Sains Malaysiana 45(6)(2016): 955–962

Effects of Alkaline Combined with Ultrasonic Pretreatment and Enzymatic Hydrolysis of Agricultural Wastes for High Reducing Sugar Production

(Kesan Gabungan Alkali dengan Ultrasonik Prarawatan dan Hidrolisis Enzim Sisa Pertanian untuk Penurunan Pengeluaran Gula yang Tinggi)

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ABSTRACT

The effects of six pretreatments of five agricultural wastes (corn cob, pineapple waste, bagasse, rice straw and water hyacinth) on the chemical composition and total reducing sugar yield were investigated. Six pretreatments were: 1% NaOH with ultrasound for 60 min; 1% NaOH with ultrasound 100% duty; 2% NaOH with ultrasound for 60 min; 2% NaOH with ultrasound 100% duty cycle; 1% NaOH by standing in the oven at 60°C for 90 min; and 2% NaOH by standing in the oven at 60°C for 90 min. Among them, the highest cellulose content of 55.15% was obtained from bagasse by pretreating with 1% NaOH with ultrasound 100% duty cycle. It subsequently yielded the highest total reducing sugar of 36.21% (36.21 g reducing sugar/100 g substrate). The lignin content of all samples significantly decreased, but ultrasonic pretreatment increased the cellulose content. However, the best pretreatment method for each sample was different. Based on SEM analysis, the morphologies of all samples were changed after pretreatment. In addition, the increase of enzyme loading from 250 to 550 CMC U/g biomass led to more than 20% increase in the total reducing sugar. It was found that the higher enzyme loading (700 CMC U/g dried biomass) did not improve the total reducing sugar for all samples.

Keywords: Agricultural waste; alkaline; hydrolysate; pretreatment; ultrasonic

ABSTRAK

Kesan enam prarawatan ke atas lima sisa pertanian (tongkol jagung, sisa nanas, hampas tebu, jerami padi dan keladi bunting) terhadap komposisi kimia dan jumlah hasil penurunan gula telah dikaji. Enam prarawatan adalah: 1% NaOH dengan ultrabunyi untuk 60 min; 1% NaOH dengan ultrabunyi tugas 100%; 2% NaOH dengan ultrabunyi untuk 60 min; 2% NaOH dengan ultrabunyi kitar tugas 100%; 1% NaOH dengan dirian di dalam relau pada suhu 60°C selama 90 min; dan 2% NaOH dengan dirian di dalam relau pada suhu 60°C selama 90 min. Antara semua, kandungan selulosa tertinggi adalah 55.15% telah diperoleh daripada hampas tebu dengan prarawatan 1% NaOH dengan ultrabunyi kitar tugas 100%. Ia kemudiannya menghasilkan jumlah penurunan gula yang paling tinggi sebanyak 36.21% (36.21 g penurunan gula/100 g substrat). Kandungan lignin daripada semua sampel menurun dengan ketara, tetapi rawatan awal ultrasonik meningkatkan kandungan selulosa. Walau bagaimanapun, kaedah rawatan awal yang terbaik bagi setiap sampel adalah berbeza. Berdasarkan analisis SEM, morfologi semua sampel telah berubah selepas rawatan awal. Di samping itu, peningkatan muatan enzim sebanyak 250-550 CMC U/g biojisim membawa kepada 20% peningkatan dalam jumlah penurunan gula. Didapati bahawa pemuatan enzim yang tinggi (700 CMC U/g biojisim kering) tidak meningkatkan jumlah penurunan gula untuk semua sampel.

Kata kunci: Alkali; hidrolisat; prarawatan; sisa pertanian; ultrasonik

INTRODUCTION

Bioethanol production from lignocellulosic materials, such as crop residues, grasses, sawdust and wood chips/shavings is more attractive because they are renewable, cheap, and abundantly available in nature. Currently, the cost of materials, which represents >40% of all process costs, is the most important factor in the ethanol production process (Sun & Cheng 2008). Thailand is an agriculture-based country producing plentiful of agricultural wastes. Among them, rice straw and corn cob contain high contents of cellulose and hemicellulose might be good raw materials for the ethanol production. Rice straw and sugarcane are major crops harvested in Thailand, which produced almost 20-50% of agricultural wastes (rice husk and sugarcane bagasse) depending on the method of harvesting. In 2010, the main agricultural wastes consisted of 10.73 million tons of rice straw, 7.64 million tons/year of bagasse and 0.96 million tons/year of corn cob, which could be utilized for energy (Department of Alternative Energy Development and Efficiency, Thailand 2010). Moreover, there were large amount of unutilised pineapple wastes every year in tropical countries, particularly in Thailand (Dacera & Babel 2007). The pineapple waste represents 0.62 million tons/year in Thailand (The Centre for Agricultural Information (CAI) and Regional Offices of Agricultural Economics 2009). Water hyacinth, a widely fast growing aquatic weed in Thailand, contains high contents of hemicellulose (33%) and cellulose (20%), which could be utilized for the ethanol production (Poddar et al. 1991). This was estimated to be 5 million tons (wet basis)/year in 2008 (Chantasiri & Chaiyopratum 2009).

