Comparison of Some Pretreatment Methods on Cellulose Recovery from Water Hyacinth (*Eichhornia Crassipe*)

Bich Thuyen Nguyen Thi, Luong H. V. Thanh, T. N. Phuong Lan, N. T. Dieu Thuy, and Yi-Hsu Ju

***Abstract*—The effects of three physical pretreatment methods (water bath, ultrasound and microwave) on the cellulose recovery of water hyacinth were investigated in this study. Before treatment, cellulose and hemicellulose content of dried water hyacinth sample was 36.04%. After treating by ultrasound (70°C for 1 h) at a ratio of deionized water (DIW) (mL) to dried sample (g) of 10:1, cellulose and hemicellulose content in the treated sample was 57.7±0.65 %. The highest yield of cellulose and hemicellulose (58.19±0.59%) obtained by water bath treament at 100°C for 1 h whereas the cellulose and hemicellulose yield was 60.42±0.07% attained by microwave pretreatment (350W, 10 min at a ratio of DIW (mL) to dried sample (g) of 10:1). Therefore, among three pretreatment methods, microwave assisted DIW pretreatment presented the best efficiency on cellulose recovery. The outcome showed that microwave assisted DIW pretreated water hyacinth is a promising raw material for bioethanol production. The effects of these treatments on the composition and structure of water hyacinth were studied by thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).**

***Index Terms*—Water hyacinth, *Eichhornia crassipes*, cellulose recovery, ultrasound, microwave and water bath.**

I. Introduction

Water hyacinth (*Eichhornia crassipes*) is a widespread aquatic weed in tropical or subtropical areas. In most countries, water hyacinth is recognized as a big threat to agriculture and aquatic ecosystem because of its rapid growth rate [1]. Xia *et al.* (2013) believed that water hyacinth is one of the top 10 worst weeds in the world. This aquatic plant causes serious environmental pollution and great economic losses. A single plant of water hyacinth can have 140 million daughter plants annually, this amount is enough to cover an area of 1.40 km^2 with a fresh biomass of 28,000 tons. This invasive plant has deteriorated native ecosystems, clogged up lakes and rivers [2]. However, water hyacinth has high cellulose and hemicellulose contents (Table I) which can provide sugars for bioconversion to fuel ethanol [1]-[10].

In the last few decades, fossil fuel sources have been exhausted, therefore, finding replaced sources is a pressing mission. The first generation sources are sugar or starch based on crops [3]. However, this has conflict with food supply [11]. Therefore, lignocellulosic materials (second generation) are gradually considered as more attractive renewable resources for fuel production owing to their easy availability and relatively low cost [3] and [11]. Many researches [5]-[7], [10] have investigated ethanol production from water hyacinth. Bioethanol production from wheat straw has been reported by Singh & Bishnoi (2013) and Guragain *et al.* (2011). Ahmed (2013) studied ethanol production from Melalueca leucadenderon. In that year, Gao *et al.* (2013) produced hydrochar from water hyacinth. Hydrogen and methane were generated from water hyacinth [4]. Study on saccharification techniques of seaweed wastes for the production of ethanol was carried out by Ge *et al.* (2011). Besides, in order to utilize waste resources, Subhedar & Gogate (2015) reported the production of ethanol from waste newspaper. Ethanol was produced using potato peel as feedstock [12].

The objective of these researches was fuel conversion from lignocellulosic materials. However, there are many barriers because many steps are required. First step is pretreatment of biomass to break down lignin–hemicelluloses–cellulose complex to make it more susceptible to hydrolysis. The second step is hydrolysis to break down cellulose and hemicellulose into sugar monomers; and the third step is fermentation of these sugars to ethanol. The final step is product recovery and concentration by distillation [6], [13]. Among these steps, pretreatment is a major challenge because of structural linkages in lignocellulose, which are difficult to break at normal conditions [1]. Therefore, pretreatment is essential to remove biomass lignin, which hinders hydrolysis reaction [14].

Pretreatments include physical, chemical, biological, and combination of these methods [1], [15]. Physical pretreatment changes biomass structure by applying mechanical shear, without adding chemical or biological reagent [1].

This study aimed at investigating the effects of various physical pretreatment methods (ultrasound, microwave and water bath) on cellulose recovery and delignification of water hyacinth.

