Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of lignocellulose

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Abstract: Grass clipping and sugarcane bagasse were pretreated with ultrasound (US), $Ca(OH)_2$, NaOH, US-$Ca(OH)_2$ and US-NaOH to enhance the enzymatic hydrolysis. The solubilization of hemicellulose and lignin and crystallinity index (CrI) of cellulose increased after US-alkaline pretreatment, leading to a significant increase of enzyme accessibility to cellulose. US-$Ca(OH)_2$ pretreatment showed the best improvement for reducing sugar yield, compared with the other four kinds of pretreatments, and the grass clipping showed higher potential for the production of reducing sugar than sugarcane bagasse by US-$Ca(OH)_2$. The reducing sugar yield of grass clipping pretreated by US-$Ca(OH)_2$ reached 273.7 mg/g, increasing by 188.7% compared with that of raw grass clipping. The US-$Ca(OH)_2$ pretreatment significantly enhanced the potential of grass clipping as a promising raw material for biofuel production.

Keywords: US-alkaline pretreatment, lignocellulosic biomass, enzymatic hydrolysis, sugar yield potential

1. Introduction

Environmental problems and energy crisis have become severer and severer as the use of fossil fuels. Regarded as a kind of clean and renewable energy, bioenergy, such as ethanol and biogas, produced from lignocellulosic biomass has gained a great amount of attention to replace liquid fuels and fossil fuels and reduce the emission of greenhouse gas (Kurian *et al*., 2013). Composed of cellulose, hemicellulose and lignin, however, the structure of lignocellulosic biomass is complex and special. Thus the hydrolysis of lignocellulosic biomass is blocked, which is the first and key step during the utilization (Jeihanipour *et al*., 2011).

Enzymatic hydrolysis of lignocellulosic biomass to turn into fermentable sugar is a crucial step for bioenergy production (Krishania *et al*., 2013). Therefore, lots of pretreatment methods were introduced to enhance the enzymatic hydrolysis efficiency of lignocellulosic biomass. Those pretreatment method co uld be classified into mechanical pretreatment, chemical pretreatment and biological pretreatment (Behera *et al*., 2014). After pretreatments, part of hemicellulose and lignin is removed, and the accessible surface area of lignocellulosic biomass is expanded, thus to improve the enzymatic hydrolysis efficiency (Behera *et al*., 2014).

Combined pretreatments got a wide range of applications in order to complement each other. Zhu *et al*. (2006) reported that the pretreated wheat straw by microwave-alkali showed increased enzymatic hydrolysis efficiency by the removal of hemicellulose and lignin. Sugarcane bagasse was pretreated by microwave (600 W, 4 min) with 1% NaOH and the reducing sugar yield reached 665.0 mg/g (Binod *et al*., 2012). Jin *et al*. (2016) pretreated catalpa sawdust with microwave assisted alkaline pretreatment to enhance the enzymatic hydrolysis, and catalpa sawdust with microwave-$Ca(OH)_2$ pretreatment showed great potential for biofuel production.

Alkaline pretreatment has received amount of research interest. Alkaline pretreatment has shown positive effect with different extent, however, it is more effective on lignocellulosic biomass containing more lignin. Saponification and cleavage of lignin-carbohydrate linkages is the main function of alkaline, which could increase the porosity and internal surface area and decrease the degree of polymerization and crystallinity.

The application of ultrasound to lignocellulosic biomass was found to enhance the efficiency of hydrolysis and subsequently increase the sugar yield (Karimi *et al*., 2014) through structural deconstruction of the materials resulting from cavitation forces. The quick collapse of cavitation bubbles generates significant shear forces in the liquid immediately surrounding the bubbles and, as a result, produces a strong stirring mechanical effect, which could intensify the mass transfer.

Grass clipping is usually used for recreation, view, sports and transportation safety, which needs clip after a certain time. Sugarcane bagasse is residue produced in large quantities by the sugar and alcohol industries. The two kinds of materials were selected in this study to examine the feasibility of ultrasound-alkaline pretreatment for improvement of enzymatic hydrolysis. Reducing sugars yield by enzymatic hydrolysis was used to evaluate the pretreatment efficiency.

