Effect of Lignocellulosic Substrate and Commercial Cellulase Loading on Reducing Sugar Concentration for Ethanol Production

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Abstract: Fuel ethanol production from lignocellulosic biomass requires a relatively high initial sugar concentration in hydrolysate. Raising the substrate and enzyme concentration in the batch hydrolysis will normally help to heighten sugar concentration. In this study, dilute sulfuric acid pretreatment was used for chemical pretreatment of rice straw. The effect of rice straw and commercial cellulase (Accellerase 1000TM) loading were investigated. It was found that an increase in rice straw from 7.5 to 15.0 g/100 mL H₂SO₄ raised the reducing sugar concentration from 27.70 ± 1.72 to 58.42 ± 1.23 g/L. At the high level of rice straw up to 20.0 g/100 mL H₂SO₄ gave a slight increase in reducing sugar of 66.17 ± 0.00 g/L. On the contrary, the rice straw conversion decreased from 343.27 ± 1.29 to 286.22 ± 2.39 mg/g DS (Dry residue substrate) and holocellulose conversion slightly decreased from 47.02 ± 0.18 to 39.21 ± 0.33% (w/w) when the rice straw increased from 15.0 to 20.0 g/100 mL H₂SO₄. The reducing sugar concentration increased from 52.98 ± 0.73 g/L to 64.61 ± 0.53 g/L and rice straw conversion changed from 293.44 ± 5.12 to 414.16 ± 0.19 mg/g DS, when the use of Accellerase 1000TM increased from 10 to 45 FPU/g DS. It also found that the large amount of Accellerase 1000TM loading (50 and 55 FPU/g DS) did not improve the reducing sugar and rice straw conversion.

Key words: Rice straw, lignocellulosic biomass, dilute sulfuric acid pretreatment, enzymatic hydrolysis, commercial cellulase (Accellerase 1000TM), ethanol production.

1. Introduction

Thailand can produce rice more than 21-25 million tones to generate huge amount of rice straw about 63-67 million tones [1]. Farmers prefer to burn it after each harvesting season leading to an increase in pollution and carbon dioxide emissions [2]. Value added of rice straw will reduce the activity of rice straw burning. Rice straw is renewable resource. The main component is lignocellulose consisting of three major complexes. They are cellulose, hemicellulose and lignin which

can be processed either chemically or biologically to form biofuels such as bioethanol, biodiesel and methane [3].

In ethanol production from lignocellulosic materials, ethanol concentration in fermentation broth should be as high as possible in order to minimize the energy consumption in evaporation and distillation [4, 5], which requires a relatively high initial sugar concentration in hydrolysate. Raising the substrate and enzyme concentration in the batch hydrolysis helps to higher sugar concentration [5, 6].

In this research, dilute sulfuric acid was used as a pretreating chemical of rice straw. The effect of rice straw and cellulase enzyme loading were investigated.

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2. Materials and Methods

2.1 Rice Straw

Rice straw was obtained from Nakhon Pathom province, Thailand. Before chemical pretreatment it was passed through vegetable chopper to cut into small pieces (< 30.00 mm) and then sieved for size of 2.00-5.00 mm. The main composition of unpretreated rice straw consisted of 5.9% (w/w) of moisture, 38.8% (w/w) of cellulose, 26.9% (w/w) of hemicellulose, and 11.4% (w/w) of lignin.

2.2 Enzyme

Accellerase 1000TM (Genencor) used in this study was commercial products bought from Siam Victory Chemicals Limited. It contains filter paper activity of 265 FPU/mL at pH 4.8 and 50 °C [7].

2.3 Dilute Sulfuric Acid Pretreatment and Enzyme Hydrolysis

Dilute sulfuric acid pretreatment of rice straw of 2.00-5.00 mm was carried out in 125 mL duran bottle. The media consisted of 1.00% (w/v) H₂SO₄ and varied rice straw from 7.0 to 20.0 g/100 mL H₂SO₄ solution. Operating temperature by autoclave was at 121 °C for 15 min and then cooled down. The medium pH was adjusted to 5.0 with 80.0% (w/v) NaOH. The activity of Accellerase 1000TM was varied from 10 to 55 FPU/g Dry residue substrate. The reaction was conducted at 50 °C in shaking incubator at 160 rpm for 72 hrs. Aliquots of 1.0 mL were periodically taken and centrifuged. The supernatants were analyzed for reducing sugars.

