Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of grass clipping

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ABSTRACT

Grass clipping, a cellulose-rich raw material, has great potential to produce biofuels, but must be firstly hydrolyzed to liberate fermentable sugars. In this study, grass clipping was pretreated with ultrasound (US), Ca(OH)₂, NaOH, US-Ca(OH)₂ and US-NaOH at relatively low temperature to enhance its enzymatic hydrolysis. The solubilization of hemicellulose and lignin, and crystallinity index of cellulose increased after US-alkaline pretreatment, leading to a significant increase of enzyme accessibility to cellulose. Compared with another four pretreatments, US-Ca(OH)₂ pretreatment of grass clipping showed the best improvement for reducing sugar yield. X-ray diffraction (XRD) determination and scanning electron microscope (SEM) observation showed that the crystallinity index of grass clipping increased and the grass clipping surface suffered from serious erosion after US-Ca(OH)₂ pretreatment. Then, the operating conditions of US-Ca(OH)₂ pretreatment and enzymatic hydrolysis were systematically optimized, and the suitable operating conditions were as follows: US power density of 0.65 W/ml, US pretreatment time of 30 min, Ca(OH)₂ concentration of 0.75%, pretreatment temperature of 75 °C, enzyme loading of 125 FPU/g, and hydrolysis time of 72 h. The reducing sugar yield of grass clipping pretreated by US-Ca(OH)₂ reached 414 mg/g, increasing by 3.5 times compared with that of raw grass clipping. The US-Ca(OH)₂ pretreatment of grass clipping at low temperature significantly enhanced the potential of grass clipping as a promising raw material to produce biofuels.

1. Introduction

With the combustion of fossil fuels, environmental pollution and energy crisis are becoming increasingly serious. The development of green renewable energy is extremely urgent. Regarded as a kind of clean and renewable energy, biofuels produced from lignocellulosic biomass, such as ethanol, biogas and pellet fuel, have gained great attention to replace liquid fuels and fossil fuels and reduce the emission of greenhouse gas [1,2]. Anaerobic digestion is a cost-effective bio-conversion technology that is widely used for commercial production of electricity, heat and compressed natural gas from organic materials. However, the lignocellulosic biomass is composed of cellulose, hemicellulose and lignin with a complex structure. Thus the hydrolysis of lignocellulosic biomass is blocked, which is normally regarded as the rate-limiting step during its utilization [3,4].

Hydrolysis of lignocellulosic biomass to fermentable sugar is a crucial step to produce biofuels [5,6]. Therefore, many pretreatment methods have been introduced to enhance the enzymatic hydrolysis

efficiency of lignocellulosic biomass. Those pretreatment methods can be classified into physical pretreatment [7,8], chemical pretreatment [9,11–17], and biological pretreatment [10]. After pretreatments, part of hemicellulose and lignin is removed, and the accessible surface area of lignocellulosic biomass is expanded, thus improving the enzymatic hydrolysis efficiency [9].

Alkaline pretreatment, a typical chemical pretreatment, has received intense research interests. Feedstock of alkaline pretreatment includes agricultural residuals [11,12], forest residuals [13–15], food waste [16], and sludge [17] et al. Chemicals used in alkaline pretreatment are usually NaOH, Ca(OH)₂, CaO, KOH and NH₃·H₂O, with a concentration of 1–10% (g/g dry matter). Alkaline pretreatment has shown positive effects in several different ways. Saponification and cleavage of lignin-carbohydrate linkages is the main function of alkaline, which increases the porosity and internal surface area and decreases the degree of polymerization and crystallinity of lignocellulosic biomass.

The application of ultrasound (US) to lignocellulosic biomass can

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enhance the hydrolysis efficiency and increase the sugar yield through structural deconstruction of the biomass resulting from cavitation forces [18]. The quick collapse of cavitation bubbles generates significant shear forces in the liquid, which can produce a strong stirring mechanical effect, thus intensifying the mass transfer; meanwhile, Local high temperature of about 5000 K and high pressure of up to 100 MPa resulted from US cavitation may significantly improve its physical-chemical effects [19]. On the other hand, researches have shown that the use of proper intensity ultrasonic waves can enhance the permeability of cell membranes and promote the catalytic activity of enzymes [20].

