Improvement of Ethanol Fermentation from Oligosaccharides in Spent Sulfite Liquor with *Pichia stipitis* by Combined Calcium Oxide and Ion Exchange Resin Treatments

Keishi Tanifuji,[a,b] Shiho Takahashi,[b] Kevin Shiell,[c] M. Sarwar Jahan,[b] Yonghao Ni,[b] and Hiroshi Ohi [a,*]

The objective of this study was to develop an effective method for the removal of inhibitory compounds without decreasing oligosaccharides in spent sulfite liquor (SSL). The oligosaccharide fermentation was subsequently conducted by using *Pichia stipitis*, which is one of the feasible strains that can produce ethanol from oligosaccharides. The effect of inhibitory compounds on ethanol fermentation from cellobiose by *P. stipitis* was investigated. No ethanol was produced from cellobiose in the presence of more than 5 g/L of acetic acid. At 1 g/L of acetic acid, 2.6 g/L of ethanol was obtained after 40 h of fermentation. The removal of acetic acid in the SSL by the combined CaO and ion exchange resin treatments was also studied. The acetic acid concentration of softwood SSL was decreased from 5.2 to 0.9 g/L without decreasing oligosaccharides concentration by the combined method. Finally, the improvement of ethanol fermentation from oligosaccharides in the SSL by using the combined CaO and ion exchange resin treatments was studied. 1.3 g/L of ethanol was obtained from the SSL treated by the combined methods, while 6.5 g/L of total oligosaccharides were consumed. No ethanol was obtained from the untreated SSL.

Keywords: Ethanol; Fermentation; Pichia stipitis; Spent sulfite liquor; Oligosaccharide; Pretreatment; CaO and ion exchange resin treatment

Contact information: a: Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572 Japan; b: Department of Chemical Engineering and Limerick Pulp and Paper Centre, University of New Brunswick, Fredericton, NB, E3B 5A3; c: Biorefinery Technology Scale-up Centre, New Brunswick Community College, Grand Falls, NB, E3Y 3W3 Canada;
** Corresponding author: oi.hiroshi.gm@u.tsukuba.ac.jp*

INTRODUCTION

Sulfite cooking is an established process in the pulp and paper industry. Several currently operational acid sulfite pulp mills can produce various chemicals and materials from wood (Rødsrud *et al.* 2012). Wood chips as raw materials provide cellulose as pulp. In addition, lignosulfonate, vanillin, and ethanol are also produced from the spent sulfite liquor (SSL). Therefore, acid sulfite cooking is one of the most notable technologies for bio-refinery and bio-ethanol production.

In a previous study (Tanifuji *et al*. 2011), when acid sulfite cooking was conducted at 150 °C for 1 to 4 h at pH 1.4, gluco-oligosaccharide and manno-oligosaccharide were obtained in softwood SSL. In addition, a softwood sulfite pulp (SP) with a kappa number of 127 had a high resolution ratio (71.4%) by enzyme treatment (Tanifuji *et al.* 2011), and the resolution ratio of SP with a kappa number 64 was higher

than that of soda-anthraquinone and kraft pulps with a kappa number 20 (Takahashi *et al*. 2010).

Several sulfite pulp mills have utilized SSL for ethanol production (Rødsrud *et al.* 2012). *Saccharomyces cerevisiae* can ferment hexose, but it cannot ferment oligosaccharides and pentose. Further hydrolysis steps such as acid or enzyme treatments are required for ethanol fermentation in order to utilize oligosaccharide in SSL, which would require additional stages and equipment.

A xylose fermentable strain *Pichia stipitis* has the genes for seven β-glucosidases, two β -mannosidases, and one endoxylanase, thus facilitating oligosaccharide utilization (Jeffries 2006; Jeffries *et al.* 2007). It has been reported that *P. stipitis* can produce ethanol from cellobiose (Parekh and Wayman 1986; Parekh *et al.* 1988) and xylan (Lee *et al.* 1986). Parekh and Wayman (1986) reported that *P. stipitis* CBS 5776 gave 10.3 g/L of ethanol from 25 g/L cellobiose within 48 h. Lee *et al.* (1986) reported that *P. stipitis* CBS 5775 can lead to the production of 2 g/L of ethanol from 2% larch wood xylan solution.

