Effect of the Two-Stage Autohydrolysis of Hardwood on the Enzymatic Saccharification and Subsequent Fermentation with an Efficient Xylose-Utilizing *Saccharomyces cerevisiae*

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To effectively utilize sugars during the fermentation process, it is important to develop a process that can minimize the generation of inhibiting compounds such as furans and acids, and a robust micro-organism that can co-ferment both glucose and xylose into products. In this study, the feasibility of efficient ethanol production was investigated using a combination of two approaches: two-stage autohydrolysis of biomass and fermentation using an engineered *Saccharomyces cerevisiae* to produce ethanol. When the hardwood chips were autohydrolyzed at 140 °C, followed by the second treatment at 180 °C, a higher yield of sugar conversion and fewer inhibitory effects on subsequent fermentation were achieved compared with the results from single-stage autohydrolysis. A higher overall yield of ethanol resulted by using an engineered yeast strain, SR8. This observation suggests the possibility of the feasible combination of two-stage autohydrolysis and the recombinant yeast.

Keywords: Two-stage autohydrolysis; Xylose-fermenting yeast; Biomass conversion to ethanol; Saccharomyces cerevisiae; Fermentation inhibitor

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INTRODUCTION

Lignocellulosic biomass is one of the most abundant and sustainable biomass resources, and extensive studies and industrial trials have been carried out to utilize it as a source for transportation fuel and platform chemicals. Challenging problems in these application processes include recalcitrance toward physical and chemical treatments, such as difficulties in its disintegration and the separation of the lignocellulosic matrix and low digestibility of the highly-organized cellulosic fraction. Although several different approaches have been taken in attempts to overcome these issues (Mosier *et al.* 2005), there is much room for improvement considering the economic and industrial feasibility of the conversion of lignocellulosic biomass.

Chemical and physicochemical approaches have been widely adapted for the pretreatment of lignocellulosic biomass prior to further biochemical processes. Through pretreatment processes, the intrinsic recalcitrance of biomass feedstock toward enzymatic hydrolysis and fermentation can be reduced, resulting in the more effective biochemical

conversion of lignocellulosic materials. During the pretreatment processes, a broad range of compounds can be formed from lignocellulosic biomass, including organic acids and furan derivatives (Larsson *et al.* 1999b). Furans can be formed during the degradation of monomeric sugars from the carbohydrate fraction (Antal *et al.* 1990; Larsson *et al.* 1999a), and acids have been obtained from hemicelluloses or degradation products of furan derivatives (Fan *et al.* 1982; Palmqvist and Hahn-Hägerdal 2000). These by-products can inhibit the subsequent conversion process by enzymes or microorganisms. Several studies have reported the inhibiting effects of furfural and hydroxymethylfurfural (HMF) on the growth of yeast and its fermenting activity (Chung and Lee 1985; Olsson and Hahn-Hägerdal 1996; Taherzadeh *et al.* 2000; Li *et al.* 2005). Acids also inhibit the fermentation of yeast (Larsson *et al.* 1999a), as well as enzymatic saccharification (Cantarella *et al.* 2004; Yu *et al.* 2010).

Several approaches have been applied to reduce undesirable by-products during pretreatment and to increase sugar conversion during enzymatic hydrolysis. Sulfur dioxide-catalyzed steam explosion has been reported to increase the yield of sugar hydrolysis, with a lesser amount of corrosive acids and fermentation-inhibiting by-products (Galbe and Zacchi 2002; Chandra *et al.* 2007). The ammonia fiber expansion (AFEX) process has also demonstrated effectiveness in the pretreatment of lignocellulosics with lower inhibitor yields (Lau *et al*. 2009). The sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) process produces a substantially lower amount of inhibitors, compared with dilute acid pretreatment (Zhu *et al.* 2009). In addition, autohydrolysis (AH), a chemical-free process that uses only water, has received attention because it is a cost-effective and environmentally benign process compared with other conventional pretreatment technologies (Garrote *et al.* 1999; Vegas *et al.* 2004). It has also been shown that the concentration of inhibitors including furfural and HMF is quite low in the liquid products after AH pretreatment (Garrote *et al.* 1999). Lee and coworkers recently demonstrated the feasibility of two-stage autohydrolysis, which enhanced the enzymatic digestibility of pretreated biomass, compared with the conventional one-stage AH pretreatment (Lee *et al.* 2010).