Combined pretreatments have been widely applied in order to complement each other. Ding et al. proposed combined pretreatments of H_2SO_4 (1% v/v) and steam heating to enhance the enzymatic hydrolysis of *Spartina anglica*, which resulted in a higher reducing sugar yield of 0.743 g/g-volatile solids (VS) [21]. Sugarcane bagasse was pretreated by microwave (600 W, 4 min) with 1% NaOH and the reducing sugar yield reached 665.0 mg/g [22]. Jin et al. pretreated catalpa sawdust with microwave assisted alkaline pretreatment to enhance the enzymatic hydrolysis, and catalpa sawdust with microwave- $\text{Ca}(\text{OH})_2$ pretreatment showed great potential for biofuel production [23]. Velmurugan and Muthukumar pretreated sugarcane bagasse by US with 2.89% NaOH and the reducing sugar yield reached 96.27% of the sugar concentration of raw materials [24]. Nakashima et al. used a combination of US and sodium percarbonate for the efficient saccharification of cellulose and hemicellulose in corn stover under mild conditions (i.e., at room temperature and atmospheric pressure), and the pretreatment greatly improved lignin removal and cellulose digestibility [25].

Grass clipping is an abundant renewable feedstock to produce bio-fuels which has several advantages, such as short growth cycle, low lignin content, and low crystallinity [26]. Previous works showed that grass clipping had high cellulose content and great potential for biofuel production and pretreatment of grass clipping with high-pressure homogenization enhanced the biogas production [26]. So, the grass clipping was used in this study to examine the feasibility of US-alkaline pretreatment for the improvement of its enzymatic hydrolysis. $\text{Ca}(\text{OH})_2$ is a cheap alkaline, however, there was few report about $\text{Ca}(\text{OH})_2$ combined with US pretreatment of lignocellulose. In addition, most of the current researches on alkaline pretreatment are mainly concentrated on using high temperature (100–180 °C) [27,28], which needs intense energy consumption and strict devices. The objective of this study is to evaluate the characteristics and efficiency of grass clipping pretreatment by US-alkaline at low temperature. Reducing sugars yield during enzymatic hydrolysis was used to evaluate the pretreatment efficiency. The changes of grass clipping caused by different pretreatments were analyzed through chemical compositions determination, X-ray diffraction (XRD) determination and scanning electron microscope (SEM) observation. In addition, the operating conditions of grass clipping pretreatment by US- $\text{Ca}(\text{OH})_2$ were optimized in order to produce more fermentable sugar with less energy and cost.

2. Materials and methods

2.1. Raw materials

Grass clipping of lawn was collected from the campus of Beijing Forestry University, Beijing, China. The sample of grass clipping was dried at room temperature for one week, and then was stored in sealed plastic bags at room temperature after being milled to approximately 0.425–0.850 mm.

2.2. Alkaline pretreatment

Triangular flask of 250 ml was used for the alkaline pretreatment reaction. The temperature of reaction was kept at a certain temperature

by water bath. Two kinds of alkaline solution, NaOH and $\text{Ca}(\text{OH})_2$, were used for pretreatment of the grass clipping. The grass clipping of 10.0 g were immersed in 200 ml alkaline solution for a certain pretreatment time. After alkaline pretreatment, the sample was cooled to room temperature, and the solid residues were recovered by filtration with a vacuum filter. The solid residues were thoroughly washed with distilled water until a neutral pH, and then dried at 105 ± 3 °C for at least 4 h. The dried solid sample was stored in sealed plastic bags at room temperature for the subsequent enzymatic hydrolysis, compositional analysis, and structural analysis.