2. Materials and methods

2.1. Raw materials

Grass clipping of lawn grass was collected from the campus of Beijing Forestry University, Beijing, China. Sugarcane bagasse was collected from a sugar factory in Guangxi, china. The sample of the grass clipping and sugarcane bagasse was dried at room temperature for one week, and then was stored in sealed plastic bags at room temperature after milling to approximately 20-40 mesh size.

2.2. Alkaline pretreatment

Triangular flask of 250 ml was used for the alkaline pretreatment reaction. The temperature of reaction was kept at 60 ℃ by water bath. Two kinds of alkaline solution, NaOH and $Ca(OH)_2$, were used for pretreatment of the two kinds of raw materials. The raw materials of 10.0 g were immersed in 200 ml 0.5% (w/v) alkaline solution for 1 hour. The solid residues after alkaline pretreatment were thoroughly washed with distilled water until reached neutral pH, then dried at 105 ± 3 ℃ for at least 4 h. The dried solid sample was stored in sealed plastic bags at room temperature.

2.3. Ultrasound assisted alkaline pretreatment

Ultrasound (US) assisted alkaline pretreatment was carried out 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. NaOH and $Ca(OH)_2$ were used for the ultrasound assisted alkaline pretreatment of two kinds of raw materials, respectively. The raw materials of 5.0 g were immersed in 100 ml water or 0.5% (w/v) alkaline solution for 1 h with the probe of ultrasound device inserted into the solution to assist alkaline pretreatment. The power of ultrasound was 500 W, and the ultrasound kept for only 30 min. The temperature of reaction was kept at 60 ℃ in water bath. After ultrasound assisted alkaline pretreatment, the followed procedure was similar to that mentioned in alkaline pretreatment.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis was conducted using commercial cellulase generated from Aspergillus niger (powder, ≥ 0.3 unit/mg solid). Enzyme solution was prepared with citrate buffer (0.05 mol/L, pH = 4.8) with concentration of 1% (w/v). 1 g sample and 40 ml citrate buffer (0.05 mol/L, pH = 4.8) was mixed in triangular flask, with enzyme loading of 30 FPU/g biomass. The triangular flasks were shaken at 50 ℃ 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..

2.5. Analysis methods

Chemical composition of raw and pretreated materials was analyzed by a fiber analyzer (A200i, Ankom, USA). Reducing sugar concentration was measured by DNS method (Miller, 1959). 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) :

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100\%$$

where I_{002} is the diffraction intensity of crystalline structure (2θ = 22.6°), and $I_{amorphous}$ is the diffraction intensity of amorphous fraction (2θ = 18.0°).

3. Results and discussion

3.1. Effects of different pretreatment methods on grass clipping and sugarcane bagasse

The reducing sugar yields were measured to evaluate the effect of pretreatment. The reducing sugar yield of grass clipping pretreated by US, $Ca(OH)_2$, NaOH, US-$Ca(OH)_2$ and US-NaOH increased by 14.9%, 144.8%, 134.5%, 188.7% and 182.6%, respectively, while sugarcane bagasse increased by 16.7%, 74.4%, 77.2%, 90.4 and 85.4%, respectively, compared with that of raw grass clipping (94.8 mg/g) and sugarcane bagasse (111.2 mg/g) (Figure 1).

Comparing the two raw materials, sugarcane bagasse could produce more reducing sugar than grass clipping during enzymatic hydrolysis. However, after pretreatment, the reducing sugar yield of grass clipping had more significantly increased than that of sugarcane bagasse; especially after US-$Ca(OH)_2$ pretreatment and US-NaOH pretreatment, reducing sugar yield of grass clipping reached 273.67 and 267.89 mg/g, respectively. It indicated that grass clipping had more sugar producing potential than sugarcane bagasse by US-alkaline pretreatment, which might be related to the structural properties of the raw materials.

The composition changes of grass clipping and sugarcane bagasse before and after alkaline, US and US-alkaline pretreatment were presented in Table 1. Obviously, grass clipping not only had larger proportion of cellulose, but also showed a greater increase in the proportion of cellulose than that of sugarcane bagasse. This might explain why grass clipping had higher reducing sugar yield than that of sugarcane bagasse after US-alkaline pretreatment.

Moreover, as for two sorts of materials, the cellulose, hemicellulose and lignin content all increased slightly after US pretreatment, however, they had little change in the proportion. Yu *et al*. (2009) reported that there were no significant changes in the main compositions (cellulose, hemicellulose, and lignin) under ultrasound irradiation. The possible reason was that US pretreatment only slightly dissolved other compositions rather than the cellulose, hemicellulose and lignin. This might also explain the phenomenon that the reducing sugar yield only increased by 14.9% after US pretreatment, which was in agreement with other researches (Yachmenev *et al*., 2009).