Another important factor for improving the overall yield of ethanol from cellulosic biomass is the efficient conversion of xylose to ethanol. Two fermentable sugars, glucose and xylose, are primarily released from the enzymatic hydrolysis of pretreated biomass. While glucose can be easily converted to ethanol by *Saccharomyces cerevisiae* (*S. cerevisiae*), xylose conversion is difficult because of its lack of xylose metabolization. The natural xylose-fermenting yeast, *Pichia stipitis*, has been used in attempts to produce ethanol from xylose. However, some characteristics of *P. stipitis*, such as poor ethanol tolerance, poor osmotic tolerance, and dependency on oxygen for growth, can be obstacles to cellulosic ethanol production. Hence, many efforts have been attempted to make *S. cerevisiae* effectively ferment xylose, including the heterologous expression of xylose metabolic enzymes from *P. stipitis*, the resolution of unbalanced cofactors' preferences between xylose metabolizing enzymes, and genetic perturbation stimulating xylose metabolic rate. Recently, a rapid and efficient xylose-fermenting *S. cerevisiae* strain, SR8, was constructed through rational and inverse metabolic engineered approaches, including strong and balanced expression of the xylose metabolic enzymes, metabolic evolution by serial-subcultures on xylose, and selective inactivation of the genes involved in by-product formation (Kim *et al.* 2013).

In this study, the feasibility of efficient ethanol production was investigated using a combination of two approaches: the two-stage autohydrolysis of hardwood chips and

the conversion of sugars into ethanol using the engineered *S. cerevisiae*. Changes in the composition of carbohydrates and by-products during the pretreatment process were monitored, and their effects on the enzymatic saccharification and the subsequent fermentation by the SR8 strain were evaluated.

EXPERIMENTAL

Sample Preparation

Mixed hardwood chips (compositional data: 45.4% glucan, 16.2% xylan, 3.6% other carbohydrates, and 25.1% Klason lignin) obtained from a pulp mill (Southeastern USA) were air-dried for autohydrolysis treatment at the water-to-wood ratio of 4 to 1 in an M/K digester (MK systems, Inc., Peabody, MA). Two strategies were used to pretreat the hardwood chips: single-stage and two-stage autohydrolyses. For single-stage autohydrolysis, the chips were pretreated at 180 °C for 0.5 or 1 h. The pretreated samples were separated into liquid hydrolysate and solid residue, and then the liquid was analyzed to quantify monomeric sugars and oligomeric sugars after acid hydrolysis using a high-performance liquid chromatography (HPLC) system (Agilent 1200, Agilent Technology, Palo Alto, CA) equipped with a Shodex SP0810 column (Showa Denko America Inc., USA). Standard curves for HPLC sugar analysis were made using glucose, xylose, galactose, arabinose, and mannose, purchased from Sigma-Aldrich (St. Louis, MO). By-products including acetic acid, formic acid, furfural, and HMF were quantified using the HPLC system with Biorad Aminex HPX-87P (Biorad Life Science Research, Hercules, CA), according to the NREL's analytical procedure (Sluiter *et al.* 2008). Standard chemicals for sugars, organic acids and furans were purchased from Sigma-Aldrich (St. Louis, MO). Separated solid residues were fiberized two times using an 8-in disk refiner. The pretreated substrates were diluted to 10% consistency and further refined using a lab-scale PFI refiner (Norwegian Pulp and Paper Institute, Oslo, Norway) at 6000 revolutions to enhance enzymatic hydrolysis.

To produce two-stage autohydrolysis substrates, the hardwood chips were pretreated at three different temperatures (120, 140, and 160 °C) for 0.5 h in the first stage of autohydrolysis. After removing the liquid hydrolysates, the collected solids were treated at 180 °C for an additional 0.5 h in the second stage of autohydrolysis. The liquid hydrolysates were analyzed in the same manner as the single-stage samples. Separated solid residues were also handled the same way: disk refining for fiberization and PFI refining for fibrillation prior to enzymatic hydrolysis. The sugar recovery from pretreated was calculated as below:

Sugar recovery (%) = [amount of sugar hydrolyzed during pretreatment]/[total amount of sugar in raw feedstock]

