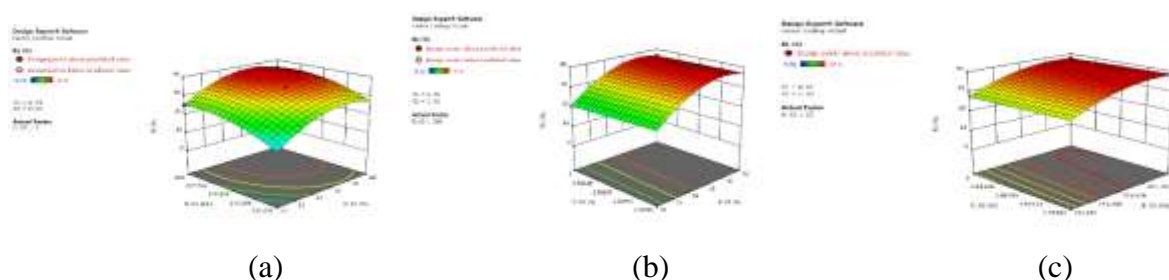
Figure 1   3D Surface Plot for Interaction of (a) Concentration and Volume of NaOH (b) Concentration of NaOH and Extraction Time (c) Volume of NaOH and Extraction Time in Lignin Extraction by Alkaline Process

Table (5) shows experimental and predicted yield of lignin from bagasse by alcoholic alkaline extraction process. It was observed that the values predicted by statistical design software were in agreement with the experimental values. A combination of alkali with ethanol treatments would liberate the recalcitrant structure of lignocelluloses, achieving a relatively high yield of lignin (RunCang Sun et al., 2002).

Table 5 Experimental and Predicted Yield of Lignin by Alcoholic Alkaline Process

Run Order	Experimental Factors			Response (lignin %)	
	X_1 (%w/v)	X_2 (mL)	X_3 (hr)	Experimental Values (Y) (% w/w)	Predicted Values (% w/w)
1	15	200	4	47.68	47.61
2	15	300	3	55.86	56.81
3	20	200	3	43.56	44.17
4	20	400	3	56.12	56.22
5	10	400	3	50.21	49.61
6	20	300	4	57.78	57.24
7	15	400	4	58.93	59.36
8	15	400	2	48.56	48.63
9	15	300	3	55.84	56.81
10	15	200	2	33.25	32.82
11	10	300	4	48.76	48.93
12	10	300	2	35.6	36.14
13	15	300	3	58.72	56.81
14	10	200	3	34.2	34.10
15	20	300	2	44.68	44.51

The model regression equation for lignin content as a function of alkali concentration, alcoholic alkaline volume and extraction time in alcoholic alkaline process is shown in equation (2).

$$\text{Lignin Content (\% w/w)} = 56.81 + 4.17X_1 + 6.89X_2 + 6.38X_3 - 0.8625X_1X_2 - 0.0150X_1X_3 - 1.02 X_2X_3 - 5.59X_1^2 - 5.19 X_2^2 - 4.51X_3^2 \quad \text{-------- eq (2)}$$

The interactive effect of alkali concentration and volume of alcoholic alkaline on lignin content at a constant extraction time of 3 hr is shown in Figure (2, a). It was found that decreasing the concentration of NaOH and rising the volume of alcoholic NaOH increased the lignin yield. Figure (2, b) pointed out that the effect of alkali concentration and extraction time at constant alcoholic alkaline volume of 303 mL. It can be observed that increasing the extraction time improved the lignin content but rising the concentration of NaOH reduced the lignin content. The effect of volume of alcoholic NaOH and extraction time on lignin content at constant alkali concentration of 15% is presented in Figure (2, c). This figure shows that the lignin content rises with an increase in volume of alcoholic NaOH and extraction time.

