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ORIGINAL PAPER

Whole slurry saccharification and fermentation of maleic acid-pretreated rice straw for ethanol production

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Abstract We evaluated the feasibility of whole slurry (pretreated lignocellulose) saccharification and fermentation for producing ethanol from maleic acid-pretreated rice straw. The optimized conditions for pretreatment were to treat rice straw at a high temperature (190 °C) with 1 % (w/v) maleic acid for a short duration (3 min ramping to 190 °C and 3 min holding at 190 °C). Enzymatic digestibility (based on theoretical glucose yield) of cellulose in the pretreated rice straw was 91.5 %. Whole slurry saccharification and fermentation of pretreated rice straw resulted in 83.2 % final yield of ethanol based on the initial quantity of glucan in untreated rice straw. These findings indicate that maleic acid pretreatment results in a high yield of ethanol from fermentation of whole slurry even without conditioning or detoxification of the slurry. Additionally, the separation of solids and liquid is not required; therefore, the economics of cellulosic ethanol fuel production are significantly improved. We also demonstrated whole slurry saccharification and fermentation of pretreated lignocellulose, which has rarely been reported.

Keywords Lignocellulose · Pretreatment · Maleic acid · Ethanol · Whole slurry fermentation

Introduction

The production of biofuels or chemicals from lignocellulose remains economically unfeasible, mainly because of the high costs associated with producing sugars from lignocellulose [1, 2]. Lignocellulose is mainly composed of cellulose surrounded by hemicellulose and lignin. The interactions between cellulose, hemicellulose, and lignin are strong, protecting lignocellulose from chemical and microbial attack, making it difficult for saccharification to proceed [3]. Acidic pretreatment of lignocellulose is often used prior to its enzymatic hydrolysis [4]. At a high temperature, acidic pretreatment releases various compounds from lignocellulose, including xylose, mannose, galactose, glucose, and acetic acid [5]. In addition, through the dehydration of sugar monomers, pentose and hexose sugars are converted into 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF), respectively [6, 7]. These compounds are further converted into organic acids, such as formic acid from furfural and HMF and levulinic acid from HMF [4]. These byproducts of pretreatment are typical fermentation inhibitors and need to be detoxified or eliminated before the fuel production step [8, 9].

Most efforts at cellulosic ethanol production have been made using washed pretreated lignocellulose [10–12]. During the washing step, all the inhibitors generated during the pretreatment process are removed. Therefore, for more realistic industrial applications of cellulosic ethanol, the use of unwashed pretreated lignocellulose slurry needs to be evaluated. There are few published studies regarding whole slurry saccharification and fermentation of pretreated lignocellulose for ethanol production [13, 14]. In these studies, a detoxification process using dilute sulfuric acid was required [13]. Additionally, pretreatment with maleic acid was not sufficiently effective for lignocellulose with a high lignin

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content [14]. In this study, saccharification and fermentation, using pretreated lignocellulose as whole slurry, were applied to rice straw. After pretreatment with maleic acid at a high temperature, pretreated rice straw was tested as a substrate for ethanol production using cellulase and *Saccharomyces cerevisiae*. We sought to determine the feasibility of whole slurry processing without the need for solid/liquid (S/L) separation, detoxification steps, or removal of pretreatment inhibitors with the overall aim of improving the process economics of cellulosic fuel production.

Materials and methods

Lignocellulosic biomass and its compositional analysis

Rice straw harvested from Yeonggwang, Korea, in 2011 was used as the lignocellulosic biomass in this study. Rice straw was washed with tap water, air-dried, milled using a cutting mill (MF 10; IKA, Staufen, Germany), and sieved to give particles of 90–1000 μm. Carbohydrates, acid-insoluble lignin, ash in the solid fraction, along with sugars and their degradation byproducts in the liquid fraction after pretreatment were analyzed following the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL; Golden, CO, USA) [15–18]. An Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) was used to quantify sugar monomers. High performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldronn, Germany) employing an HPX-87H column (Bio-Rad) was used to measure levels of furfural, HMF, acetic acid, formic acid, levulinic acid, glycerol, and ethanol as previously described [1].