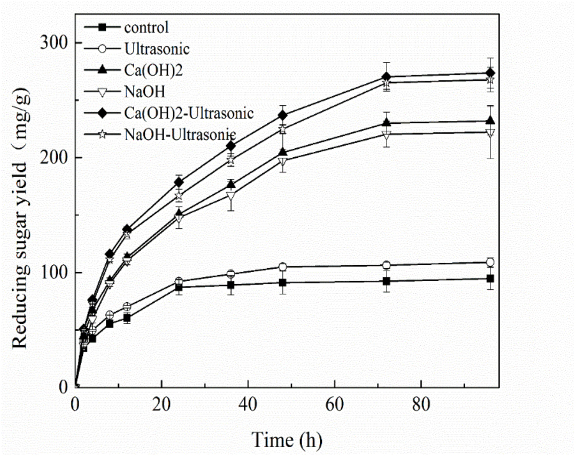

Figure 1a. Reducing sugar yield of grass clipping before and after US, alkaline and US-alkaline pretreatment (US power density of 5 w/ml, US pretreatment time of 30 min, temperature of 60 ℃, solid content of 5% (w/v), NaOH or $Ca(OH)_2$ dosage of 0.5% (w/v), NaOH or $Ca(OH)_2$ pretreatment time of 1 h, enzyme loading of 30 FPU/g, and enzymatic hydrolysis time of 96 h).

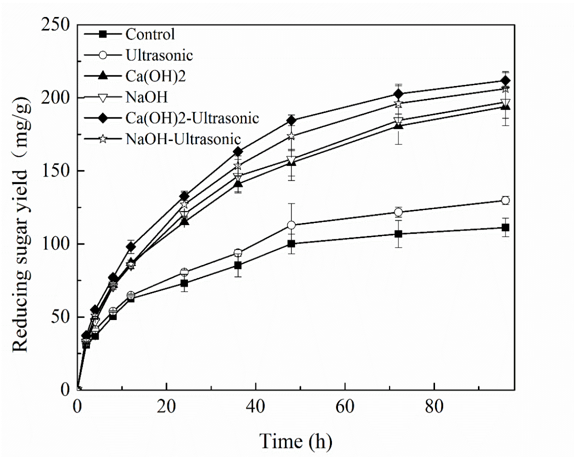

Figure 1b. Reducing sugar yield of sugarcane bagasse before and after US, alkali and US-alkali pretreatment (US power density of 5 w/ml, US pretreatment time of 30 min, temperature of 60 ℃, solid content of 5% (w/v), NaOH or $Ca(OH)_2$ dosage of 0.5% (w/v), NaOH or $Ca(OH)_2$ pretreatment time of 1 h, enzyme loading of 30 FPU/g, and enzymatic hydrolysis time of 96 h).

However, the hemicellulose and lignin contents both decreased after $Ca(OH)_2$, NaOH, US-$Ca(OH)_2$ and US-NaOH pretreatment. It was reported that the removal rate of lignin could be enhanced by 23% and the degradation rate of the hemicellulose could be increased by 12% in samples of alkaline pretreated feedstock (Zhang *et al*., 2008). It was suggested that ultrasound waves could release the lignin and hemicellulose by hydrolyzing the linkages between lignin and carbohydrate. At the same time, it could strongly affect the degradation and/or removal of hemicellulose and lignin if used along with the alkaline pretreatment (Zhang *et al*., 2008). In addition, the required negative pressure to produce cavitation was proportional to the tensile strength of liquid, and thus depended on the type and purity of liquid (Chowdhury and Viraraghavan, 2009). Hence, the addition of alkaline would promote the formation of cavitation, so as to enhance the effect of ultrasound. In conclusion, US-alkaline pretreatment had better effect on removal of hemicellulose and lignin than alkaline pretreatment alone. It might be due to that slight shock and cavitation collapse produced ultrasound in alkaline solutions could not only enhance the lignin removal rate, but also greatly increase the hemicellulose degradation rate (Karimi *et al*., 2014). The hemicellulose and lignin were the main components of cellulose-hemicelluloses-lignin network, which was the major barrier for effective enzymatic hydrolysis of cellulose (Karimi *et al*., 2014). After hemicellulose hydrolysis, the cellulose-hemicelluloses-lignin network was destroyed and the hydrolysis efficiency increased accordingly. Another obstacle for efficient enzymatic hydrolysis of lignocellulosic biomass was the existence of lignin. The hemicellulose and lignin content both decreased after US-alkaline pretreatment, which could significantly enhance the enzymatic hydrolysis. This might be the reason why the samples could produce more reducing sugar after US-alkaline pretreatment

