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Study on Technology of Reducing Sugar Production from Bermuda Grass by Combination of Dilute Acid and Ultrasound

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ABSTRACT
Bermuda grass is rich in cellulose, which can be degraded into reducing sugar, providing sufficient and cheap carbon source for ethanol fermentation. Employing acid pretreatment assisted by ultrasound to degrade Bermuda grass, this study studied effects of temperature, time, liquid-solid ratio, ultrasound power, acid concentration on reducing sugar yield, by which orthogonal experiment was designed to optimize acid-ultrasound pretreatment technology of Bermuda grass. Results showed that optimum conditions of acid-ultrasound pretreatment were as follows. 80°C for temperature, 100 min for time, 12 mL g^{-1} for liquid-solid ratio, 80 W for ultrasound power and 2% for acid concentration led to the reducing sugar yield of 36.89%.

Key words: Bermuda grass, ultrasound, dilute acid, pretreatment, reducing sugar

INTRODUCTION

Currently, in energy structure worldwide, biomass resource plays an important role (Yang *et al*., 2007; Li *et al*., 2009). For one thing, biomass resource is renewable, for another, carbon dioxide emitting from its utilization, can be absorbed during the course of growth, which ranks among pollution free energy resources. With the increasing shortage of traditional energy, exploiting new technology, by which biomass can be converted to liquid fuel and chemical products, is essential to realize sustainable development (Balat, 2008; Qian and Zhu, 2009).

Bioethanol is one of good substitutes for fossil energy. Originally, starch-based material is the mainstay of bioethanol production. The United States is the biggest bioethanol producer throughout the world. In addition, US Energy Act targets the output of bioethanol, produced from corn, to be 15 billion gallon in 2015 (Liu, 2009). Thus, bioethanol production is in close connection with grain supply, which will affect grain price (Zhao and Pu, 2008), therefore, increasing scholars question and resist its development, promoting its development to a new stage, namely, non-grain feedstock for ethanol production. In this stage, researchers focus on biomass resource, such as straw of all kinds, leaves, wood chips and so on, all these materials are rich in cellulose and semi-cellulose, which can be degraded into reducing sugar, providing enough material for ethanol production economically and environmentally. Up to now, several countries with large amount of ethanol yield, America, Brazil and Canada included, pay more attention to converting biomass resource into ethanol by pretreatment, enzyme hydrolysis and fermentation (Zhao and Hou, 2011; Tang *et al*., 2005).

In the degradation process of cellulosic material, pretreatment is the key procedure, which directly affects results and economic benefit of follow-up process. Pretreatment methods consist of physical method, chemical method and biological method. Acid pretreatment is well developed among all methods, which functions well in destroying crystallization structure, breaking the linkage between lignin and cellulose and dissolving semi-cellulose, etc (Silverstein *et al*., 2007).

When ultrasound acts on biomass material, a variety of effects produces, including cavitation effect, turbulent effect, perturbation effect and so on, which do well in decreasing crystallinity and increasing amorphous region. With more reaction sites exposed, therefore follow-up reaction is accelerated (Kang *et al*., 2009).

Ultrasound pretreatment can shorten reaction time and reduce energy consumption, hence, the combination of ultrasound with other methods is considered as suitable technology for cellulose degradation (Peng *et al*., 2008).

Bermuda grass is rich in cellulose and semi-cellulose, which can be degraded into reducing sugar effectively by appropriate method. By acid-ultrasound pretreatment, this study analyzes effects of acid concentration, reaction temperature and time, liquid-solid ratio, ultrasound power on reducing sugar yield from Burmuda grass, by which orthogonal experiment is designed to optimize reaction condition, laying groundwork for subsequent enzyme hydrolysis and fermentation.

MATERIALS AND METHODS

Material: Select mature Bermuda grass in Campus of Henan University of Urban Construction, wash and dry, then shatter materials over 60-80 mesh screen.