(a)   (b)   (c)
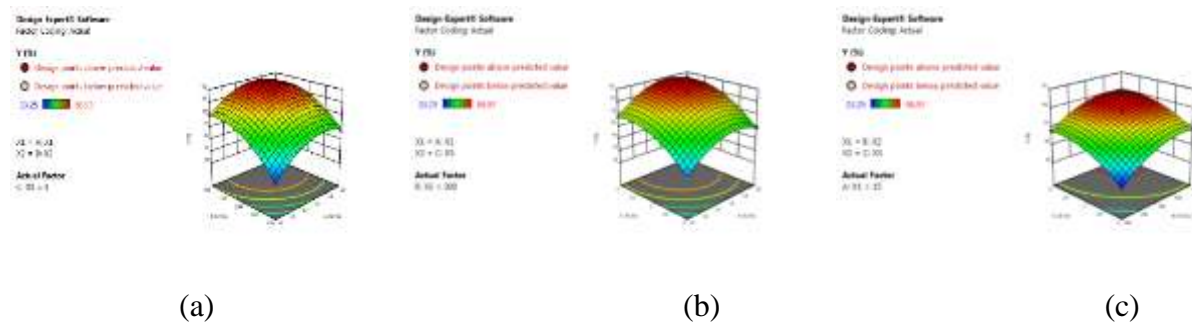
Figure 2 3D Surface Plot for Interaction of (a) Concentration of NaOH and Volume of Alcoholic NaOH (b) Concentration of NaOH and Extraction Time (c) Volume of Alcoholic NaOH and Extraction Time in Lignin Extraction by Alcoholic Alkaline Process

Table (6) shows the actual values and predicted values of lignin under optimum conditions. The optimum conditions in alkaline process were 304 mL of 15% of NaOH and 2 hr extraction time to obtain 35.62% of lignin and that in alcoholic alkaline process were 303 mL of 15% of NaOH and 3 hr extraction time to achieve 57.19% of lignin. The optimum conditions under predicted values were validated by repeating the experiments about 3 times. As can be seen in the Table (6), lignin contents are (37.13±0.34 (% w/w)) for alkaline process and (56.75±0.03 (% w/w)) for alcoholic alkaline process. It was indicated that alcoholic alkaline process has a positive effect on lignin yield.

Table 6 Actual Values and Predicted Values of Lignin under Optimum Conditions

| Sr No.	Extraction Process	Concentration (%)	Volume (mL)	Reaction Time (hr)	Predicted Value (% w/w)	Actual Value (% w/w)
1.	Alkaline	15	304	2	35.62	37.13±0.34
2.	Alcoholic Alkaline	15	303	3	57.19	56.75±0.03

The physico-chemical characteristics of lignin such as moisture, ash and melting point are shown in Table (7). According to the results, the moisture content and melting point of lignin extracted by alkaline process were higher than that of lignin extracted by alcoholic alkaline process. Most of the extracted lignin did not provide sharp melting point and the melting point depends on the extraction process and molecular weight of lignin. The ash content of lignin from alkaline process was lower than that from alcoholic alkaline process. The ash content of lignin sample depends on the biomass type and extraction process (Chen et al., 2013).

Table 7 Physico-chemical Characteristics of Lignin

| Sr No.	Extraction Process	Moisture (% w/w)	Ash (% w/w)	Melting Point (°C)
1.	Alkaline	2.39 ± 0.79	1.24 ± 0.11	263-269
2.	Alcoholic Alkaline	3.07 ± 0.15	1.07 ± 0.04	200-206