2.4 Analytical Methods

The cellulose, hemicellulose and lignin contents were determined by the methods described by the Technical Association of Pulp and Paper Industry (TAPPI) [8-10]. The concentration of reducing sugar in the pretreatment and hydrolysate were analyzed by DNS method [11]. Ethanol was analyzed by Gas chromatography method (GC) with HP-INNOWAX

19091 N-133 column a length 30 m, outer diameter 0.251 mm and inner diameter 0.25 μ at column temperature increased with rate of 15 °C/min to 120 °C, inject temperature of 220 °C, carrier gas (Helium) flow rate of 50 mL/min and analysis by Flame-Ionized detector (FID).

The percentage of rice straw saccharification was calculated as follow:

Rice straw conversion developed from Sun et al.

$$[12], \text{Saha et al. [13]} = \frac{\text{Total reducing sugar (g)}}{\text{Dry rice straw (g)}}$$

Holocellulose conversion developed from Chen et

$$\text{al. [5]} = \frac{\text{Total reducing sugar (g)} \times 0.9 \times 100}{\text{Holocellulose (g)}}$$

Cellulose conversion developed from Chen et al. [5]

$$= \frac{\text{Reducing sugar from enzyme hydrolysis (g)} \times 0.9 \times 100}{\text{Cellulose (g)}}$$

All experiments were carried out in triplicate. The data were expressed as the mean values ± standard deviation (S.D.). The composition of rice straw or its hydrolysate residue was expressed on dry basis throughout this work.

3. Results and Discussion

3.1 Effect of Substrate Loading

Effects of rice straw loading on pretreatment and enzymatic hydrolysis were investigated as shown in Fig. 1. Reducing sugar concentration (g/L) highly increased as the initial rice straw was increased. When rice straw increased from 7.5 to 15.0 g/100 mL H₂SO₄, the reducing sugar increased from 27.70 ± 1.72 to 58.42 ± 1.23 g/L. At high rice straw contents of 20.0 g/100 mL H₂SO₄ the reducing sugar obtained was up to 66.17 ± 0.00 g/L. Although reducing sugar concentration would however increase, rice straw conversion (mg/g Ds) and holocellulose conversion slightly decreased when rice straw contents were over 15 g/100 mL H₂SO₄. The rice straw conversion

Fig. 1 Effect of rice straw concentration (7.5-20.0 g/100 mL H₂SO₄) on enzymatic hydrolysis (Acellerase 1000TM), 20 FPU/g Dry residue substrate at pH 5.0 in incubator shaker, 50 °C and 160 rpm for 72 hrs.

decreased from 343.27 ± 1.29 to 286.22 ± 2.39 mg/g Ds and the holocellulose conversion decreased from 47.02 ± 0.18 to $39.21 \pm 0.33\%$ (w/w), when rice straw was increased from 15.0 to 20.0 g/100 mL H₂SO₄. This may be a bad mixing between acid, enzyme, and rice straw.

Rice straw and Holocellulose conversions showed a similar variation trend as reducing sugar concentration (g/L) showed in the opposite (Fig. 1) corresponding to Chen et al. [5] and Wen et al. [14]. This shows that the hemicellulose and cellulose residues remain a lot after pretreatment and hydrolysis at high rice straw contents. This may come from the high rice straw content causing high viscosity in mixture leading to decrease in an efficiency of binding between sulfuric acid, cellulase enzyme and composition of rice straw.

However, final cellulose conversion (72 hrs of hydrolysis) showed slight difference when rice straw increased (Fig. 1). This shows that the efficiency of cellulase enzyme in order to convert cellulose to reducing sugar does not reduce, although mixture viscosity increases. The decrease in rice straw conversion and cellulose conversion may not come from enzyme hydrolysis. This may come from pretreatment with sulfuric acid. It can notice from

initial holocellulose conversion (0 hr of hydrolysis) in Fig. 2. When the rice straw increases, holocellulose conversion decreased particularly at higher rice straw concentration of 15 g/100 mL H₂SO₄. This may be cause of rice straw increased as unchanged sulfuric acid concentration. The acid concentration may not be enough to digest hemicelluloses of the rice straw. The pretreatment had no agitation leading to bad mixing between acid and rice straw.

The volume variation trend of solid residue showed the opposite to the hydrolysate when the rice straw increased. Solid residue after hydrolysis increased as the hydrolysate volume decreased. This may be a negative effect. There is low hydrolysis of holocellulose to other products such as xylose and glucose. The hydrolysate is absorbed by the rice straw residue. It is quite clear that conversion of rice straw to reducing sugar has low efficiency particularly at higher rice straw concentration of 15 g/100 mL H₂SO₄.