Enzymatic Saccharification

Samples from the single-stage and the two-stage autohydrolyses were hydrolyzed to produce fermentable sugars by cellulolytic enzymes. This was performed in an acetate buffer (100 mM, pH 4.8) with 0.3% sodium azide, which was added to inhibit the undesirable growth of microorganisms during incubation. The consistency of the samples with the buffer was 5%. CTec2 enzymes provided by Novozymes North America Inc. (Franklinton, NC, USA) were used and loaded at different dosages: 5, 10, and 15 FPU/g

of substrates. The liquid hydrolysate was aliqoted periodically and analyzed using HPLC to quantify the sugar yield from enzymatic hydrolysis. All saccharification experiments were performed in duplicate. The sugar recovery from enzymatic hydrolysis was also calculated in the same manner as the calculation for sugars in liquid hydrolysates, on the basis of total carbohydrates in raw feedstock.

Ethanol Fermentation of Liquid Hydrolysates with Engineered *S. cerevisiae*

S. cerevisiae SR8 was used as the ethanol producing strain in the fermentation of liquid hydrolysates. YP medium (10 g/L yeast extract, 20 g/L Bacto peptone, pH 6.7) with 20 g/L glucose was used for seed cultivation and pre-cultivation. Seed cultivation and pre-cultivation were carried out at 30 °C and 250 rpm. Yeast cells at exponential growth (12 h of cultivation) in the pre-cultivation were harvested and used in the fermentation of liquid hydrolysates.

The fermentation of liquid hydrolysates was carried out in 125-mL flasks containing 25 mL of YP medium with 90% of liquid hydrolysates at 30 °C and 80 rpm to maintain the oxygen-limited condition. Because of the low pH (4.5 to 4.8) of the liquid hydrolysates, the initial pH of the medium was adjusted to 6.7, the same pH as the YP medium for seed cultivation and pre-cultivation. As the control medium, YP medium with mixed sugars (50 g/L glucose, 20 g/L xylose, and 4 g/L acetate, pH 6.7) was used. The initial concentration of the yeast cells was adjusted to 1.0 of optical density at 600 nm. All fermentation experiments were performed in duplicate.

RESULTS AND DISCUSSION

Sugars and By-Products in Liquid Hydrolysate after Autohydrolysis

The concentration of monomeric and oligomeric sugars in liquid hydrolysate after different autohydrolysis conditions was analyzed (Fig. 1).

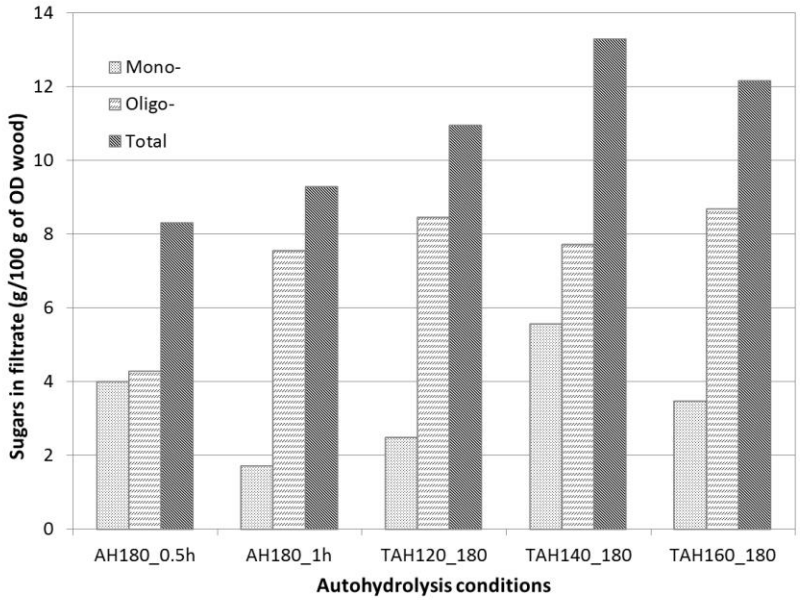

Fig. 1. Monomeric and oligomeric sugars in liquid hydrolysates after the autohydrolysis of hardwood pulp

