Water Hyacinth (*Eichhornia crassipes*) Biomass as a Biofuel Feedstock by Enzymatic Hydrolysis

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Water hyacinth (*Eichhornia crassipes*) is an invasive floating plant that has caused many environmental problems in Asia. Efficiently removing and utilizing this biomass has become an urgent issue. In this work, the composition of water hyacinth biomass (WHB) was analyzed with the Van Soest method. The combined cellulose and hemicellulose content reached 58.6%, and the lignin content was very low compared with other biomass. An efficient alkali pretreatment technology for WHB was developed, and the enzymatic hydrolysis of WHB to reducing sugars was investigated. With favorable hydrolysis conditions for the alkali-pretreated WHB, the cellulose conversion rate reached almost 100%. Structural changes resulting from WHB pretreatment and hydrolysis were analyzed by Fourier-transform infrared spectrometry and scanning electron microscopy. This work demonstrates that WHB is an alternative cellulose source for bioenergy production.

Keywords: Water hyacinth biomass; Cellulose; Biomass utilization; Lignocellulose

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a fast growing, perennial aquatic weed. In favorable environmental conditions, an individual water hyacinth can produce 140 million daughter plants annually; these plants cover 1.40 km^2 water area with a fresh biomass of 28,000 tons (Lu *et al.* 2007). These aquatic plants deplete dissolved oxygen in the water and jeopardize the health of aquatic animals. They also block rivers, drainage system, and water transportation. As an invasive floating plant, it has caused an enormous ecological and economic disaster worldwide (Lu *et al.* 2007; Villamagna and Murphy 2010). In southern China, these plants seriously threaten the aquatic systems of lakes and rivers (Chu *et al.* 2006), and they must be removed every year. Thus, efficiently utilizing water hyacinth biomass (WHB) is an urgent issue.

Economical and societal development has driven the demand for alternative energy sources. Fossil fuels are still the dominant sources of energy, but they cannot support the global energy demand due to inherent defects such as non-renewability and atmospheric carbon emissions. These issues have prompted efforts toward sustainable energy sources. Among various options, biofuel produced from biomass appears to be a feasible alternative energy source. Cheap and abundant lignocellulosic biomasses are alternative feedstock for biofuel production (Balat 2011; Wi *et al.* 2013; Lee and Kuan 2015). In addition to terrestrial lignocellulosic biomass such as sugarcane bagasse and crop straw, WHB is a potential feedstock for biofuels.

WHB has been evaluated for bioethanol production (Cheng *et al.* 2014; Pothiraj *et al.* 2014; Yan *et al.* 2015). But the fundamental information about water hyacinth composition and WHB hydrolysis to fermentable sugars has not been determined. In this report, the chemical composition of water hyacinth was analyzed, and an efficient process to hydrolyze WHB to fermentable sugars with cellulase was developed.

EXPERIMENTAL

WHB and Materials

Fresh water hyacinth plants were collected from Yang-Shuwan Lake in Yichang City, China (30.70°N, 111.30°E). The roots of the plant were removed, the leaf and body were washed to remove dirt and dried in sunlight. They were further dried overnight at 70 °C in an oven to remove residual moisture. The dried biomass was milled to powder and passed through a 0.425 mm mesh sieve. *Trichoderma reesei* cellulase was purchased from the Imperial Jade Bio-technology Co., LTD (Yinchuan, China). This multiple component enzyme contains three activity components: endoglucanase, exoglucanase, and beta-glucosidase, with 100,000 FPU/mL (as measured by the method in Adney and Baker (1996)).

WHB Pretreatment

Lignin-hemicellulose bonds are barriers to the hydrolysis of lignocelluloses into fermentable sugars, and pretreatment is essential to breaking these bonds. In this study, the alkali or dilute acid pretreatment methods were compared. Briefly, 5 g of sample was pretreated with NaOH (1 to 10 g/L) or sulfuric acid (0.1 to 0.5%) with a 10:1 to 30:1 liquid-solid ratio (mL acid to g WHB) at varied temperatures (60 to 135 °C) for 30 to 90 min. After cooling, the samples were collected by filtration. The filter residue was washed to neutral pH followed by a final rinse in deionized water. The samples were dried at room temperature, and the pretreated feedstock was either used immediately for hydrolysis or stored in a desiccator.

To evaluate the pretreatment efficiency, the pretreated biomass was hydrolyzed with *Trichoderma reesei* cellulase in a general reaction at 50 °C for 20 h. The medium for hydrolysis contains 200 mg pretreated biomass, 100 FPU/g biomass, and 10 mL citrate buffer pH 4.8.

