EFFECT OF CELLOBIASE AND SURFACTANT SUPPLEMENTATION ON THE ENZYMATIC HYDROLYSIS OF PRETREATED WHEAT STRAW

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Wheat straw is a suitable raw material for ethanol production since it has high cellulose content. The objective of this work was to evaluate the effect of cellobiase and surfactant on the enzymatic hydrolysis of lignocellulosic materials. For this purpose, wheat straw was first pretreated by organosolv digestion. The chemical compositions of raw and pretreated wheat straw were analyzed. Much of the hemicellulose and lignin were removed, and the relative cellulose content of pretreated wheat straw was 26.57% higher when compared to untreated wheat straw. Cellobiase was added into hydrolysate to improve the hydrolysis efficiency. Through experiments and analysis, the optimum cellobiase dosage was found to be 1/10 of the cellulase loading. Surfactant was also added into hydrolysate. Nonionic surfactant (Tween 80) exhibited better effect on improving enzymatic hydrolysis. When 0.06 g/g dry solids (DS) Tween 80 was also added into hydrolysate, the yield of glucose in hydrolyzate could reach 486 g/kg DS.

Keywords: Wheat straw; Organosolv pretreatment; Cellobiase; Surfactant; Enzymatic hydrolysis

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INTRODUCTION

Lignocellulosics such as wheat straw are an abundant renewable resource in the biosphere (Chen et al. 2008). Because its cellulose content is high (roughly 34% of dry weight), it is a good source of sugar to use in the bioprocess of making ethanol for use as a fuel extender (Alfani et al. 2000; Roozbeh et al. 2010). However, due to the native association of cellulose, hemicellulose, and lignin, it is not readily available as a biomass source unless the lignin is modified or removed by chemical and/or biological methods (Freer and Detroy 1982; Keller et al. 2003; Frank and Kevin 2007). Ethanol is produced from lignocelluloses by an integrated process involving basically three steps: pretreatment, hydrolysis, and fermentation. The final objective of both pretreatment and hydrolysis is to break down the carbohydrate polymers, which are present in plant cell walls, into low-molecular-weight sugars so that microorganisms can ferment them to ethanol (Cara et al. 2007; Zhu and Pan 2010).

In the case of terrestrial plants, a high content of cellulose and hemicellulose does not lead directly to a high yield of ethanol, but a pretreatment step is required to improve sugar solubility and enzymatic digestion (Viola et al. 2008). Compared to the steam explosion and SPORL pretreatment methods, the ethanol organosolv pretreatment has

several advantages: (1) a separate size-reduction step is not necessary even when pretreatment is directly applied to commercial wood chips; (2) it produces a readily digestible cellulose substrate from almost all kinds of feedstock; (3) it also produces very high purity and quality lignin with the potential of high-value utilizations; and (4) the reaction temperature and the pretreatment energy are lower (Söderström et al. 2004; Pan et al. 2006; Zhu et al. 2009). In summary, the organosolv process is a unique and promising biomass fractionation and pretreatment process. Consequently, the organosolv digestion pretreatment is one of the more used because of its delignification and defibration abilities (Sun and Chen 2008; Araque et al. 2009; Park et al. 2010).

It is well known that the presence of cellobiose inhibits the enzymatic hydrolysis of lignocelluloses (Shi et al. 2009; Andric et al. 2010; Lammirato et al. 2010). If the cellobiose concentration is too high in the hydrolysate solution, the rate of enzymatic hydrolysis will slow down. Consequently, cellobiase is added into hydrolysate to accelerate the rate of enzymatic hydrolysis and produce higher glucose concentration. Zhao and Xia (2009) demonstrated that using cellobiose could reduce the feedback inhibition caused by cellobiose accumulation to the cellulase reaction. They indicated that synergetic hydrolysis by a more balanced cellulase complex (2 FPU: 1 CBU) could effectively avoid cellobiose accumulation, and the suitable enzyme loading was identified as 20 FPU/g substrate, i.e. an enzyme complex including cellulase at a level of 20 FPU/g substrate and cellobiase at 10 CBU/g substrate (Zhao and Xia 2009).

