Bioprocess Biosyst Eng (2014) 37:1337–1344
DOI 10.1007/s00449-013-1106-0

ORIGINAL PAPER

Study of chemical pretreatment and enzymatic saccharification for producing fermentable sugars from rice straw

Wen-Hsing Chen · Yi-Chun Chen · Jih-Gaw Lin

Received: 26 July 2013 / Accepted: 29 November 2013 / Published online: 18 December 2013

Abstract This study evaluated a cost-effective approach for the conversion of rice straw into fermentable sugars. The composition of rice straw pretreated with 1 % sulfuric acid or 1 % sodium hydroxide solution was compared to rice straw with no chemical pretreatment. Enzymatic saccharification experiments on non-pretreated rice straw (NPRS), pretreated rice straw (PRS), and pretreated rice straw with acid hydrolysate (PRSAH) were conducted in a series of batch reactors. The results indicated that pretreating the rice straw with dilute acid and base increased the cellulose content from 38 % to over 50 %. During enzymatic saccharification, straight aliphatic cellulose was hydrolyzed before branched hemicellulose, and glucose was the major hydrolysis product. The glucose yield was 0.52 g glucose/g for NPRS and was comparable to the yields of 0.50 g glucose/g for PRS and 0.58 g glucose/g for PRSAH. The hydrolysis of rice straw to produce glucose can be described by a first-order reaction with a rate constant of 0.0550 d^{-1} for NPRS, 0.0653 d^{-1} for PRSAH, and 0.0654 d^{-1} for PRS. Overall, the production of fermentable sugars from ground rice straw will be more cost effective if the straw is not pretreated with chemicals.

W.-H. Chen (✉)
Department of Environmental Engineering, National Ilan University, Yilan 260, Taiwan
e-mail: albert@niu.edu.tw

Y.-C. Chen · J.-G. Lin (✉)
Institute of Environmental Engineering, National Chiao Tung University, Hsinchu 30010, Taiwan
e-mail: jglin@mail.nctu.edu.tw

Y.-C. Chen
e-mail: laura40511.ev98g@nctu.edu.tw

Keywords Process integration · Chemical pretreatment · Rice straw · Glucose · Cellulase · Enzymatic saccharification

Introduction

Rice is a major crop in Taiwan. The country has 254,590 ha of planted rice fields, with an annual harvest of 6.2 tons/ha. The harvest of rice leads to the production of a significant quantity of rice straw that is mainly composed of carbohydrates. If adequately processed biologically, these carbohydrates may offer a cost-effective and environmentally friendly renewable resource for sustainable biofuels. The fermentation of carbohydrates for ethanol production is a commercial biotechnology currently used in industrial applications [1–4]. In addition to ethanol fermentation, acetone–butanol–ethanol (ABE) fermentation technology was also developed in the early 20th century [5–8].

Rice straw is composed of complex carbohydrates, including cellulose, hemicellulose, and lignin. Due to its structural complexity, rice straw is not typically directly fermented for biofuel production without pretreatment and saccharification processes to convert the polysaccharides into monosaccharides. This pretreatment functions to reduce the size of feedstock, to open up the hemicellulose–lignin matrix that surrounds the cellulose, and to break the cellulose crystal structure [9]. The chemical alteration makes cellulose and hemicellulose more accessible to enzymes that disrupt carbohydrate polymers into fermentable sugars [10]. Different pretreatments have been used to treat rice straw, including biological treatments, physical treatments, and chemical processes. For instance, the treatment of rice straw with *Cyathus stercoreus* TY-2

resulted in a five-fold increase in the yield of enzymatic saccharification as compared to that in the absence of the fungus [11]. This strain of fungus required 25 days to achieve this performance. For physical pretreatment, Hideno et al. [12] reported that wet disk milling is more effective than dry ball milling and hot-compressed water pretreatments in terms of glucose and xylose yields, inhibitor production, and energy consumption. Their results indicated that the energy input was significantly reduced when using wet disk milling to treat rice straw. However, energy consumption is always a pivotal parameter driving the economic feasibility of physical pretreatment.