2.3. US assisted alkaline pretreatment

US assisted alkaline pretreatment was carried out by using ultrasonic cell crusher (SCIENTZ, JY92-IIN) equipped with a probe (diameter of 6 mm) with frequency of 25 kHz and constant ultrasonic power of 60–650 W. Both NaOH and $\text{Ca}(\text{OH})_2$ were used for the US assisted alkaline pretreatment of the grass clipping. The grass clipping of 5.0 g were immersed in 100 ml water or alkaline solution, at the same time the probe of US device was inserted into the solution to assist alkaline pretreatment with a certain US power and a certain pretreatment time. The temperature of reaction was kept with a water bath. After US assisted alkaline pretreatment, the followed procedures were similar to that mentioned in alkaline pretreatment.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis was conducted by using commercial cellulase generated from *Aspergillus niger* (powder, ≥ 0.3 unit/mg solid), and purchased from Sigma-Aldrich Co. LLC. Enzyme solution was prepared using citrate buffer (0.05 mol/L, pH = 4.8) with a concentration of 1%. 1 g sample and 40 ml citrate buffer (0.05 mol/L, pH = 4.8) were mixed in triangular flask, with enzyme loading of 30 FPU/g biomass [26]. The triangular flask was shaken at 50 °C for 96 h in an air bath thermostat oscillator (100 r/min). The supernatant of sample was taken at certain time interval for the measurement of reducing sugar yield. The experiments were triply performed and average value was reported.

2.5. Analysis methods

Chemical composition of raw and pretreated materials was analyzed by a fiber analyzer (A200i, Ankom, ultrasound A). Reducing sugar concentration was measured in DNS method [29]. The surface morphology of different residual solids were observed by SEM (S-3400N II, Hitachi, Japan) and performed at a beam accelerating voltage of 10 kV. Crystallinity of materials was examined by an X-ray diffractometer (D8 Advance, Bruker, Germany), and the samples were scanned in 2θ ranged from 5° to 40° with a step of 0.2°. The crystallinity index (CrI) was calculated by Eq. (1):

$$\text{CrI} = \frac{I_{002} - I_{\text{amorphous}}}{I_{002}} \times 100\% \quad (1)$$

where I_{002} is the diffraction intensity of crystalline structure ($2\theta = 22.6^\circ$), and $I_{\text{amorphous}}$ is the diffraction intensity of amorphous fraction ($2\theta = 18.0^\circ$).

3. Results and discussion

3.1. Effects of pretreatment methods on enzymatic hydrolysis of grass clipping

After US pretreatment, the reducing sugar yield of grass clipping increased by only 0.15 times (Fig. 1), which was in agreement with other researches. Nakashima et al. found that the US pretreatment of corn stover increased the glucose concentration from 1.15 to 1.29 mg/

Fig. 1. Reducing sugar yield of grass clipping before and after US, alkaline and US-alkaline pretreatment (US power density of 5 W/ml, US pretreatment time of 30 min, temperature of 60 °C, alkaline concentration of 0.5%, enzyme loading of 30 FPU/g, and enzymatic hydrolysis time of 96 h).

ml after enzymatic hydrolysis [20]. US could not significantly increase the reducing sugar yield [25]. Alkaline pretreatment was confirmed to be effective to enhance the enzymatic hydrolysis of lignocellulosic biomass [30]. The reducing sugar yield of grass clipping pretreated by $\text{Ca}(\text{OH})_2$ and NaOH increased by 1.45 and 1.34 times respectively, compared with that of raw material (Fig. 1). Obviously, compared with alkaline pretreatment, US-alkaline had better effect on the reducing sugar yield, and the reducing sugar yield of grass clipping with US- $\text{Ca}(\text{OH})_2$ and US- NaOH pretreatment increased by 1.89 and 1.83 times, respectively (Fig. 1). Zhang et al. also found that the decomposition rate of corn stover pretreated by US-alkaline was higher than that of single alkaline pretreatment [19]. US- $\text{Ca}(\text{OH})_2$ pretreatment was more economical considering its lower chemical price and higher reducing sugar yield than US- NaOH pretreatment.