In single-stage autohydrolysis for 0.5 h at a temperature of 180 °C, the amount of monomeric and oligomeric sugars showed similar values of 4.0 and 4.3 g/100 g of oven-dried (OD) feedstock, respectively. For 1 h of reaction time, however, the amount of monomeric sugars decreased substantially to 1.7 g, and that of oligomeric sugars increased to 7.6 g, resulting in the slight increase of the total amount of sugars from 8.3 to 9.3 g/100 g of OD hardwood. This decrease in monomeric sugars indicated that they were degraded more severely with an extended residence time of 1 h, while the longer residence time produced more oligomeric sugars. This statement can be supported by the observation of the substantially lower amount of monomeric sugars in the liquid from AH180-1h and by the higher yield of total by-products presented in Table 1. A noticeable increase in by-products such as acids and furans was observed, rising from 2.96 to 7.86 g/100 g of OD feedstock with a longer residence time.

Table 1. Experimental Conditions for Autohydrolysis, pH of Liquid Hydrolysates, and the Yield of By-Products (g/100 g of OD Feedstock)

	Single-stage		Two-stage					
	AH180_0.5h	AH180_1h	TAH120_180		TAH140_180		TAH160_180	
Temp. (°C)	180	180	120	180	140	180	160	180
Time (min)	30	60	30	30	30	30	30	30
pH	3.51	3.25	5.16	3.67	4.61	3.5	4.14	3.52
Formic Acid	0.49	1.17	0.01	0.35	0.05	0.5	0.14	0.29
Acetic Acid	1.62	4.13	0.02	1.43	0.07	1.79	0.22	1.51
HMF	0.09	0.46	0	0.08	0	0.11	0.03	0.07
Furfural	0.77	2.1	0	0.63	0	0.98	0.02	0.72
Total	2.96	7.86	0.03	2.49	0.12	3.23	0.41	2.59

As for the two-stage autohydrolysis, the treatment temperature during the first stage of autohydrolysis affected the overall product yield. Compared to the amount of soluble sugars after autohydrolysis for 1 h at 180 °C (AH180_1h), the two-stage samples treated with the first-stage temperature at 120 and 140 °C (TAH120_180 and TAH140_180) showed increasing trends in the amount of monomeric and total sugars, with substantially less by-products (shown in Fig. 1 and Table 1). The two-stage sample treated at 160 and 180 °C (TAH160_180) showed a decreased amount of sugars compared with that of TAH140_180, though it was still higher than that of TAH120_180.

An interesting observation is that all of the two-stage treated samples showed substantially lower amounts of by-products compared to the single-stage samples treated at 180 °C. This decrease in by-product was due to the removal of liquid hydrolysate, which is rich in monomeric sugars, from the first-stage treatment at a lower temperature. It can be seen from Table 1 that the first-stage hydrolysates contained low amounts of acids and furans, ranging from 0.03 to 0.41 g/g of OD feedstock. These levels implied that the conversion of sugars into by-products might be less dominant at temperatures lower than 160 °C. However, it was observed that the treatment at 180 °C could generate more by-products (2.59 g/g of OD feedstock). Hence, it is considered that the removal of hydrolyzed monomeric sugars after the first stage of autohydrolysis was responsible for

the decrease in the overall amount of by-products after the second stage autohydrolysis, compared to that of the AH180_1h sample.

Enzymatic Hydrolysis for Solid Residues after Autohydrolysis

Solid residues from the single- and the two-stage autohydrolyses were hydrolyzed using a cellulolytic enzyme cocktail for 48 and 96 h. Their percentages of sugar recovery with different enzyme dosages are compared in Fig. 2.