Main reagents:

- DNS reagent
- Dilute hydrochloric acid with concentrations from 0.5-3%
- Phosphate Buffered Saline (PBS)
- Enzymes: xylanase and cellulase (hereafter it is called double enzyme)

Apparatus: Plant crusher, pH apparatus, Paradigm 722 spectrophotometer, electric drying oven with wind-drumming, thermostat water bath, KQ5200DE ultrasound cleaner, etc.

Method: Add dilute hydrochloric acid into Burmuda grass, put it into ultrasound cleaner, after which double enzyme is used to degrade cellulose, when the reaction is over, measure reducing sugar yield (Liu *et al*., 2012). Concentration of dilute hydrochloric acid, liquid-solid ratio, temperature, time and ultrasound power are all single factors affecting pretreatment results. On the basis of single factor experiment, orthogonal experiment is designed to optimize pretreatment conditions, with a view to heightening cellulose degradation efficiency.

RESULTS AND ANALYSIS

Single factor experiment

Effect of temperature on reducing sugar yield: Ultrasound frequency: 40 kHz, dilute hydrochloric acid: 1.5%, liquid-solid ratio: 8 mL g^{-1} ultrasound power: 200 W, time: 60 min. Temperature was from 40-80°C with the gradient of 10°C. Effect of temperature on reducing sugar yield was shown in Fig. 1.

As shown in Fig. 1, reducing sugar yield increased with the temperature going up. Between 60 and 70°C, reducing sugar yield experienced a rapid increase, then, slow increase appeared, taking energy consumption into consideration, appropriate temperature is 70°C.

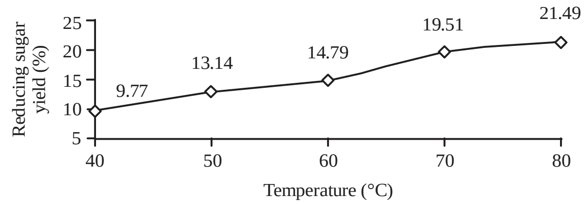

Fig. 1: Effect of ultrasound temperature on reducing sugar yield

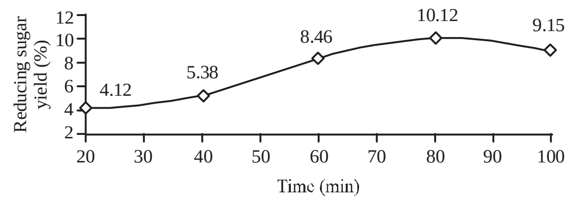

Fig. 2: Effect of time on reducing sugar yield

Effect of time on reducing sugar yield: Ultrasound frequency: 40 kHz, dilute hydrochloric acid: 1.5%, liquid-solid ratio: 8 mL g^{-1} ultrasound power 200 W, temperature: 80°C, time was from 20-100 min with gradient of 20 min. Effect of time on reducing sugar yield was shown in Fig. 2.

Figure 2 indicated that reducing sugar yield increased with the time going on, 80 min later, the result decreased. In the system, combination of high temperature and pressure from ultrasound destroyed cellulose structure. References showed that acid reacted with cellulose, which was degraded into monosaccharide, lowering its polymerization. Therefore, with the time continuing, more cellulose was degraded, reducing sugar yield increased. With the accumulation of reducing sugar, feedback inhibition impeded reaction, thus, the result went down.

Effect of liquid-solid ratio on reducing sugar yield: Ultrasound frequency: 40 kHz, dilute hydrochloric acid: 1.5%, ultrasound power 200 W, temperature: 80°C, time: 60 min. Liquid-solid ratio was from 4-12 with gradient of 2 mL g^{-1}. Effect of liquid-solid ratio on reducing sugar yield was shown in Fig. 3.