The yield of reducing sugars was calculated as a percentage conversion of the pretreated biomass, assuming a theoretical yield of 1.12 g sugar/g pretreated biomass.

Enzymatic Saccharification of WHB

A given amount of pretreated WHB was incubated with *Trichoderma reesei* cellulase (20 FPU) at 20 to 60 °C, in a stoppered 50-mL plastic tube with gentle agitation (150 rpm) in an air bath shaker (HZ200LB, Wuhan Ruihua Instrument & Equipment Co., LTD, Wuhan China).

In general, the saccharification reaction was performed in a 10 mmol/L sodium-citrate buffer (pH 4.8) containing 50 mg/L penicillin to inhibit microbial growth. Different biomass loading (varied from 200 to 1000 mg) and saccharification conditions, including temperature (varied from 20 to 60 °C), pH (varied from 4.0 to 6.4), and incubation time (varied from 1 to 48 h), were investigated.

Analytical Methods

WHB composition

Cellulose, hemicellulose, and lignin contents in WHB were determined by the detergent extraction method (Van Soest *et al*. 1991). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and ash were also determined by this method. The hemicellulose content was calculated as the difference between NDF and ADF, and the cellulose content was calculated from the difference between ADF and ADL.

Saccharification efficiency and sugar estimation

Saccharification efficiency was represented by the cellulose conversion rate, which was calculated as the percentage conversion of cellulose in the pretreated biomass to reducing sugars, assuming a theoretical yield of 1.12 g sugar/g pretreated biomass. Total sugar was estimated by the dinitrosalicylic acid (DNS) reagent method (Miller 1959).

Fourier-transform infrared spectroscopy (FTIR)

FTIR was used to explore chemical changes due to WHB pretreatment and hydrolysis using a Bruker spectrometer (VERTEX 70, Bruker Corporation, Ettlingen, Germany). First, 3.0 mg of the sample was dispersed in 300 mg of spectroscopic grade KBr and then pressed into disks at 10 MPa for 3 min. The spectra were obtained with an average of 80 scans and a resolution of 16 cm^{-1} within the range of 4,000 to 400 cm^{-1}.

Scanning electron microscopy (SEM)

To examine structure changes in WHB after pretreatment and hydrolysis, SEM analysis was performed using a Nova NanoSEM 400 (FEI, Oregon, USA) operating at 20 kV accelerating voltage and 5000× magnification.

RESULTS AND DISCUSSION

WHB Composition

The composition of biomass from the water hyacinth is the essential information needed for effective utilization of this biomass.

Table 1. Composition of Various Lignocelluloses Biomass

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Reference
WHB	24.5	34.1	8.6	1.5	This work
	18.2	29.3	2.8	1.2	Ma *et al.* 2010
	17.3	24.7	1.1	-	Lay *et al.* 2013
Sugarcane Bagasse	23.7	25.4	14.2	1.3	This work
	34	27	18	4	Binod *et al.* 2012
Corn Stover	35.5	33.4	19.2	2.6	This work
	37	31.3	17.8	7.3	Saha *et al.* 2013
Wheat Straw	28.6	20.5	15.4	2.3	This work
	38.36	19.71	16.86	5	Rajan *et al.* 2014
Rice Straw	26.5	32.6	13.5	2.4	This work
	41.7	18.3	16.6	-	Zheng *et al.* 2013
Alkali-Pretreated WHB	59.9	18.2	1.2	1.4	This work
-: Not given					

The cellulose, hemicellulose, lignin, and ash contents of WHB were analyzed by the Van Soest method on a dry weight basis (Van Soest *et al.* 1991). The published composition data from other common biomass sources is compared to WHB in Table 1. The combined cellulose and hemicellulose content of WHB was, respectively, 24.5% and 34.1%, which is similar to values from other biomasses, but WHB lignin content was the lowest of the compared sources. There were some small differences in the biomass contents, which may be due to different biomass origins and growth conditions. It was concluded that WHB is a favorable raw material for use in the bioenergy industry.