II. Materials and Methods

Fresh Eichhornia crassipe plants used in this study were harvested from Hau river, Viet Nam. The dried sample was ground and screened through 0.7 mm wire-mesh sieve. The sample was either used immediately or stored in airtight plastic bags at 4°C until used.

In order to compare the efficiency between pretreatment methods on cellulose recovery, the best pretreatment condition of each method was firstly selected based on cellulose yield and reducing sugars of the pretreated sample.

Manuscript received November 9, 2015; revised May 19, 2016.

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Thermalgravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used for examining the composition and structure of the treated samples.

TABLE I: LIGNOCELLULOSIC CONTENT OF WATER HYACINTH (% DRY BIOMASS)

Cellulose	Hemicellulose	Lignin	References	Treatment methods
18.3	23.3	17.7	[9]	untreated
24.3	22.5	8.6	[9]	DMSO, 120° C, 24h
	36	6.5	[1]	untreated
	49	not shown	[1]	ultrasound, 20 min
	36.04	2.51	this study	untreated
	58.19	1.37	this study	water bath, DIW, 100° C, 1h
	60.42	0.63	this study	microwave, DIW, 350W, 10 min

A. Water Bath Pretreatment

Dried sample (1g) was mixed with deionized water (DIW) and incubated in a water bath. The conditions in this experiment were as follows: ratio of DIW (mL) to dry biomass (g) of 7:1; 10:1; 15:1 or 20:1, treatment time of 30, 60, 90 or 120 min and treatment temperature of 70, 80 or 100°C.

B. Ultrasound Pretreatment

An ultrasonic bath was used in this trial (model SB-5200 DTN; temperature control range: room temperature (RT) –70 ℃; frequency (kHz): 10). The experiment was conducted at a ratio of DIW (mL) to dry biomass (g) of 7:1; 10:1; 15:1 or 20:1 and treatment time of 15, 30, 60 or 90 min. The bath was operated at 70°C, 25 kHz and a maximum power of 200 W.

C. Microwave Pretreatment

Microwave – assisted pretreatment on dried water hyacinth was carried out in microwave oven (Tatung, model TMO−7D−BK, power consumption: 120V~60Hz, 1200W, operation frequency: 2,450 MHz; 30 minute timer).

The experiment was carried out at various times (5, 10, 15, 20 and 30 min) and various ratio of DIW (mL) to dry biomass (g) of 7:1; 10:1; 15:1 or 20:1, respectively.

After pretreatments, the solid was collected by filtration and washed by DIW. The dried solid was analyzed for its lignocellulosic content by TGA. Reducing sugar compositions in the liquid were analyzed by DNS (3,5-dinitrosalicylic acid) method [16]. Each experiment was done at least in duplicates.

D. Thermal Analysis

Dried water hyacinth sample (5-7 mg) was pyrolyzed by a TGA (Model：Perkin Elmer, temperature range: 30-800°C). The increasing rate of temperature was 10°C/min. Nitrogen atmosphere (40mL/min) was employed in this experiment.

E. Fourier Transform Infrared Spectroscopy (FTIR)

A FTIR Bio-Rad FTS-3500 spectroscopy was employed to determine the alteration of chemical component of the treated sample. The spectrum was obtained in the transmission mode and wave number range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} with 40 scans per sample.

F. Scanning Electron Microscopy (SEM)

Surface morphology of the sample was observed by a scanning electron microscopy (SEM, JSM-6390LV, JEOL, Japan) at an accelerated voltage of 15−20 kV after gold or platinum coating by a JEOL JFC−1100 E sputtering device for 85 seconds prior to SEM observation.

