Evaluation of Microwave Assisted Alkaline Pretreatment for Extraction of Cellulose from Selected Lignocellulosic Materials

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Abstract:

The objective of the present study is to evaluate the performance of the microwave assisted pretreatment method by subjecting five selected biomasses; Corn leaves, Corn Husks, Bagasse, Guinea grass and Sugar cane leaves, to obtain the maximum cellulose percentages with optimum pretreatment concentration and minimum exposure time on selected range. This method was performed by using a domestic microwave oven. Different concentrations of three solutions; NaOH - 1.25mol/L, 2.5 mol/L and 5 mol/L were used as pretreatment solutions. Total of 25 g of selected biomass with 250 ml of pretreatment solution was added in a 500 ml beaker and irradiated under microwave power of 170 W for 15minutes, 30 minutes and 45minutes. Forty five treatments consisted of combinations of five biomasses, three NaOH solutions and three expose time for irradiation. Percentages of weight losses of pretreated fibers were measured. In addition, Percentages of cellulose of treaded fibers were evaluated using chlorination method. Data was analyzed by using Minitab 14 version. Additionally, pretreated fibers with the highest percentage of cellulose obtained after evaluation of samples were analyzed using Fourier Transform Infra-Red Spectrometry (FTIR) to confirm extracted fibers were cellulose. Analysis results indicated that bio mass, NaOH concentration, time and (Bio mass × NaOH concentration) were significant for percentage weight loss. Except for time and (NaOH concentration × Time), other factors were significant for percentage cellulose extracted ($P < 0.05$). According to the results of main and interaction effects graphs of percentage of cellulose, all pretreated fibers comprised 65% - 85% of cellulose from pretreatment comprising 5 mol/L NaOH and 15 minutes of 170W microwave dose. FTIR analysis of extracted cellulose fibers from 5 mol/L NaOH and 15 minute treatment confirmed that extracted fibers were cellulose. Best condition for microwave alkaline pretreatment for a selected biomasses was found to be 5 mol/L NaOH and 15 minutes of 170W microwave dose.

Keywords: *Microwave Assisted Pretreatment, Lignocellulosic materials, Cellulose*

1.INTRODUCTION

Lignocellulosic materials are the most abundant biopolymer available in nature showing potential for ethanol production including agricultural residues such as corn straw, wheat straw bagasse, sugar cane leaves and guinea grass which are widely available in Sri Lanka. Using these, several researches have been initiated for production of activated carbon, bio ethanol and gasification by extraction of cellulose. Lignocellulosic plant materials are a matrix of hemicelluloses wrapped around long chains of cellulose encased in lignin (figure 1). Compositions of cellulose, hemicellulose and lignin are different from each other. Table 1

indicates the different percentages of chemical composition of mostly available lignocellulosic material in Sri Lanka. This paper discusses extraction of cellulose from selected lingocellulosic materials with an effective method. After extraction cellulose fibers were processed to produce cellulose based biodegradable Super Absorbent Polymers for different agricultural practices in Sri Lanka. Super Absorbent Polymers (SAPs) are mostly used to retain available water in the soil which enables the plants to survive longer under water stress and enhanced water holding capacity of the plant(Barbucci et al., 2000).

Pretreatment methods are necessary to remove or alter the lignin from lignocellulosic materials (figure1). An effective pretreatment of these lignocellulosic materials is needed to liberate the cellulose from the lignin seal and at the same time to reduce the lignin content, to reduce cellulose crystallinity and to increase cellulose porosity (Zhu et al., 2006 and Zhao et al., 2008). Pretreatment methods are suffered from relatively low sugar yields, severe reaction conditions, large capital investment, high processing costs and great investment risks (Alvira et al., 2010, Kumar et al., 2009). Acid pretreatment is able to hydrolyze the cellulose and hemicelluloses but capital cost is high because of the formation of inhibitors and equipment corrosion problems (Wyman, 1996). Oxidative pretreatment usually results in losses of cellulose and hemicellulose due to the fact that all oxidants used are non-selective (Hendriks et al., 2009). Pretreatment with organic solvents is too expensive to be employed for biomass though pure lignin could be obtained as a byproduct (Zhao et al., 2009). Biological pretreatment which commonly involves the use of the white rot fungus to degrade lignocelluloses requires low energy input, low capital cost and mild environmental conditions. However, it is otherwise unattractive at industrial scale because of slow conversion rates (Aita et al., 2010). Extensive reviews on pretreatment process methods and the use of these technologies for pretreatment of various lignocellulosic biomasses are given by (Mosier et al., 2005, Alvira et al., 2010, Kumar et al., 2009, Aita et al., 2010, and Taherzadeh et al., 2007).

Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Advantages of microwave based technologies include reduction of process energy requirements, uniform and selective processing, and the ability to start and stop the process instantaneously (Datta et al., 2001 and Hu et al., 2008). The earliest known study involving microwave pretreatment examined the effect of microwave radiation on rice straw and bagasse immersed in water and reported an improvement in total reducing sugar production by a factor of 1.6 for rice straw and 3.2 for bagasse in comparison to untreated biomass (Ooshima et al., 1984). Some studies have shown that microwave irradiation could change the ultra structure of cellulose (Xiong et al., 2000.) degrade lignin and hemicelluloses in lignocellulosic materials, and increase the enzymatic susceptibility of lignocellulosic materials (Hu et al., 2008, . Xiong et al., 2000, and Azuma et al., 1984). Most microwave pretreatment is generally carried out at elevated temperature (>160°C). Some previous studies have shown that application of microwave irradiation pretreatment may significantly increase the conversion of starch materials to glucose (Zhu et al., 2006 and Palav et al., 2006). Combination microwave treatment with either acid or alkali or combined acid/alkali might be an alternative for pretreatment of lignocellulosic materials has been recently explored (Zhu et al., 2006, Hu et al., 2008 and Binodet al., 2012).