Chemical pretreatment does not require substantial energy input but does require the addition of chemicals. Chemical pretreatment can efficiently modify the crystalline matrix of lignocelluloses to an amorphous structure. Among various chemical pretreatments, ionic liquids are chemical compounds used to dissolve cellulose from lignocellulosic materials [13]. A recently published report found that the glucan in 1-butyl-3-methylimidazolium acetate ([BMIM][OAc])-treated rice straw is completely converted to glucose during enzymatic saccharification [14]. However, the high cost of ionic liquids is a disadvantage impeding large-scale applications. However, acid or base pretreatment is more cost effective than ionic liquid pretreatment. Dilute acid pretreatment is a proven chemical method that can significantly improve the hydrolysis of cellulose [2, 15]. Hsu et al. [16] found that the dilute acid pretreatment will increase the pore volume of solid rice straw residue by two fold, resulting in a 20 % increase in the glucose yield. This increase in glucose yield was also observed by Ranjan and Moholkar [17]. In addition, dilute base pretreatment can accomplish the same results as dilute acid pretreatment, which increases the internal surface area of the biomass, decreases the polymerization and crystallinity of the cellulose, and disrupts the lignin structure [10]. Furthermore, dilute base pretreatment removes lignin more efficiently than dilute acid pretreatment [18].

During subsequent enzymatic saccharification, cellulase and hemicellulase break down cellulose and hemicellulose into five- and six-carbon sugars. Cellulase contains endoglucanase, exoglucanase, and β-glucosidase to initiate the degradation of cellulose into glucose; hemicellulase hydrolyzes hemicellulose to release pentose, hexose, and uronic acids. The activity of the enzymes is affected by the incubation pH and temperature. Abedinifar et al. [1] reported that the maximum activity of a commercial cellulase enzyme from *Trichoderma reesei* was achieved at pH 5 when the temperature was maintained at 50 °C. Currently, enzymatic saccharification coupled with the dilute acid/base pretreatment has been widely used to convert agriculture residues into fermentable sugars. Glucose yields of 0.6–0.8 g/g of glucan have been reported from rice straw, 0.3–0.4 g/g from bagasse, and 0.35–0.55 g/g from silver grass [2]. Pretreatment with a dilute acid/base can lead to the production of furfural and hydroxymethyl furfural (HMF) [19, 20], which might inhibit enzymatic saccharification. Therefore, the practice of pretreating rice straw before it is subject to the saccharification process must be re-evaluated based on necessity and cost effectiveness. This study was initiated to examine a cost-effective approach for the conversion of rice straw into fermentable sugars. The pretreatment of rice straw with a dilute acid or base was evaluated by examining the effectiveness of changing the composition of the treated rice straw. Kinetic analyses of the sugar production from non-pretreated rice straw (NPRS), pretreated rice straw (PRS), and pretreated rice straw with acid hydrolysate (PRSAH) were performed to determine the potential benefit of integrating pretreatment into the saccharification process.

Materials and methods

Raw materials

Rice straw was supplied by the Hsinchu County Department of Agriculture, Taiwan. The raw material was milled through 30 mesh sieves for an average size of approximately 0.2–0.4 mm. Then, the rice straw was dried in an oven at 105 °C to ensure a consistent weight before it was used for the subsequent acid or base pretreatment.

Dilute acid/base pretreatment

The dry rice straw (DRS) was added to 1 % dilute sulfuric acid for the dilute acid pretreatment and a 1 % sodium hydroxide solution for the dilute base pretreatment. Two sets of samples were prepared, i.e., 2.4 and 10 %, which were defined as the weight of the DRS mass (in grams) soaked in 1 L of a 1 % acid or base solution. The samples were then autoclaved at 121 °C for 30 min. After cooling to room temperature, the samples were immediately filtered to separate the solid and liquid portions. The solid portion was washed with distilled water several times and then oven dried at 105 °C.

Three types of samples, NPRS, PRS, and PRSAH, were prepared for the enzymatic saccharification experiments. Five grams of DRS was used to make one of the three types of samples. NPRS was DRS without chemical pretreatment. PRS was DRS pretreated with a 1 % sulfuric acid solution. The acid hydrolysate was discarded, and the solid portion was washed using distilled and deionized (DI) water several times until the final pH exceeded 4. To prepare PRSAH, DRS was subjected to the same acid pretreatment.

However, the acid hydrolysate was not removed from the solid portion; the pH of PRSAH was adjusted to 5 by adding a 5 N NaOH solution. All samples were oven dried at 105 °C before saccharification treatment.

Enzymatic saccharification

The enzymatic saccharification experiments were conducted in a series of 500 mL serum bottles under sterile condition. The prepared samples were placed in the serum bottles containing 250 mL of an acetate buffer solution with the initial pH controlled at 5.0 ± 0.1 using a sodium hydroxide or hydrochloric acid solution. Each liter of the acetate buffer solution consisted of 357 mL of 0.1 M acetic acid (UN2789, Scharlau, Spain) and 643 mL of 0.1 M sodium acetate (J.T. Baker 3470, Avantor, USA). Cellulase, hemicellulase, and cellobiase purchased from Sigma-Aldrich were used for enzymatic saccharification. Table 1 lists the major characteristics of the enzymes made by Sigma-Aldrich. The enzymatic saccharification experiments were performed with different levels of enzyme loadings (Table 2). These enzyme loadings were determined based on our preliminary tests. The loaded serum bottles were incubated in a shaker at 50 ± 1 °C and 2.8 Hz. The samples to examine enzymatic saccharification were withdrawn every 24 h. All experiments were performed in duplicate.