The SSL also contains furfural, acetic acid, and sulfite ion (Sixta 2006), which inhibit the ethanol production from monosaccharides with microorganisms (Agbogbo and Coward-Kelly 2008). *P. stipitis* is more sensitive to inhibitory compounds than *S. cerevisiae* (Delgenes *et al.* 1996; Palmqvist and Hahn-Hagerdal 2000). However, the effect of inhibitory compounds on oligosaccharide fermentation is not well understood. Moreover, it is necessary to remove inhibitory compounds from SSL to achieve effective ethanol fermentation.

The removal of inhibitory compounds such as lignin, furfural, and acetic acid from pulping waste liquors *e.g.* prehydrolysis liquor (PHL), based on the concepts of flocculation and adsorption, has been reported (Liu *et al*. 2011, 2012; Shen *et al*. 2011, 2012; Shi *et al*. 2011, 2012; Takahashi *et al*. 2012, 2013).

As an example of the flocculation concept, Shen *et al*. (2012), by using lime to treat PHL, found that the concentration of furfural and lignin in PHL can be decreased by 100% and 25-30%, respectively, by lime treatment. Shi *et al*. (2011, 2012) focused on the combined acidification/poly ethylene oxide (PEO) flocculation process of PHL; this process was found to be effective in removing lignin from PHL.

Activated carbon has been used as adsorbent to treat PHL and SSL. Takahashi *et al*. (2012) studied the simultaneous detoxification and fermentation (*in-situ* detoxification) of SSL using activated carbon; half of the acetic acid in model softwood SSL was removed by activated carbon treatment. Liu *et al.* (2011) reported that the peroxide- or sulfuric acid-modified activated carbons have increased adsorption capacities for hemicellulose, lignin, and furfural present in the PHL.

In this study, we followed the concept of removing acetic acid by the combination of CaO and ion exchange resin treatments. In the previous study, we treated hardwood SSL, which contained monosaccharides but did not contain oligosaccharides, by the combined methods, and found that the combined treatments of CaO, CO_2, and two-stage strong base ion exchange resin (OH^- form) for 4 min decreased acetic acid concentration from 11.2 g/L to 0.9 g/L (Takahashi *et al.* 2013).

The objective of this study was to apply the same concept to SSL, which contains oligosaccharides, that is, to develop an effective method to improve the ethanol production from oligosaccharides in SSL. This was achieved by the removal of inhibitory compounds with effect on oligosaccharides so that the ethanol production is maximized. Firstly, the effect of inhibitory compounds on ethanol fermentation from cellobiose by using *P. stipitis* was clarified. Secondly, the removal of acetic acid in softwood SSL

containing oligosaccharides by the combined CaO and ion exchange resin treatment was studied. Thirdly, the improvement of ethanol production from oligosaccharides in softwood SSL by using the combined CaO and ion exchange resin treatments was studied.

EXPERIMENTAL

SSL and Waste Liquor Samples

The softwood SSL and the hardwood waste liquor from alkaline sulfite pulp mill were obtained from pulp mills in Eastern Canada. The softwood SSL was prepared as followed: pH; 3.5 to 5.5, maximum temperature; 164 ^{o}C for 2 h, wood to liquor ratio; 4.5 L/kg, and magnesium bisulfite charge; 24% as SO_2. The waste liquor was derived from xylanase treatment of hardwood alkaline sulfite pulp and alkaline extraction of that. The chemical composition of waste liquor and SSL are shown in Table 1 and Table 2, respectively.

Ion Exchange Resins

The strong base ion exchange resin (Diaion PA408 Cl^- form) was obtained from Mitsubishi Chemical and used for the experiments. The OH^- form of PA408 was prepared as follows; the Cl^- form resin was soaked in 2 N NaOH for 30 min, and then filtered. This treatment was repeated 5 times; subsequently the resin was washed by distilled water 5 times. The OH^- form of PA408 was kept in distilled water to prevent oxidation and denaturation (Takahashi *et al*. 2013).