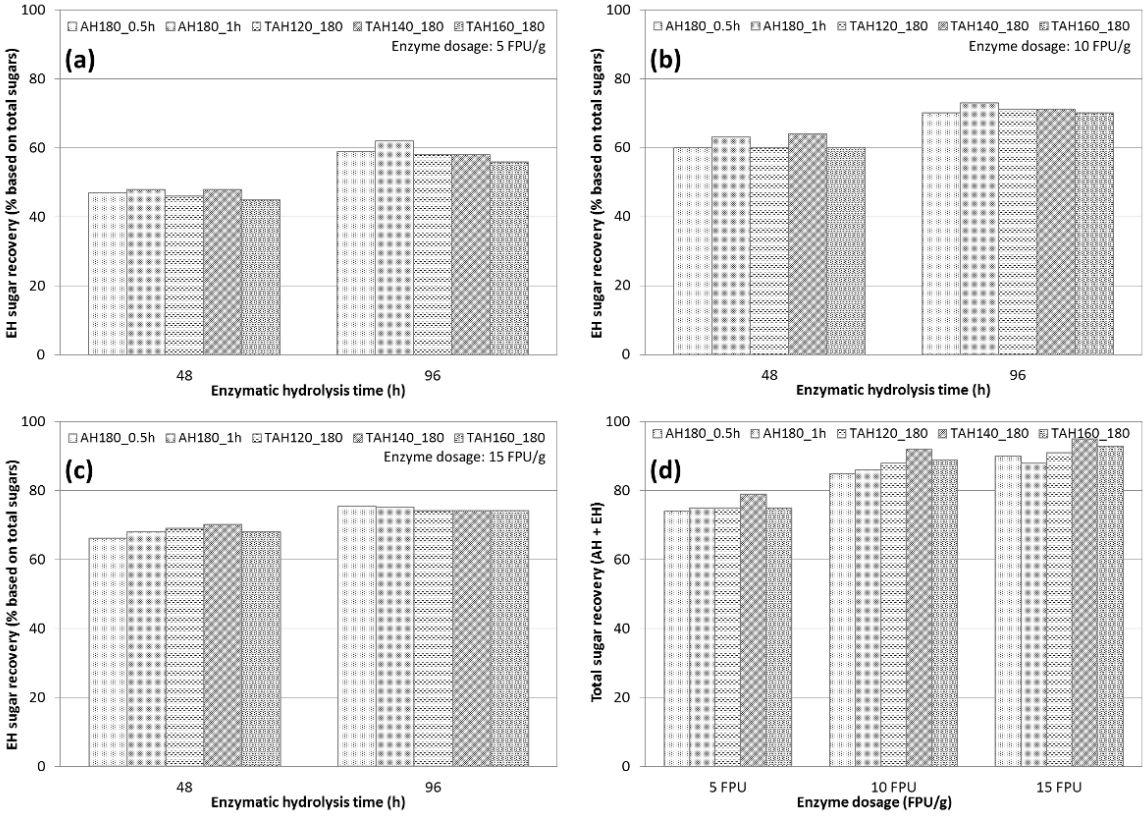

Fig. 2. Sugar recovery in enzymatic hydrolysates from pretreated hardwood pulps at different enzyme loadings (a, b, and c); and total sugar recovery that is the sum of sugars in enzymatic hydrolysate and liquids from autohydrolysis (d)

The sugar recovery after enzymatic hydrolysis showed a positive correlation with the enzyme dosage and hydrolysis time. The increase in sugar conversion from 48 h to 96 h of incubation time became smaller with the higher enzyme dosage, and the sugars released during the enzymatic hydrolysis with 15 FPU/g loading presented less than a 7% increase in most samples. The substantial difference of enzymatic sugar recovery among the autohydrolyzed samples was not clear, but AH180_1h and TAH140_180 samples presented a slightly higher yield after enzymatic hydrolysis with three different loadings of enzyme, especially at 48 h of incubation time. The treatment conditions for TAH-160_180 might be too severe, as it showed the lowest enzymatic sugar recovery among all of the samples. In most cases presented in Fig. 2a-c, enzymatic sugar recovery of TAH-160_180 was lower than even AH180_0.5h. This could be due to a greater amount of solubilized and/or degraded carbohydrates from the first hydrolysis at 160 °C, and the corresponding decrease of carbohydrate fraction in pretreated biomass after two-stage

autohydrolysis. In addition, there was difference in reactivity of pretreated biomass, resulting in less recovery after 48 h of incubation time for TAH-160_180, compared to that of TAH-140_180. This difference became less after 96 h of incubation, and in case of 15 FPU/g of enzyme loading, almost no difference observed between the two samples. Further investigation about the difference in reactivity of pretreated biomass need to be followed in future studies.

As for the total sugar recovery, which included sugars in the liquid hydrolysates after autohydrolysis and after enzymatic saccharification, the TAH140_180 sample showed the highest yield of all three enzyme charges tested. This high yield was due to the benefit of its higher sugar amount in the liquid after autohydrolysis, which was 21% based on the total sugars in feedstock (Table 2).