Analytical methods

Solid content was measured according to the standard methods for the examination of water and wastewater [21]. The cellulose, hemicellulose, lignin, and ash content in the rice straw were determined based on the methods proposed by Van Soest et al. [22]. Hexose and pentose were analyzed using high-pressure liquid chromatography (HPLC) equipped with a carbohydrate analysis column (3.9 × 300 mm, Waters), pump (Hitachi L-2130), and refractive index detector (Waters 410). The method has been described elsewhere with some modifications [15, 16]. The analysis was performed using an 80 % acetonitrile solution as an eluent at a flow rate of 1 mL/min with the temperature controlled at 35 °C. Before injection into the HPLC, the samples were diluted two fold with acetonitrile and then filtered through 0.45 μm syringe filters (30416250, Advantec, Japan) composed of mixed cellulose esters.

Data analysis

The modified Gompertz equation, Eq. (1), which is a sigmoid function, has been successfully used to delineate the kinetic analyses of biohydrogen production [23] and biobutanol production [24, 25] experiments. In this study, the cumulative glucose production curves with respect to time were obtained first from the enzymatic saccharification experiments. Then, the modified Gompertz equation was employed to determine the glucose production potential, glucose production rate, and lag phase.

$$G(t) = G \cdot \exp\left\{-\exp\left[\frac{R_g \cdot e}{G}(\lambda - t) + 1\right]\right\} \quad (1)$$

where $G(t)$ is the cumulative glucose production (g) at time t, λ is the time of lag phase (h), G is the glucose production potential (g), R_g is the glucose production rate (g/d), and e is exp(1), i.e., 2.71828.

In this study, curves relating sugar production to time as obtained from the enzymatic saccharification experiments were fitted using the linear form of the first-order kinetics (Eq. 2) to determine the rate constant (k).

$$\log C_t = -kt + \log C_i \log C_t = kt + \log C_i \quad (2)$$

Table 1 Characteristics of cellulase, hemicellulase, and cellobiase

Enzymes	Cellulase from *Aspergillus niger*	Hemicellulase from *Aspergillus niger*	Cellobiase from *Aspergillus niger*
Synonym	1,4-(1,3:1,4)-β-D-Glucan 4-glucanohydrolase	–	Novozyme 188
Brand (product number)	Sigma (C1184)	Sigma (H2125)	Sigma (C6105)
Density (g/mL)	–	–	~1.2
Unit definition	One unit will liberate 1.0 μmol of glucose from cellulose in 1 h at pH 5.0 and 37 °C (2 h incubation time).	One unit will produce a relative fluidity change of 1 per 5 min using locust bean gum as a substrate at pH 4.5 and 40 °C.	One unit is defined as 2 μmol of glucose produced per minute at pH 5 and 40 °C.
Activity (Unit/g enzyme)	1,400	1,500	≥250

Table 2 Experimental designs for enzymatic saccharification and the corresponding sugar productions

Experiments	Materials	pH/Temp. (°C)	Enzyme loading			Agitation (Hz)	Incubation[a] time (h)	Sugar productivity (mmol/L/h)	Yield (g sugar/g PRS)
			Cellulase (kU/g DRS)	Hemicellulase (kU/g DRS)	Cellobiase (kU/g DRS)				
Enzyme loading test	PRS	5/50	0.14	0.14	0.36	2.8	165	0.16	0.20
			0.28	0.28	0.72		171	0.27	0.27
			0.56	0.56	1.44		171	0.41	0.47
			1.93	1.93	4.97		168	1.28	0.94

PRS Pretreated rice straw, *DRS* dry rice straw

[a] Time to achieve the maximum sugar concentration during the saccharification experiments

where C_i represents the initial total sugar concentration (mol/L), C_t is the total sugar concentration (mol/L) at time t, and k is the rate constant of sugar production (d^{-1}).