In Fig. 3, it was obvious that reducing sugar yield got to 11.35% with the liquid-solid ratio of 10:1, when it continued to go up, the result decreased. Low liquid-solid ratio led to low result, which may be due to two reasons. Firstly, substrate concentration was relatively high with low liquid-solid ratio, energy provided by ultrasound was fixed, therefore, energy absorbed by substrate per gram couldn't meet the demand of pretreatment. Secondly, high concentration of substrate

Table 1: Factors and levels of orthogonal experiment

Acid concentration (%) (A)	Liquid-solid ratio (mL g^{-1}) (B)	Temperature (°C) (C)	Time (min) (D)	Ultrasound power (W) (E)
1.0	6	50	40	80
1.5	8	60	60	120
2.0	10	70	80	140
2.5	12	80	100	180

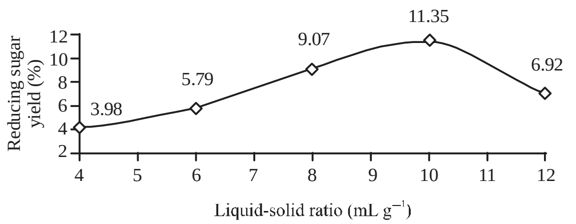

Fig. 3: Effect of liquid-solid ratio on reducing sugar yield

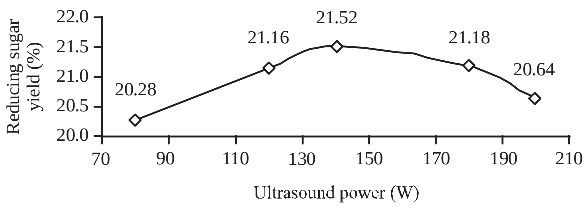

Fig. 4: Effect of ultrasound power on reducing sugar yield

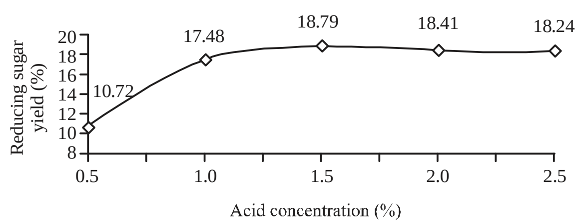

Fig. 5: Effect of acid concentration on reducing sugar yield

resulted in insufficient transmission medium of ultrasound, which led to "no ultrasound function", hence, energy provided couldn't be used with high efficiency. If liquid-solid ratio was too high, low substrate concentration made energy dissipation occur in the medium, which weakened pretreatment effect.

Effect of ultrasound power on reducing sugar yield: Ultrasound frequency: 40 kHz, dilute hydrochloric acid: 1.5%, ultrasound power 200 W, temperature: 80°C, time: 60 min, liquid-solid ratio: 8 mL g^{-1}, ultrasound power was 80, 120, 140, 180 and 200 W, respectively. Effect of ultrasound power on reducing sugar yield was shown in Fig. 4.

Figure 4 suggested that reducing sugar yield increased when the ultrasound power was below 140 W, whose increase rate was significant between 80-120 W. When ultrasound frequency and reaction time were fixed to 40 kHz and 60 min, respectively, crushing rate of substrate depended on ultrasound power. For substrate concentration was a constant, the higher ultrasound power was, the stronger mechanical vibration worked, until substrate was saturated by ultrasound of 140 W, hereafter, reducing sugar yield reduced.

Effect of acid concentration on reducing sugar yield: Ultrasound frequency: 40 kHz, ultrasound power: 200 W, temperature: 80°C, time: 60 min. liquid-solid ratio: 8 mL g^{-1}, hydrochloric acid concentration was from 0.5-2.5% with gradient of 0.5%. Effect of acid concentration on reducing sugar yield was shown in Fig. 5.

Figure 5 revealed that reducing sugar yield took on rapid increase with acid concentration increasing, when it reached 1.0%, increase rate showed the sign of leveling off. Concentration of 1.5% contributed to the uttermost reducing sugar yield, after which the result dropped.

Acid acted on semi-cellulose degradation, enhancement appeared when acid concentration increased, along with the increasing reducing sugar yield. When substrate was saturated by acid, its increasing concentration didn't promote reaction further. Furthermore, high reducing sugar concentration brought about remarkable feedback inhibition, resulting in decrease in result.

Technology optimization: On the basis of single factor experiment, orthogonal experiment was designed to optimize pretreatment technology. Design and results of orthogonal experiment were shown in Table 1 and 2.