The present study focused on extraction of cellulose from selected five biomasses using microwave assisted alkaline pretreatment. A domestic microwave oven with low microwave power - 170W - was used for this experiment with pretreatment solution being Sodium hydroxide. Study was carried out to evaluate the performance of the microwave assisted pretreatment method by subjecting five selected biomasses; Corn leaves, Corn Husks, Bagasse, Guinea grass and Sugar cane leaves, to obtain the maximum cellulose percentage with optimum pretreatment concentration and minimum exposure time on selected range.

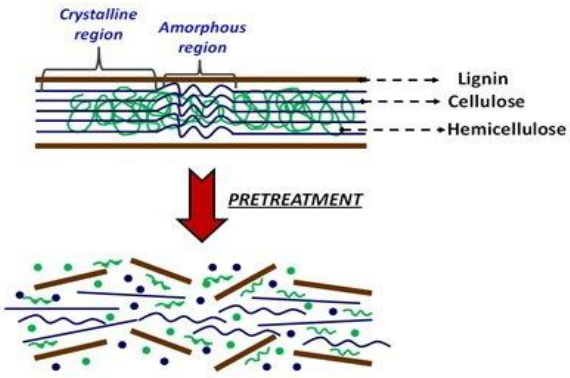

Figure 1: Structure of lignocellulosic materials

Table 1: Chemical composition of agro-industrial waste available

Agro-Industrial waste	Chemical composition (%W/W)					
	Moisture	Total solid	Ash	Cellulose	Hemicelluloses	Lignin
Corn straw	1.92	97.78	10.8	61.2	19.3	6.9
Sugar cane Bagasse	8.34	91.66	1.9	30.2	56.7	13.4
Guinea grass	-	-	-	31.0	22	7

Notes: (%w/w) = percentage based on dry weight.
Source: (Rice straw, Corn straw and Sugar can bagasse (SciELO, 2014) and guinea grasses (Odedireet al., 2008).

2. MATERIALS AND METHODOLOGY

2.1 Materials

Sodium hydroxide (NaOH)
99% Hydrochloric acid (HCl)
Sodium Chlorite ($NaClO_2$)
Acetic acid, Acetone
Five selected biomasses-(Bagasse, Sugar cane leaves, Corn leaves, Corn husks, Guinea grass)
Desiccator
Microwave oven and Electric oven
Electric grinder
Analytical balance

2.2 Methodology

This method was performed by using a domestic microwave oven. Different concentrations of three pretreatment solutions (NaOH) and selected five different biomasses were used for the experiment.

Three prepared pretreatment (NaOH) solutions
(1) - 1.25 mol/L NaOH
(2) - 2.5 mol/L NaOH
(3) - 5.0 mol/L NaOH

Five biomasses;
(1) - Bagasse
(2) - Sugarcane leaves
(3) - Corn leaves
(4) - Guinea grass
(5) - Corn husk

Selected biomasses (Bagasse, Sugarcane leaves, Corn leaves, Guinea grass and Corn husk) were dried at 105^{o}C using electric oven until a constant weight was observed and then ground using a normal grinder. Forty five treatments consisting of combinations of five biomass, selected three NaOH concentrations and selected three exposed time durations of microwave irradiation– (1)15minutes (2)30 minutes and (3)45 minutes were used. Each treatment was carried out three times. 10 ml from each prepared NaOH solution was added to 1g of biomass. According to that total of 25 g of biomass with 250 ml of NaOH solution was added to 500 ml beaker. Selected biomasses were immersed separately in 1.25mol/L NaOH, 2.5mol/L NaOH and 5 mol/L NaOH solutions and irradiated under a microwave power of 170W for 15, 30 and 45minutes. The lower power level of domestic microwave ovens is 170 W was chosen following the results of work carried out by Galema et al., (1997). According to their findings, low power rate was allowed for sufficient lengths of pretreatment time without drastic volumetric losses of the liquid phase and no charring of the solid sample occurred during 45 minutes of treatment time. After pretreatment, bio masses were washed with tap water. The mixtures were kept immersed in 10% HCl for 4 hours, washed with tap water once and heated to 60^{0}C using an electric oven and kept for 24 hours in a desiccator until a constant weight was reached. Percentages of weight losses were evaluated as weight different of before and after pretreatment of each biomass using an analytical balance. Cellulose percentages were determined using chlorination method as described by the Silverstein et al., (2007). 1g of sample was placed in a thermos flask (300ml) and 150 ml of distilled water was added. 1g of sodium chlorite ($NaClO_2$) and 0.2 ml of acetic acid were added while shaking slowly inside a shaking water bath with the flask covered with a glass lid and boiled at 70 ^{0}C to 80 ^{0}C for 60 minutes. Again, 1 g of $NaClO_2$ and 0.2 ml of acetic acid were added and boiled three more times. After cooling, the sample was filtered using filter papers and washed with hot water until free of acid. Afterward, the insoluble portion was dried in an oven at 60 ^{0}C for 4 h, cooled in a desiccator and weighed repeatedly until a constant weight was obtained. Holocellulose (combination of hemicelluloses and cellulose) content was calculated as follows.

Holocellulose content (%) = (Final weight after treatment/Initial weight of sample) 100

Amount of cellulose was calculated after determination of holocellulose content by further treating the fiber obtained with sodium hydroxide and acetic acid. Hemicellulose content was calculated by subtracting the cellulose content from the holocellulose content.
In addition, percentages of weight losses and cellulose were evaluated using Minitab version 14. Samples of Pretreated fibers with highest cellulose content were analyzed using Fourier Transformer Infra-Red Spectrometry (FTIR) to confirm extracted fibers were cellulose (sample were dried in an oven at 60 ^{0}C for 4 h, kept in a desiccator and weighed repeatedly until a constant weight was obtained prior to FTIR analysis).