III. RESULTS AND DISCUSSION

A. Effect of DIW to Dried Sample Ratio on Reducing Sugar Content

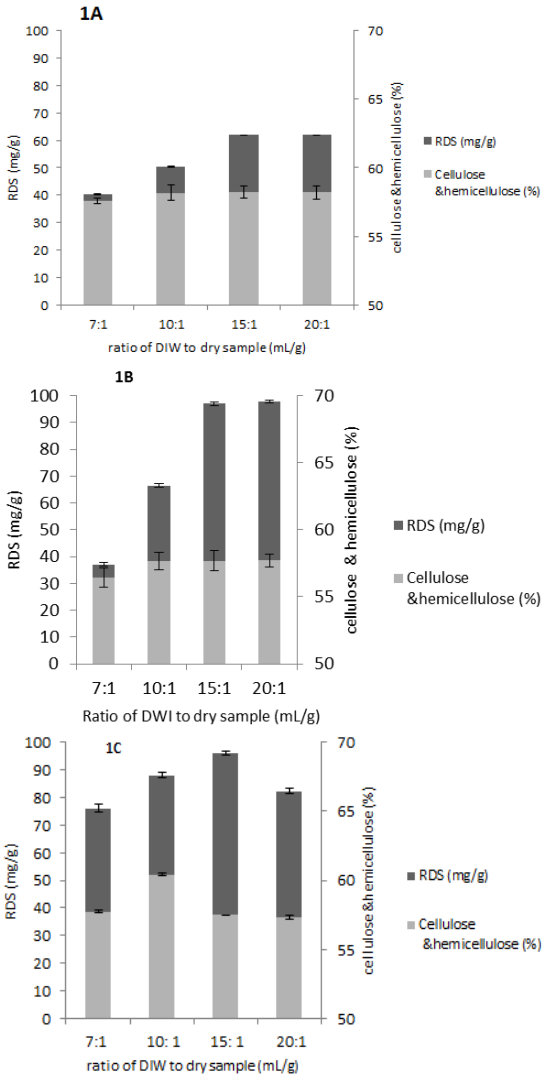


Fig. 1. Effect of DIW to dried sample ratio (mL/g) on the lignocellulose and reducing sugar yield of water hyacinth attained from: (1A) water bath pretreatment (at 100°C, 60 min); (1B) ultrasound pretreatment (at 70°C, 60 min); (1C) microwave pretreatment (at 350W, 10 min).

For water bath pretreatment, Fig. 1A shows that reducing sugar yield increased from 40.31 mg/g to 61.93 mg/g as DIW to dried biomass ratio was changed from 7:1 to 15:1 mL/g. This may be attributed to the role of water in the degradation of lignocellulosic materials leading to releasing of sugars from biomass matrix. More water resulted in higher penetration. A ratio of 7:1 of DIW to dried biomass was not

enough water to solubilize carbohydrates leading to low sugar release after a week digestion. The increase of DIW to dried biomass ratio to 15:1 mL/g resulted in high yield of sugars (61.93 mg/g). However, increasing the ratio of DIW to dried sample to 20:1 mL/g did not increase reducing sugar yield, which 61.98 mg/g. Similar phenomena were observed in the ultrasound pretreatment (Fig. 1B). In contrast, in microwave-assisted pretreatment, sugars content was decreased from 96.08 mg/g to 82.33 mg/g as the ratio of DIW to dried biomass was increased from 15:1 to 20:1 mL/g (Fig. 1C). This is because unlike conventional heating, microwave irradiation directly and quickly permeated into the inner particles to selectively heat sensitive polar molecules, such as water [2]. Microwave oscillation caused polar water molecules to collide with each other, thereby generating a large amount of heat to effectively break lignocellulose materials and crystal structures [2]. Therefore, more water leads to more generated heat leading to degradation of sugars into smaller molecular such as acetic acid, furfural or hydroxyl methyl furfural (HMF) [2].

B. *Effect of DIW to Dried Sample Ratio on Lignocellulose Content*

Fig. 1A shows that water bath treatment at 100°C for 1h dramatically decomposed lignin compounds in biomass matrices. Untreated water hyacinth contains 2.51% lignin, 36.04% cellulose and hemicellulsose. After pretreatment using a ratio of DIW to dried sample 7:1 mL/g, cellulose and hemicellulose content in residue solid was 57.59%, with a corresponding lignin content of 1.43%. Cellulose and hemicellulose content inreased to 58.19% when the ratio of DIW to biomass was increased to 10:1 mL/g. The reason was that at this ratio (10:1), lignin content in the residue was 1.37% which means that better lignin removal than at a ratio of 7:1 (1.43%) leading to expelling of cellulose and hemicellulose from matrices. However, cellulose and hemicellulose content remained almost constant though DIW to dried biomass ratio was increased from 10:1 to 20:1 because while cellulose and hemicellulose were generated by pretreatment, they were subsequently converted to sugars including glucose, xylose, galactose or arabinose [1], [2], [4], [6], [7], [9], [14], [17]; hence the productivity of sugars increased as DIW to dried biomass ratio was increased from 10:1 to 20:1 (Fig. 1A). This observation was also found in ultrasound pretreatment (Fig. 1B). The highest yield of cellulose and hemicellulose in solid residue (57.70%) was obtained at a DIW to dried biomass ratio of 10:1, 70°C and 1 h. This value stabilized even as DIW to biomass ratio was increased from 10:1 to 20:1.