It has been reported that additives such as nonionic surfactants can drastically enhance the enzymatic conversion of cellulose into soluble fermentable sugars, and they also can lower the amount of cellulolytic enzymes required for obtaining a given sugar yield (Qi et al. 2010). The positive effect of surfactant addition on enzymatic digestibility of lignocellulosics is generally believed to be attributable to the prevention of unproductive adsorption of cellulase to the lignin fraction of the pretreated lignocellulosic material, which results in a higher amount of free cellulolytic enzymes that would be available for hydrolyzing the substrate (Eriksson et al. 2002; Kristensen et al. 2007).

In this work, both the optimum cellobiase dosage and the time of enzymatic hydrolysis, combined with cellobiase for producing higher sugar concentration, were studied. The effect of different surfactants on enzymatic hydrolysis of lignocelluloses was also investigated.

EXPERIMENTAL

Raw Materials and Enzymes

The wheat straw used in this work was obtained from a local farm in Wuji, Hebei Province, China. Before any pretreatment, the wheat straw was thoroughly washed to remove any extraneous impurities until the washings were clean and colorless. The straw was then air-dried and stored in a dry and cool room for further treatment. The moisture content of the straw was 7.21%. For organosolv digestion experiments, the wheat straw was chopped into pieces of about 5 cm in length by a crop chopper.

Cellulase was purchased from Zaozhuang Jienuo Biotech. Corp., China, with a filter paper activity (FPA) of 43.5 FPU/g. Cellobiase (NS5001) was provided by Novozymes and the enzyme activity was 250 CBU/g. All other chemicals used in this study were of analytical grade.

Organosolv Pretreatment

Organosolv pretreatment using H_2SO_4 as a catalytic agent was carried out in a 15 L digester (ZQS_1, manufactured by Shaanxi University of Science & Technology, China). 600 g (oven dry) of wheat straw was filled manually into the reactor. The cooking liquor was prepared by mixing water with industrial alcohol (93% to 95%, v/v) purchased from the market. The pretreatment conditions were: ethanol concentration 65%, catalytic agent dosage 1.2% (w/w dry weight), cooking temperature 180 ºC, cooking time 120 min, and solid-liquor ratio 1:6 (g/mL).

Enzymatic Hydrolysis

After organosolv pretreatment, the water-insoluble fibre was enzymatically hydrolyzed to determine the glucose yield. The experiment for enzymatic hydrolysis was carried out in a 250 mL conical flask. The experiment conditions were fixed in an incubator shaker with a total working volume of 100 mL at 50 ºC, 160 rpm, acetate buffer at pH 4.8, and dry matter content 2%.

The cellulase was added at a dosage of 25 IU/g dry weight together with the cellobiase at 1/5, 1/10, 1/15, and 1/20 (v/v) of cellulase loading. Samples were withdrawn after the reaction time of 3, 6, 12, 24, 36, 48, 60, 72, and 84 h, then centrifuged, and analyzed for sugar concentration.

The effect of surfactant on enzymatic hydrolysis was studied subsequently. Three kinds of surfactant were used: nonionic (Tween 80), cationic (quaternary ammonium salt), and anionic (sodium dodecyl sulfate). The surfactant dosage was 0.04 g/g dry weight and the samples were withdrawn when reacted for 60 h, then centrifuged, and analyzed for sugar yield.

Analytical Methods

The chemical composition of wheat straw and organosolv pretreated wheat straw were determined using the following TAPPI standards: T 429 cm-01 for cellulose content, T 223 cm-01 for hemicellulose content, T 222 om-02 for lignin content, T 211 om-02 for ash content, and T 204 cm-97 for alcohol-benzene extractive content.

Sugar content in the samples withdrawn at different times was measured by a Biosensing analyzer (SBA-40C, manufactured by Biology Institute of Shandong Academy of Sciences, China).