2.3 RESULT AND DISCUSSION

Visual inspection shows that extracted fibers are thinner and straight after pretreatment. Normally formation of cellulose is of wool type, Hu et al., (2008) mentioned that this

formation indicates that the lignin degraded and increased the exposure of cellulose and hemicelluloses in the lignocellolsic materials after microwave assisted pretreatment.
The result of analyzed data for characteristic of percentage of weight losses as shown in table 2; biomasses, NaOH concentration, time and interaction effect of (biomasses × NaOH concentration) were significant for percentage weight losses. According to the result of percentage of cellulose as show in table 3 indicated that except for exposure time to irradiation and (NaOH concentration × Time), other data were significant for percentage cellulose.
Figure 2 and 3 indicated variation of "main effect plot" and "interaction plot" of percentage of weight loss of selected plant bio materials after microwave assisted alkaline pretreatment. According to those different percentage of weight losses occurred due to different composition characteristics of selected fibers as described in SciELO,(2014) and Odedireet al., (2008). Corn leaves and corn husk show highest weight losses and bagasse had lower percentage of weight loss due to effects of predominant presence of lignin content of native biomass. Also concentrations of NaOH affected percentage of weight losses. The present study shows that increasing NaOH concentration up to 1.25mol/L, 2.5mol/L to 5 mol/L increased the percentage of weight loss, Fang et al, (1999) has confirmed that the increasing alkaline condition in pretreatment solution resulted in an increase in the amount of lignin and hemicellulose solubilizing. The result of present study confirms their findings. The "main effect plot" and "interaction plot of percentage of cellulose "are shown in figures 2 & 4 and 3 & 5. According to that fibers extracted from corn leaves and corn husk show highest percentage of weight loss and lowest percentage of cellulose. The low content of lignin in corn straw may be the reason for highest amount of degradation of biomass due to solubilizing of lignin, hemicelluloses and also cellulose (syifarobbani., 2016). The results of present study indicated that NaOH concentration of 5mol/L applied treatment resulted in the highest amount of cellulose when compared to those with concentration of 1.25 mol/L and 2.5 mol/L. During delignification, the NaOH breaks the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass (Silverstein et.al., 2007) and also increasing the amount of lignin and hemicellulose solubilizing. Sugarcane leaves show highest percentage cellulose and bagasse show lowest percentage of cellulose compared to those of other biomasses. All pretreated fibers using pretreatment of 5mol/L NaoH solution and 15 minute duration resulted in 65.2% - 85% of cellulose. Also preheating times of 15, 30 and 45 minutes only affected the loss of lignin, hemicelluloses and cellulose and there was no significant effect on percentage of cellulose. According to the result of this study, the optimum pretreatment condition for selected biomasses was found to be 5 mol/L NaOH and 15 minutes of 170W microwave dose. Comparison of results of FTIR analysis of extracted cellulose fibers from 5 mol/L NaOH and 15 minute pretreatment shown in figure 6(a,b) to 10 (a,b), confirmed that the structural changes of native and microwave assisted alkaline pretreated fibers compares well with those wave numbers in the findings of Wang et al.,(2007) and Pandey et al.,(2004) as shown in table 4. Major changes observed were broadening of band at 3200-3400 cm^{-1} which was associated with the O-H stretching of the hydrogen bond in cellulose. The peak of $-CH_2$ stretching near 2900 cm^{-1} were easily distinguishable from native as well as microwave assisted alkaline pretreated fibers in present study. According to the Sun et al., 2008, band at 1000 – 1200 cm^{-1} were related to structural features of cellulose and hemicelluloses. The enhancement of absorption peak at

1000- 1100cm^{-1} after pretreatment indicate the increase in cellulose content in solid residue. The peak of O-H bonds at 3400 cm-1 stretching at 3300 cm-1 are the distinguished features of cellulose. The O-H bond at 3400 cm^{-1} is affected by microwave assisted alkaline pretreatment and its intensity is decreased in all extracted fibers. It has been reported that microwave irradiation enhances the saponification of intermolecular ester bonds cross-linking xylan the O-H band intensity tend to decrease due to its consumption in this reaction.

Table 2: Variation of probability values (P value) of percentage weight losses of selected bio masses

Factors	P value
Bio mass	0.031
NaOH concentration	0.019
Time	0.035
Bio mass* NaOH concentration	0.000
Bio mass*Time	0.577
NaOH concentration*Time	0.261

Table 3: Variation of probability values (P value) of percentage of cellulose of selected bio masses

Factors	P value
Bio mass	0.031
NaOH concentration	0.005
Time	0.180
Bio mass* NaOH concentration	0.002
Bio mass*Time	0.000
NaOH concentration*Time	0.062

Table 4: Variation of FTIR characteristics of cellulose (Wang et al., , 2007 and Pandey et al 2004)

Wave number (cm−1)	Compound
3336	O-H streching
2880 - 2940	C-H stretching in methyl and methylene groups
1734	C=O stretching in unconjugated ketones, carbonyls and in ester groups, frequently of carbohydrate origin.
1598	Aromatic skeletal vibration plus C=O stretch
1502	Aromatic skeletal vibration plus C=O stretch
1372	CH deformation in cellulose and hemicellulose
1316	C-H vibration in cellulose
1270	C-O stretch in lignin; C-O linkages in guaiacyl aromatic methoxy groups.
1235	C = stretch in lignin and xylan
1157	C-O-C vibration in cellulose and hemicellulose
1034	Aromatic C-H in plane deformation, C-O deformation; primary alcohol
897	β-glycosidic linkages