Water bath pretreatment degrades lignocellulosic materials by conventional heating while ultrasound decomposes the materials by formation and explosion of bubbles [18]. Unlike these two methods, microwave irradiation digests biomass by electromagnetic wave [2] which generates energy by collisions of polar molecules like water. Therefore, increasing DIW to biomass ratio from 10:1 to 20:1 produced more energy, leading to degradation of cellulose and hemicellulose to sugars. Therefore, cellulose and hemicellulose content dropped form 60.42% to 57.30% (Fig. 1C).

For calculating a theoretical maximum reducing sugar yield. It was assumed that cellulose and hemicellulose in water hyacinth completely hydrolyze into hexose (viz. glucose) and pentose (viz. xylose). The weight ratio of glucose molecule to cellulose was 1.111 and that of xylose to hemicellulose was 1.136. Given that the water hyacinth sample contained 36.04% cellulose and hemicellulose, the theoretical maximum glucose and xylose hydrolyze that can be originated from 100 g of the sample were 40.49 g/100 g biomass [2].

In this study the highest reducing sugars yield obtained was 96.08 mg/g which was only 23.70% of the theoretical value whereas the highest cellulose and hemicellulose recovery after treatment was 60.42%, which is 134.18% of the untreated sample. Therefore, further experiments were based on the optimal conditions for cellulose and hemicellulose recovery.

C. *Effect of Time on Reducing Sugar Content*

The effect of treatment time on reducing sugar and lignocellulose content was conducted based on the optimal conditions for cellulose and hemicellulose recovery in Section III.A.

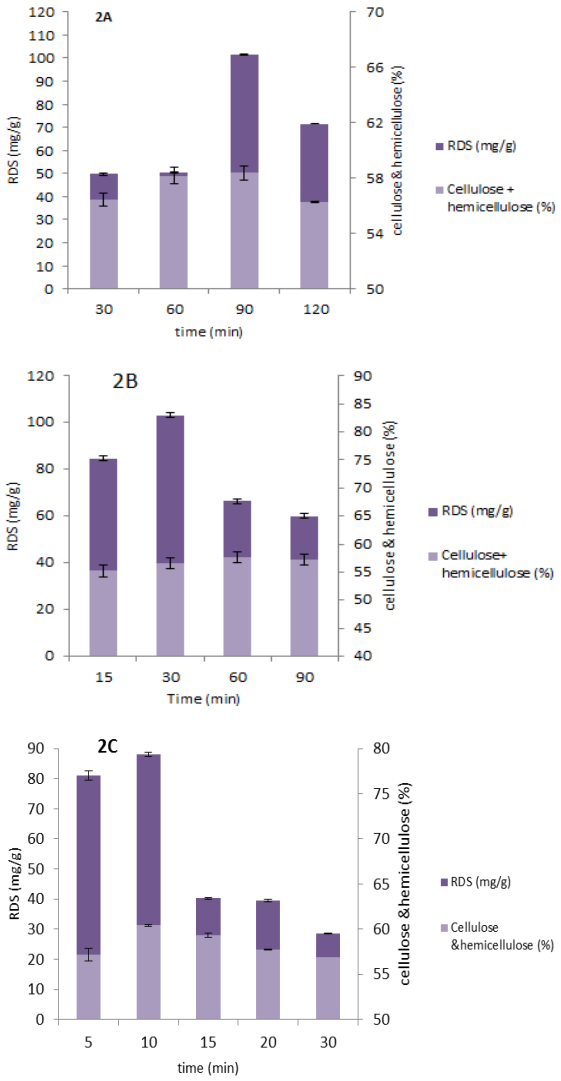


Fig. 2. Effect of time on the lignocellulose and reducing sugar yield of water hyacinth attained from: (2A) water bath pretreatment at 100°C, DIW (mL) to dried sample (g) ratio of 10:1; (2B) ultrasound pretreatment at 70°C, DIW (mL) to dried sample (g) ratio of 10:1; (2C) microwave pretreatment at 350W, DIW (mL) to dried sample (g) ratio of 10:1.