Combined Treatment of CaO and Strong Base Ion Exchange Resin Treatments

The CaO treatment was conducted as follows: the pH of softwood SSL and hardwood waste liquor were adjusted to 10.5 with 100 g/L of CaO slurry and then treated at 70 ^{o}C for 15 min in a water bath (Howard 1932; Kuroishi 1983). The mixture was separated with filtration, and the filtrate was used for further experiments. The CaO treated samples were neutralized with CO_2 to pH of 6.8 and were subsequently treated with the ion exchange resin for up to 5 min at 30 ^{o}C with 150 rpm. The resin dosage was 20% on sample in all the experiments (Takahashi *et al*. 2013) .

Microorganism

P. stipitis CBS 6054 was obtained from the USDA. Stock cultures were kept on a YPDX agar plate which contained 10 g/L of yeast extract, 20 g/L of peptone, 10 g/L of D-glucose, 20 g/L of D-xylose, and 10 g/L of agar (Amartey and Jeffries 1994).

Inoculum Preparation

Inoculum was prepared by transferring a loopful of colonies from the agar plate into 50 mL of media which contained peptone, 3.5 g/L; yeast extract, 3 g/L; KH_2PO_4, 2 g/L; $MgSO_4$, 1 g/L; $(NH_4)_2SO_4$, 1 g/L; and xylose, 80 g/L, pH 5.0 in 125 mL of Erlenmeyer flask. Incubation was conducted at 30 ^{o}C and 150 rpm for 48 h. The yeast was collected by centrifugation at 5000 rpm for 5 min and washed with sterile distilled water twice (Amartey and Jeffries 1994).

Fermentation

In the model fermentation, cellobiose (15 g/L) solution in the presence of various acetic acid, furfural, and SO_2 concentrations (0.01, 0.1, 1, 5, and 10 g/L) were prepared. In the fermentation of SSL, the untreated, CaO-treated, and ion exchange resin-treated SSLs were used. These SSL samples were concentrated by a vacuum evaporator to make the total oligosaccharides concentration 15 g/L. The peptone, yeast extract, and ions, which were the same amount as those used for the inoculum preparation, were added to samples. About 1 g/L of dry cell weight was used. All of the fermentation experiments were conducted at 30 ^{o}C with 225 rpm, and the initial pH was adjusted to 5.0. (Takahashi *et al*. 2013).

Analytical Methods

Monosaccharides concentrations were measured using an ion chromatography unit equipped with CarboPac® PA1 column (Dionex-300, Dionex Corporation, USA) and a pulsed amperometric detector (PAD) (Shen *et al.* 2011). Oligosaccharides were hydrolyzed by 4% H_2SO_4 at 121 ^{o}C for 1 h and then measured by the ion chromatography unit. Oligosaccharides concentration was calculated by the subtract sugar concentration before hydrolysis from that after hydrolysis. The furfural, acetic acid, and ethanol concentrations were determined by the ^{1}H-NMR method as described in the literature (Saeed *et al.* 2011). The area of signal at 1.2 ppm was used for ethanol determination. The lignosulfonate concentration was determined by the absorbance at 205 nm with UV spectrometry (Browning 1967). A calibration curve was prepared by using commercial lignosulfonate. Sulfite and sulfate ions analyses were conducted according to TAPPI test method T699 OM-87. The cellobiose concentration was determined using an HPLC equipped with RI detector (Shimadzu). Separations were performed at 65 ^{o}C on Rezex ROA-organic Acid H^{+} column (phenomenex). Injection volume was 20 μL. The mobile phase was 5 mM H_2SO_4. The flow rate was 0.6 mL/min.