Lignocellulosic materials were being investigated to produce fermentable sugar as a potential substrate for the bioethanol production. Lignocellulosic materials contain cellulose and hemicelluloses in a complex crystalline structure covered with lignin, which severely restricts enzyme hydrolysis. Pretreatment process is required to break the lignin seal and disrupt the crystalline structure of cellulose and hemicelluloses in order to improve accessibility of the enzyme to cellulose (Sun & Cheng 2008). Alkaline pretreatment, usually employing sodium hydroxide (NaOH), is known to separate lignin from lignocellulosic material by breaking ester bond, causing cellulose swelling and partial decrystallization of cellulose (Binod et al. 2012; Mcintosh & Vancov 2010). Ultrasound produces sonochemical and mechanoacoustic effects due to its caviation phenomenon, the pressure differentials within a solution. Mechanoacoustic effects augment the microjets from bubble collapse resulting in the disruption of cell wall and the increase in the usability of biomass in the heterogeneous solid-liquid environment. On the contrary, sonochemical phenomenon creates free radicals resulting in faster oxidation reaction at lower temperature and chemical attack on the components of lignocelluloses (Madeleine & Dongke 2013). There were several reports on the comparison of different pretreatment methods for enzymatic digestibility, in which the alkaline pretreatment was found to be the best pretreatment method (Cao et al. 2012; Chen et al. 2006; Taherzadea & Karimi 2008). Although all alkaline pretreatment processes are effective, they are relatively complex and time-consuming. Therefore, the combination of two pretreatment techniques (alkaline and ultrasonic) may be more effective and faster than separate single one.

The objectives of this study were to investigate the effect of various combined alkaline and ultrasonic pretreatment on the chemical composition of agricultural wastes (corn cob, pinapple waste, bagasse, rice straw and water hyacinth), the yield of reducing sugar by enzymatic hydrolysis process and the effect of cellulase loading on the total reducing sugar yield.

MATERIALS AND METHODS

RAW MATERIAL

Five agricultural wastes, such as corn cob, rice straw, bagasse, pineapple waste and water hyacinth were obtained from a local farm and market (Nakhon Pathom Province, Thailand) in 2012. All samples were washed using tap water, sun-dried and then hot-oven dried at 60°C for 48 h. All samples were milled and kept in the desiccators at room temperature prior to the determination of chemical composition, such as cellulose, hemicelluloses and lignin content, according to the method of National Renewable Energy Laboratory (NREL) (Sluiter et al. 2008).

COMBINATION OF ALKALINE AND ULTRASONIC PRETREATMENT FOR THE REDUCING SUGAR PRODUCTION

The samples were pretreated by the combination of sodium hydroxide and ultrasound at frequency 44 kHz (Ultrasonik 104H, Lukadent, Germany) and initial solids: liquid ratio of 1:20 (w/v). All samples were pretreated as follows:

Method	Procedure
A	1% NaOH with ultrasound for 60 min
B	1% NaOH with ultrasound 100% duty cycle (It was obtained by sonicating for 30 min followed by resting for 30 min and then sonicating for 30 min)
C	2% NaOH with ultrasound for 60 min
D	2% NaOH with ultrasound 100% duty cycle
E	1% NaOH by standing in the oven at 60°C for 90 min
F	2% NaOH by standing in the oven at 60°C for 90 min

After pretreatment, half of the samples were assigned to analyze acid and neutral detergent fiber by centrifuging and washing using distilled water until they reach neutral pH and drying at 60°C for 72 h. The remaining half samples were used for the determination of reducing sugar production by adjusting pH to 4.8 with sulfuric acid and subsequently hydrolyzed using cellulase (Accellerase 1500, Genecor, CA, USA) at 550 CMC U/g biomass in a shaking incubator (180 rpm) at 50°C for 24 h. The total reducing sugar concentration was determined according to dinitrosalicylic acid (DNS) method (Miller 1959). Total reducing sugar yield was calculated as g/100 g substrate or %.