Considering the suppressed amount of by-products and 79% of the total sugar yield at the enzyme dosage of 5 FPU/g, which is comparable with the reported value of the alkali pretreatment (Gu *et al.* 2012), the TAH140_180 sample appears to be a good candidate for a subsequent fermentation process to produce value-added chemicals from lignocellulosic biomass. Hence, the enzymatic hydrolysis with 5 FPU/g loading on TAH140_180 sample was selected for further studies on ethanol fermentation.

Table 2. Sugar Recovery from Autohydrolysis and Enzymatic Hydrolysis with Enzyme Loading of 5 FPU/g Biomass (%, Based on Total Carbohydrates in Raw Biomass), and By-Products Yield (g/100 g of Raw Biomass)

	Sugars in AH hydrolysate	Sugars in EH hydrolysate	Total sugar recovery	By-products yield
AH180_0.5h	15	59	74	2.96
AH180_1h	13	62	75	7.86
TAH120_180	17	58	75	2.52
TAH140_180	21	58	79	3.35
TAH160_180	19	56	75	3.00

Ethanol Fermentation of Liquid Hydrolysate with a Genetically Modified Yeast Strain

It has been reported that an engineered yeast strain, SR8, demonstrated the fastest and the most efficient xylose fermentation compared to other engineered yeast strains in terms of ethanol yield, ethanol productivity, and by-product yield from xylose (Kim *et al.* 2013). With this engineered yeast, combined liquid hydrolysates from autohydrolysis and subsequent enzymatic hydrolysis were tested to verify the fermentability of sugars in the liquid. The composition of liquid hydrolysates and corresponding fermented products after 33 h are summarized in Table 3.

It was observed that the liquid hydrolysates from autohydrolysis contained a substantial amount of xylose, resulting in a xylose content of 14.7 g/L in AH180_1h with its autohydrolysis liquid, compared to the 4 g/L xylose in the enzymatic hydrolysate from the AH180_1h sample. TAH140_180 with its autohydrolysis liquid presented the highest amount of xylose of 19.0 g/L, but its amount of glucose was the lowest (45.7 g/L), compared to AH180_1h with and without autohydolysis liquid.

Table 3. The Composition of Sugars and Other Products, with the Yield of Ethanol Before/After Yeast Fermentation of Enzymatic Hydrolysates (with and without the Addition of Corresponding Liquid Hydrolysates from Autohydrolysis)

	Concentration of sugars and by-products (g/L)						Ethanol (g/L)	Ethanol Yield[a]
	Cellobiose	Glucose	Xylose	Xylitol	Glycerol	Acetate		
AH180_1h (+ AH liquid)	(3.5)[b] 3.5	(50.0) 0.1	(14.7) 3.2	(0) 0.1	(0) 0.7	(5.6) 4.1	22.8	0.42
TAH140_180 (+ AH liquid)	(3.7) 3.7	(45.7) 0.1	(19.0) 1.2	(0) 0.3	(0) 1.0	(5.4) 4.1	24.2	0.43
AH180_1h	(1.8) 2.0	(52.3) 0.2	(4.0) 0.2	(0) 0	(0) 0.8	(5.6) 3.4	21.3	0.43
Control	(0) 1.0	(50.0) 0	(20.0) 0.2	(0) 0.5	(0) 1.3	(4.0) 3.1	27.2	0.41

[a]Ethanol yield: g of produced ethanol per g of consumed sugar
[b]Values in parentheses: composition of sugars and by-products in liquids before fermentation