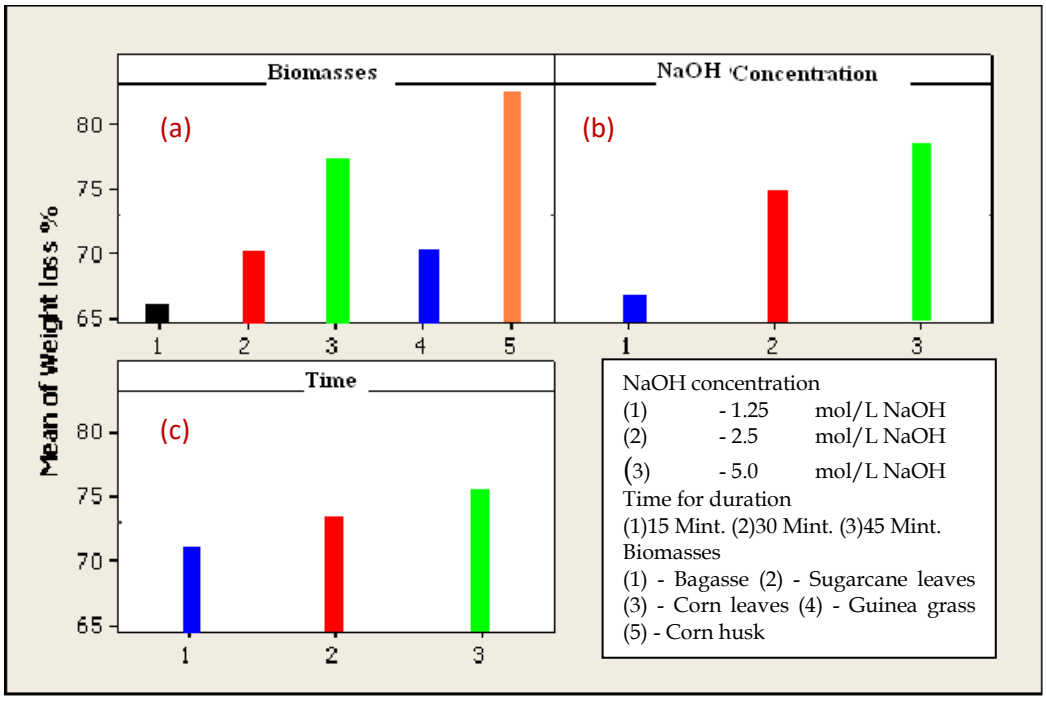

Figure 2: Variation of mean percentage weight losses with (a) biomass, (b) NaOH concentration and (c) time duration

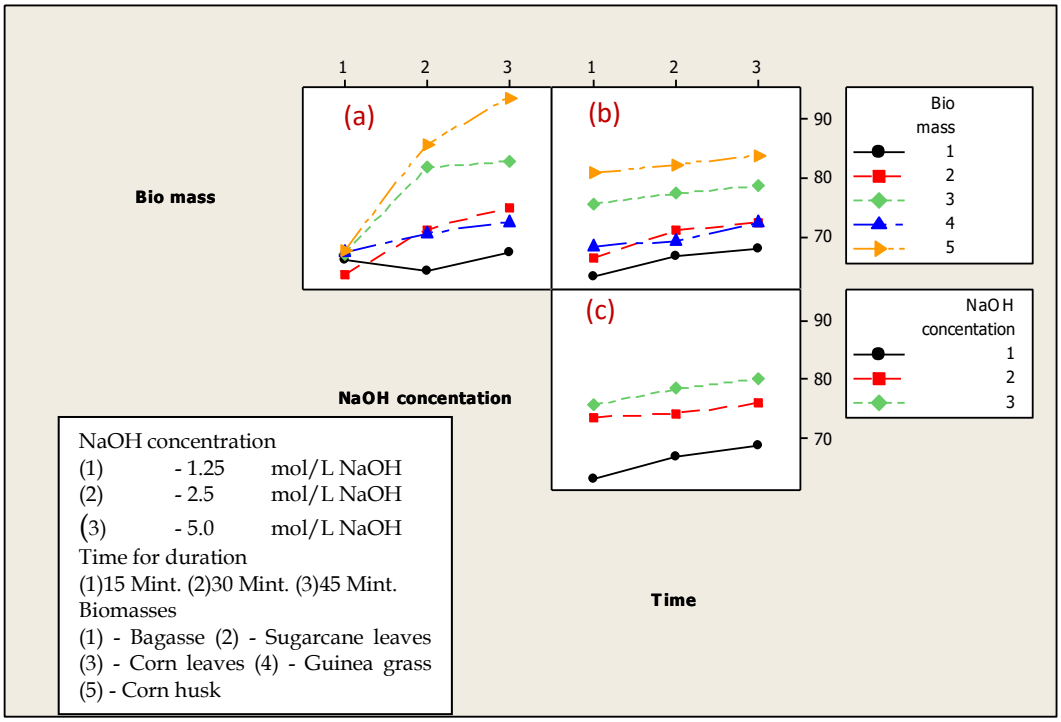

Figure 3: Variation of interaction plot percentage of weight loss a) (biomass × NaOH concentration), (b) (biomass × time) (c) (time × NaOH concentration)

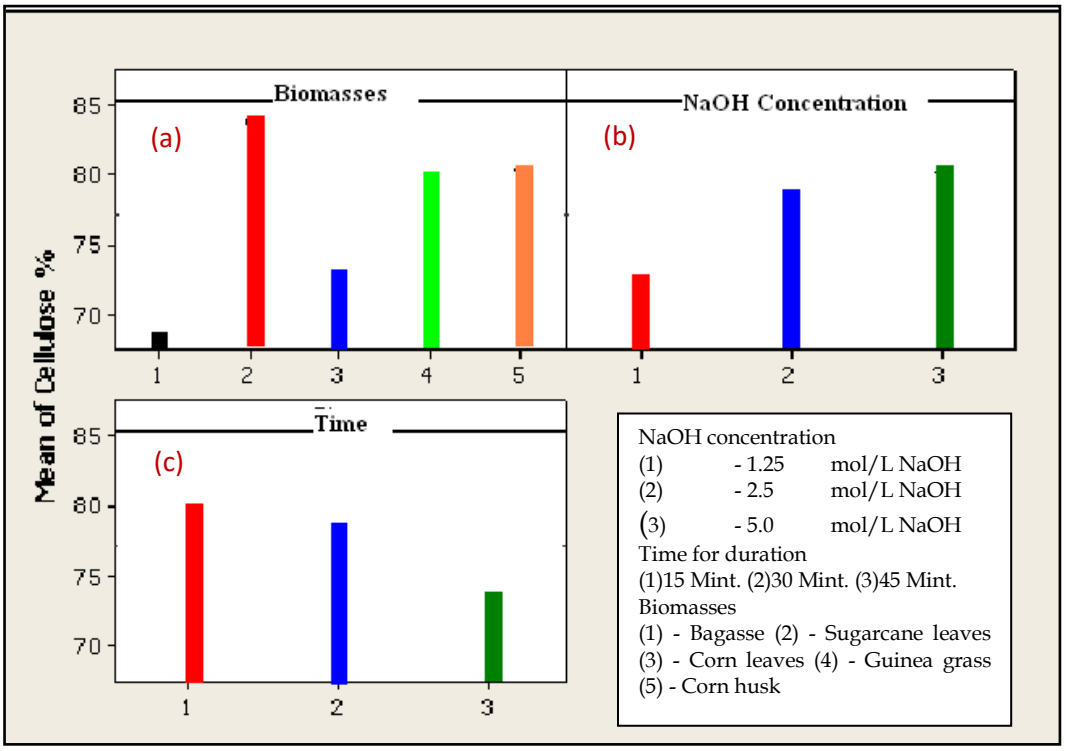

Figure 4: Variation of mean percentage cellulose with (a) biomass, (b) NaOH concentration and (c) time duration