EFFECT OF ENZYME LOADING ON TOTAL REDUCING SUGAR CONCENTRATION

After pretreatment, the samples were hydrolyzed at different enzyme loadings (250, 400, 550, 700 CMC U/g biomass) in a shaking incubator (180 rpm) at 50°C for 42 h. The samples were collected every 6 h. The total reducing sugar concentration was determined according to DNS method (Miller 1959).

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

A scanning electron microscopy (JEOL, JSM-5410LV, Jeol Company, Japan) was used to observe the physical changes of unpretreated and pretreated sample with the best condition. All samples were gold sputter-coated using sputter coater SC7640 (Fison Instruments, Polaron, UK) prior to imaging with a scanning electron microscope.

ANALYTICAL METHOD

Carboxymethyl cellulase activity (CMC) was determined according to the standard IUPAC (International Union of

Pure and Applied Chemistry) procedure. One CMC unit was defined to liberate 1 μmol of reducing sugar (expressed as glucose equivalents) in 1 min at 50°C and pH4.8. All experiments were carried out in triplicate. The data were analyzed for statistical significance using one-way analysis of variance (ANOVA) at the significance level of 0.05 followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

EFFECT OF COMBINED ALKALINE AND ULTRASONIC PRETREATMENT ON THE CHEMICAL COMPOSITION

The pretreatments were usually used to degrade lignocellulosic structure, reduce the degree of crystallinity of the cellulose and improve the enzymatic hydrolysis of cellulose (Kumar et al. 2009). In this study, the combined effects of alkaline and ultrasonic treatments on the chemical composition were determined (Table 1). Cellulose was the main component of untreated and pretreated samples. The pretreatment by the combination of alkaline and ultrasound significantly changed the chemical composition of all samples, which was better than sole alkaline pretreatment. Each pretreatment method resulted in different compositions of celluloses and hemicelluloses. The highest cellulose content of corn cob, pineapple waste, bagasse, rice straw and water hyacinth were obtained by the pretreatment method C, C, B, A and D, respectively. Different types of lignocellulosic materials will respond differently to ultrasonic pretreatment under the same conditions (Madeleine & Dongke 2013). Each lignocellulosic material has its own typical structure and digestion ceiling. There is a significant variation in the contents of cellulose, hemicellulose and lignin depending on its derivation. There are four types of chemical bonds that provide linkages within the individual components of lignocellulose and connect the different components to form the complex internal structure. Therefore, the same condition of ultrasonic pretreatment often results in different responses to different types of lignocellulosic materials. The initial rates of digestion also vary widely among different materials.

The lignin contents of all pretreated samples significantly decreased more than 50% of delignification. The pretreatment reduced the dry matter losses from 25 to 53% of initial materials after pretreatment. The dry matter samples pretreated by the combination of alkaline and ultrasonic was lost significantly more than by alkaline pretreatment in the oven. In addition, the dry matter loss by 2% NaOH pretreatment was significantly higher than that of 1% NaOH pretreatment. The dry matter loss of pretreated water hyacinth was lowest among all samples.

The pretreatment changed the chemical composition of samples by decomposing and converting into other soluble components. Alkaline pretreatment of lignocellulosic biomass has been found to cause swelling, disruption of the lignin structure and increase in internal surface area by breaking the link between lignin monomers or between lignin and polysaccharide (Cao et al. 2012). Ultrasonic waves produce different pressure within a solution in order to increase physical (mechanoacoustic) and chemical (sonochemical) processes. The mechanoacoustic and sonochemical processes cause the separation, depolymerization and degradation of cellulose and hemicelluloses. This results in an accumulation of hydroxyl radicals, generating shear forces and pyrolytic degradation of hydrophobic polymer in the hot area around the collapsing bubbles. The viscosity of the solution pretreated by the combination of alkaline and ultrasound might affect the cavitational threshold and physical properties, such as improvement of mass transfer and presence of shear forces of the solution. Moreover, it involved in the bubble collapse which generates different radicals and species to attack the lignocellulose structure (Madeleine & Dongke 2013).

The ultrasonic treatment might be a promising process in term of energy consumption comparing with alternative treatments. The energy requirements for pretreatment in an autoclave, steam explosion and ultrasound were 23.3 × 10^4 J/g, 9.9 × 10^4 J/g and 7.2 × 10^4 J/g, respectively (Velmurugan & Muthukumar 2012). It was agreed well with the pretreatment of cassava chips, in which ultrasonic was preferred to heat pretreatment. The energy consumption for ultrasound was 11 kJ compared with 22 kJ for heat pretreatment (Nitayavardhana et al. 2010). The costs of ultrasound could be reduced when combined with other technologies such as chemical pretreatment, ultraviolet radiation and ozone addition.