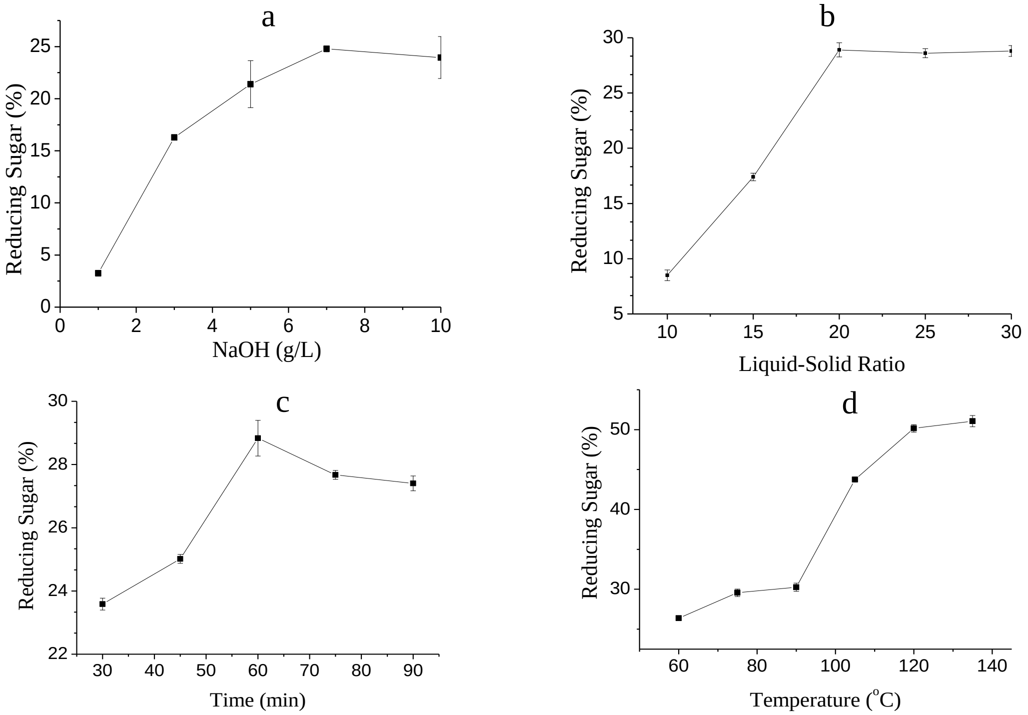

Fig. 1. Yield of reducing sugar from alkali-pretreated WHB where the pretreatment conditions were varied; a: NaOH concentration, b: liquid-solid ratio, c: time, d: temperature

Chemical Pretreatment of Water Hyacinth

Pretreatment of WHB with alkali and dilute acid

To break bonds linking hemicelluloses and lignin, WHB was pretreated with an alkaline solution containing sodium hydroxide. The general conditions for the pretreatment were 7 g/L of NaOH, a liquid-solid ratio of 20:1, and 90 °C for 60 min. The effects of pretreatment conditions, *i.e.* NaOH concentration, liquid-solid ratio, time, and temperature, on the efficiency of the pretreatment process was investigated (Fig. 1). Results indicated that they all conditions influenced the efficiency of WHB pretreatment. When the NaOH concentration was increased, the yield of reduced sugar rose, and it became stable at greater than 7 g/L NaOH. The high NaOH concentration increased the removal of hemicellulose and lignin, and the surface of WHB became rougher. Thus, more cellulose surfaces were exposed to cellulase and degraded (Liu *et al.* 2014). The liquid-solid ratio similarly affected pretreatment efficiency, as the yield of reduced sugar increased up to the liquid-solid ratio of 20. For pretreatment time, 60 min was sufficient. When the temperature was raised from 60 °C to 120 °C, the yield of reduced sugar increased from 26.4% to 50.1%. There was a sharp increase in reducing sugar above 90 °C. The reason is that the higher temperature can improve the breakage of the bond between lignin and reduce the degree of polymerization in the biomass. In summary, the optimal conditions for alkali pretreatment were 7 g/L of NaOH, 20% liquid-solid ratio, 60 min, and 120 °C. The reducing sugar yield from these conditions was 50.1 %.

WHB was also pretreated with dilute H_2SO_4. The highest yield of reduced sugar was about 32.2%, and when the H_2SO_4 concentration and pretreatment time were increased, the yield of reducing sugar was lower. Hence, dilute acid pretreatment was not as efficient as alkali pretreatment, probably because H_2SO_4 produces some byproducts, such as formaldehyde, which will influence the further hydrolysis (Liu *et al.* 2014).