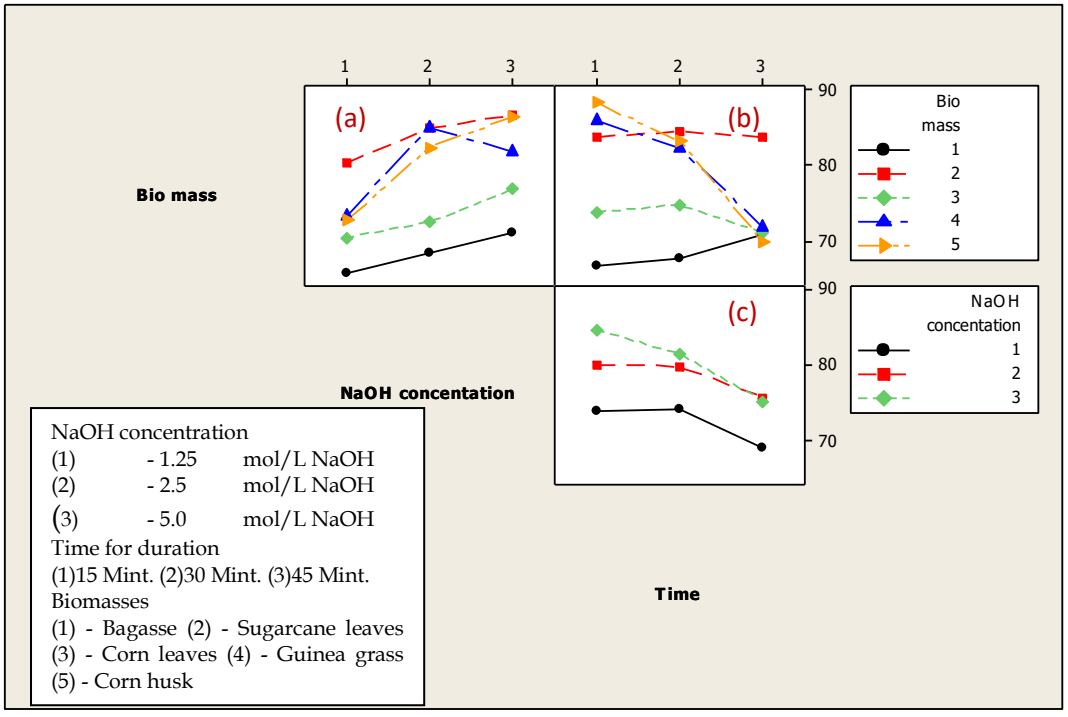

Figure 5: Variation of Interaction plot of percentage of cellulose (a) (Biomass ×NaOH concentration, (b) (Biomass × time) (c) (Time duration× NaOH concentration)