Acidic pretreatment of rice straw

Ground dry rice straw (2 g) was suspended in 20 mL of maleic acid at various concentrations (0–2 %, w/v) in 100-mL vessels (SK-12 type; Milestone, Shelton, CT, USA) equipped with a thermocouple. The mixture of rice straw and maleic acid was allowed to digest for various holding times after 3 min ramping to a set temperature in an ETHOS EZ microwave oven (Milestone). Insoluble solids that separated from pretreated slurry were washed and filtered through a filter cloth (22–25 μm pore size; Calbiochem, La Jolla, CA, USA) with approximately 1 L of distilled water until the pH of the filtrate was 6–7. A portion of the representative insoluble solids was transferred to aluminum dishes and dried at 45 °C for 3 days for subsequent analysis of biomass composition. The remaining insoluble solids were stored at −20 °C until required. For whole slurry fermentation, the solid and liquid fractions of the pretreated slurry were not separated.

Enzymatic digestibility of pretreated rice straw

To evaluate the effectiveness of pretreatment, pretreated and washed rice straw was enzymatically hydrolyzed using 15 filter paper units (FPU) of Accellerase 1000 (Genencor, Rochester, NY, USA) per g glucan in pretreated and washed rice straw. By the definition of FPU, 0.1875 FPU of cellulase in a 0.5 mL assay aliquot is supposed to liberate 20 mg of glucose from 50 mg of filter paper in 60 min at 50 °C at pH 4.8 [19]. Pretreated or untreated rice straw at a final glucan concentration of 1 % (w/v) was added to 10 mL of 0.05 M sodium citrate buffer (pH 4.8) following the LAP of the NREL [20]. Enzymatic digestibility was expressed as the percentage of the theoretical maximum yield of glucose produced from the initial biomass. To determine the statistical significance of differences among experimental variables, one-way analysis of variance (ANOVA) in conjunction with the post hoc least significant difference test was performed using Statistica version 7.1 (StatSoft, Tulsa, OK, USA).

Simultaneous saccharification and fermentation (SSF) of rice straw

We used SSF to produce ethanol from untreated or pretreated rice straw. A final glucan concentration of 3 % (w/v) for untreated or pretreated washed rice straw was used following the NREL protocol for the SSF of lignocellulose [21]. A total biomass concentration of 6 % (w/v) based on untreated rice straw was used for whole slurry fermentation. For whole slurry SSF of pretreated rice straw, the pH of the SSF medium was adjusted to 4.8 (±0.2) using 28 % (w/w) ammonia solution (Junsei Chemical, Tokyo, Japan). After autoclaving the SSF media at 121 °C for 20 min, 15 FPU/g glucan of Accellerase 1000 was added to the SSF medium. The SSF medium was then inoculated with 5 % (v/v) *S. cerevisiae* D_5A (ATCC 200062) that had been growing in YPD medium [1 % (w/v) yeast extract, 2 % (w/v) peptone, 2 % (w/v) glucose]. SSF was conducted for 60 h in a flask with a needle-pierced silicone stopper to vent CO_2 produced during fermentation; flasks were incubated at 38 °C and 170 rpm. Ethanol yields were expressed as the percentage theoretical maximum based on the glucan content in the initial biomass before pretreatment.

Results and discussion

Effects of pretreatment temperature and time on the enzymatic digestibility of rice straw
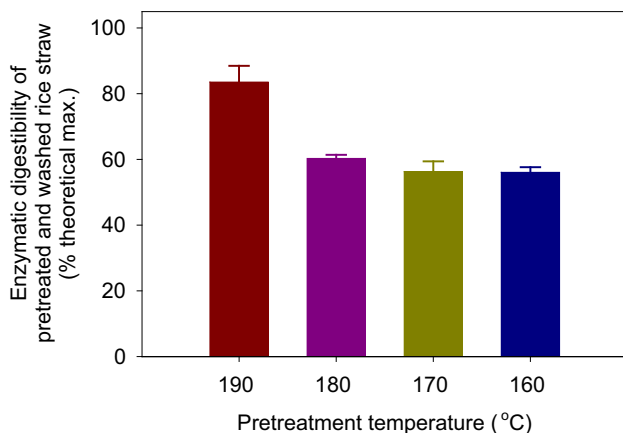
The effects of pretreatment temperature on the enzymatic digestibility of rice straw were investigated at 160–190 °C.