After fermentation with the engineered yeast strain, TAH140_180 with AH hydrolysate demonstrated the highest ethanol production among the tested samples, despite the smaller amount of glucose compared to the other two samples. This high production might be due to its higher xylose content from AH hydrolysate, which can be converted to ethanol by the xylose-fermenting yeast, SR8. AH180_1h with AH hydrolysate had the same amount of fermentable sugars (64.7 g/L including glucose and xylose) as TAH140_180 with AH hydrolysate, but the overall ethanol production after fermentation was lower: 22.8 compared to 24.2 g/L. Considering the typically higher yield of ethanol from glucose than from xylose by fermentation and the larger initial amount of glucose in AH180_1h with AH hydrolysate, this lower ethanol yield after fermentation might be due to the higher concentration of by-products in the AH hydrolysate of AH180_1h. Similar results have already been reported in several studies in which the negative effects of furans and acids on the activity of enzymatic hydrolysis and fermentation processes were observed (Chung and Lee 1985; Larsson *et al.* 1999a; Cantarella *et al.* 2004). It was also observed that a larger fraction of xylose (3.2 g/L out of 14.7 g/L of the initial amount of xylose) remained in AH180_1h with AH hydrolysate, compared to TAH140_180 with AH hydrolysate (1.2 g/L out of 19.0 g/L of the initial xylose). This difference implied that the negative effect of inhibitors on the ethanol fermentation had a more substantial impact on the xylose fermentation. In the case of ethanol fermentation with glucose, it was shown that most of the glucose was consumed in all samples. As for AH180_1h, a low amount of ethanol (21.3 g/L) was produced from a low amount of initial sugar (56.3 g/L), but the overall ethanol yield was as high as the yield from TAH140_180 with AH liquid. This might be due to the low amount of fermentation inhibitors in the AH180_1h sample, as shown in Table 3.

The consumption of sugars, the yield of ethanol from fermentation, and the microbial growth are plotted as a function of incubation time in Fig. 3. It is presented that the negative effect of the by-products from autohydrolysis at a temperature higher than 180 °C affected the cellular metabolism during the fermentation of the sugars. As for the two-stage autohydrolyzed sample, TAH140_180 (Fig. 3b), and the enzymatic hydrolysate of AH180_1h (Fig. 3c), as well as the control sample with refined sugars and acetate (Fig. 3d), most glucose was consumed within 12 h, giving rapid rises in ethanol yield and cell growth. AH180_1h with AH hydrolysate (Fig. 3a), however, presented a longer lag

time of cell growth (initial 6.5 h), and glucose was depleted between 12 to 24 h of fermentation. This implied that the negative effect of the by-products in AH liquid from TAH140_180 was not strong enough to impede the overall fermentation process compared to the AH liquid from AH180_1h, though it could still retard the utilization of glucose by SR8, to a certain extent, at the early stage of fermentation. One interesting observation was that the initial rate of glucose consumption and cell growth in the AH180_1h sample (initial 6.5 h of fermentation) was faster than that of TAH140_180 in Fig. 3, and was comparable with that of the control sample (mixture of pure sugars). This faster sugar consumption and ethanol production in AH180_1h indicated that inhibitory compounds were not formed during enzymatic hydrolysis of the autohydrolyzed samples.