Composition of WHB after pretreatment

The composition of WHB was analyzed after the alkali pretreatment (Table 1). After pretreatment, cellulose content increased from 24.5% to 59.9%, and the hemicellulose and lignin contents were correspondingly decreased from 34.1% and 8.6% to 18.2% and 1.2%, respectively. A total of 46.7% and 85.9% of hemicellulose and lignin, respectively, were removed. Thus, pretreatment enhanced cellulose content percentage and removed hemicellulose and lignin, which greatly improved the contact between cellulose and cellulase and consequently improved the efficiency of the cellulose hydrolysis.

Enzymatic Saccharification of WHB

Pretreated WHB was broken down to fermentable sugars by cellulase hydrolysis. These sugars are the feedstock for biofuels or other fermentation products. The hydrolysis reaction conditions including time, substrate load, pH, and temperature were investigated for their effects on hydrolysis. Saccharification efficiency was represented by the cellulose conversion rate (Fig. 2).

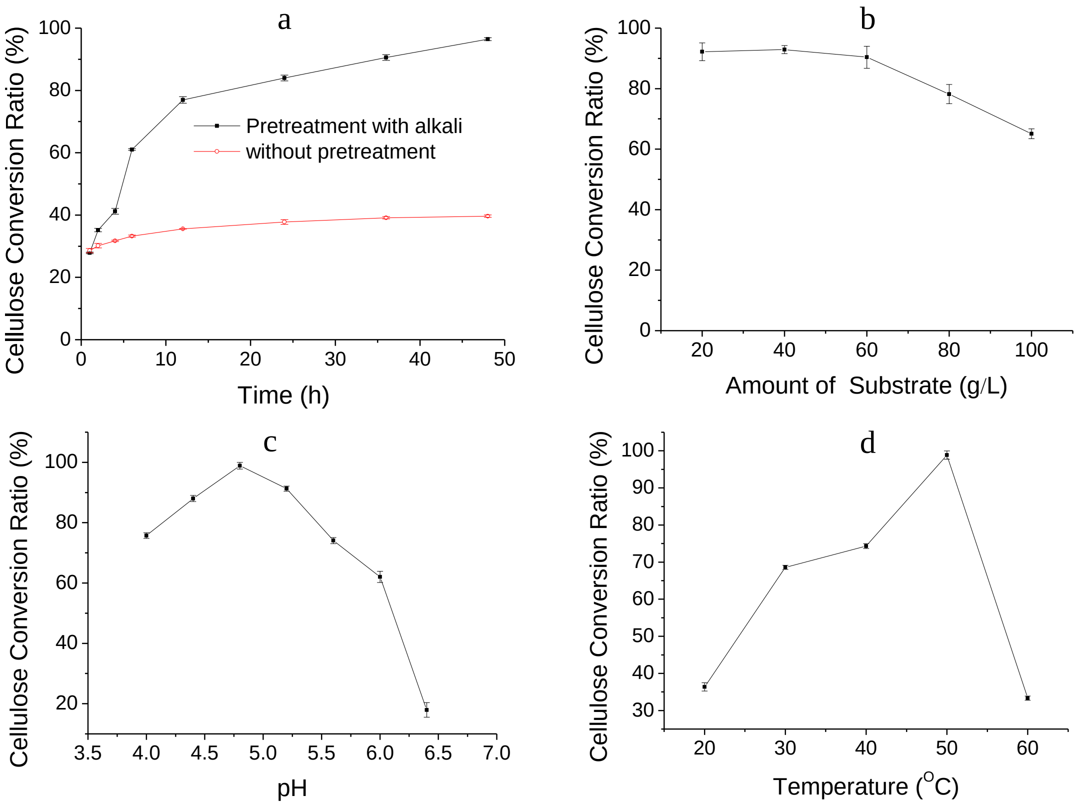

Fig. 2. Cellulose conversion with different hydrolysis conditions. a: time, b: substrate concentration, c: pH, d: temperature