Statistical Analyses

All experiments were carried out in duplicate, and the data reported were expressed as mean values. Experimental errors, which were calculated as the relative standard deviation, were shown by the error bars in the figures.

RESULTS AND DISCUSSION

Chemical Compositions

The chemical composition of raw and pretreated wheat straw are summarized in Table 1.

Table 1. Weight Percentage of Lignocellulosic Components in Native and Organosolv Pretreated Wheat Straw (% of dry weight)

Component	Raw wheat straw	Pretreatment by ethanol
Cellulose	36.14	62.71
Hemicellulose	23.16	13.25
Lignin	17.74	7.32
Ash	7.13	5.87
Alcohol-benzene extractive	3.26	15.96

Results revealed that the content of cellulose in the ethanol pretreated straw increased by 26.57% when compared to untreated wheat straw. Pretreated wheat straw contained a higher proportion of cellulose (62.71%), since a significant proportion of the hemicellulose was hydrolyzed and degraded during the pretreatment.

The concentration of hemicellulose decreased from 23.16% to 13.25%, as a result of organosolv pretreatment preferentially attacking the hemicellulose components (Saha 2003). Pretreatment gave rise to higher solubilization of hemicellulose, which degraded into monomeric sugars afterwards.

Furthermore, the organosolv pretreatment induced a substantial delignification yield, as lignin level decreased from 17.74% to 7.32% of the pretreated wheat straw. Most of the lignin was converted to ethanol soluble organosolv lignin, which was directly removed during the pretreatment, but some insoluble lignin remained in the cellulosic pulp (Heiss-Blanquet et al. 2011). Therefore, the organosolv pretreatment was an effective method for lignin solubilization (Wörmeyer et al. 2011).

When compared to untreated wheat straw, the content of ash in pretreated wheat straw dropped from 7.13% to 5.87%. However, after the organosolv pretreatment, the content of alcohol-benzene extractive in pretreated wheat straw increased sharply from 3.26% to 15.96%.

Effect of Cellobiase on Enzymatic Hydrolysis

In the process of lignocellulosic enzymatic hydrolysis, cellulose would be hydrolyzed into glucose and cellobiose. Meanwhile, part of cellobiose was hydrolyzed into glucose. If cellobiose concentration was too high in the hydrolysate, it would pose an obstruction for cellulose to be hydrolyzed into glucose, and the glucose yield would become lower. In order to eliminate the negative influence of cellobiose on the system of enzymatic hydrolysis, cellobiase was added into the hydrolysate.

Figure 1 shows the effect of cellobiase on the release of glucose from enzymatic hydrolysis at 2% (w/v) solids loading. The dosage of cellobiase was 1/5, 1/10, 1/15, and 1/20 of cellulase loading.

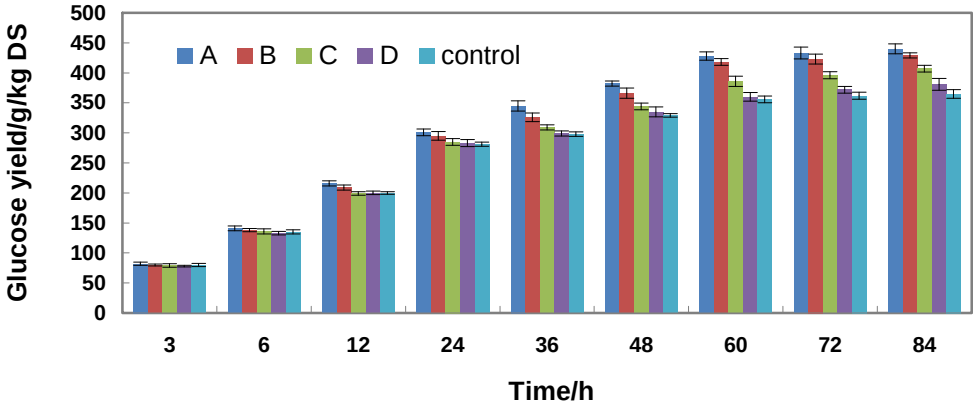

Fig. 1. Effect of different cellobiase dosages on enzymatic hydrolysis
(A, dosage was 1/5 of cellulase loading; B, dosage was 1/10 of cellulase loading;
C, dosage was 1/15 of cellulase loading; D, dosage was 1/20 of cellulase loading)