There was no difference in cellulose conversion during 72 hrs of hydrolysis time when initial rice straw contents increased (Figs. 1 and 2). At the first period of its hydrolysis between 12 and 24 hrs, there was obviously decrease in cellulose conversion as initial rice straw increased from 17.5 and 20.0 g/100 mL

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Fig. 2 Time course of enzyme hydrolysis at different rice straw contents (between 7.5-20.0 g/100 mL H₂SO₄), hydrolysis using Accellerase 1000™ content of 20 FPU/g Dry residue substrate at pH 5.0 in incubator shaker, 50 °C, 160 rpm.

H₂SO₄ (Fig. 2). The result shows a low efficiency of enzyme at high initial rice straw contents. The high viscosity of mixture leads to the decrease in an efficiency of binding between the cellulase enzyme and composition of rice straw.

Reducing sugar quickly increased in the first period. There was slightly increased in the reducing sugar when hydrolysis time was prolonged beyond 48 hours giving the cellulose conversion around 32% (w/w). The result shows that there is a small change for cellulose to reducing sugar. This may be due to the end-product feedback inhibition caused by high reducing sugar concentration [5, 14]. The result leads to a little increase in holocellulose and cellulose conversion (Fig. 2).

From above result, the rice straw content of 15 g/100 mL H₂SO₄ was optimal substrate concentration

because of highest reducing sugar concentration obtained, no change in Holocellulose and cellulose conversion when rice straw contents less than 15 g/100 mL H₂SO₄.

Biomass pretreatments with dilute acid 1.0% (w/v), most of them normally use solid-to-liquid ratio of less than 1:10 such as corncob pretreatment used solid-to-liquid ratio of 1:6 [5], wheat straw pretreatment used wheat straw/demineralised water in a ratio of 1/10 (g/g) [15], rye straw pretreatment used solid loading of 10% (w/w) [12] and oil palm empty fruit bunch (OPEFB) pretreatment used 1 g OPEFB fiber/8 g liquor on dry basis [16].

Although biomass pretreatment with dilute acid at lower concentration of 1%:10% (w/v), it would achieve high biomass conversion or giving high efficiency for digestion of hemicelluloses and lignin.

Reducing sugar concentration obtained was however low such as OPEFB pretreatment giving xylose concentration only 30 g/L [16] leading to achievement of low ethanol concentration. Ethanol production from wheat straw also obtained lower concentration of 20 g/L [13].

In this work, rice straw pretreatment with dilute acid at lower concentration of 1%:10% (w/v), the rice straw conversion achieved was also higher than the use of higher ratio. The reducing sugar achieved was however very low. It was not therefore suitable for ethanol production.

Rice straw pretreatment with dilute acid as ratio of rice straw: acid solution was over 1%:10% (w/v), rice straw conversion decreased. The reducing sugar concentration obtained however increased. In order to increase efficiency of rice straw pretreatment, some techniques such as agitation during pretreatment to make well mixing between acid and rice straw will be done in further work.

3.2 Effect of Enzyme Loading

As the cost of cellulase enzyme contributes significantly to the total cost of biomass conversion process, the cellulase dosage should be minimized as little as possible [5]. Hydrolysis experiments were

performed with 15.0 g/100 mL H₂SO₄ and different dosages of Accellerase 1000TM at pH 5.0, 50 °C, 160 rpm for 72 hrs. The results are shown in Fig. 3. Reducing sugar (g/L) and rice straw conversion (mg/g Ds) showed a similar variation trend, both of them sharply increased with the Accellerase 1000TM dosage varied from 10 to 45 FPU/g Dry residue substrate. The reducing sugar concentration increased from 52.98 ± 0.73 g/L to 64.61 ± 0.53 g/L as the rice straw conversion increased from 293.44 ± 5.12 to 414.16 ± 0.19 mg/g Ds. At a high Accellerase 1000TM dosage (50 and 55 FPU/g Dry residue substrate), it could not help to increase reducing sugar and rice straw conversion (Fig. 3). Cellulose conversion gradually increased as the Accellerase 1000TM dosage varied from 10 to 35 FPU/g Dry residue substrate. This may be caused from enzyme activity inhibited by the reducing sugar due to tiny the Accellerase 1000TM use. However, cellulose conversion sharply increased (Figs. 3 and 4) as the Accellerase 1000TM was up to 45 FPU/g Dry residue substrate. This may be no inhibition of reducing sugar because of enough enzyme presence.

However, holocellulose and cellulose conversion merely gave a half conversion. The highest holocellulose conversion was 58.54 ± 0.85% (w/w)

Fig. 3 Effect of enzyme loading (10-55 FPU/g Dry residue substrate) on rice straw (15 g/100 mL H₂SO₄) hydrolysis at pH 5.0 in incubator shaker, 50 °C, 160 rpm for 72 hrs.