3.2. Mechanisms of grass clipping pretreatment

3.2.1. Chemical composition change of grass clipping

The composition changes of grass clipping before and after various pretreatments are presented in Table 1. Please note that after pretreatment, some solid mass was hydrolyzed into liquid phase, so the solid mass of cellulose, hemicellulose and lignin decreased at different rates. Obviously, the grass clipping had high cellulose content and the cellulose proportion even increased to over 50% after alkaline and US-alkaline pretreatments. It was beneficial for sugar production since cellulose is the most important raw material for reducing sugar production. As the literature stated, US alone virtually did not change the chemical composition of grass clipping, which might explain the almost unchanged reducing sugar yield after single US pretreatment [20].

Table 1
Chemical composition changes of grass clipping after pretreatment.

Pretreatment method	Total solid reduction (%)	Composition							
		Cellulose (%)	Cellulose reduction (%)	Hemicellulose (%)	Hemicellulose reduction (%)	Lignin (%)	Lignin reduction (%)	Others (%)	Other reduction (%)
Control	–	42.21	–	34.25	–	6.17	–	16.03	–
US	8.32	45.88	0.35	36.10	0.35	6.55	2.67	11.47	34.40
$\text{Ca}(\text{OH})_2$	26.67	50.22	12.75	28.36	12.75	5.77	31.42	15.65	28.41
NaOH	32.37	50.21	19.55	28.48	19.55	5.82	36.21	15.49	34.65
US- $\text{Ca}(\text{OH})_2$	30.28	53.51	11.62	25.57	11.62	5.61	36.61	15.31	33.41
US- NaOH	36.45	53.47	19.50	26.30	19.50	5.78	40.47	14.45	42.71

Moreover, the proportion of both hemicellulose and lignin decreased and the reduction of hemicellulose and lignin increased after alkaline and US-alkaline pretreatment (Table 1). It was reported that the lignin removal enhanced by 23% and the hemicellulose degradation increased by 12% when alkaline pretreated corn stover [19]. That might be due to that alkaline can effectively remove lignin and hemicellulose [30]. US-alkaline pretreatment had better effect on removing hemicellulose and lignin than alkaline pretreatment. Similar studies also showed that US waves could strongly affect degradation of hemicellulose and lignin if used along with alkaline [19,31]. It might be due to that slight shock and cavitation collapse produced by US improved the contact of alkaline and lignocellulose, thus enhancing the removal of hemicellulose and lignin. The hemicellulose and lignin were the main components of cellulose-hemicelluloses-lignin network, which was the major barrier for effective enzymatic hydrolysis of cellulose [18]. After lignin removal and hemicellulose degradation, the cellulose-hemicelluloses-lignin network was destroyed and the hydrolysis efficiency increased accordingly. This might be the reason why the samples could produce more reducing sugar after US-alkaline pretreatment.

In addition, the intensity of ultrasonic cavitation depends not only on the tensile strength of liquid, but also on the type and purity of liquid [32]. The addition of $\text{Ca}(\text{OH})_2$ might affect the tensile strength of the liquid, thus promoting the formation of cavitation and enhancing the effect of US.