Table 1 Chemical composition of samples

Sample	pretreatment method	Chemical composition (%)			
		Cellulose	Hemicellulose	Lignin	Others
Grass clipping	Control	42.21	34.25	6.17	16.03
	US	45.88	36.10	6.55	11.47
	$Ca(OH)_2$	50.22	28.36	5.77	15.65
	NaOH	50.21	28.48	5.82	15.49
	US- $Ca(OH)_2$	53.51	25.57	5.61	15.31
	US- NaOH	53.47	26.30	5.78	14.45
Sugarcane bagasse	control	27.97	35.29	3.27	33.47
	US	31.01	36.31	3.64	29.04
	$Ca(OH)_2$	32.81	32.84	2.83	31.52
	NaOH	31.92	33.82	3.06	31.20
	US- $Ca(OH)_2$	34.34	32.22	2.72	30.72
	US- NaOH	33.22	33.18	2.93	30.67

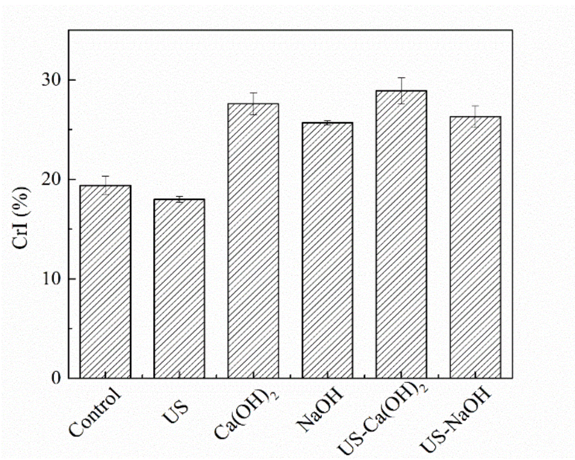

Figure 2. Crystallinity index (CrI) of grass clipping before and after US, alkaline and US-alkaline pretreatment (US power density of 5 w/ml, US pretreatment time of 30 min, temperature of 60 ℃, solid content of 5% (w/v), NaOH or $Ca(OH)_2$ dosage of 0.5% (w/v), NaOH or $Ca(OH)_2$ pretreatment time of 1 h, enzyme loading of 30 FPU/g, and enzymatic hydrolysis time of 96 h).

3.2. Crystallinity

Crystallinity index (CrI), the ratio of crystalline cellulose among the lignocellulosic biomass, is a significant factor affecting the enzymatic hydrolysis. X-ray diffraction measurement of CrI is the best option to estimate the impact of chemical pretreatment on biomass crystallinity (Kumar *et al*., 2009). The CrI changes of grass clipping was shown in Fig. 2. There was almost no change in the crystallinity of grass clipping after US pretreatment compared to that without pretreatment. Similar results were also obtained that both the degree and size of crystalline cellulose did not change so much after ultrasound treatment (Tang *et al*., 2005; Eblaghi *et al*., 2016). However, The CrI of grass clipping pretreated by $Ca(OH)_2$, NaOH, US-$Ca(OH)_2$ and US-NaOH increased to 27.6%, 25.7%, 28.9% and 26.3%, respectively, compared with that of raw grass clipping (19.4%) and raw sugarcane bagasse (23.2%). Other researches also reported increase of CrI of lignocellulosic biomass pretreated by alkaline (Gu *et al*., 2015), and the enzymatic hydrolysis efficiency increased accordingly. Moreover, Eblaghi *et al*. (2016) found that the CrI increased from the control of 53% to 60 and 65% after combined ultrasound 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 a relative increase in the proportion of cellulose (Li *et al*., 2010). In this study, there was increase of crystallinity of US-alkaline pretreated grass clipping, compared to that pretreated by alkaline only. To sum up, ultrasonic could promote alkaline to release hemicellulose and lignin of the amorphous parts, thus to increase the crystallinity of raw materials, which was in agreement with the results in Table 1.