The concentration of maleic acid was 1 % (w/v), and solids loading was 10 % (w/v) with 3 min ramping to the desired temperature and a 90 s hold (Fig. 1). Upon increasing the temperature from 180 to 190 °C, a significant increase in enzymatic hydrolysis yield from 60.3 to 83.5 %, was observed with a 99 % confidence level. This can be attributed to the increased rate of chemical reactions at a higher temperature as previously demonstrated [13, 14]. Pretreatment at 190 °C resulted in the greatest levels of digestibility. Because of limitation in the equipment used, a further increase in temperature, beyond 190 °C, was not possible. Therefore, 190 °C was selected as the optimum temperature for pretreatment.
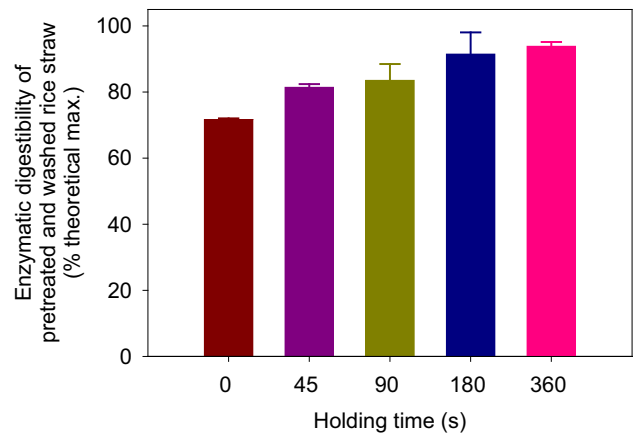
Pretreatment of rice straw over different holding times was performed after ramping for 3 min to 190 °C with a solids loading of 10 % (w/v) and 1 % (w/v) maleic acid (Fig. 2). A holding time of 180 s resulted in an increase of enzymatic digestibility from 71.7 to 91.5 %. However, a further increase in holding time to 360 s did not significantly increase enzymatic digestibility. Therefore, a 180-s holding time was selected as the optimal pretreatment time.

Effects of maleic acid concentrations on the enzymatic digestibility of rice straw

The optimal maleic acid concentration for the pretreatment of rice straw was determined by varying the maleic acid concentration from 0.1 to 2.0 % (w/v). Solid loading was 10 % (w/v) for a 3 min holding time with 3 min ramping to 190 °C (Fig. 3). Enzymatic digestibility of 91.5 % was obtained when the concentration of maleic acid was 1.0 %
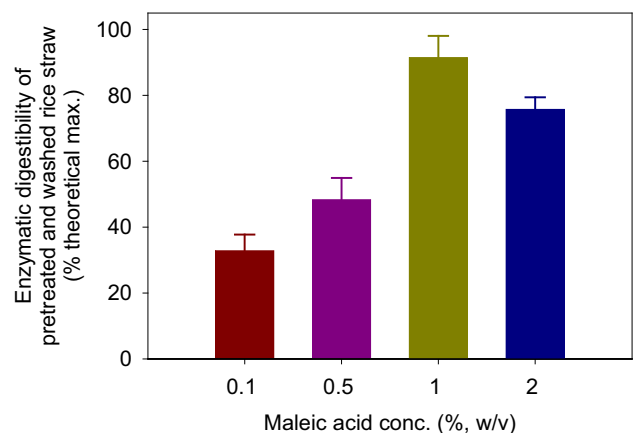

Fig. 2 Effects of pretreatment time on enzymatic digestibility. Pretreatment was conducted using 1 % (w/v) maleic acid and solids loading of 10 % (w/v) for different holding times after 3 min ramping to 190 °C. Enzymatic hydrolysis was conducted using 15 FPU of Accellerase 1000/g glucan at pH 4.8, 50 °C, and 200 rpm for 50 h

(w/v). In previous studies, the optimal concentrations of maleic acid for the hydrolysis of cellobiose and wheat straw were 0.6 and 0.5 % (w/v) at 160 and 170 °C, respectively [22, 23]. In another study, the optimal maleic acid concentration for pretreatment of oil palm empty fruit bunches (EFB) was 1 % (w/v) [14].

Compositional analysis of rice straw

Untreated rice straw was composed of 35.8 % glucan, 11.3 % xylan, 3.3 % galactan, 3.4 % arabinan, 18.1 % acid insoluble lignin, and 11.9 % ash on a dry weight basis (Table 1). This composition was comparable to that of rice

Fig. 1 Effects of pretreatment temperature on enzymatic digestibility. Pretreatment was conducted using 1 % (w/v) maleic acid, a solid loading of 10 % (w/v), and a 90 s hold with 3 min ramping to the set temperature. Enzymatic hydrolysis was conducted using 15 FPU of Accellerase 1000/g glucan at pH 4.8, 50 °C, and 200 rpm for 50 h