In conclusion, alkaline solution acts like peeling which cleaves glycosidic linkages of polysaccharides and delignifies. In addition, ultrasound produces oxidizing radicals to degrade the carbohydrate. Based on the principle of alkaline and ultrasound pretreatment, both treatments improved the cellulose and hemicelluloses contents and delignification.

EFFECT OF COMBINED ALKALINE AND ULTRASONIC PRETREATMENT ON TOTAL REDUCING SUGAR YIELD

Total reducing sugar concentration of the enzymatic hydrolysates was determined at different alkaline and ultrasonic pretreatment conditions (Table 2). Based on types of raw materials, bagasse exerted the highest total reducing sugar yield of 36.21±0.46%, which might resulted in high conversion of celluloses and hemicelluloses to sugar by hydrolysis process. The highest total reducing sugar yield of each pretreated sample was well matched with cellulose content. The total reducing sugar yields of pretreatment methods were A=B > C > D > E > F, in that order. Sodium hydroxide and ultrasound pretreatment yielded higher total reducing sugar than did the non-sonicated methods. Pretreatment method A as well as method B yielded the highest total reducing sugar yield among all enzymatic hydrolysates. The ultrasound duty cycle did not strongly affect sugar release. In all cases, 1% NaOH yielded total reducing sugar higher than did 2% NaOH. Eblaghi et al. (2015) reported that ultrasound pretreatment of bagasse

TABLE 1. Effect of the combined alkaline pretreatment with ultrasonic on the chemical composition of five different agricultural wastes

Sample	Method	Cellulose(%)	Hemicellulose(%)	Lignin(%)	Dry matter loss(%)
Corn cob	A	43.68 ± 0.45^{b}	30.30 ±1.47^{c}	6.21 ±0.28^{cd}	49.07±0.68^{b}
	B	41.16 ±0.76^{c}	28.16 ±0.91^{d}	7.48 ±0.12^{b}	49.40±0.95^{b}
	C	47.06 ±0.95^{a}	32.63 ±0.57^{ab}	6.44 ±0.05^{c}	51.33±0.40^{a}
	D	42.90 ±0.79^{b}	30.79 ±0.37^{bc}	6.48 ±0.33^{c}	51.70±0.66^{a}
	E	31.51 ±0.74^{d}	33.66 ±0.14^{a}	5.80 ±0.05^{d}	46.13±1.46^{c}
	F	30.16 ±0.48^{e}	30.43 ±0.25^{bc}	6.48 ±0.33^{c}	47.43±0.72^{c}
	Control	30.49 ±0.48^{de}	32.22 ±2.49^{abc}	12.28 ±0.61^{a}	Null
Pineapple waste	A	34.19 ±0.72^{c}	21.62 ±0.24^{de}	3.05 ±0.30^{d}	48.93±1.24^{b}
	B	31.64 ±0.77^{d}	19.86 ±1.01^{e}	3.64 ±0.09^{c}	49.37±0.99^{b}
	C	41.32 ±0.28^{a}	24.69 ±0.97^{bc}	4.05 ±0.04^{b}	52.90±0.36^{a}
	D	23.86 ±0.91^{f}	25.96 ±2.40^{ab}	4.12 ±0.08^{b}	51.67±0.65^{a}
	E	26.17 ±0.91^{e}	27.34 ±0.53^{a}	4.14 ±0.06^{b}	46.37±0.60^{c}
	F	38.37 ±1.75^{b}	23.13 ±1.57^{cd}	4.12 ±0.07^{b}	47.10±0.40^{c}
	Control	22.49 ±0.14^{f}	22.86 ±0.58^{cd}	11.41 ±0.23^{a}	Null
Bagasse	A	50.70 ±0.31^{b}	18.72 ±1.78^{bc}	6.85 ±0.05^{c}	33.20±0.26^{bc}
	B	55.15 ±1.04^{a}	20.02 ±0.75^{ab}	7.23 ±0.11^{bc}	32.73±0.85^{c}
	C	47.06 ±0.61^{c}	20.45 ±0.31^{a}	6.61 ±0.05^{c}	35.23±0.72^{a}
	D	42.69 ±1.89^{d}	19.91 ±0.16^{ab}	7.53 ±0.14^{b}	34.23±0.71^{ab}
	E	39.59 ±1.92^{e}	19.37 ±0.45^{ab}	7.76 ±0.02^{b}	31.10±0.66^{d}
	F	38.40 ±0.54^{e}	17.65 ±0.30^{c}	7.54 ±0.14^{b}	31.13±0.65^{d}
	Control	31.23 ±0.85^{f}	18.57 ±0.47^{bc}	15.75 ±0.90^{a}	Null
Rice straw	A	51.63 ±0.15^{a}	16.23 ±0.32^{e}	6.41 ±0.28^{d}	30.10±0.70^{c}
	B	49.46 ±0.88^{b}	17.68 ±0.46^{d}	6.31 ±0.07^{d}	30.27±0.40^{c}
	C	44.35 ±0.51^{c}	17.40 ±0.42^{d}	6.78 ±0.06^{c}	34.97±0.90^{b}
	D	40.76 ±0.77^{e}	16.59 ±0.20^{e}	7.18 ±0.15^{b}	37.60±0.92^{a}
	E	44.07 ±1.38^{c}	22.15 ±0.08^{b}	7.06 ±0.09^{b}	28.37±0.67^{d}
	F	42.48 ±0.37^{d}	21.16 ±0.34^{c}	7.19 ±0.15^{b}	27.77±0.59^{d}
	Control	36.52 ±0.75^{f}	23.88 ±1.00^{a}	16.53 ±0.17^{a}	Null
Water hyacinth	A	46.94 ±0.97^{c}	18.76 ±1.50^{b}	2.31 ±0.15^{d}	27.23±0.47^{ab}
	B	50.97 ±0.97^{ab}	16.02 ±1.04^{c}	2.58 ±0.09^{cd}	27.50±0.70^{ab}
	C	49.33 ±0.29^{b}	19.62 ±0.40^{ab}	2.40 ±0.06^{d}	28.93±0.70^{a}
	D	52.52 ±3.01^{a}	17.16 ±0.50^{c}	3.32 ±0.11^{b}	28.63±0.97^{a}
	E	42.83 ±1.08^{d}	17.20 ±0.68^{c}	2.84 ±0.05^{c}	24.63±1.79^{c}
	F	44.60 ±0.74^{cd}	14.12 ±0.26^{d}	3.33 ±0.11^{b}	25.97±0.70^{bc}
	Control	33.19 ±0.29^{e}	20.47 ±0.27^{a}	10.97 ±0.55^{a}	Null