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and the highest cellulose conversion was $46.52 \pm 1.48\%$ (w/w) at Accellerase 1000TM dosage of 50 and 45 FPU/g Dry residue substrate respectively.

Variation trend of solid residue was opposition to hydrolysate volume. When Accellerase 1000TM increased, solid residue after hydrolysis decreased as the hydrolysate volume increased. This was a positive effect. The decrease in solid residue suggests that holocellulose (hemicellulose + cellulose) is highly hydrolyzed to other products such as xylose and glucose. The increase in hydrolysis volume suggests that rice straw is highly digested. As good digestion of rice straw, then solution is well separated from rice straw leading to high hydrolysate volume.

The reducing sugar, holocellulose and cellulose conversions quickly increased in first period of hydrolysis as shown in Fig. 4. When hydrolysis time

prolonged beyond 24 hours, there was only a little increase in hydrolysis. This may be due to the end-product feedback inhibition caused by high reducing sugar concentration [5]. Change in reducing sugar and holocellulose conversion can be divided into 3 groups; the 1st group (Accellerase 1000TM used less than 10 FPU/g Dry residue substrate) gave the least of reducing sugar and holocellulose conversion, the 2nd group (Accellerase 1000TM used between 15-35 FPU/g Dry residue substrate) gave the medium of reducing sugar and holocellulose conversion, and the 3rd group (Accellerase 1000TM used more than 45 FPU/g Dry residue substrate) gave the highest reducing sugar and holocellulose conversion. Changing of cellulose conversion can be however divided into 2 groups; the 1st group the use of enzyme was less than 35 FPU/g Dry residue substrate, and the

Fig. 4 Time course of enzyme hydrolysis at rice straw contents of 15.0 g/100 mL H₂SO₄ and hydrolysis by using Accellerase 1000 contents of 10-55 FPU/g Dry residue substrate at pH 5.0 in incubator shaker, 50 °C, 160 rpm.

other the use of enzyme was more than 45 FPU/g Dry residue substrate. These results showed that the enzyme (Accellerase 1000TM) contents of 45 FPU/g Dry residue substrate was the optimal enzyme loading.

Optimum enzyme in this research was rather high comparing to other works (e.g. the optimum enzyme of corncob hydrolysis was only 20 FPU/g substrate [5], animal manure hydrolysis was 13 FPU/g substrate [14] and rye straw hydrolysis was 25 FPU/g substrate [12]. This may be cause from high rice straw loading (15 g/100 mL H₂SO₄) leading to high viscosity. Therefore enzyme had to be high. In order to minimize enzyme used, pretreatment development at high rice straw use will be however done for our further work.

In this research we tried to achieve the maximum reducing sugar concentration. However, reducing sugar obtained only 64.61 ± 0.53 g/L. Half of reducing sugar obtained from pretreatment was low quality for ethanol production. The low concentration of ethanol may be not valuable enough for energy consumption in distillation.

This study could be a guideline for an increase in reducing sugar from rice straw. Severe conditions for pretreatment and some technique (e.g., an increase in mixing during pretreatment may enhance a pretreatment efficiency) could lead to lower enzyme use for further work.

4. Conclusion

Reducing sugar concentration (g/L) highly increased as the initial rice straw was increased. When rice straw increased from 7.5 to 15.0 g/100 mL H₂SO₄, the reducing sugar increased from 27.70 ± 1.72 to 58.42 ± 1.23 g/L. At high rice straw contents of 20.0 g/100 mL H₂SO₄, the reducing sugar obtained was up to 66.17 ± 0.00 g/L.

At high rice straw contents, rice straw and Holocellulose conversions showed a similar variation trend as reducing sugar concentration (g/L) showed in the opposite. The rice straw conversion decreased

from 343.27 ± 1.29 to 286.22 ± 2.39 mg/g Ds and the holocellulose conversion decreased from 47.02 ± 0.18 to 39.21 ± 0.33% (w/w), when rice straw was increased from 15.0 to 20.0 g/100 mL H₂SO₄.

Reducing sugar (g/L) and rice straw conversion (mg/g Ds) showed a similar variation trend, both of them sharply increased with the use of increasing Accellerase 1000TM. When the Accellerase 1000TM dosage varied from 10 to 45 FPU/g Dry residue substrate, the reducing sugar concentration increased from 52.98 ± 0.73 g/L to 64.61 ± 0.53 g/L as the rice straw conversion increased from 293.44 ± 5.12 to 414.16 ± 0.19 mg/g Ds.

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