Results and discussion

Rice straw composition

The results of the laboratory analyses indicated that the rice straw used in this research contained 38 % cellulose, 35 % hemicellulose, 7 % lignin, and 4 % ash, adding up to 84 % of the total mass of straw analyzed. The observation that 16 % of the total mass was non-measurable is consistent with a report by Abedinifar et al. [1]. A comparison of the composition of other lignocellulosic biomasses reported in the literature is summarized in Table 3. Cellulose and hemicellulose are the main components of the rice straw, whereas lignin accounts for only a small portion of the total mass. Rice straw contains a considerably smaller portion of lignin than wheat straw, corn cob, bagasse, or silver grass. In plant tissues, lignin surrounds cellulose microfibrils and strengthens the cell wall; therefore, pretreatment with a dilute acid or base will loosen the structure of the lignocellulosic biomass to facilitate hydrolysis [10]. Due to its low lignin content, rice straw might not require the acid or base pretreatment for effective hydrolysis and subsequent enzymatic processing. Thus, rice straw is more advantageous than other types of lignocellulosic biomass for use as a raw material to produce biofuels.

Different pretreatment methods

Table 4 compares the rice straw composition before and after the various different pretreatments; the results indicate that pretreatment changes the composition of the material. The content of cellulose increased by more than 50 % for both sets of the 2.4 and 10 % samples. In particular, dilute base pretreatment of the 10 % rice straw sample increased the percentage of cellulose to as high as 70 %. Comparatively, the 35 % hemicellulose content in the non-pretreated rice straw was reduced to 7 and 14 % in the 2.4 and 10 % sample sets, respectively, after pretreatment of the rice straw with the 1 % sulfuric acid solution. The results of reducing the hemicellulose contents after dilute acid pretreatment were consistent with the findings of Abedinifar et al. [1], who reported that 3.01 g/L of xylose, 1.95 g/L of glucose, and 1.88 g/L of galactose were detected in the hydrolysate solution. These monosaccharides are the building blocks of hemicellulose, which is a branched polymer, unlike the linear cellulose polymer. Thus, the structure of hemicellulose is more heterogeneous than cellulose, and chemical treatment, such as a dilute acid solution, breaks down hemicellulose more easily than cellulose. However, concerning the dilute base pretreatment, the results revealed that the hemicellulose content was not significantly reduced by pretreatment with 1 % sodium hydroxide solution, and no significant quantity of reduced sugars was detected in the base hydrolysate.

Table 3 Various lignocellulosic biomass and their compositions

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Rice straw	38	35	7	This study
	39	27	12	[4]
	24	38	8	[1]
	27	30	26	[26]
Wheat straw	35–40	20–50	20	[27]
Corn cob	45	35	15	[28]
Bagasse	37	29	19	[29]
Silver grass	34	28	19	[29]

Data are reported as the percentage of dry weight

Enzymatic saccharification

The PRS with a solid content of 2 % was used to conduct enzymatic saccharification experiments using different

Table 4 Rice straw composition before and after pretreatment

Component (%)	Original DRS	Dilute acid pretreatment		Dilute base pretreatment	
		(2.4 %)[a]	(10 %)[a]	(2.4 %)[a]	(10 %)[a]
Cellulose	38	56	50	59	70
Hemicellulose	37	8	14	31	28
Lignin	7	26	25	4	3
Ash	4	7	7	0.5	4

Data are reported as the percentage of dry weight

[a] Solid contents (w/v) in the dilute acid or base solution

enzyme loadings. The results listed in Table 2 present that the sugar productivity, which is determined by the maximum sugar concentration and the saccharification time, increases with increasing enzyme loading. The maximum sugar productivity was 1.28 mmol/L/h for the enzyme loading of 1.93 kU cellulase/g DRS, 1.93 kU hemicellulase/g DRS, and 4.97 kU cellobiase/g DRS. This result is comparable to the 1.20 mmol/L/h reported by Abedinifar et al. [1]. At this level of enzyme loading, the maximum sugar yield of 0.94 g sugar/g PRS can be obtained.

Based on the results of the enzyme loading tests, the enzymatic saccharification experiments for NPRS, PRS, and PRSAH were performed using enzyme loadings of 1.93 kU cellulase/g DRS, 1.93 kU hemicellulase/g DRS, and 4.97 kU cellobiase/g DRS. A 47 % loss of rice straw was observed during the preparation of PRS before the enzymatic saccharification experiments. This mass loss was also reported by Cara et al. [30]. Figure 1 presents the cumulative sugar concentrations for NPRS, PRS, and PRSAH during the enzymatic saccharification. Saccharification performance is highly related to the pretreatment. Glucose, galactose, xylose, and arabinose were produced during the enzymatic saccharification, with glucose being the main product. As revealed in Fig. 1a, the maximum glucose concentration of 14.05 g/L from the NPRS was achieved at the end of the saccharification. The glucose concentration reaches 79 % of its maximum level in 48 h without lags. However, galactose and arabinose were first detected after 48 and 114 h, respectively. At the end of the saccharification, the concentrations of galactose and arabinose were 4.99 and 1.37 g/L, respectively. These observations suggest that cellulose is instantly hydrolyzed to form glucose, resulting in the sharp increase in the glucose concentration; subsequently, galactose and arabinose are gradually produced due to the hydrolysis of hemicellulose. The hydrolysis of hemicellulose also leads to the production of a small quantity of glucose that is identified by the slow increase of the glucose concentration after 48 h of saccharification.