3.2.2. Crystallinity

CrI, the ratio of crystalline cellulose among the lignocellulosic biomass, is a significant factor affecting the enzymatic hydrolysis. If the aim of pretreatment is to remove lignin and hemicellulose, higher crystallinity would show better pretreatment effect. However, if the aim is to break the cellulose crystallization zone, lower crystallinity would indicate better pretreatment effect. In this study, the former is what we need, thus higher crystallinity means better pretreatment effect. X-ray diffraction measurement of CrI is the best option to estimate the impact of chemical pretreatment on biomass crystallinity [33,34]. The CrI changes of grass clipping are shown in Fig. 2. There was almost no change in the crystallinity of grass clipping after US pretreatment. Similar results were obtained that both the degree and size of crystalline cellulose did not change after US treatment [31,35]. However, all the CrIs of grass clipping pretreated by $\text{Ca}(\text{OH})_2$, NaOH , US- $\text{Ca}(\text{OH})_2$ and US- NaOH increased compared with that of raw grass clipping. Other researches also reported the increase of CrI of lignocellulosic biomass pretreated by alkaline [36,37]. Eblaghi et al. (2016) found that the CrI increased from 53% of the control to 60% and 65% after combined pretreatment of US with 1% and 3% NaOH solution, respectively. The observed increase of CrI might be caused by the release of amorphous parts, such as hemicellulose and lignin, which, in turn, led to the increase of cellulose proportion [38]. This agreed with the results in Table 1, namely, the alkaline and US-alkaline pretreated samples contained less lignin and hemicellulose than the US pretreated samples. Hence, higher CrI might improve enzymatic hydrolysis. Jin et al. reported that the CrI of catalpa sawdust increased by 35.8% after

Fig. 2. Crystallinity index (CrI) of grass clipping before and after US, alkaline and US-alkaline pretreatment (US power density of 5 W/ml, US pretreatment time of 30 min, temperature of 60 °C, alkaline concentration of 0.5%).

microwave-Ca(OH)₂ pretreatment, and the corresponding enzymatic digestibility increased more than three times [23].

3.2.3. SEM observation

Pretreatment can damage the structure of grass clipping. The morphological changes induced by US, alkaline, and US-alkaline

pretreatment were observed by SEM, which are shown in Fig. 3. The raw grass clipping had relatively smooth surface without any erosion trace. After US pretreatment, the surface of grass clipping showed jagged erosion traces. Eblaghi et al. reported that the apparent physical changes in distant layer during US pretreatment were caused by the effect of cavitation [35]. After alkaline pretreatment, the surface of grass clipping became more rough. The explanation might be that alkaline pretreatment caused the release of lignin and hemicellulose, therefore, the porosity of biomass increased [39]. As shown in Fig. 3(e) and (f), the most severe structure disruption achieved after the US-alkaline pretreatment, leading to the increase of enzymatic accessibility. Such effects were also obtained in other researches [35,40]. Both the enhancement of enzymatic accessibility to cellulose and rupture of grass clipping fibers contributed to the increase of reducing sugar yield during enzymatic hydrolysis.

3.3. Optimization of US-Ca(OH)₂ pretreatment

The results in Fig. 1 indicated that the US-Ca(OH)₂ pretreatment was the most effective for reducing sugar yield of grass clipping. Meanwhile, Ca(OH)₂ is one of the cheapest alkalis [41]. Therefore, US-Ca(OH)₂ can provide a low-cost and effective pretreatment for grass clipping, and the optimum of operating conditions is necessary for US-Ca(OH)₂ pretreatment. The enzyme hydrolysis time was set at 72 h in the following optimization experiments considering the little differences in reducing sugar yield between 72 h and 96 h hydrolysis (see Fig. 1).

Fig. 3. SEM micrographs of grass clipping before pretreatment (a) and after pretreatment with US (b), Ca(OH)₂ (c), NaOH (d), US-Ca(OH)₂ (e) and US-NaOH (f) (US power density of 5 W/ml, US pretreatment time of 30 min, temperature of 60 °C, alkaline concentration of 0.5%).

Fig. 4. Effects of US time and power density on reducing sugar yield of grass clipping ($\text{Ca}(\text{OH})_2$ concentration of 0.5%, pretreatment temperature of 30 °C, enzyme loading of 30 FPU/g).

Fig. 5. Effects of $\text{Ca}(\text{OH})_2$ concentration on reducing sugar yield of grass clipping and delignification efficiency (US pretreatment time of 30 min, US power density of 6.5 W/ml, pretreatment temperature of 30 °C, enzyme loading of 30 FPU/g).