In Fig. 1, it was clearly deduced that at different dosages of cellobiase, the yield of glucose in hydrolysate increased with the reaction time, which was also true for the control sample. The results showed that the yield of glucose did not significantly change after 60 h. This reaction time was assumed to be an optimum value for all the samples in enzymatic hydrolysis. As could be seen from Fig.1, a higher glucose concentration was obtained as the more cellobiase was added. The glucose yields corresponding to conditions A, B, C, D, and the control sample were 428, 418, 386, 360, and 356 g/kg DS at reaction time 60 h, respectively. The glucose yield of A was 10 g/kg DS higher than that of B (Fig.1), however, the difference of glucose yield between A and B was quite small. When cost is considered, it was concluded that 1/10 of cellulase loading (B) was the optimum dosage of cellobiase. Under the given experimental conditions of enzymatic hydrolysis, the glucose yield of sample B was 62 g/kg DS higher than that of the control sample.

Effect of Surfactant on Enzymatic Hydrolysis

Adding surfactant to hydrolysate could not only reduce the ineffective combination between lignin and cellulase, but could restrain the denaturation of the substrate (Kurakake et al. 1994). In order to get a higher glucose yield, surfactant was added to hydrolysate.

Figure 2 shows the effect of different kinds of surfactant on the release of glucose from lignocellulosic enzymatic hydrolysis at 2% (w/v) solids loading. The dosage of cellobiase was 1/10 that of the cellulase loading, and the enzymatic hydrolysis time was 60 h. From Fig. 2 one can find that different kinds of surfactant had distinct effects on the glucose yield of lignocellulosic enzymatic hydrolysis. When surfactant was added into hydrolysate, the nonionic surfactant (Tween 80) exhibited a positive effect. The glucose yield was 476 g/kg DS, which was 58 g/kg DS higher than that of control sample. However, the cationic (quaternary ammonium salt) and anionic (sodium dodecyl sulfate) surfactants showed negative effects on enzymatic hydrolysis.

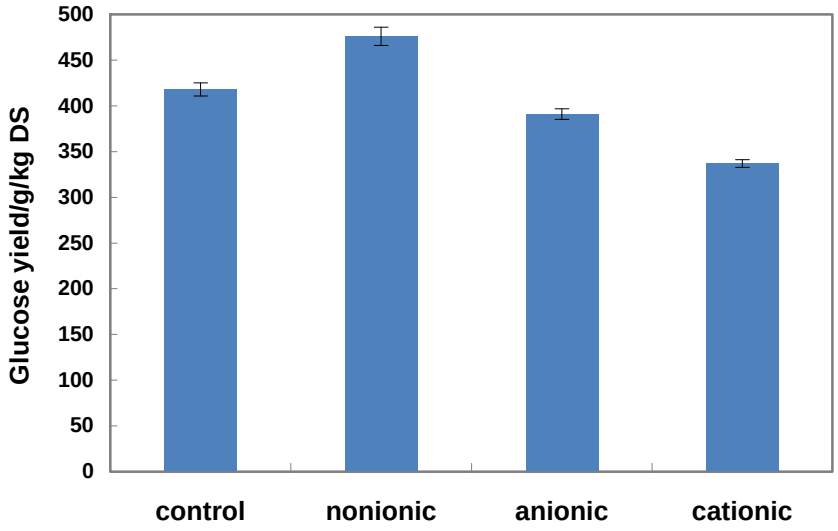

Fig. 2. Effect of surfactants on enzymatic hydrolysis

The glucose yields were 337 and 391 g/kg DS, which were 81 and 27 g/kg DS lower than control sample, respectively. Thus, nonionic surfactant was more suitable for enzymatic hydrolysis of lignocellulosics than cationic and anionic surfactants. This might be due to the fact that the cationic and anionic surfactants had a higher toxicity to enzymatic hydrolysis than the nonionic surfactant. Denaturation of enzymes was the probable cause for the decreased conversion when cationic and anionic surfactants were used.