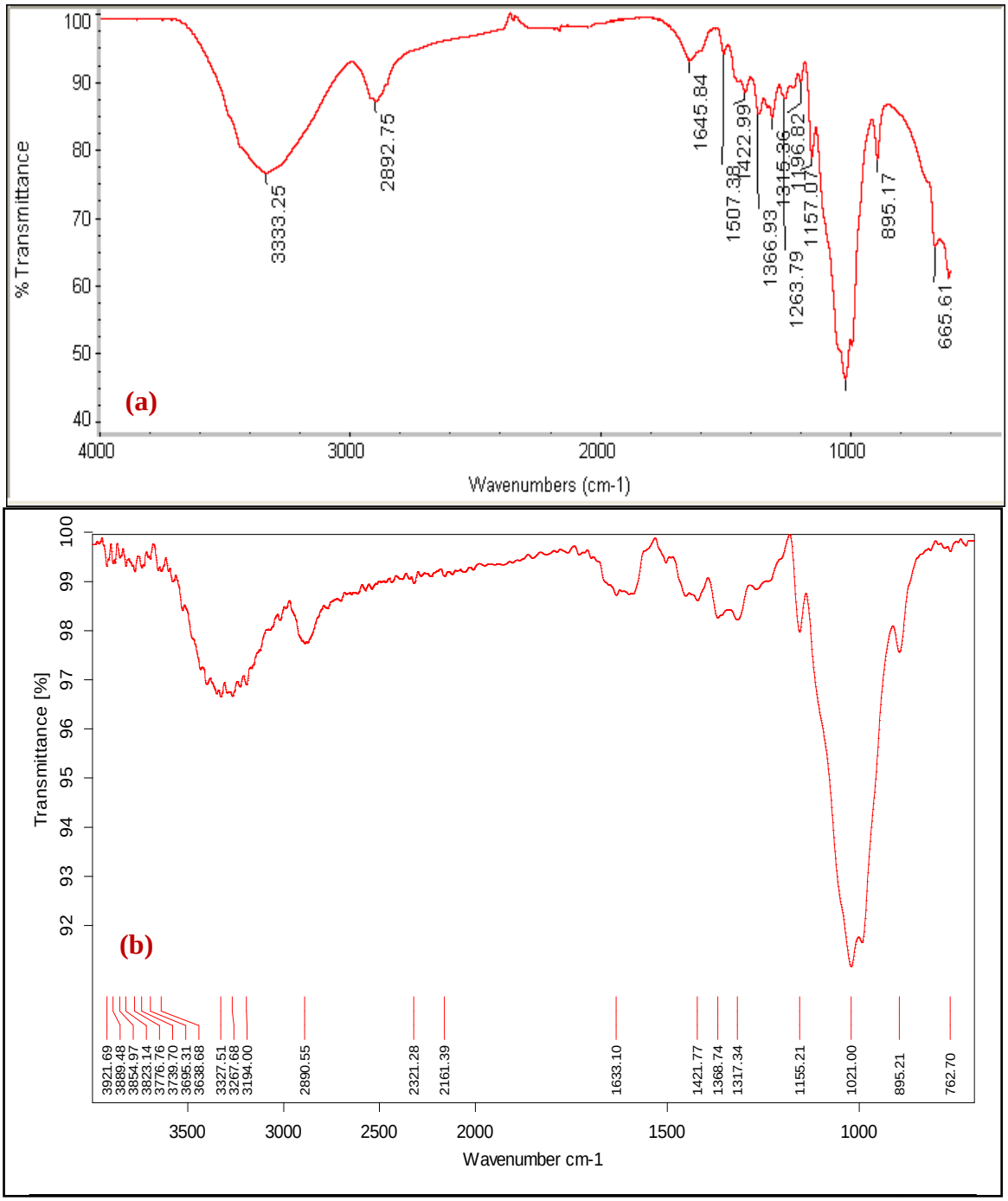

Figure 6: FTIR results (a) Raw bagasse (b) extracted fibers from 5 mol/L NaOH pretreatment solution and 15 minutes

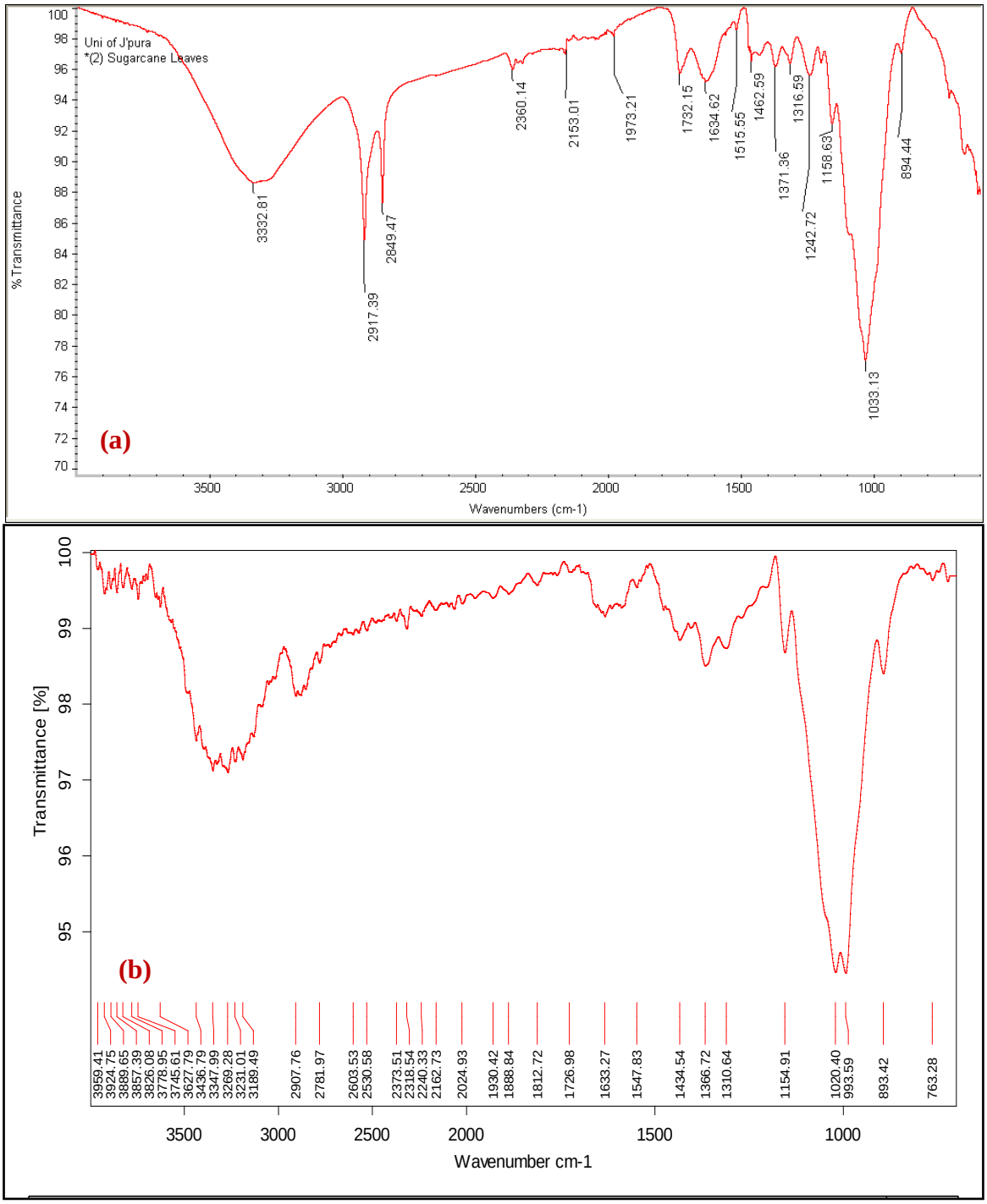

Figure 7: FTIR results (a) Raw Sugurecane leaves (b) extracted fibers from 5 mol/L NaOH pretreatment solution and 15 minutes