Similar results were also observed for the PRS and PRSAH, as shown in Fig. 1b and c, respectively. The production of galactose and arabinose from the PRS are consistent with that from NPRS. However, galactose and arabinose were detected 24 h earlier from the PRSAH than

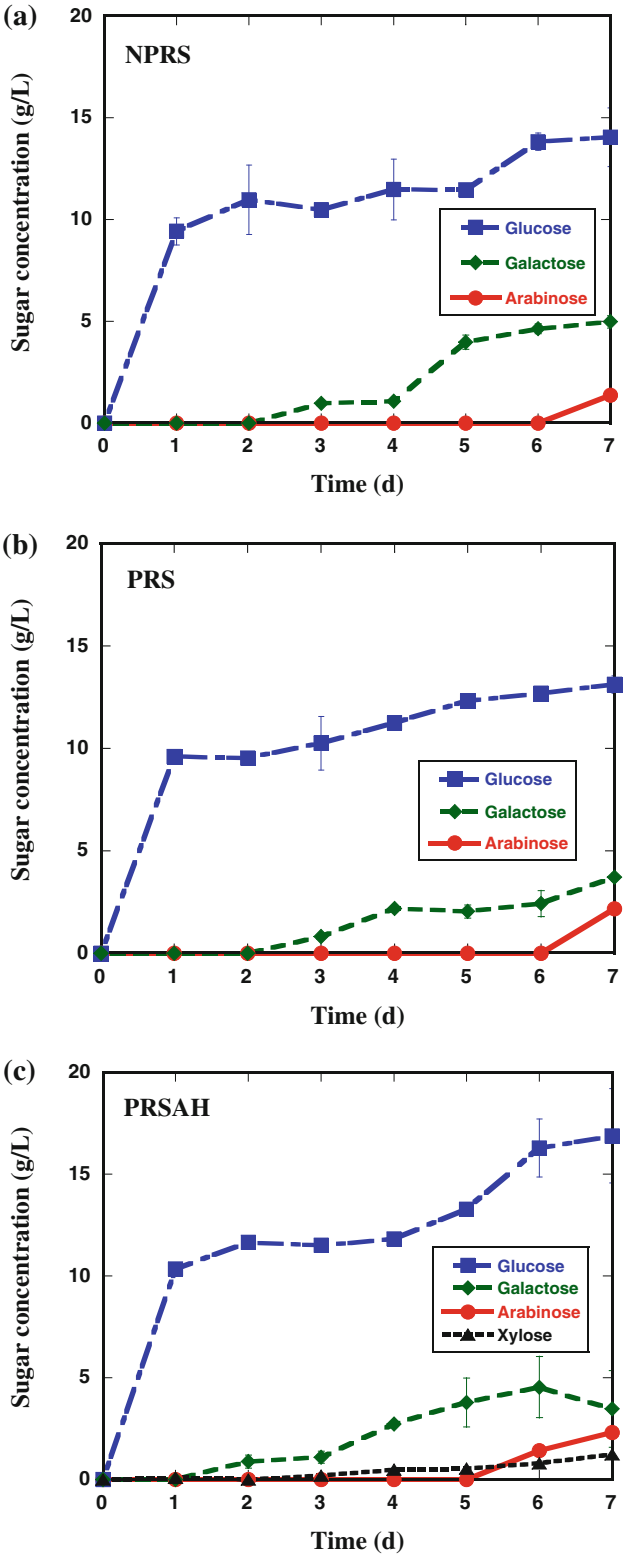

Fig. 1 Sugar production from the rice straw subjected to various pretreatments **a** NPRS; **b** PRS; **c** PRSAH