Similar trend was found on the effect of time on reducing sugar and lignocellulose content for all three pretreatment methods. Prolonging reaction time resulted in decreasing sugars as well as cellulose and hemicellulose contents because of degradation of these compounds to smaller molecular.

For ultrasound pretreatment (Fig. 2B), 30 min treatment time gave the highest amount of reducing sugar (103.04 mg/g) which was only 25.44% of the theoretical value. An increase of time to 60 min resulted in decreasing reducing sugar content sharply to 66.46 mg/g since sugars continued to decompose to smaller molecular such as formic acid, pentanoic acid, acetic acid, furfural or hydroxyl methyl furfural (HMF) and propiolic acid [2]. Treatment by water bath generated the highest reducing sugars at 90 min (101.57 mg/g) and this value tumbled to 71.53 mg/g when time was raised to 120 min (Fig. 2A). For microwave-assisted treatment, reducing sugars content dropped considerably from 88.15 mg/g to 28.56 mg/g as treatment time was increased from 10 min to 30 min (Fig. 2C).

The finding of this study agrees with the results of Harun *et al.* (2011) who reported that increasing steaming time of water hyacinth from 30 min to 90 min decreased reducing sugars from 37.62 mg/g to 22.74 mg/g. In the same study, the authors showed that reducing sugars dropped from 116.52 mg/g to 97.25 mg/g as ultrasonication time was increased from 20 min to 30 min [1]. Xia *et al.* (2013) evidenced that reducing sugars of water hyacinth declined due to producing of furfural, 5-methyl-2-furaldehyde, and 5-hydroxymethyl-2-furaldehyde, formic acid, 2-methylpropanal and propiolic acid as microwave irradiation time was prolonged from 15 min to 45 min [2].

D. Effect of Time on Lignocellulose Content

For ultrasonication pretreatment (Fig. 2B), maximal yield of cellulose and hemicellulose was obtained at 60 min (57.70%). Prolonging of time to 90 min did not improve cellulose and hemicellulose yield because cellulose was converted to hexoses (glucose, galactose) and hemicellulose produced glucose and pentoses such as xylose, arabinose [1], [2], [4], [6], [7], [9], [13], [14].

The maximum cellulose and hemicellulose content (58.37%) was obtained at 90 min for water bath treatment and 60.42% at 10 min from microwave irradiation, corresponding to 1.38% and 0.63% lignin yield, respectively. For water bath pretreatment, increasing reaction time from 90 min to 120 min decreased lignin content from 1.38% to 1.28%, but cellulose and hemicellulose content also dropped from 58.37% to 56.29% because of cellulose and hemicellulose digestion to sugars (Fig. 2A). This finding was observed similarly in microwave pretreatment where the removal of lignin at 10 min was 0.63% and this value decreased to 0.62% as time was lengthened to 15 min but cellulose and hemicellulose content did not increase with increasing time after 10 min (Fig. 2C).

This observation agrees with that of Xia *et al.* (2013) [2] who believed that prolonged microwave irradiation time of water hyacinth from 5 min to 45 min resulted in decreasing weight percentages of residual solid biomass (46.20%−36.85%), residual hemicellulose (25.25%−0%; complete degradation), residual cellulose (83.48%−62.67%), and lignin (89.86%−60.75%).

E. Effect of Temperature on Reducing Sugar and Lignocellulose Content

For water bath pretreatment, the experiments investigated at 70°C, 80°C and 100°C. Treating at lower than 100°C (in 60 min and a ratio of DIW (mL) to dried biomass (g) of 10:1) did not give satisfactory yields of reducing sugars, cellulose and hemicellulose because apparently temperatures lower than 100°C did not supply enough heat to digest the sample.

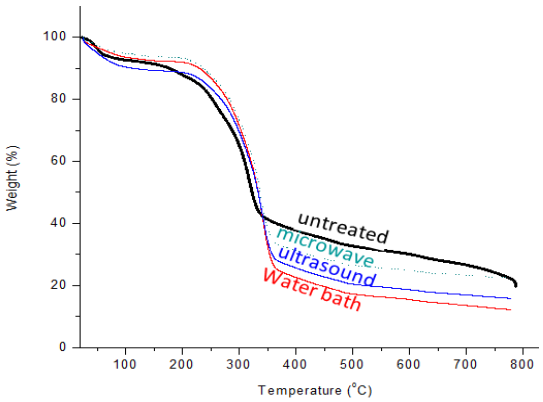


Fig. 3. TGA curves of water hyacinth.