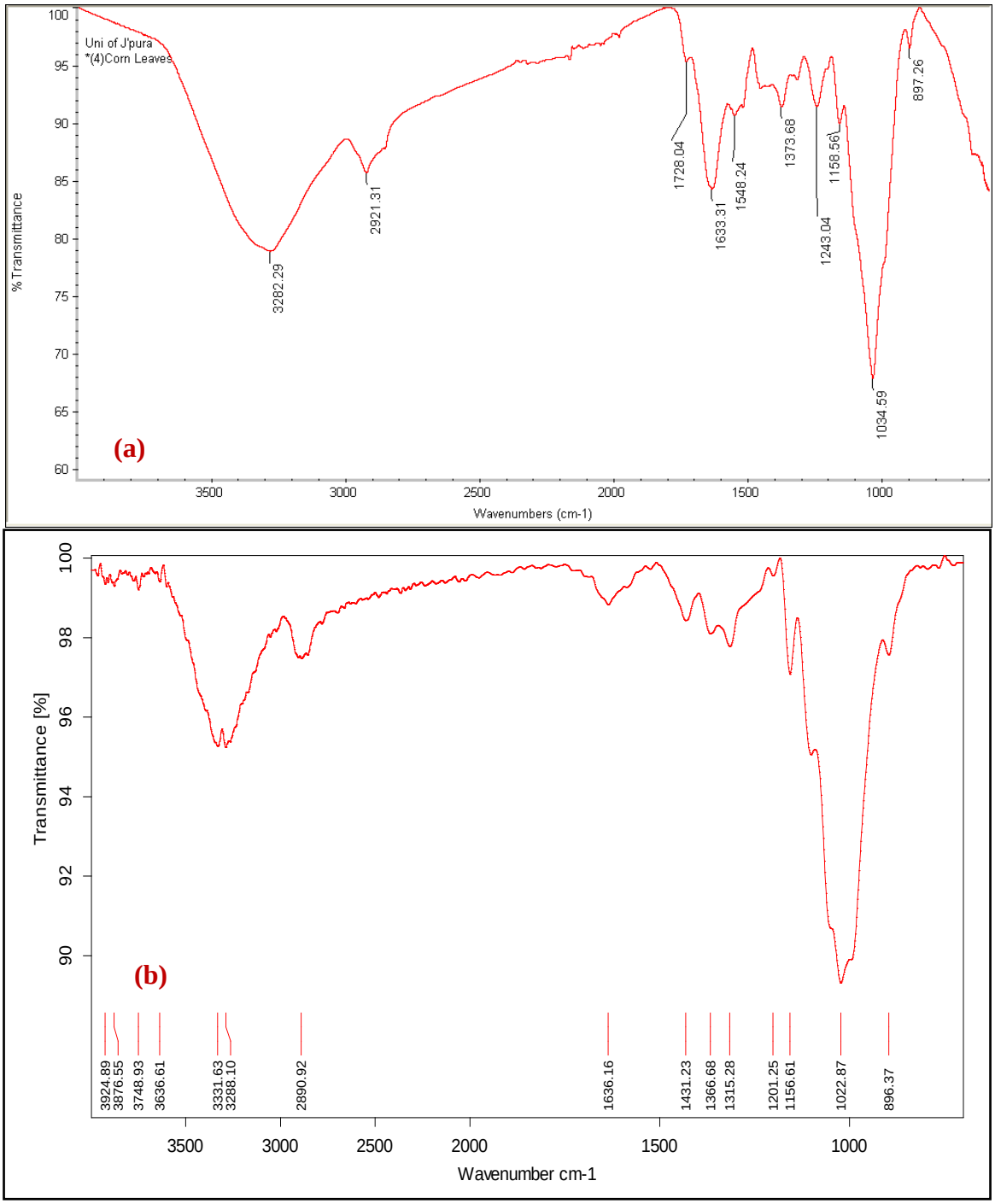

Figure 8: FTIR results (a) Raw Cornleaves (b) Extracted fibers from 5 mol/L NaOH pretreatment solution and 15 minutes