RESULTS AND DISCUSSION

Effect of Inhibitory Compounds on Ethanol Production from Cellobiose by Using *P. stipitis*

We first used cellobiose with or without the inhibitory compounds (acetic acid, furfural, sulfite ions) on the ethanol fermentation using *P. stipitis*, and the results are shown in Fig. 1 (ethanol production) and Fig. 2 (cellobiose consumption). The maximum ethanol concentration from the control (without inhibitory compounds) during fermentation was 2.9 g/L. It was found that the presence of more than 5 g/L of acetic acid in media totally inhibited ethanol production from cellobiose and no ethanol was produced (Fig. 1 A).

The presence of 1 g/L of acetic acid inhibited ethanol production until 24 h, but after 40 h of fermentation, 2.6 g/L of ethanol was obtained, which was almost the same as that of the control. At this time, the amount of cellobiose consumption increased significantly from 24 h to 40 h (Fig. 2 A). The above results indicated that the inhibition of cellobiose fermentation caused by acetic acid is negligible in the late stage of fermentation at the presence of 1 g/L acetic acid. Glucose was not found from all of fermented samples.

The maximum ethanol concentrations with 1 g/L of furfural and 0.1 g/L of sulfite ion were 1.9 g/L and 1.3 g/L, respectively (Figs. 1 B and C). Both still show strong inhibition even at the late stage of fermentation (40 h). Also, cellobiose in 1 g/L of furfural and 0.1 g/L of sulfite ion model solutions was not completely assimilated at the late stage of fermentation and its concentrations were 2.2 g/L and 3.3 g/L, respectively; while all of the cellobiose in the control was assimilated (Figs. 2 B and C).

Figure 3 shows the change of acetic acid and furfural concentrations during fermentation. The acetic acid concentration was reduced after 16 h of fermentation. On the other hand, furfural concentration only changed marginally. Implied here is that *P. stipitis* consumed acetic acid during fermentation. This is the reason why the inhibitory effect of acetic acid was reduced in the late stage of fermentation.

In addition, the ethanol production from 1 g/L acetic acid model solution was higher than that from 1 g/L furfural solution (Figs. 1 A and B), and less furfural was assimilated than acetic acid (Fig. 3). Even 0.1 g/L of sulfite ion inhibited ethanol production (Fig. 1 C). These results indicate that the negative effect of acetic acid on the inhibition of cellobiose fermentation is less than that of furfural or sulfite ion.