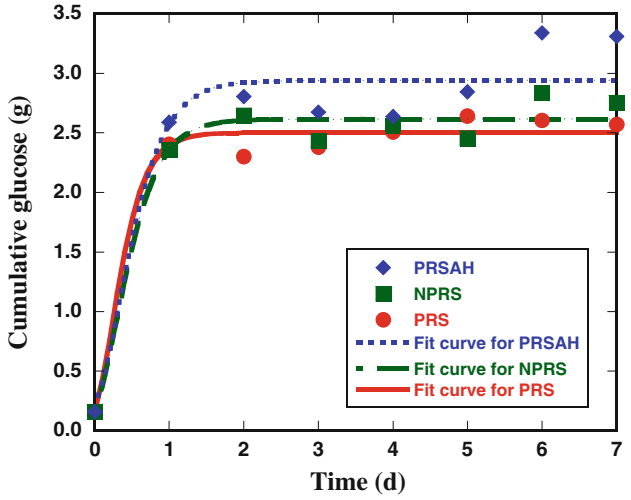

Fig. 2 Cumulative glucose production from NPRS, PRS, and PRSAH. (Markers—experimental data; nonlinear lines—data estimated by Eq. (1))

the NPRS. This result might be due to the remaining sulfuric acid in the PRSAH, which could continuously break down the rice straw during enzymatic saccharification. Thus, the more open structure of the rice straw facilitated the hydrolysis of hemicellulose [10]. In addition, 1.24 g/L of xylose was detected from the PRSAH, whereas none was detected from the PRS. This observation reflects the fact that the acid hydrolysate in the PRSAH contains some xylose. Overall, glucose was the main product from the PRS and PRSAH during saccharification. The maximum glucose concentrations were 13.12 and 16.89 g/L for the PRS and PRSAH, respectively. The glucose concentrations from both PRS and PRSAH reached approximately 70 % of their respective maximum levels after 48 h.

Results using the modified Gompertz equation to fit the kinetics of glucose production are shown in Fig. 2. The values of the kinetic parameters estimated using Eq. (1) are listed in Table 5. As shown in the table, glucose production is well fitted by the modified Gompertz equation ($R^2 > 0.93$). The glucose production potential and glucose production rate were 2.62 g and 3.14 g/d for the NPRS, 2.50 g and 3.81 g/d for the PRS, and 2.94 g and 3.26 g/d for the PRSAH. During the hydrolysis of all three rice straw samples, the reactions displayed no lag time. The results revealed that the PRS and PRSAH have higher glucose production rates than the NPRS. These observations confirm that soaking the rice straw in dilute sulfuric acid solution enhances subsequent biocatalysis and accelerates enzymatic saccharification. However, there is no solid evidence to confirm that dilute acid pretreatment enhances the potential of glucose production from rice straw because similar glucose yields were observed for all three rice straw samples. The glucose yield was calculated from the glucose

Table 5 Estimated parameters of the modified Gompertz equation for glucose productions from NPRS, PRS, and PRSAH

Rice straw	G (g)	R_g (g/d)	Glucose yield (g/g DRS)	R^2
NPRS	2.62	3.14	0.52	0.98
PRS	2.50	3.81	0.50	0.98
PRSAH	2.94	3.26	0.58	0.93

Table 6 Summary of glucose yields in previous studies evaluating the effect of different pretreatments on the enzymatic saccharification of rice straw

Pretreatment	Temperature (°C)	Duration	Glucose yield (g/g PRS)	Reference
Acid (1 % H_2SO_4)	160	5 (min)	0.50	[16]
Acid (85 % H_3PO_4)	50	30 (min)	0.35	[31]
Base (12 % NaOH)	0	180 (min)	0.41	[31]
Base (2.0 % NaOH)	85	60 (min)	0.55	[32]
Base (3.0 % NaOH)	82	57 (min)	0.25	[33]
Cutter milling	~	~	0.52	[34]
Hot-compressed water	150	60 (min)	0.71	[34]
Hot-compressed water followed by wet disk milling	135	60 (min)	0.94	[34]
Cyathus stercoreus	~	25 (day)	0.57	[35]
Green liquor (20 % sulfidity)	140	60 (min)	0.32	[36]
Ionic liquid (20 % [Ch][Lys]IL)	90	60 (min)	0.57	[37]

PRS Pretreated rice straw

production potential and DRS used in the experiments. The glucose yields were 0.52, 0.50, and 0.58 glucose/g DRS for the NPRS, PRS, and PRSAH, respectively. In addition, Table 6 summarizes the glucose yields from previous studies exploring the effect of various pretreatments on enzymatic saccharification. The results for glucose yields vary from 0.25 to 0.94 g glucose/g PRS. The glucose yields obtained in this study are comparable to these investigations. Accordingly, pretreating the rice straw with acid may be omitted to increase the cost effectiveness of the process.