Fig. 6. Effect of pretreatment temperature of grass clipping on reducing sugar yield (US pretreatment time of 30 min, US power density of 6.5 W/ml, $\text{Ca}(\text{OH})_2$ concentration of 0.75%, enzyme loading of 30 FPU/g).

Fig. 7. Effect of enzyme loading on reducing sugar yield of grass clipping (US pretreatment time of 30 min, US power density of 6.5 W/ml, pretreatment temperature of 75 °C, $\text{Ca}(\text{OH})_2$ concentration of 0.75%).

3.3.1. US power density and US pretreatment time

Fig. 4 shows the effects of US power density and US pretreatment time on the reducing sugar yield of grass clipping. The reducing sugar yield significantly increased with the increase of US pretreatment time from 15 to 30 min, and reached its peak value when the pretreatment time was 30 min. Longer pretreatment hampered the reducing sugar yield. Similarly, Wang et al. pretreated microcrystalline cellulose with US of 500 W and 20 kHz, and the specific surface area reached the peak value when the pretreatment time was 15 min [42]. This phenomenon might be due to the adverse effect of US cavitation on the structure and crystalline arrangement [43]. Moreover, reducing sugar yield kept increasing with the increase of US power density from 2.0 to 6.5 W/ml. The further increase in power density to 8.0 W/ml did not show significant effect. Higher power density might be unnecessary for better results, especially in the case of enzymatic reactions [44]. Taking into account the energy consumption, 6.5 W/ml was appropriate for pretreatment. Therefore, the suitable US pretreatment time and US power density for grass clipping were 30 min and 6.5 W/ml.

3.3.2. $\text{Ca}(\text{OH})_2$ concentration

Effects of alkaline concentration on the enzymatic hydrolysis are shown in Fig. 5. The enzymatic hydrolysis efficiency increased along with the increase of $\text{Ca}(\text{OH})_2$ concentration till 0.75% and then decreased. Similarly, the lignin removal efficiency was limited at higher $\text{Ca}(\text{OH})_2$ concentration, and showed little increase when the $\text{Ca}(\text{OH})_2$ concentration was higher than 0.75%. Ishiguro & Endo also reported that the increase of $\text{Ca}(\text{OH})_2$ dosage from 0.1 g/g TS to 0.2 g/g TS did not benefit the lignin reduction in eucalyptus [45]. Considering the effects of lignin removal and reducing sugar yield, $\text{Ca}(\text{OH})_2$ concentration of 0.75% would be the suitable choice in this study.

3.3.3. Pretreatment temperature

Fig. 6 shows the reducing sugar yield of grass clipping after pretreated at various temperatures. Obviously, higher temperature was more beneficial to the reducing sugar yield at the same reaction time. When the temperature reached 75 °C, the maximum reducing sugar yield was 325.7 mg/g. High temperature helped to disrupt the strong solute matrix which encompassed van der Waals forces, hydrogen bonding and dipole attraction [40]. In addition, through $\text{Ca}(\text{OH})_2$ pretreatment, increasing the pretreatment temperature led to the enhancement of lignin removal efficiency, which was beneficial to reducing sugar yield [46]. The temperature didn't further increase since higher temperature meant more energy and extreme equipment

Table 2Comparison of enzymatic hydrolysis efficiency of US-Ca(OH)₂ pretreating grass clipping and other methods pretreating lignocellulosic biomass.