4. Conclusions

The grass clipping was a promising lignocellulosic biomass for biofuel production, and the US-alkaline pretreatment was effective to improve enzymatic hydrolysis of grass clipping. The US-alkaline pretreatments significantly improved solubilization of hemicellulose and lignin with the crystallinity index (CrI) of cellulose increased, and $Ca(OH)_2$ was superior to NaOH for the enzymatic hydrolysis of grass clipping.

References

Behera S., Arora R., Nandhagopal N. and Kumar, S. (2014), Importance of chemical pretreatment for bioconversion of lignocellulosic biomass, *Renewable Sustainable Energy Reviews*, **36**, 91-106.

Binod P., Satyanagalakshmi K., Sindhu R., Janu K.U., Sukumaran R.K. and Pandey A. (2012), Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse, *Renewable Energy*, **37**, 109-116.

Chowdhury P. and Viraraghavan T. (2009), Sonochemical degradation of chlorinated organic compounds, phenolic compounds and organic dyes - a review, *Science of Total Environment*, **407 (8)**, 2474-2492.

Eblaghi M., Niakousari M., Sarshar M., Mesbahi G.R. (2016), Combining Ultrasound with Mild Alkaline Solutions as an Effective Pretreatment to Boost the Release of Sugar Trapped in Sugarcane Bagasse for Bioethanol Production, *Journal of Food Process Engineering*, **39 (3)**, 273-282.

Jeihanipour A., Niklasson C. and Taherzadeh M.J. (2011), Enhancement of solubilization rate of cellulose in anaerobic digestion and its drawbacks, *Process Biochemistry*, **46**, 1509-1514.

Jin S., Zhang G., Zhang P., Li F., Wang S., Fan S. and Zhou S. (2016), Microwave assisted alkaline pretreatment to enhance enzymatic saccharification of catalpa sawdust, *Bioresource Technology*, **221**, 26-30.

Karimi M., Jenkins B. and Stroeve P. (2014), Ultrasound irradiation in the production of ethanol from biomass, *Renewable and Sustainable Energy Reviews*, **40**, 400-421.

Krishania M., Kumar V., Vijay V.K. and Malik A. (2013), Analysis of different techniques used for improvement of biomethanation process: a review, *Fuel*, **106**, 1-9.

Kumar R., Mago G., Balan V. and Wyman C.E. (2009), Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies, *Bioresource Technology*, **100 (17)**, 3948-3962.

Kurian J.K., Nair G.R., Hussain A. and Raghavan V.G.S. (2013), Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: a comprehensive review, *Renewable and Sustainable Energy Reviews*, **25**, 205-219.

Li M.F., Fan Y.M., Xu F., Sun R.C. and Zhang X.L. (2010), Cold sodium hydroxide/urea based pretreatment of bamboo for bioethanol production: Characterization of the cellulose rich fraction, *Industrial Crops and Products*, **32 (3)**, 551-559.

Miller G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Analytical Chemistry*, **31**, 426-428.

Tang A., Zhang H., Gang C., Xie G. and Liang W. (2005), Influence of ultrasound treatment on accessibility and regioselective oxidation reactivity of cellulose, *Ultrasonics Sonochemistry*, **12 (6)**, 467-472.

Yachmenev V., Condon B., Klasson T. and Lambert A. (2009), Acceleration of the enzymatic hydrolysis of corn stover and sugar cane bagasse celluloses by low intensity uniform ultrasound, *Journal of Biobased Materials and Bioenergy*, **3**, 1-7.

Yu J., Zhang J., He J., Liu Z. and Yu Z. (2009), Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull, *Bioresource Technology*, **100**, 903-908.

Zhang Y.Q., Fu E.H. and Liang J.H. (2008), Effect of Ultrasonic Waves on the Saccharification Processes of Lignocellulose. *Chemical Engineering & Technology*, **31 (10)**, 1510-1515.

Zhu S., Wu Y., Yu Z., Chen Q., Wu G., Yu F., Wang C. and Jin S. (2006), Microwave-assisted alkali pre-treatment of wheat straw and its enzymatic hydrolysis, *Biosystems Engineering*, **94 (3)**, 437-442.