Subsequently, the effect of different dosages of nonionic surfactant (Tween 80) on enzymatic hydrolysis of lignocellulosics (Fig. 3) was studied.

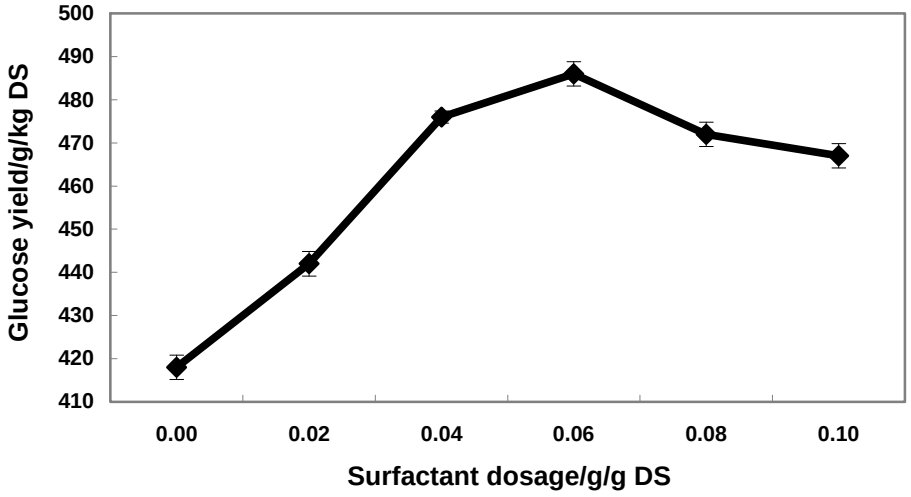

Fig. 3. Effect of different dosages of Tween 80 on enzymatic hydrolysis

As shown in Fig. 3, as the dosage of surfactant increased from 0 to 0.06 g/g DS, the glucose yield increased rapidly (from 418 to 486 g/kg DS). This was because when

surfactant dosage increased, more and more lignin was covered, and the ineffective combination between enzymes and lignin was reduced. As the dosage increased, more enzymes could attack cellulose, which led to a large amount of glucose being released from cellulose. When the dosage of surfactant came to 0.06 g/g DS, the glucose yield reached 486 g/kg DS, which was 68 g/kg DS higher than that of the control sample. However, when the dosage of surfactant exceeded 0.06 g/g DS, the glucose yield was reduced slightly (from 486 to 467 g/kg DS). This might have resulted from superfluous surfactant addition. Micelle can be expected to form by auto-agglomeration of the surfactant, which restrained the enzyme from contacting with the fibre, so the glucose yield was reduced. The optimum dosage of Tween 80 was 0.06 g/g DS.

CONCLUSIONS

1. This study revealed that organosolv pretreatment was an effective way to treat wheat straw for lignin solubilization; the lignin content was reduced from 17.74% to 7.32% of dry weight.
2. To produce high-concentration fermentable sugars, cellobiase and surfactant were added in the system of hydrolyzation. Results and analysis indicated that both additives had positive effects on enzymatic hydrolysis of lignocellulosics.
3. When cellobiase dosage was 1/10 of cellulase loading at a hydrolysis time of 60 h, it made it possible to achieve a glucose yield of 418 g/kg DS, which was 62 g/kg DS higher than that of the control sample. Furthermore, as 0.06 g/g DS Tween 80 was added into hydrolysate together with cellobiase, the glucose yield reached 486 g/kg DS.

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