During alkaline and alcoholic alkaline treatments, holocellulose was broken down and the lignin was separated. Figures 3 (a) shows UV-spectrum of lignin extracted from bagasse by alkaline process. It was found that the phenolic hydroxyl group contributes to the absorption band of lignin at 282 nm in alkaline process. Figure 3 (b) points out UV-spectrum of lignin extracted from bagasse by alcoholic alkaline process. It can be seen that the hydroxyl group gives the absorption band of lignin at 238 nm and also phenolic hydroxyl group supplies the wavelength of lignin at 288 nm in alcoholic alkaline process. The characteristic absorption minimum of lignin provides the etherified hydroxyl groups near 280 nm. The absorption peaks within the range from 277 to 282 nm supply the unconjugated quaiacyl and 3,4-dimethoxy-phenyl model compounds (Jablonský et al., 2015).
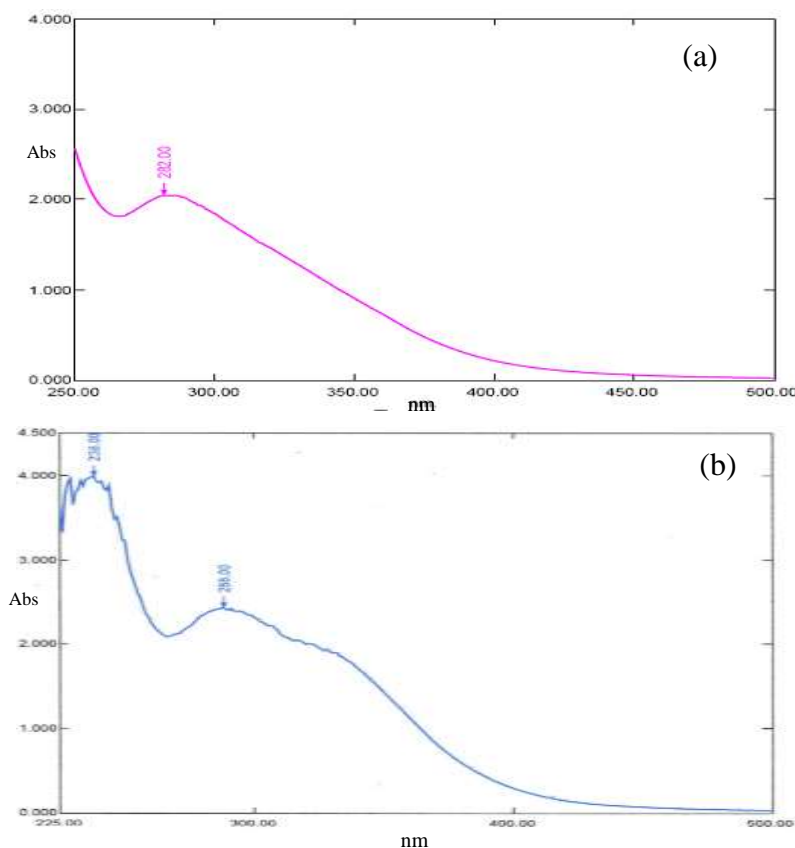
Figure 3   UV-Spectrum of Lignin Extracted by (a) Alkaline Process and (b) Alcoholic Alkaline
Process