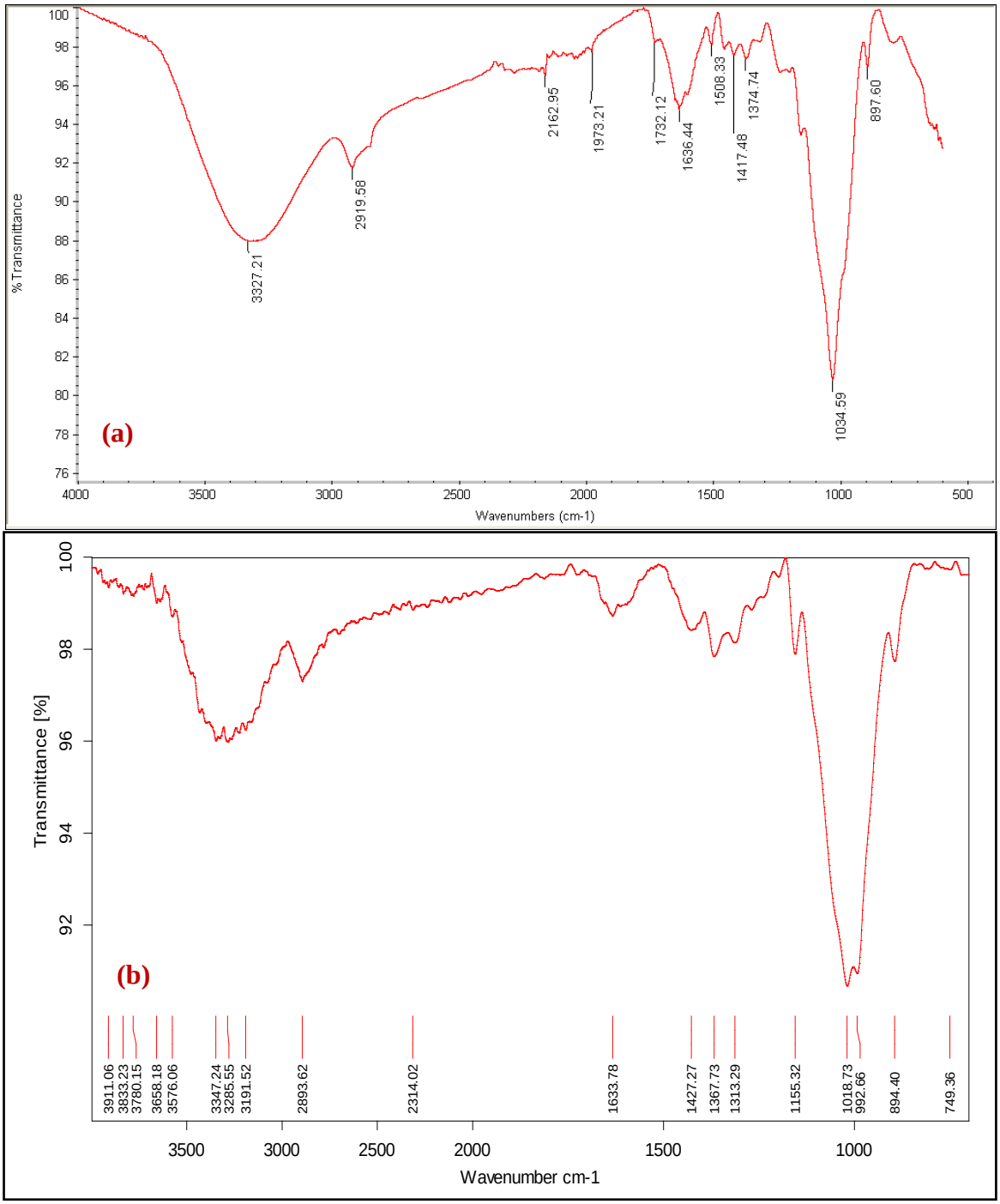

Figure 9: FTIR results (a) Raw Corn husk (b) Extracted fibers from 5 mol/L NaOH pretreatment solution and 15 minutes

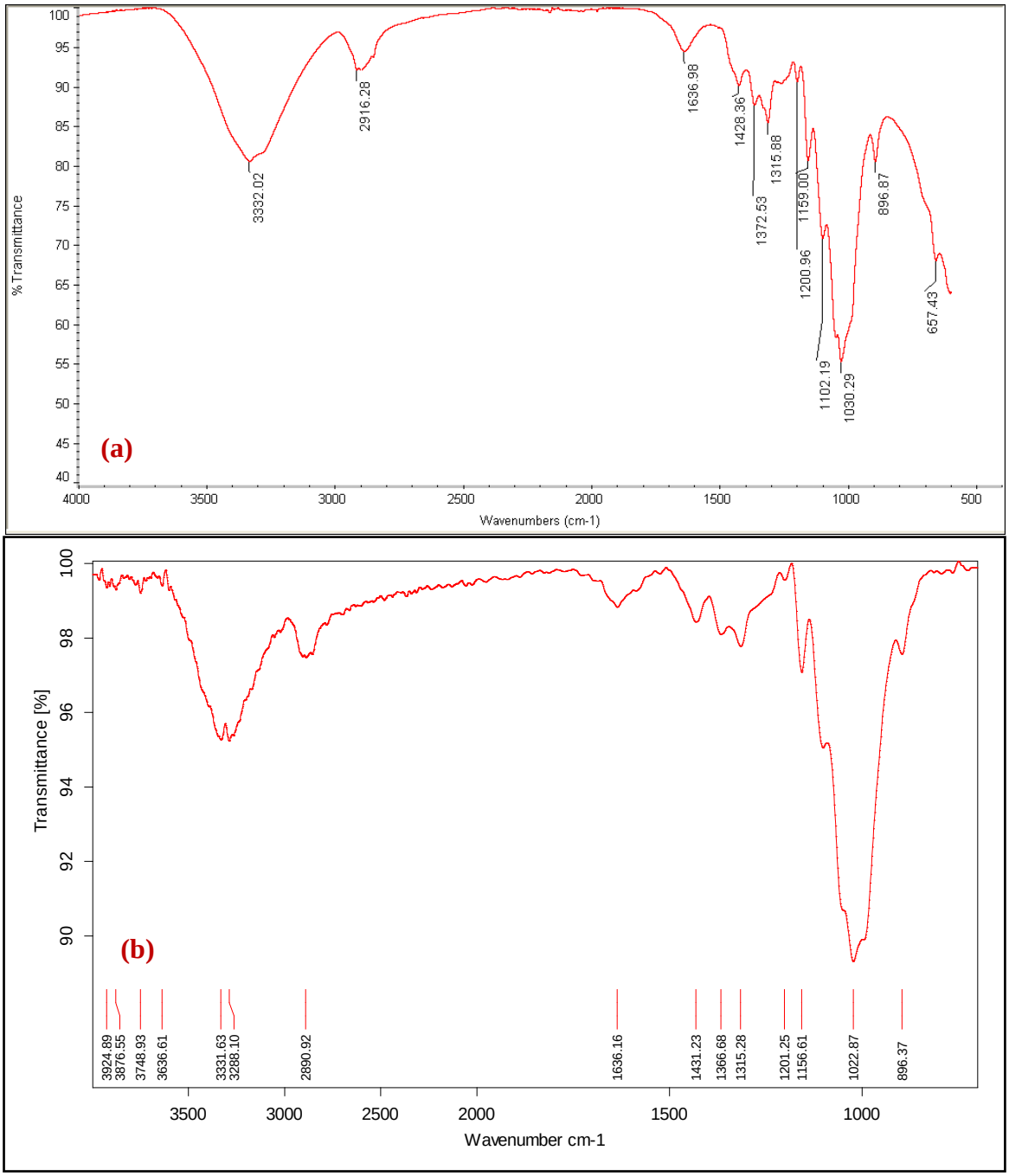

Figure 10: FTIR results (a) Raw Guinea grass (b) Extracted fibers from 5 mol/L NaOH pretreatment solution and 15 minutes

CONCLUSIONS

Composition characteristics of native bio masses affected percentage of weight losses and percentage of cellulose after microwave assisted alkaline pretreatment. Increasing of alkaline concentration of pretreatment solutions resulted in high weight losses of the bio masses. Highest weight losses of plant biomaterials occurred in pretreated corn leaves and corn husk which also shows lower percentage of cellulose. All pretreated fibers using pretreatment 5mol/L NaoH solution and 15 minutes consisted of 65% - 85% of cellulose. Among them sugarcane leaves show the highest (84%) and bagasses (65%) show the lowest values.

Exposed time for irradiation was not significant in the formation of percentage of cellulose. According to the results, the optimum pretreatment condition for selected biomasses was found to be 5 mol/L NaOH and 15 minutes of 170W microwave dose within the pretreatment concentrations exposure to microwave radiation.

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