Figure 3 presents the first-order kinetics of total sugar production from rice straw, which is the sum of the monosaccharides, including pentose and hexose, produced during the course of the saccharification experiments. As is

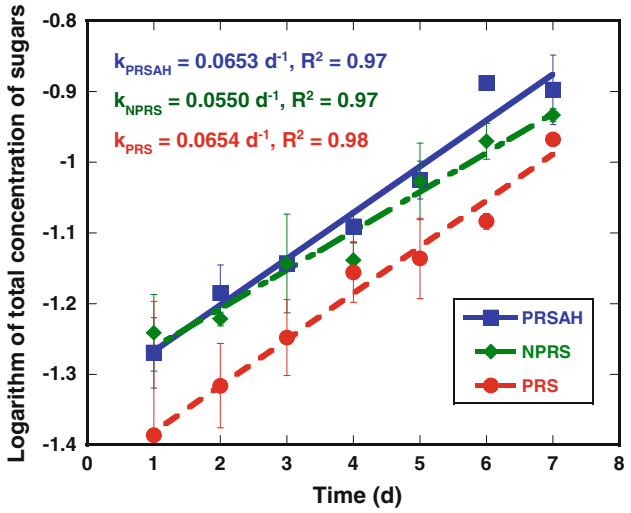

Fig. 3 First-order kinetics of the sugar production from NPRS, PRS, and PRSAH

evident from the figure, the total sugar concentration is well described by first-order kinetics, with all coefficients of determination exceeding 0.97. The rate constants were 0.0550, 0.0653, and 0.0654 d^{-1} for the NPRS, PRSAH, and PRS, respectively. There is no discrepancy between the k values for the PRS and PRSAH. As stated earlier, the acid hydrolysate was removed from the PRS sample, whereas it remained in the PRSAH samples. The similar *k* values for the PRS and PRSAH suggest that the acid hydrolysate did not interfere with the activities of the enzymes. The step of decanting the acid hydrolysate prior to hydrolysis can be neglected to simplify the pretreatment process. In addition, the *k* value for the NPRS is 84 % of that for the PRS or PRSAH, indicating that the hydrolysis rate of rice straw might be slightly accelerated by dilute acid pretreatment. This result suggests that although the time for enzymatic saccharification can be reduced if the rice straw is subjected to dilute acid pretreatment, the overall reaction time including the pretreatment and saccharification processes will not be significantly decreased as compared to rice straw without pretreatment.

Conclusions

This study examined the practice of pretreating rice straw with an acid or base before the enzymatic saccharification of the straw for the production of fermentable sugars. Dilute acid pretreatment results in the reduction of hemicelluloses, whereas dilute base pretreatment will not cause the reduction of hemicellulose. During enzymatic saccharification, the straight polymeric cellulose is hydrolyzed before the branched polymeric hemicellulose. In addition, enzymatic saccharification is not significantly inhibited by residual acid hydrolysate from the dilute acid pretreatment. Overall, omitting the acid–base treatment in the production of fermentable sugars from ground rice straw will increase the cost effectiveness of the process.

Acknowledgments This study was supported by a grant from the Taiwan National Science Council (NSC 101-2221-E-197-009).

References

1. Abedinifar S, Karimi K, Khanahmadi M, Taherzadeh MJ (2009) Ethanol production by *Mucor indicus* and *Rhizopus oryzae* from rice straw by separate hydrolysis and fermentation. Biomass Bioenergy 33:828–833
2. Guo GL, Hsu DC, Chen WH, Chen WH, Hwang WS (2009) Characterization of enzymatic saccharification for acid-pretreated lignocellulosic materials with different lignin composition. Enzyme Microb Technol 45:80–87
3. Huang CF, Lin TH, Guo GL, Hwang WS (2009) Enhanced ethanol production by fermentation of rice straw hydrolysate without detoxification using a newly adapted strain of *Pichia stipitis*. Bioresour Technol 100:3914–3920
4. Karimi K, Emtiazi G, Taherzadeh MJ (2006) Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with *Mucor indicus*, *Rhizopus oryzae*, and *Saccharomyces cerevisiae*. Enzyme Microb Technol 40:138–144
5. Liu FF, Liu L, Feng XS (2005) Separation of acetone–butanol–ethanol (ABE) from dilute aqueous solutions by pervaporation. Sep Purif Technol 42:273–282
6. Liu JH, Wu M, Wang M (2009) Simulation of the process for producing butanol from corn fermentation. I EC Res 48: 5551–5557
7. Qureshi N, Saha BC, Dien B, Hector RE, Cotta MA (2010) Production of butanol (a biofuel) from agricultural residues: part I use of barley straw hydrolysate. Biomass Bioenergy 34:559–565
8. Qureshi N, Saha BC, Hector RE, Dien B, Hughes S, Liu S, Iten L, Bowman MJ, Sarath G, Cotta MA (2010) Production of butanol (a biofuel) from agricultural residues: part II use of corn stover and switchgrass hydrolysates. Biomass Bioenergy 34:566–571
9. Sheehan JJ (1994) Bioconversion for production of renewable transportation fuels in the United States. Enzymatic conversion of biomass for fuels production: American Chemical Society, p 1–52
10. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686
11. Yamagishi K, Kimura T, Watanabe T (2011) Treatment of rice straw with selected *Cyathus stercoreus* strains to improve enzymatic saccharification. Bioresour Technol 102:6937–6943
12. Hideno A, Inoue H, Tsukahara K, Fujimoto S, Minowa T, Inoue S, Endo T, Sawayama S (2009) Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. Bioresour Technol 100:2706–2711
13. Zhu S (2008) Use of ionic liquids for the efficient utilization of lignocellulosic materials. J Chem Technol Biotechnol 83:777–779
14. Poornejad N, Karimi K, Behzad T (2013) Improvement of saccharification and ethanol production from rice straw by NMMO and [BMIM][OAc] pretreatments. Ind Crops Prod 41:408–413
15. Vancov T, Mclntosh S (2012) Mild acid pretreatment and enzyme saccharification of *Sorghum bicolor* straw. Appl Energy 92:421–428
16. Hsu TC, Guo GL, Chen WH, Hwang WS (2010) Effect of dilute acid pretreatment of rice straw on structural properties and enzyme hydrolysis. Bioresour Technol 101:4907–4913