Raw material	Pretreatment conditions	Reducing sugar yield (mg/g)	Reference
Water hyacinth	Microwave assisted NaOH (0.2 wt.%) pretreatment at 190 °C for 10 min	296	[51]
Sugarcane trash	Biological pretreatment with <i>Bacillus macerans</i> for 48 h	12	[52]
Corn straw	Ca(OH) ₂ (15%), NaOH (2%) pretreatment at 83.92 °C for 6 h + H ₂ O ₂ (3%) pretreatment at 50 °C for 2 h	349	[53]
Elephant grass	Ammonia (15%) pretreatment at 60 °C for 6 h	134	[27]
Rice straw	US assisted NaOH (1.0%) pretreatment at room temperature for 60 min	298	[54]
Grass clipping	US assisted Ca(OH) ₂ (0.75%) pretreatment at 75 °C for 30 min	414	This study

corruption (high temperature and high alkalinity). Furthermore, high temperature led to production of inhibitors and degradation of carbohydrates, which might eventually decrease the reducing sugar yield [40,47]. So 75 °C was the suitable temperature for reducing sugar yield of US-Ca(OH)₂ pretreating grass clipping.

3.4. Optimization of enzyme loading

Enzyme loading could affect the enzymatic hydrolysis, thus optimal ratio of enzyme and substrate was very important for the hydrolysis efficiency of biomass [48]. Fig. 7 shows the effect of enzyme loading on the reducing sugar yield of grass clipping. As shown in Fig. 7, the increase of enzyme loading resulted in a faster hydrolysis efficiency and higher reducing sugar yield. As the enzyme loading increased from 25 to 125 FPU/g, the reducing sugar yield increased from 287.6 to 414.0 mg/g after 72 h. However, when the enzyme loading further increased from 125 to 200 FPU/g, the reducing sugar yield only increased during the first 12 h. Especially, the reducing sugar yield at enzyme loading of 175 and 100 FPU/g was lower than that at 125 FPU/g. For a certain amount of substrate, the amount of enzyme required for hydrolysis reaction remained fixed. The reducing sugar yield could not rise just by increasing the amount of enzyme, and higher enzyme loadings did not always bring better results. Similar findings were also reported by Chen et al. [49]. The possible reason was that reducing sugar, as the product of hydrolysis, might induce feedback inhibition for continuous hydrolysis [50]. Hence, 125 FPU/g was considered as the suitable enzyme loading for this study.

Based on the above results, US pretreatment time of 30 min, US power density of 6.5 W/ml, Ca(OH)₂ concentration of 0.75%, pretreatment temperature of 75 °C, and enzymatic loading of 125 FPU/g were optimal for US-Ca(OH)₂ pretreatment of grass clipping. Under these conditions, the reducing sugar yield was 414 mg/g, increasing by 3.5 folds compared with that of raw grass clipping.

The hydrolysis efficiencies of grass clipping pretreated by US-Ca(OH)₂ and lignocellulosic biomass pretreated by other methods are compared in Table 2. The sample with suitable operating conditions in this study showed a high reducing sugar yield. So, the US-Ca(OH)₂ pretreatment of grass clipping significantly enhanced the potential of grass clipping as a promising raw material for biofuel production.

The reactor volume in laboratory scale was so small that the energy produced by US was prone to be transferred to the outside, thus causing great energy loss. When the US-Ca(OH)₂ pretreatment system is scaled up, the energy consumption will reduce. In the future pilot study, the scale-up continuous flow reactor can be used to reduce energy loss. In addition, Economic analysis should take into account reducing sugar yield and addition value created by reducing sugar based on continuous pilot study or application.

4. Conclusions

The grass clipping was a promising lignocellulosic biomass for biofuel production, and the US-Ca(OH)₂ pretreatment was effective to improve enzymatic hydrolysis of grass clipping. The US-alkaline pretreatments significantly improved solubilization of hemicellulose and lignin, and crystallinity index (CrI) of cellulose. Ca(OH)₂ was superior

to NaOH for the enzymatic hydrolysis of grass clipping due to its lower price and better pretreatment effect. The optimal operating conditions were found as following: US pretreatment time of 30 min, US power density of 6.5 W/ml, Ca(OH)₂ dosage of 0.75%, pretreatment temperature of 75 °C and enzymatic loading of 125 FPU/g. The maximum reducing sugar yield reached 414 mg/g under these conditions.

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