$^{a\text{-}f}$The mean in the same column with different superscripts are significant different at $p<0.05$

TABLE 2. Effect of the combined alkaline and ultrasonic pretreatment on total reducing sugar yield

Sample	Total reducing sugar yield (%)						
	Pretreatment A	Pretreatment B	Pretreatment C	Pretreatment D	Pretreatment E	Pretreatment F	Control
Corn cob	20.01±0.17^{c,z}	18.85±0.19^{d,y}	21.44±0.06^{a,z}	20.57±0.14^{b,z}	12.95±0.54^{e,z}	11.49±0.10^{f,z}	3.06±0.07^{g,z}
Pinapple waste	18.75±0.11^{c,z}	17.74±0.08^{d,z}	21.06±0.14^{a,z}	20.37±0.21^{b,z}	12.81±0.39^{e,z}	11.79±0.14^{f,z}	2.63±0.11^{g,y}
Bagasse	34.53±0.37^{b,w}	36.21±0.46^{a,v}	32.89±0.38^{c,w}	25.75±0.23^{de,x}	26.73±1.28^{d,x}	25.10±0.39^{e,x}	6.14±0.11^{f,w}
Rice straw	32.72±1.60^{a,x}	31.06±0.46^{b,x}	23.66±0.31^{f,y}	22.10±0.08^{e,y}	27.79±0.64^{c,x}	25.25±0.50^{d,x}	6.39±0.23^{g,w}
Water hyacinth	29.70±0.23^{b,y}	31.71±0.31^{a,w}	31.79±0.29^{a,x}	32.31±0.36^{a,w}	23.64±0.57^{c,y}	22.25±0.53^{d,y}	5.24±0.26^{e,x}

$^{a\text{-}g}$The mean in the same row with different superscripts are significant different at $p<0.05$

$^{v\text{-}z}$The mean in the same column with different superscripts are significant different at $p<0.05$

prior to hydrolysis step resulted in increased sugar yield comparing with sole alkaline pretreatment. The ultrasound-assisted alkaline (at 3% NaOH) showed maximum sugar yield was 33.73 g sugar/100 g biomass. Moreover, mechanistic in ultrasound-assisted alkaline delignification was investigated (Singh et al. 2014). The best value of delignification parameter with ultrasound have been identified as temperature at 30°C, 1.5% (w/v) NaOH and 2%(w/v) biomass concentration. *Parthenium hysterophorus* (carrot grass) was used as the model biomass and resulted in maximum reducing sugar yield of 30.84% after 84 h of hydrolysis.