17. Ranjan A, Moholkar VS (2013) Comparative study of various pretreatment techniques for rice straw saccharification for the production of alcoholic biofuels. Fuel 112:567–571
18. Nlewem KC, Thrash ME Jr (2010) Comparison of different pretreatment methods based on residual lignin effect on the enzymatic hydrolysis of switchgrass. Bioresour Technol 101: 5426–5430
19. Ezeji T, Qureshi N, Blaschek HP (2007) Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. Biotechnol Bioeng 97:1460–1469
20. Heinonen J, Tamminen A, Uusitalo J, Sainio T (2012) Ethanol production from wood via concentrated acid hydrolysis, chromatographic separation, and fermentation. J Chem Technol Biotechnol 87:689–696
21. APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC
22. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597
23. Chen WH, Chen SY, Kumar KS, Sung S (2006) Kinetic study of biological hydrogen production by anaerobic fermentation. Int J Hydrogen Energy 31:2170–2178
24. Chen WH, Jian ZC (2013) Evaluation of recycling the effluent of hydrogen fermentation for biobutanol production: kinetic study with butyrate and sucrose concentrations. Chemosphere 93: 597–603
25. Chen WH, Chen YC, Lin JG (2013) Evaluation of biobutanol production from non-pretreated rice straw hydrolysate under non-sterile environmental conditions. Bioresour Technol 135:262–268
26. Teramoto Y, Lee SH, Endo T (2009) Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification. Bioresour Technol 100:4783–4789
27. Qureshi N, Saha BC, Cotta MA (2007) Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*. Bioprocess Biosyst Eng 30:419–427
28. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11
29. Hwang WS (2007) The pretreatment for cellulosic ethanol at INER. In: INER (ed) Longtan, Taoyuan County
30. Cara C, Ruiz E, Oliva JM, Sáez F, Castro E (2008) Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification. Bioresour Technol 99:1869–1876
31. Moradi F, Amiri H, Soleimanian-Zad S, Ehsani MR, Karimi K (2013) Improvement of acetone, butanol and ethanol production from rice straw by acid and alkaline pretreatments. Fuel 112:8–13
32. Jeya M, Zhang YW, Kim IW, Lee JK (2009) Enhanced saccharification of alkali-treated rice straw by cellulase from *Trametes hirsuta* and statistical optimization of hydrolysis conditions by RSM. Bioresour Technol 100:5155–5161
33. Kim I, Han JI (2012) Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass Bioenergy 46:210–217
34. Hideno A, Inoue H, Yanagida T, Tsukahara K, Endo T (2012) Combination of hot compressed water treatment and wet disk milling for high sugar recovery yield in enzymatic hydrolysis of rice straw. Bioresour Technol 104:743–748
35. Yamagishi K, Kimura T, Watanabe T (2011) Treatment of rice straw with selected *Cyathus stercoreus* strains to improve enzymatic saccharification. Bioresour Technol 102:6937–6943
36. Gu F, Wang W, Jine L, Jin Y (2013) Effects of green liquor pretreatment on the chemical composition and enzymatic digestibility of rice straw. Bioresour Technol 149:375–382
37. Hou XD, Li N, Zong MH (2013) Significantly enhancing enzymatic hydrolysis of rice straw after pretreatment using renewable ionic liquid-water mixtures. Bioresour Technol 136:469–474