Ultrasonication technique usually is used at low temperature (ambient to 70°C). This method breaks material's contents mainly based on formation and explosion of bubbles. In this study, treating sample at 60°C did not give satisfactory results.

For microwave treatment, the highest yields of reducing sugar, cellulose and hemicellulose were obtained at 350 W for 10 min with a DIW to dried sample ratio of 10:1(mL/g). Treatment at 450 W decreased dramatically the productivity since the sample was burn at high power (data not shown).

Briefly, the optimal conditions for cellulose and hemicellulose recovery in this study were found at DIW to dried biomass ratio of 10:1 (mL/g) for all three pretreatments; the temperature of 100°C, 60 min and for water bath pretreatment; 70°C, 60 min for ultrasound pretreatment and 350 W, 10 min for microwave irradiation.

Among the three studied pretreatment methods, microwave assisted DIW pretreatment seems to be the best method for cellulose and hemicellulose recovery from water hyacinth.

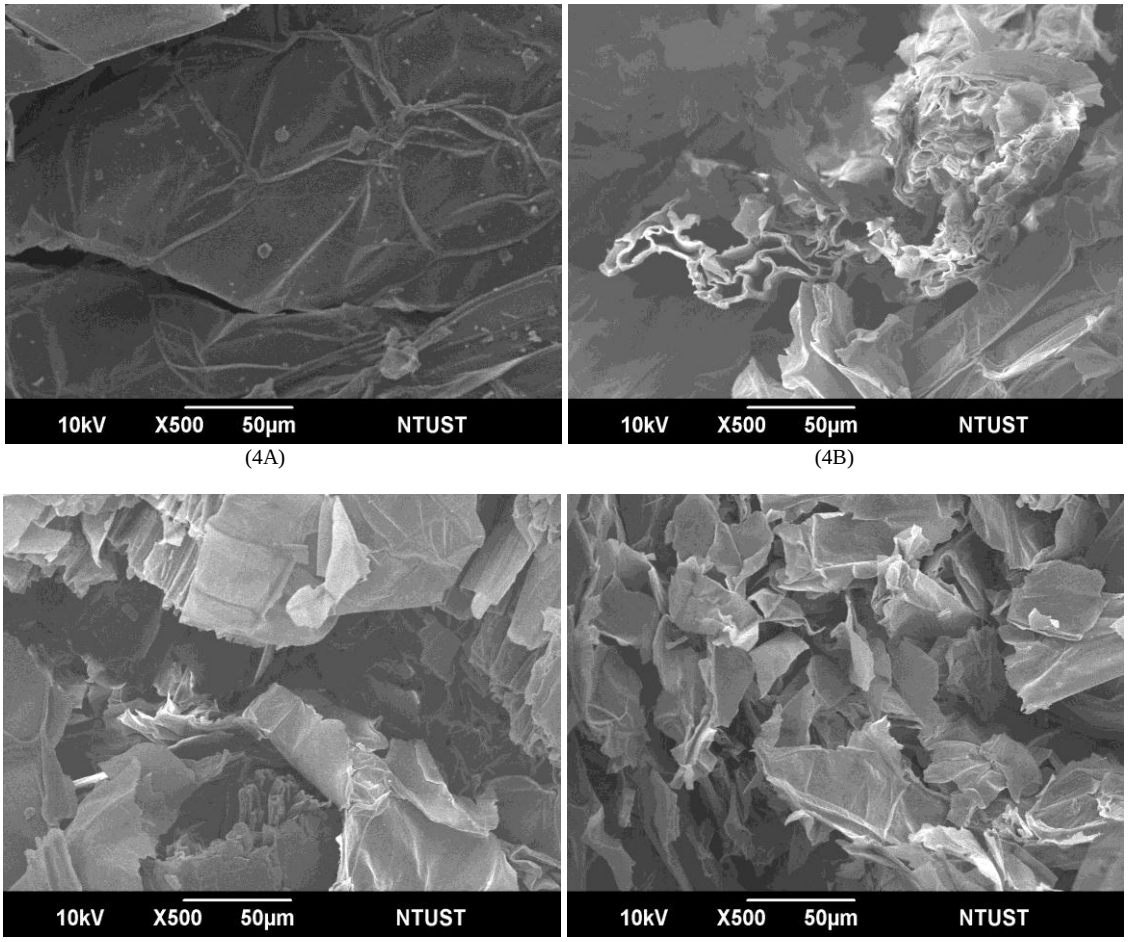
F. Thermal Analysis

Hemicellulose and cellulose are decomposed at 200−350°C, lignin decomposition occurs at 350−470°C [1]. Fig. 3 indicates that hemicellulose was digested at 265°C while the main peak that appeares at 326°C was attributed to cellulose degradation. However, almost all sample curves show that hemicellulose peak is the only shoulder in the derivative thermogravimetric curve which means hemicellulose was the minor component in water hyacinth. The small peak appeared at approximately 468°C was contributed to lignin. This observation agrees with that of Harun, 2011.

G. Scanning Electron Microscopy (SEM)

Fig. 4A shows that the untreated sample's surface was still smooth and tightly random. The structure was changed after

treating by ultrasonic wave irradiation at 70°C for 1 h. As seen from Fig. 4B, broken structure was observed due to the applied ultrasonic waves that generated micro bubbles which exploded eventually and caused the sample to break down leading to releasing of cellulose and hemicellulose form matrices. For this reason, cellulose and hemicellulose content in the treated sample was higher than that of the non-treated one. This was also observed in water bath pretreatment at 100°C for 1 h which shows that cracked ditch appeared in the treated sample (Fig. 4C). For the microwave-treated sample, the surface of water hyacinth became crumbled. This finding indicates that the intact structure of lignocellulose was dramatically ruined and cellulose was completely expelled by microwave pretreatment (Fig. 4D). Thus, the yield of cellulose, hemicellulose and reducing sugar was improved compared to other pretreatments.