Figure 4 (a) and (b) represent the FTIR spectra of lignin extracted from bagasse by alkaline process and alcoholic alkaline process. The absorption peaks of both lignins at (3855 cm^{-1}) belonged to the stretching vibration of hydrogen bonded OH group in alcohol. A wide absorption bands at 3375 cm^{-1} for alkaline process and 3381 cm^{-1} for alcoholic alkaline process are assigned to hydroxyl groups in phenolic and aliphatic structures. The absorption peaks at (2939 and 2908 cm^{-1}) in two processes predominantly arise from CH stretching in aromatic methoxy group and in methyl and methylene groups of side chains. The bands (2337, 2110,1950 cm^{-1}) in alkaline process and 2386 cm^{-1} in alcoholic alkaline process arise C-H-stretching in the lignin molecules. The bands at (1710 cm^{-1}) was observed in alcoholic alkaline process but it was absent in alkaline process. In the carbonyl/carboxyl region, weak medium bands were found at 1705–1720 cm^{-1} that indicates the stretching of C=O group in these lignin (Boeriu et al*.,* 2004). The weak absorption peak at 1699 cm^{-1} in alkaline process resulting in the asymmetry and broadening of the more intense bands may originate from both protein impurity and water associated with lignin. This peak was not observed in alcoholic alkaline process which indicated that lignin extracted by this process has no impurity. The bands at (1599, 1514, 1512, 1462, 1425 cm^{-1}) are assigned to C–H deformation combined with aromatic ring vibration. The bands at (1329 cm^{-1} in alkaline process and 1327 cm^{-1} in alcoholic alkaline process) belong to O-H stretching vibrations in alcohol and phenol. Spectra of hardwood and all non-wood lignins show a band at 1326 cm^{-1}, which is characteristic for syringyl (S) ring plus guaiacyl (G) ring. The bands at (1041, 993, 987, 895,835, 638 cm^{-1}) to COC group out of plane deformation (trans) which further indicated that the lignin with conjugated units was isolated by aqueous alkaline solution (Boeriu et al., 2004). From the analysis of IR-spectra of lignin, it may be inferred that lignins consist of ionizable groups (carboxylic acid and phenol groups) and polar functional groups (hydroxyl, carbonyl and methoxy group) abundantly.
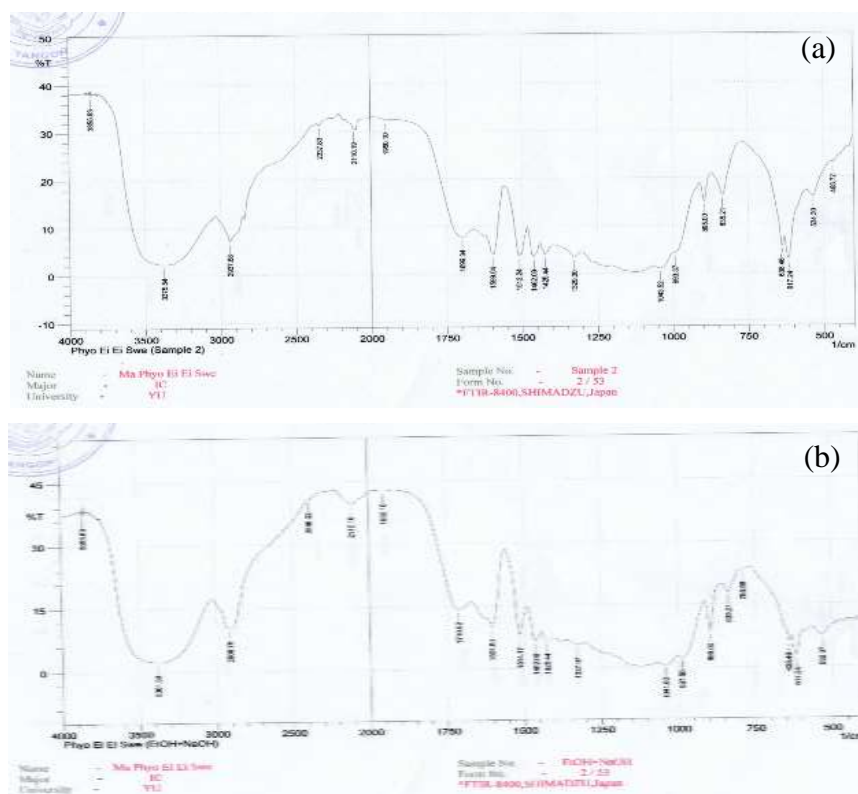
Figure 4   FT-IR Spectrum of Lignin Extracted by (a) Alkaline Process (b) Alcoholic Alkaline Process

Conclusion

In this research work, lignin was extracted from sugarcane bagasse by using alkaline process and alcoholic alkaline process. Box-Behnken Design was used to obtain the optimum conditions of lignin extraction by two processes. From the economical point of view, alcoholic alkaline extraction process was found to be the most suitable process because of the maximum yield of lignin and also ethanol can be recovered from black liquor by distillation. The analysis of UV and FT-IR spectra of lignin indicated that lignins extracted by both processes consisted of ionizable groups (carboxylic acid and phenyl) and polar functional groups (hydroxyl and methoxy). The lignin in alcoholic alkaline process also contains C=O stretching vibration of unconjugated ketone, carbonyl and ester at the wave number around 1710 cm^{-1}. Moreover, the band around 1699cm^{-1} indicating the presence of a small amount of impurities was not found in lignin from alcoholic alkaline process but this peak was observed in lignin from alkaline process.

Acknowledgements

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