The alkaline pretreatment causes the degradation of ester and glycosidic side chains, resulting in structural alteration of lignin and cellulose and swelling partial decrystallization of cellulose (Cheng et al. 2010). Sodium hydroxide could disrupt the lignin and increase the accessibility of enzyme to cellulose and hemicellulose. Commonly, the alkaline pretreatment requires longer time at room temperature (Sun et al. 1995). It has been reported that the water hyacinth was needed to be pretreated with 1% (w/v) NaOH at room temperature for 12 h, followed by the slow addition of 31% (w/v) H_2O_2 to the final concentration of H_2O_2 of 1% (w/v) and left for another 12 h. The pretreated water hyacinth was autoclaved at 121ºC for 20 h, followed by saccharification with an enzyme for 96 h (Mishima et al. 2008). Moreover, the increase in alkaline concentration yields higher sugar release (Teater et al. 2011). On the contrary, in this study, lower alkaline concentration yielded higher total reducing sugar concentration and reduced process time. Cellulose and hemicellulose might be destroyed at higher NaOH concentration (2%) which resulted in the loss of carbohydrates and the reducing sugar concentration. Therefore, the use of ultrasound with alkaline pretreatment might improve the extractability of celluloses and hemicelluloses, which shortened processing time at low alkaline concentration. Temperature and pressure increases during ultrasound due to collapse of cavitational bubbles which led to the generation of free radicals to promote certain reactions (Kunaver et al. 2012). It has been reported that the combined sonication and alkaline pretreatment was more efficient than non-sonicated treatment (Filson & Dawson-Andoh 2009). Moreover, sonicated treatments shorten processing time and reduce the requirement of alkaline (Zhang et al. 2008).

In this study, all agricultural wastes in Thailand produced high amount of fermentable sugar and consequently could be utilized as raw materials for the ethanol production. In addition, the pretreatment method of sodium hydroxide solution with ultrasound could

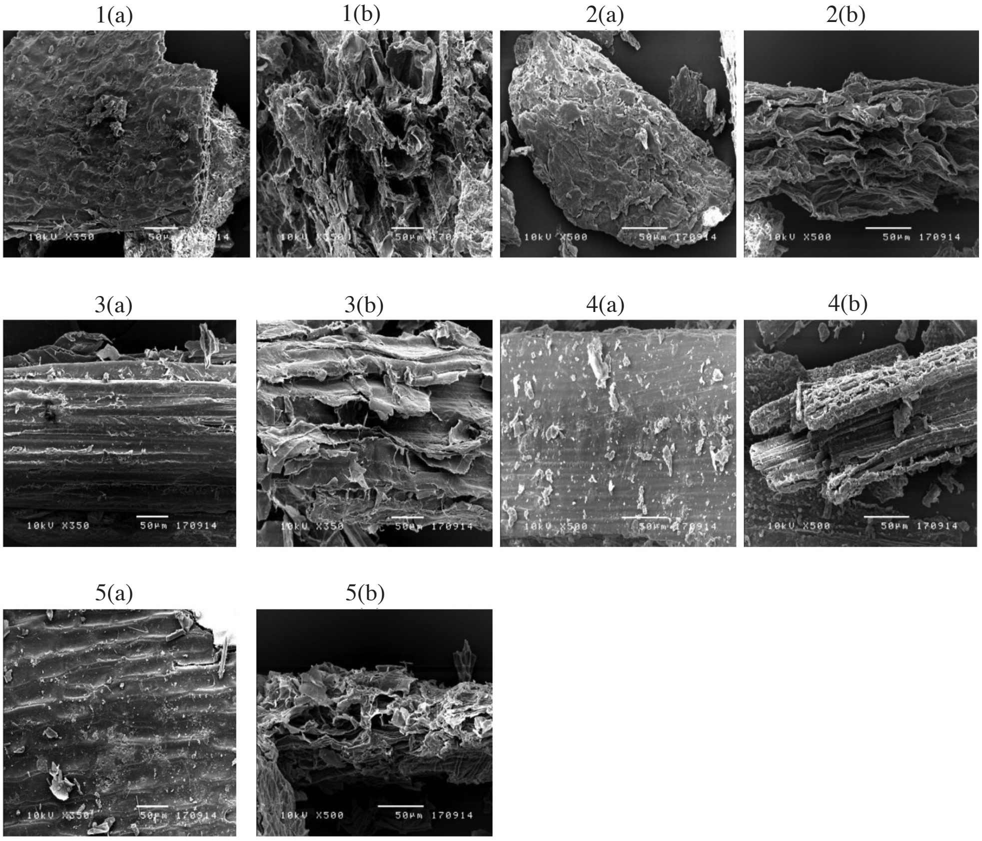

FIGURE 1. Scanning electron microscope (SEM) micrographs of five different agricultural wastes: (1) corn cob, (2) pineapple waste, (3) bagasse, (4) rice straw and (5) water hyacinth; (a) untreated samples and (b) pretreated samples

improve sugar production. Therefore, this pretreatment method is possible to upgrade for industrial scale.