Intuitive analysis showed that suitable combination was A3B2C4D3E1 by which reducing sugar yield was 35.52%. Range analysis indicated that temperature was the most insensitive factor and optimum combination attributed to A3B4C4D4E1.

Verification experiment indicated that A3B4C4D4E1 was the optimum technology, namely reducing sugar yield reached 36.89% by acid concentration of 2%, liquid-solid ratio of 12 mL g^{-1}, temperature of 80°C, reaction time of 100 min and ultrasound power of 80 W.

DISCUSSION

Ultrasound technology is widely used in large molecules' separation and purification currently, for the differences in kinds, contents, structures and existing forms of these large molecules in various materials, corresponding results are illustrated.

Experiment on rice straw (Kang *et al*., 2009) showed that reducing sugar yield was 26.4 g L^{-1} with saccharification time of 108 h at 45°C and pH 4.8 by acid and ultrasound, however,

Table 2: Results of orthogonal experiment

Acid concentration (%) (A)	Liquid-solid ratio (B)	Temperature (°C) (C)	Time (min) (D)	Ultrasound power (W) (E)	Reducing sugar yield (%)
1	1	1	1	1	8.60
1	2	2	2	2	12.30
1	3	3	3	3	21.87
1	4	4	4	4	30.21
2	1	2	3	4	15.85
2	2	1	4	3	16.88
2	3	4	1	2	27.27
2	4	3	2	1	32.46
3	1	3	4	2	33.68
3	2	4	3	1	35.52
3	3	1	2	4	19.04
3	4	2	1	3	18.10
4	1	4	2	3	34.98
4	2	3	1	4	20.09
4	3	2	4	1	25.61
4	4	1	3	2	14.24
k1	18.245	23.277	14.690	18.515	25.548
k2	23.115	21.198	17.965	24.695	21.872
k3	26.585	23.448	27.025	21.870	22.957
k4	23.730	23.753	31.995	26.595	21.297
R	8.340	2.555	17.305	8.080	4.251

under the same conditions without ultrasound, reducing sugar yield got to 26.2 g L^{-1} with saccharification time of 120 h.

Taking corn straw as material, by alkali and ultrasound, Yang *et al*. (2007) illustrated that reducing sugar yield was 13%, which was higher than that of simple alkali pretreatment. As to wheat straw, the same experiment was carried out, which showed that reducing sugar yield climbed to 72.6% by the degradation of lignin under the conditions as followed: alkali concentration of 1.54%, ultrasound power of 1160 W, reaction time of 50 min and the temperature of 78.94°C (Wang *et al*., 2010).

Qin *et al*. (2009) indicated that the time of reaction in alkali solution assisted by ultrasound was shortened obviously comparing with simple alkali pretreatment. Also total carbohydrate yield was increased by 59.6%. Zhang *et al*. (2007) suggested that ultrasound functioned well in improving saccharification yield of enzyme hydrolysis, whose result increased by 70% in contrast with that of traditional method. Reducing sugar yield got to 80.72% from peanut shell by ultrasound and acid, which increased by 27.38% comparing with the pretreatment without ultrasound under the same conditions at 61.77°C for 124.14 min with acid concentration of 5.88% (Yu *et al*., 2011).

Taking wheat straw as material, by ultrasound and propionic acid, research (Tian and Ma, 2011) showed that reducing sugar yield was 91% 18 h later, while that of simple propionic acid pretreatment was 84% after 36 h. Taking Bermuda grass as material, this paper analyzed effects of temperature, reaction time, liquid-solid ratio, ultrasound power, acid concentration on reducing sugar yield, on which optimum conditions were find out. Under the above conditions, reducing sugar yield was much more than simple acid pretreatment, which was only 14.05% (Liu and Yin, 2010).

However, in the operation, material were at the bottom of tubes, which made it difficult for full contact of material and reagent, hence, vibration was needed to heighten the reducing sugar yield. Also, by this method, degradation ratio of the reducing sugar was high, leading to the waste of the product (Wang *et al*., 2011), therefore measures should be taken to protect product from degradation.

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