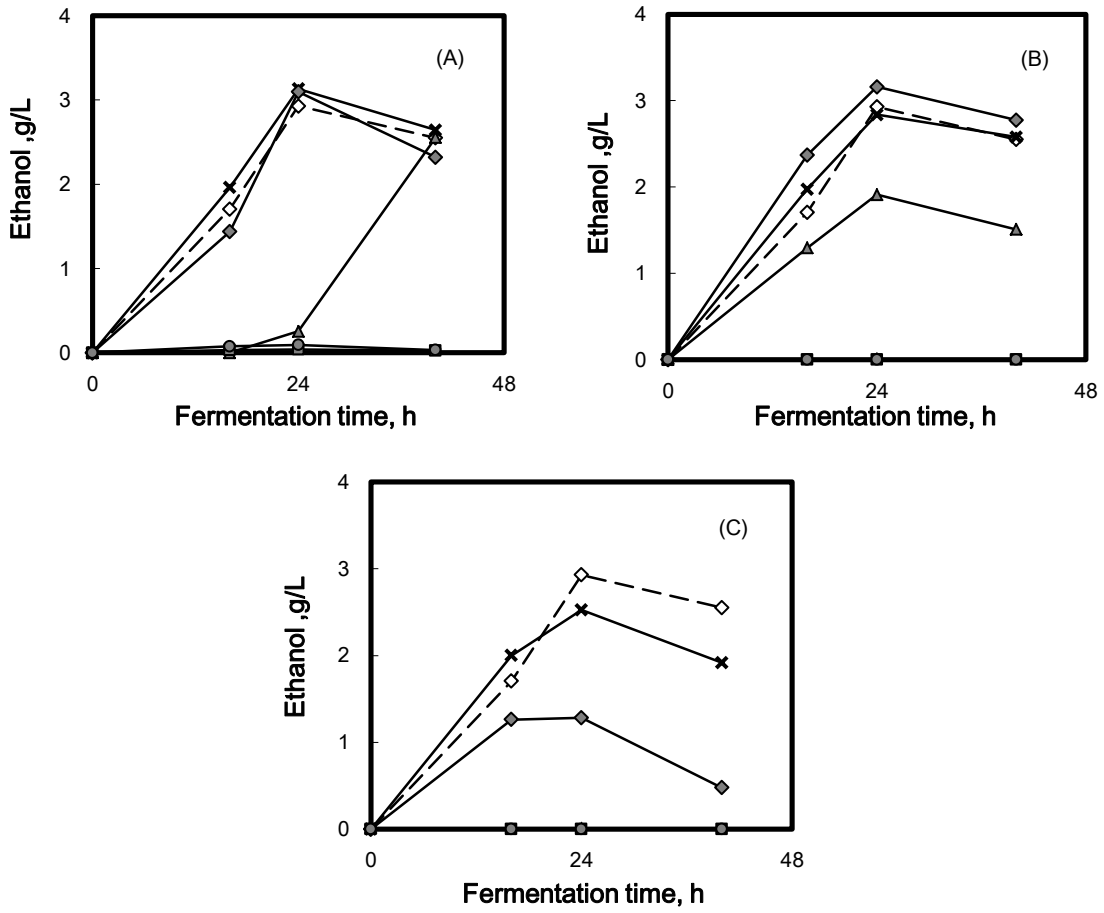

Fig. 1. Ethanol production from cellobiose by *P. stipitis* in the presence of inhibitory compounds: (A) acetic acid; (B) furfural; (C) sulfite ion. Legends: (◇) control; (×) 0.01 g/L; (◆) 0.1 g/L; (▲) 1 g/L; (■) 5 g/L; (●) 10 g/L