The cellulose conversion rate steadily increased with reaction time, up to almost 100% at 48 h (Fig. 2a). For untreated WHB, the maximum cellulose conversion rate was only 40% with 48-h hydrolysis. There are two reasons for the difference in hydrolysis between pretreated and untreated WHB. In untreated WHB, the cellulose is mainly crystalline, which is difficult to hydrolyze. Secondly, lignin in untreated WHB inhibits the cellulase enzyme (Rahikainen *et al*. 2013). For substrate loading at less than 60 g/L, a high conversion rate was obtained (almost 90%), but cellulose conversion decreased with further increases in WHB loading (Fig. 2b). Experiments using varied pH and temperature showed that the maximum conversion rate occurred in pH 4.8 and 50 °C. The pH and reaction temperature are two key reaction conditions to an enzymatic hydrolysis reaction. These conditions depend on the cellulase which type was used. To this cellulose in this experiment, the pH 4.8 and 50 °C are its optimal conditions. If further increase the pH and temperature, the enzyme will inactivate, then the cellulose conversion decrease very sharp. These results are consistent with the temperature-dependent adsorption behavior of cellulase in lignocellulosic biomass (Zheng *et al.* 2013). Taken together, the most favorable hydrolysis conditions for alkali-pretreated WHB were 60 g/L of WHB, pH 4.8, 50 °C and 48 h.

Structural Changes in WHB

FTIR and SEM were used to explore the structural changes in WHB during pretreatment and hydrolysis. FTIR spectra for untreated WHB before and after hydrolysis were very similar, which indicated that hydrolysis alone caused few structural changes in untreated WHB (Fig. 3a, b).

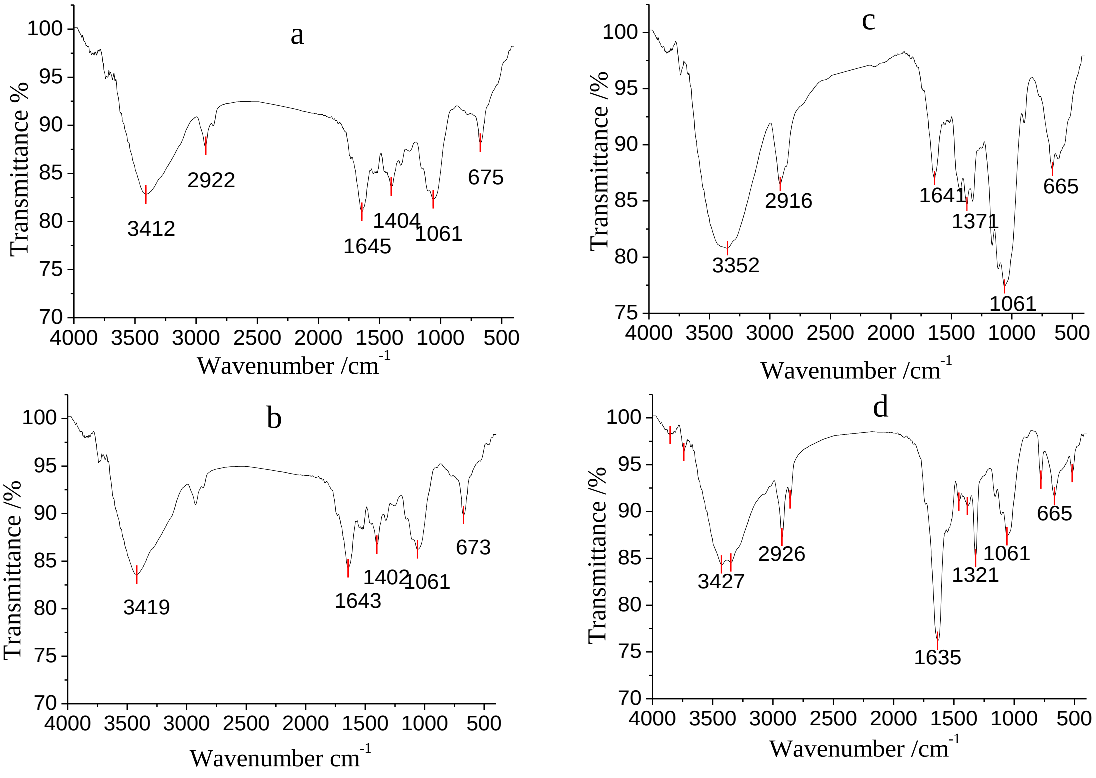

Fig. 3. FTIR spectra for a: untreated WHB, b: hydrolyzed untreated WHB, c: alkali-pretreated WHB, d: hydrolyzed alkali-pretreated WHB

This result confirms that hydrolysis was inefficient in untreated WHB. In contrast, pretreated WHB spectra showed different characteristics, demonstrating that pretreatment and hydrolysis changed WHB structure (Fig. 3c, d). These changes occurred from 2000 to 1500 cm^{-1} and from 1500 to 1000 cm^{-1}; these regions correspond to the phenyl ring bond in lignin and the C-H bond in cellulose and hemicellulose, respectively (Li *et al.* 2012; Sundari and Ramesh 2012). The wavenumber around 3340 cm^{-1} was assigned to hydrogen bonded (O–H) stretching absorption. Reduction in the peak of 3340 cm^{-1} indicates a reduction in the hydrogen bond of cellulose of the pretreated WHB.