Fig. 3 Effects of maleic acid concentration on enzymatic digestibility. Pretreatment was conducted using various concentrations of maleic acid, solid loading of 10 % (w/v), and a 3 min hold with 3 min ramping to 190 °C. Enzymatic hydrolysis was conducted using 15 FPU of Accellerase 1000/g glucan at pH 4.8, 50 °C, and 200 rpm for 50 h

Table 1 Biomass composition of untreated and maleic acid-pretreated rice straw

	Pretreated[a]	Untreated
Component from insoluble solids (g per 100 g rice straw before pretreatment)[b]		
Glucan	30.7 ± 0.5	35.8 ± 1.5
Xylan	1.9 ± 0.5	11.3 ± 0.4
Galactan + arabinan	0.8 ± 0.5	6.6 ± 0.5
Lignin	10.7 ± 0.2	18.1 ± 1.3
Ash	8.8 ± 0.4	11.9 ± 0.8
Component from dissolved solids (g per 100 g rice straw before pretreatment)[b]		
Glucose	5.9 ± 0.1	NA
Xylose + galactose + arabinose	13.8 ± 0.4	NA
Acetic acid	1.2 ± 0.2	NA
Furfural	0.6 ± 0.2	NA
Glycerol	0.4 ± 0.1	NA

Sugar masses are expressed with respect to its monomeric form. Values presented are the mean ± standard deviation

NA not applicable

[a] Pretreatment conditions were 3 min ramping to 190 °C with a 3 min holding time, 1 % (w/v) maleic acid, and 10 % (w/v) solid loading

[b] The recovery yield of insoluble solids after pretreatment was 53.2 % (w/w)

straw used in other studies [11, 24]. After rice straw was pretreated at the optimal conditions we determined, insoluble solids recovery yields were 53.2 % (w/w). Approximately, 100 % of mass balance closure was obtained for glucan, including glucose in the dissolved solids after maleic acid pretreatment. Most of the glucan was retained in the insoluble solids of pretreated rice straw (Table 1). Unlike glucan, most hemicellulose (85.7 %) was solubilized in the liquid fraction of pretreated rice straw. Therefore, the dissolved sugars in the liquid phase should be utilized. According to a previous study, maleic acid pretreatment of wheat straw resulted in nearly 100 and 90 % recovery of glucan and xylan, respectively, from solids and liquid [22]. These previous results, taken together with our results, indicate higher recovery and lower degradation of sugars for maleic acid pretreatment compared with sulfuric acid pretreatment [13]. Moreover, 40.6 % of lignin was removed from rice straw after maleic acid pretreatment. This degree of delignification of rice straw was much higher than that from EFB pretreated using maleic acid (27.4 %). This is possibly because rice straw is less complex, with weaker interactions between lignocellulose components than EFB. Maleic acid pretreatment resulted in lower levels of byproducts, such as acetic acid and furfural, compared with those generated when sulfuric acid was used during pretreatment [25]. This can mainly be attributed to the catalytic nature of maleic acid, which is more favorable for the hydrolysis of cellulose or hemicellulose, as opposed to monomer degradation [23, 26–28].
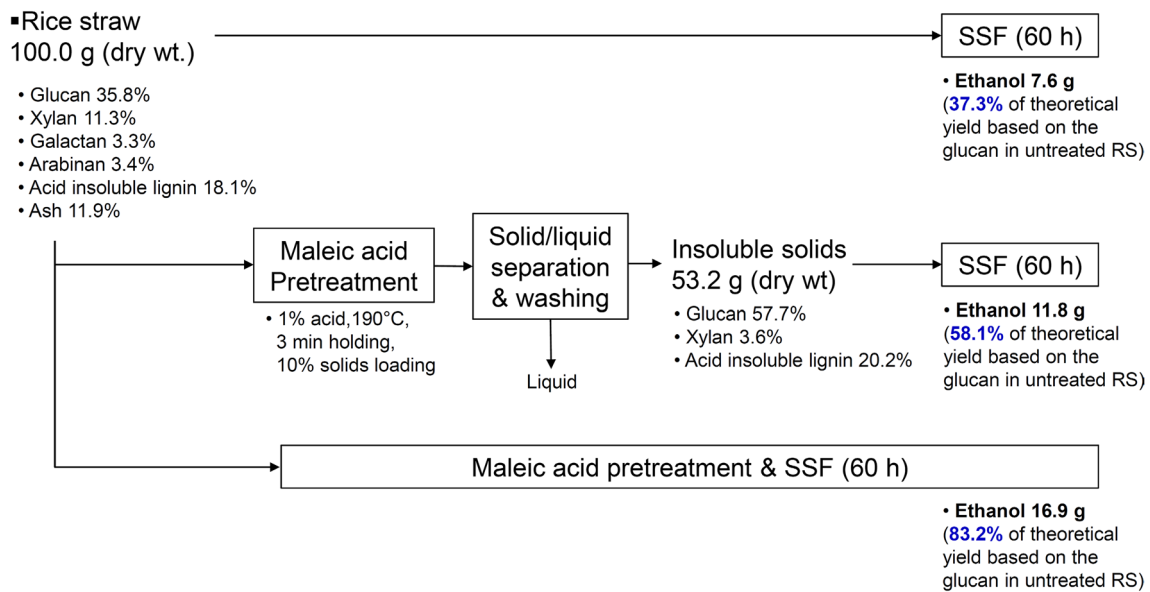
Whole slurry SSF of maleic acid-pretreated rice straw