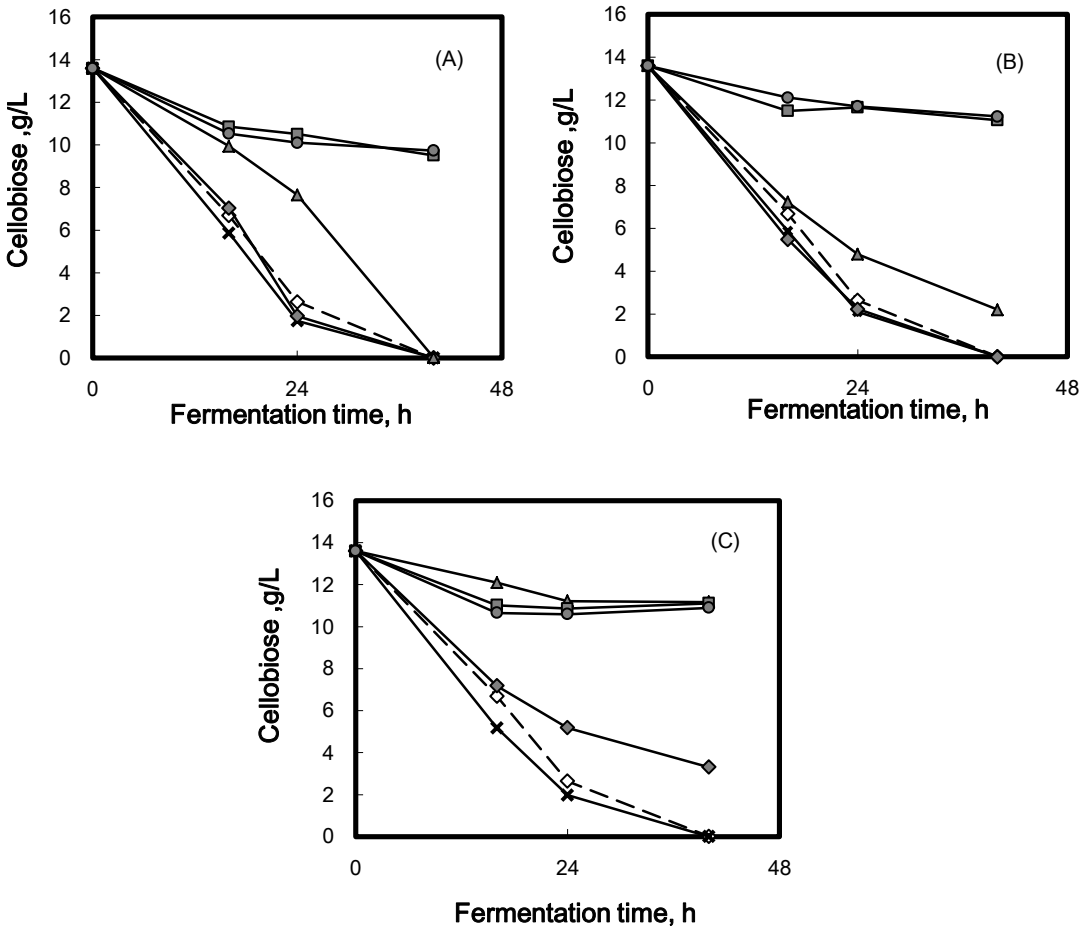

Fig. 2. Cellobiose consumption during fermentation in the presence of inhibitory compounds: (A) acetic acid; (B) furfural; (C) sulfite ion. Legends: (◇) control; (×) 0.01 g/L; (◆) 0.1 g/L; (▲) 1 g/L; (■) 5 g/L; (●) 10 g/L

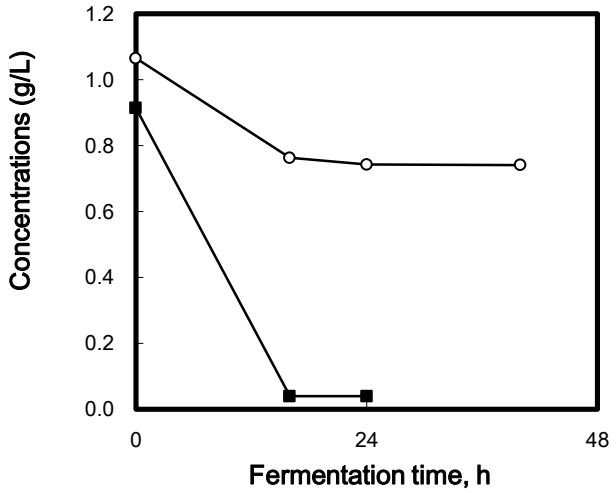

Fig. 3. Change of acetic acid and furfural concentrations during cellobiose fermentation with *P. stipitis*. Legends: (○) furfural; (■) acetic acid

Removal of Inhibitory Compounds by Combination of CaO and Ion Exchange Resin Treatments

In the previous study hardwood SSL was treated by the combined methods; such liquor contained monosaccharides but did not contain oligosaccharides. It was found that the combined treatments of CaO, CO_2, and two-stage strong base ion exchange resin (OH- form) for 4 min decreased acetic acid concentration from 11.2 g/L to 0.9 g/L. However, total monosaccharides concentration also decreased to 15.9 g/L from 35.9 g/L (Takahashi *et al*. 2013).

In this study, we followed the same concept for removing acetic acid from softwood SSL, which contained oligosaccharides, and studied the effect of this method on decrease of acetic acid and oligosaccharides concentrations. The results are shown in Fig. 4, and it can be found that the acetic acid concentration in softwood SSL was decreased from 5.2 g/L to 0.9 g/L by the combined CaO treatment and one-stage ion exchange treatment for 2 min. The total monosaccharides concentration was also decreased by this treatment (1.8 g/L). However, there was little change in total oligosaccharides concentration.