SEM ANALYSIS

The structural changes and surface characteristics of pretreated samples were analyzed using SEM (Figure 1). The untreated and pretreated samples of corn cob, pineapple waste, bagasse, rice straw and water hyacinth were treated using method C, C, B, A and D, respectively. All untreated samples had a smooth surface and compact structure. Pretreatment significantly destroyed and changed the structures of all samples to be roughened and loosened. The combined alkaline and ultrasonic pretreatment removed external fibers, exposed internal structure and generated some irregular cracks and pores. The SEM results of this study were agreed well with the ultrasound-assisted alkaline pretreatment of corn stover and carrot grass (Singh et al. 2014; Zhang et al. 2008). Their structures became loose. In addition, the fracture and roughness of the surface with the increase of fiber surface area for ultrasound-treated biomass was occurred due to erosion and attrition. Therefore, the structural changes improved the enzymatic accessibility of the cellulose and hemicelluloses for the enzymatic hydrolysis.

EFFECT OF ENZYME LOADING

The cost of cellulase represents one of the cost factors for the conversion of cellulosic biomass to sugars for the

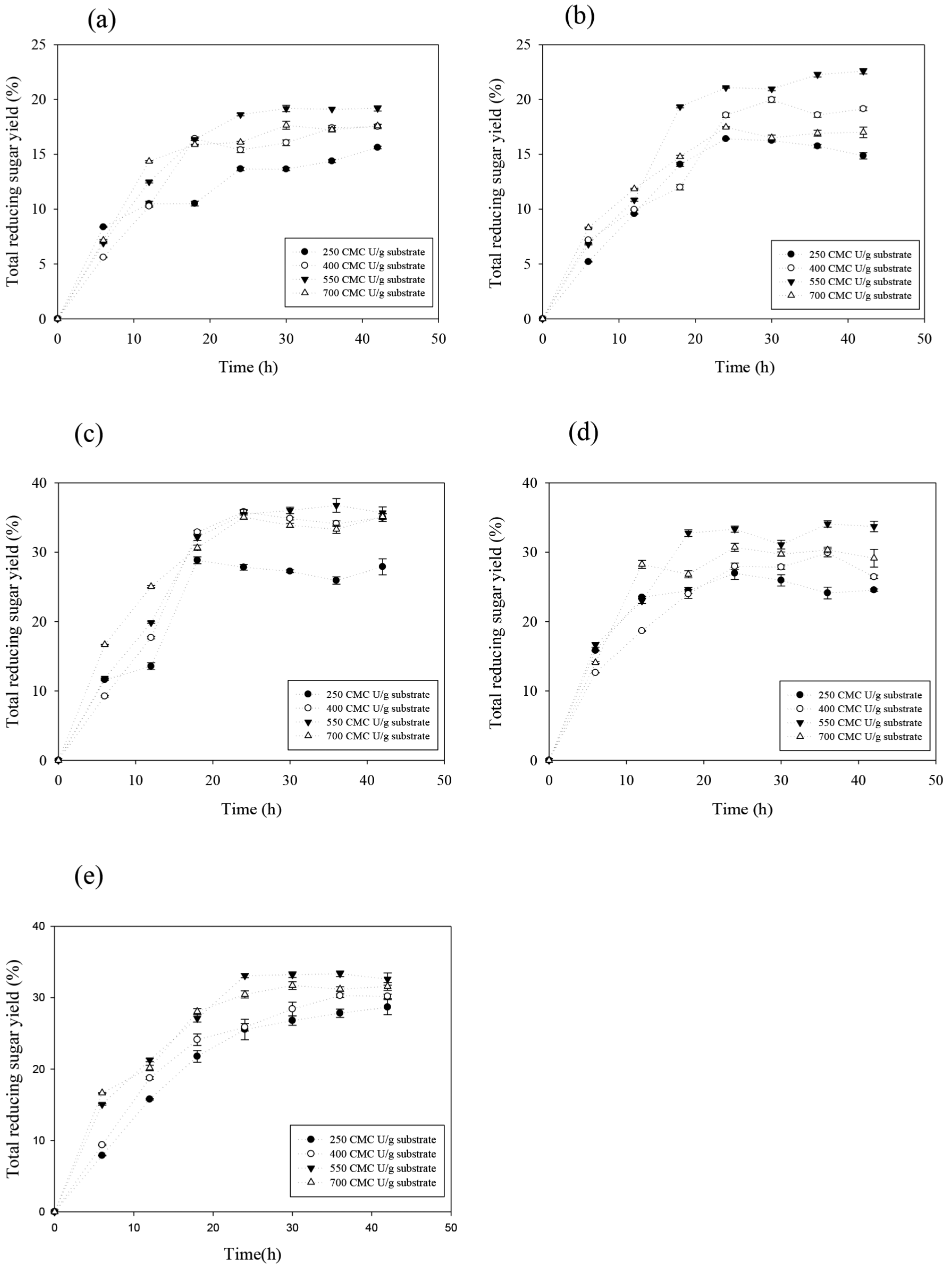

FIGURE 2. Effect of enzyme loading on the total reducing sugar yield (a) corn cob, (b) pineapple waste, (c) bagasse, (d) rice straw and (e) water hyacinth hydrolysis in a shaking incubator (180 rpm) at 50°C for 42 h

ethanol production which should be minimized as little as possible (Chen et al. 2006). The best pretreatment method of each sample was used in this experiment. After pretreatment, all samples were hydrolyzed by cellulase at different enzyme loadings (250, 400, 550, 700 CMC U/g biomass) and then were heated at 50°C for 42 h. The effect of enzyme loading on the total reducing sugar yield of hydrolysates is shown in Figure 2(a)-2(e). The increasing of enzymatic loading from 250 to 550 CMC U/g biomass increased the total reducing sugar yield of all enzymatic hydrolysates. Total reducing sugar yield of corn cob, pineapple waste, bagasse, rice straw and water hyacinth were 36.3, 28.45, 27.45, 23.64 and 22.80%, respectively, when increased enzyme loading from 250 to 550 CMC U/g biomass. Total reducing sugar yield has reached to the highest concentration at 24 h and thereafter was constant. The highest total reducing sugar of all hydrolysate