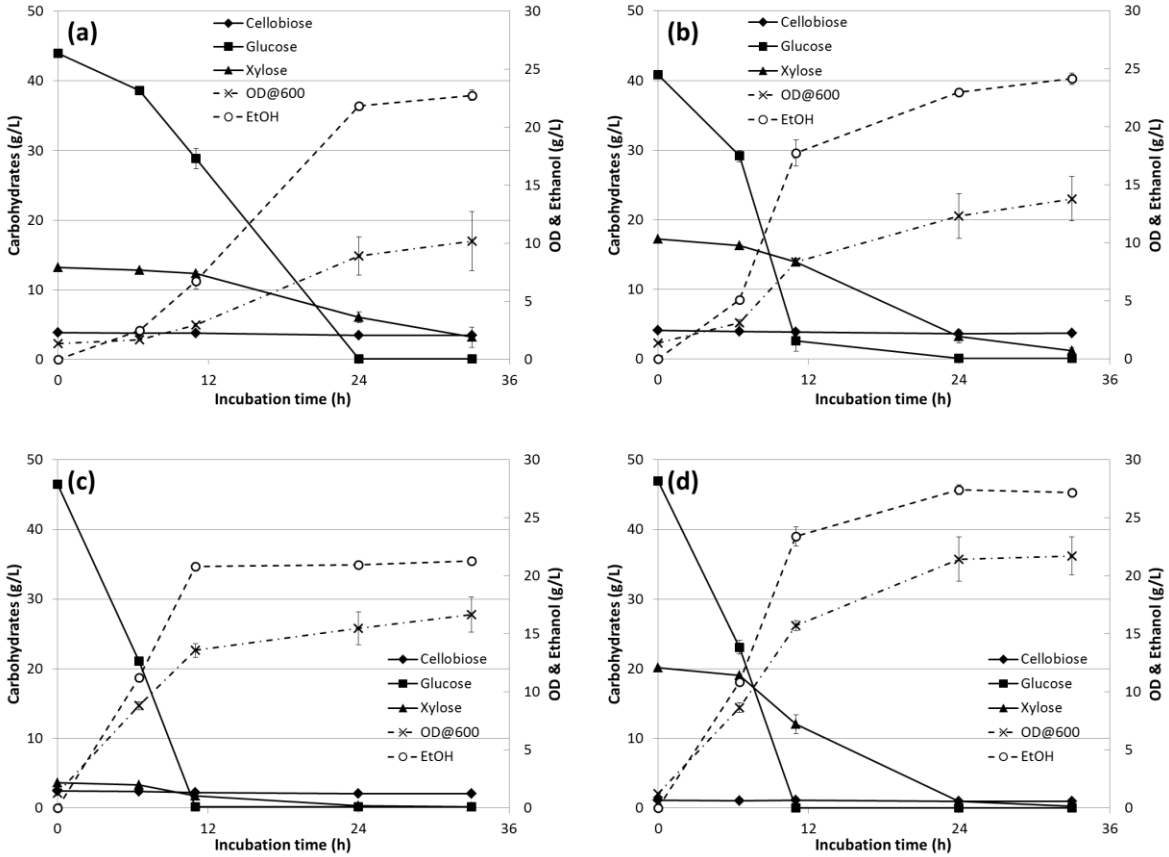

Fig. 3. Consumption of sugars and corresponding yield of ethanol with the growth of the SR8 strain expressed as the optical density (OD) @ 600 nm wavelength: (a) AH180_1h, enzymatic hydrolysate + autohydrolysis liquid; (b) TAH140_180, enzymatic hydrolysate + autohydrolysis liquid; (c) AH180_1h, enzymatic hydrolysate only, and (d) control samples with pure sugars

Compared to the preferred utilization of glucose during fermentation, slower utilization of xylose was observed. Even small decreases in the xylose concentrations during the initial 6.5 h of fermentation were observed in all of the samples, and most of the initial xylose was consumed after the initial glucose was completely consumed. In particular, xylose utilization rates in the fermentations of enzymatic hydrolysates with AH liquids (AH180_1h with AH liquid and TAH140_180, Figs. 3a and 3b) were approximately 40% and 70% of xylose utilization rate in the fermentation of the control sample (Fig. 3d). Meanwhile, glucose utilization rates in AH180_1h with AH liquid and TAH140_180 were 50% and 80% of the rate in the control sample. These results

indicated that by-products from AH liquids could inhibit xylose metabolism of SR8 more severely than glucose metabolism. These results also suggested that sequential utilization of glucose and xylose by the engineered yeast should be resolved to increase ethanol yield and productivity from xylose. Compared to approximately 80 to 95% of xylose consumption after 24 h of fermentation for the other three samples, only half of the initial xylose was consumed in AH180_1h with AH hydrolysate. This markedly slower rate of xylose utilization suggested that the formation of inhibitory compounds was stimulated by autohydrolysis at a high temperature.

Considering the total sugar recovery and the ethanol yield from the subsequent fermentation process, the two-stage autohydrolysis at 140 and 180 °C is a promising strategy to achieve both high sugar and low by-products, resulting in high ethanol yields, compared to the single-stage process. In addition, the recombinant yeast strain SR8 in this study showed good potential as a xylose-fermenting microorganism, which can be suitable for the development of an economically viable process to produce bioethanol from lignocellulosic biomass.

CONCLUSIONS

1. The experimental results demonstrated that two-stage autohydrolysis of biomass generated fewer undesirable by-products and facilitated successful saccharification and fermentation from cellulosic biomass using the engineered yeast strain SR8.
2. When the feedstock biomass was autohydrolyzed at 140 °C, followed by the second treatment at 180 °C, a higher yield of sugar conversion and a less inhibitory effect on subsequent fermentation was achieved, resulting in the higher overall yield of ethanol.
3. This result suggested the possibilities of the feasible combination of autohydrolysis, which is a cost-effective and environmentally benign process, and the recombinant yeast, which effectively utilized xylose into ethanol.

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