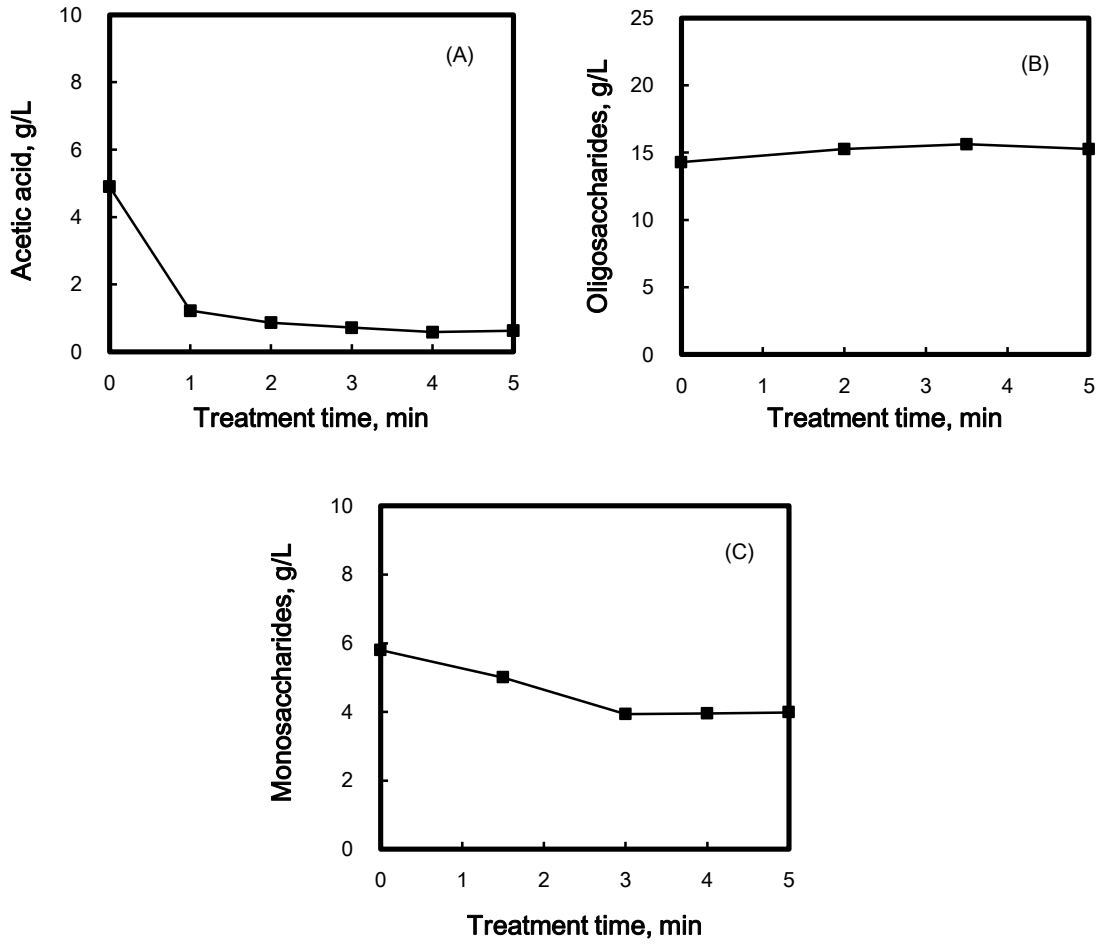

Fig. 4. Time dependence of removal of acetic acid and sugars in softwood SSL by strong base ion exchange resin (PA408) treatment: (A) acetic acid; (B) oligosaccharides; (C) monosaccharides

Table 1 shows the results of the combined treatment of hardwood waste liquor that contained 23.1 g/L of acetic acid and 7.9 g/L of xylo-oligosaccharide. The acetic acid content of the waste liquor treated by combined CaO and the first-stage of ion exchange resin treatments was 14.9 g/L, and further ion exchange treatments were required to remove more acetic acid. The third-stage of ion exchange resin treatment decreased acetic acid content in the waste liquor to 5.9 g/L; furthermore the decrease of oligosaccharides with the third-stage treatment was marginal.

It was reported that the OH^- form of strong base anion exchange resin could adsorb monosaccharides and consequently decompose them (Koizumi and Okada 1980; Phillips and Pollard 1953; Turton and Pacsu 1955). Oligosaccharides could hardly be adsorbed to ion exchange resin, thus their decomposition was minimum. It was found that the acetic acid can be removed by the ion exchange resin treatment without decreasing oligosaccharide from softwood SSL and hardwood waste liquor.