(4A) (4B)

(4C) (4D)

Fig. 4. SEM images of water hyacinth. (4A) untreated sample; (4B) treated by ultrasound at 70°C for 1h with DIW to dried biomass ratio of 10:1 (mL/g); (4C) treated by water bath at 100°C for 1 h with DIW to dried biomass ratio of 10:1 (mL/g); (4D) treated by microwave at 350 W for 10 min with DIW to dried biomass ratio of 10:1 (mL/g).

H. Fourier Transform Infrared Spectroscopy (FTIR)

Fig. 5 shows FTIR spectra of the untreated water hyacinth and the treated samples. The peaks were observed at 1240 and 1346 cm^{-1} are those of C−O stretching of syringyl lignin and C−O−C of hemicellulose oscillation in anomeric region [19]. The peak at 1500 cm^{-1} indicates the C=C stretching of aromatic rings of lignin [20]. These peaks were observed in untreated water hyacinth but in the treated samples were observed weakly. The peaks at 1346 cm^{-1} and 1500 cm^{-1} were disappeared in the microwave treated sample, indicating that lignin was removed. This is in agreement with Abral, 2014 [19] and Sundari, 2012 [20]. These observations indicate that microwave treatment removed lignin leading to expelling of cellulose and hemicellulose from tight matrices. Therefore, cellulose and hemicellulose content of the microwave treated sample was higher than that of the untreated sample and the others treated water hyacinth.

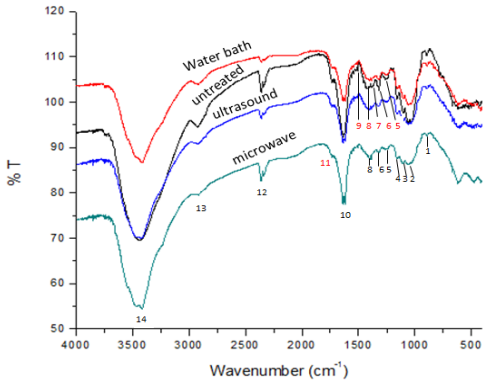


Fig. 5. FTIR spectra of untreated and treated water hyacinth.

IV. Conclusion

Among three pretreatment methods, microwave pretreatment presented the best ability on cellulose and

hemicellulose recovery. Under conditions of 350 W, DIW to dried biomass ratio (mL/g) of 10:1 for 10 min, cellulose and hemicellulose recovery was 60.42% which is 134.18% of the untreated sample. Morphology and functional groups of all samples were inspected by SEM, TGA and FTIR. Results indicate degradation of lignocellulosic materials which explains the enhancement of cellulose and hemicellulose yield. Based on the results of this study, bioethanol production from water hyacinth will be investigated.

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