samples was obtained at 550 CMC U/g biomass. Total reducing sugar yields of 5 types of biomass hydrolyzed at 550 CMC U/g biomass were 18.63±0.11, 21.10±0.06, 35.47±0.68, 33.33±0.44 and 27.10±0.17% for corn cob, pineapple waste, bagasse, rice straw and water hyacinth, respectively. The total reducing sugar yield of hydrolyzed bagasse at 550 CMC U/g biomass after 24 h was 35.47±0.68%. High enzyme loading (700 CMC U/g substrate) did not increase total reducing sugar concentration which might be due to the end-product feedback inhibition caused by high concentration of reducing sugar or the limitation of substrate utilization. In addition, enzyme loading higher than a certain critical value does not improve hydrolysis because the excess of enzyme adsorbed into the substrate restricts the diffusion process through the structure (Martin et al. 2012). However, an obvious decrease in solid residue and increase in hydrolysate volume were observed when cellulose and hemicelluloses were hydrolyzed to fermentable sugars (glucose and xylose) (Teerapatr et al. 2012). Therefore, enzyme loading of 550 CMC U/g at temperature of 50°C was the optimum enzymatic hydrolysis process.

Conclusion

The combined alkaline and ultrasonic treatment was applied to pretreat five different lignocellulosic agricultural materials. The ultrasonic assistance reduced the processing time and alkaline concentration. There were significant delignification and compositional and structural changes of pretreated samples. The consistent results of cellulose content and total reducing sugar concentration were also obtained. Total reducing sugar concentrations of corn cob, pineapple waste, bagasse, rice straw and water hyacinth, increased 36.3, 28.45, 27.45, 42.12, 22.80%, respectively, when cellulase enzyme loading from 250 to 550 CMC U/g biomass. Hence, it was possible to increase reducing sugar production through the combination of ultrasound and alkaline pretreatment followed by subsequent enzymatic hydrolysis. All lignocellulosic materials in this study resulted in high reducing sugar concentrations. Therefore, they can be used as alternative substrates for ethanol production using yeast fermentation.

ACKNOWLEDGEMENTS

This research was supported by Kasetsart University scholarship for Doctoral student (contract number 5421224) and Research Promotion and Technology.

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Received: 10 February 2015
Accepted: 11 January 2016