Scanning electron microscopy (SEM) was used to observe micro-structural changes during the pretreatment and hydrolysis of WHB (Fig. 4). The structures in untreated WHB before and after hydrolysis were very similar; this results further confirms the poor hydrolysis of untreated WHB. Pretreatment and hydrolysis in many changes to the WHB surface, including holes (Fig. 4 c, d).

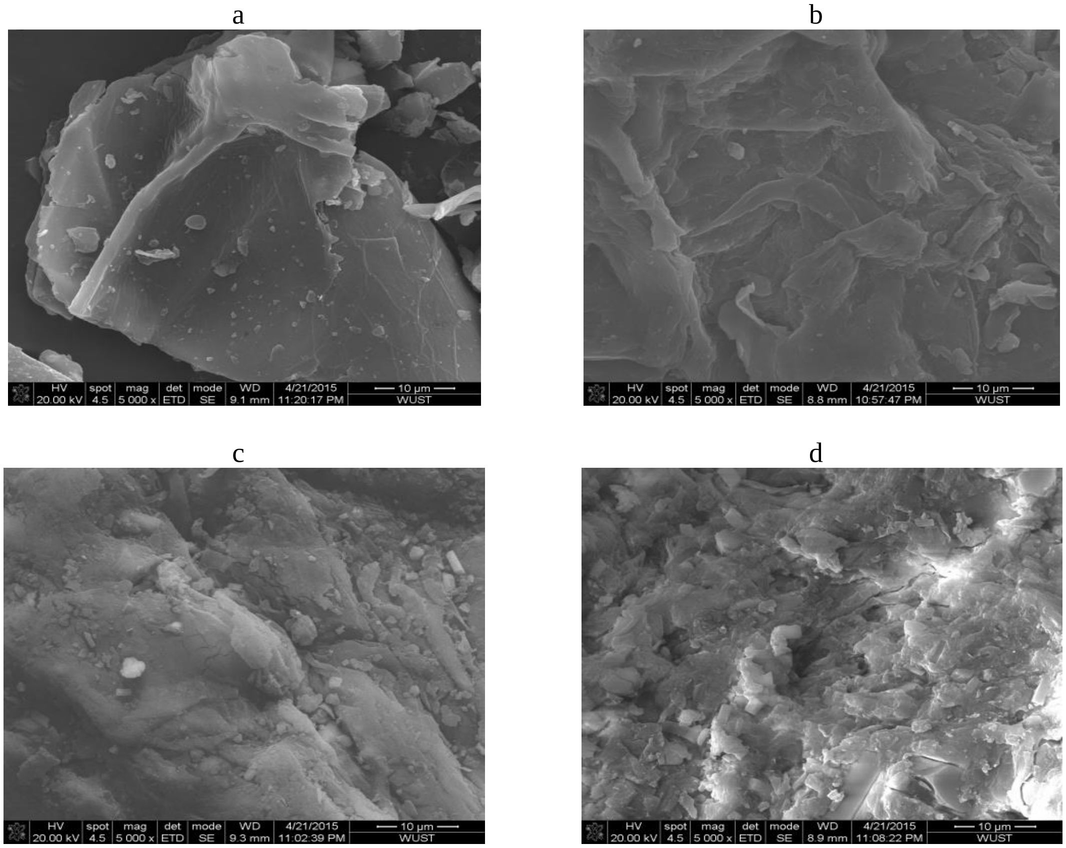

Fig. 4. SEM of a: untreated WHB, b: hydrolyzed untreated WHB, c: alkali-pretreated WHB, d: hydrolyzed alkali-pretreated WHB

CONCLUSIONS

1. Water hyacinth biomass is an alternative cellulose source for bioenergy production. Its combined cellulose and hemicellulose content is 58.6%, and it has a low lignin content.
2. Alkali pretreatment is essential for WHB hydrolysis *via* cellulase. The optimal conditions for alkali pretreatment were 7 g/L NaOH, a liquid-solid ratio of 20, 120 °C, and 60 min.

3. Favorable hydrolysis conditions for alkali-pretreated WHB were 60 g/L of substrate, pH 4.8, 50 °C, and 48 h. The cellulose conversion rate almost reached 100%.

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