To compare the fermentability of the pretreated whole slurry of rice straw with pretreated washed solid rice straw, SSF was conducted (Fig. 4). Ethanol yields were expressed as the percentage of the theoretical maximum yield of ethanol based on total glucan content in the untreated biomass. Given that ethanol production was determined on the basis of untreated biomass, yields were low when only pretreated washed biomass was used in SSF. Ethanol yields after 60 h were 58.1 and 37.3 % for pretreated washed and untreated rice straw, respectively. Use of whole slurry in SSF resulted in an ethanol yield of 83.2 %, 1.4-fold higher than that for pretreated washed rice straw (Fig. 4). This increase in ethanol yield was due to the increased use of glucan and glucose in the solid and liquid fractions of the pretreated whole slurry.

Based on the results obtained from this study, untreated rice straw (100 g) produces 7.6 g of ethanol by SSF, based on total glucan content in the initial biomass (Fig. 5). When solid rice straw is pretreated with maleic acid, 11.8 g of ethanol can be obtained from 100 g of biomass. However, when SSF is carried out using a whole slurry of pretreated rice straw without S/L separation, 16.9 g of ethanol is produced from 100 g of rice straw.
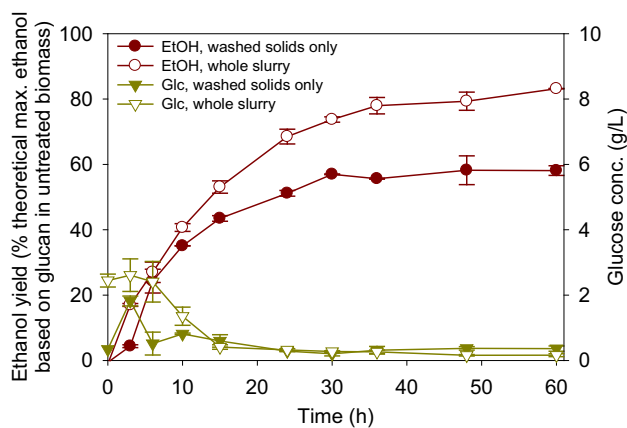
Fig. 4 Simultaneous saccharification and ethanol fermentation (SSF) of pretreated rice straw. In the SSF media, 3 % (w/v) glucan for untreated or pretreated washed rice straw was used. A total biomass concentration of 6 % (w/v) based on untreated rice straw was delivered for SSFs of the whole slurry of the maleic acid-pretreated rice straw. For SSF, rice straw was incubated with *S. cerevisiae* D$_5$A and 15 FPU of Accellerase 1000/g of glucan at pH 4.8, 38 °C, and 170 rpm for 60 h

Fig. 5 Mass balance for the conversion of rice straw into ethanol using whole slurry or washed solids of pretreated rice straw. Rice straw was pretreated with 1 % (w/v) maleic acid at 190 °C for 3 min with 10 % solids loading. Pretreated rice straw was hydrolyzed with 15 FPU Accellerase 1000/g glucan and fermented with *S. cerevisiae* D$_5$A at 38 °C and 170 rpm for 60 h

In conclusion, maleic acid pretreatment of rice straw to generate a whole slurry can be effectively used in SSF without S/L separation. SSF of the whole slurry of maleic acid-pretreated rice straw resulted in an 83.2 % theoretical ethanol yield, which was higher than that when untreated rice straw was used. Therefore, whole slurry SSF using maleic acid-pretreated rice straw is a viable alternative for the ethanol production industry. It is more economical to use this whole slurry because S/L separation is not required, detoxification is not necessary, and the utilization of available sugars in the pretreated lignocellulosic biomass is maximal.

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