The CaO treatment of SSL was applied for the recovery of lignosulfonate in pulp mills (Howard 1932). The sulfate and sulfite ions can also be removed from the SSL by the CaO treatment (Howard 1932; Kuroishi 1983). In this study, the sulfite ion content of softwood SSL was 12.7 g/L, and no sulfite ion was found after CaO treatment of SSL (Table 2). Other studies have also shown that furfural in the pre-hydrolyzates or SSL can be removed by lime treatment (Nigam 2001; Purwadi *et al*. 2004), and the furfural in the hardwood waste liquor (0.7 g/L) was removed by CaO treatment (Table 1). On the other hand, in this study we found that the combined CaO and ion exchange resin treatments can decrease the acetic acid level to a fermentable level, while having minimum effect on oligosaccharides.

Table 1. Change of Chemical Composition of Hardwood Waste Liquor with the Combined Method

Treatment	Oligosaccharides** (g/L)					Inhibitory compounds** (g/L)	
	Glc	Man	Xyl	Ara	Gal	Acetic acid	Furfural
Untreated	0.6	0.3	7.9	0.4	0.5	23.1	0.7
CaO	0.6	0.3	7.9	0.4	0.5	19.9	0
1st ion exchange*	0.5	0.3	7.9	0.3	0.4	14.9	0
2nd ion exchange*	0.5	0.3	7.9	0.3	0.4	9.7	0
3rd ion exchange*	0.4	0.3	7.9	0.3	0.4	5.9	0
* Each treatment time was 5 min. ** No monosaccharides and sulfite ion were found.							

Enhanced Ethanol Production from Oligosaccharides in Softwood SSL by Combined CaO and Ion Exchange Resin Treatments

Table 2 shows the chemical composition of SSL samples before and after fermentation. The fermentation results are shown in Fig. 5. When the fermentation with *P. stipitis* was conducted for 24 h, 1.3 g/L of ethanol was obtained in the SSL treated by the combined CaO and ion exchange resin treatments. All of monosaccharides were consumed at 16 h of fermentation. In addition, 3.3 g/L manno-oligosaccharides, 1.9 g/L gluco-oligosaccharides, and 0.2 g/L of xylo-oligosaccharides were consumed (Table 2). *P. stipitis* has the genes for seven β-glucosidases, two β-mannosidases, and one endoxylanase (Jeffries 2006; Jeffries *et al.* 2007). Oligosaccharides were hydrolyzed by these enzymes and consequently consumed by the strain. Several reports have shown that *P. stipitis* can produce ethanol from cellobiose (Parekh and Wayman 1986; Parekh *et al.* 1988) and xylan (Lee *et al.* 1986), however limited results are available on utilization of

manno-oligosaccharides by *P. stipitis*. The present results confirmed that *P. stipitis* has an ability to metabolize manno-oligosaccharide. As shown in Fig. 5, further oligosaccharides consumption from the SSL treated with combined CaO and ion exchange resin did not occur after 16 h of fermentation. β-glucosidases and β-mannosidases, which are produced by *P. stipitis*, have high specificities to hydrolyze cellobiose and mannobiose.

Table 2. Chemical Composition of Softwood SSL Samples Before and After Fermentation

	Monosaccharides (g/L)					Oligosaccharides(g/L)					Inhibitory compounds*(g/L)	
	Glc	Man	Xyl	Ara	Gal	Glc	Man	Xyl	Ara	Gal	Acetic acid	Sulfite ion
Untreated	1.0	3.3	0.8	0	0.7	3.9	9.1	0.3	0	1.5	5.2	12.7
CaO	0.9	2.3	0.5	0	0.5	3.9	9.1	0.4	0	1.4	4.9	0
Ion exchange	0.7	1.8	0.4	0	0.4	3.9	9.1	0.4	0	1.4	0.9	0
After 24 h fermentation	0	0	0	0	0	2.0	5.8	0.2	0	1.3	0	0
*No furfural was found.												

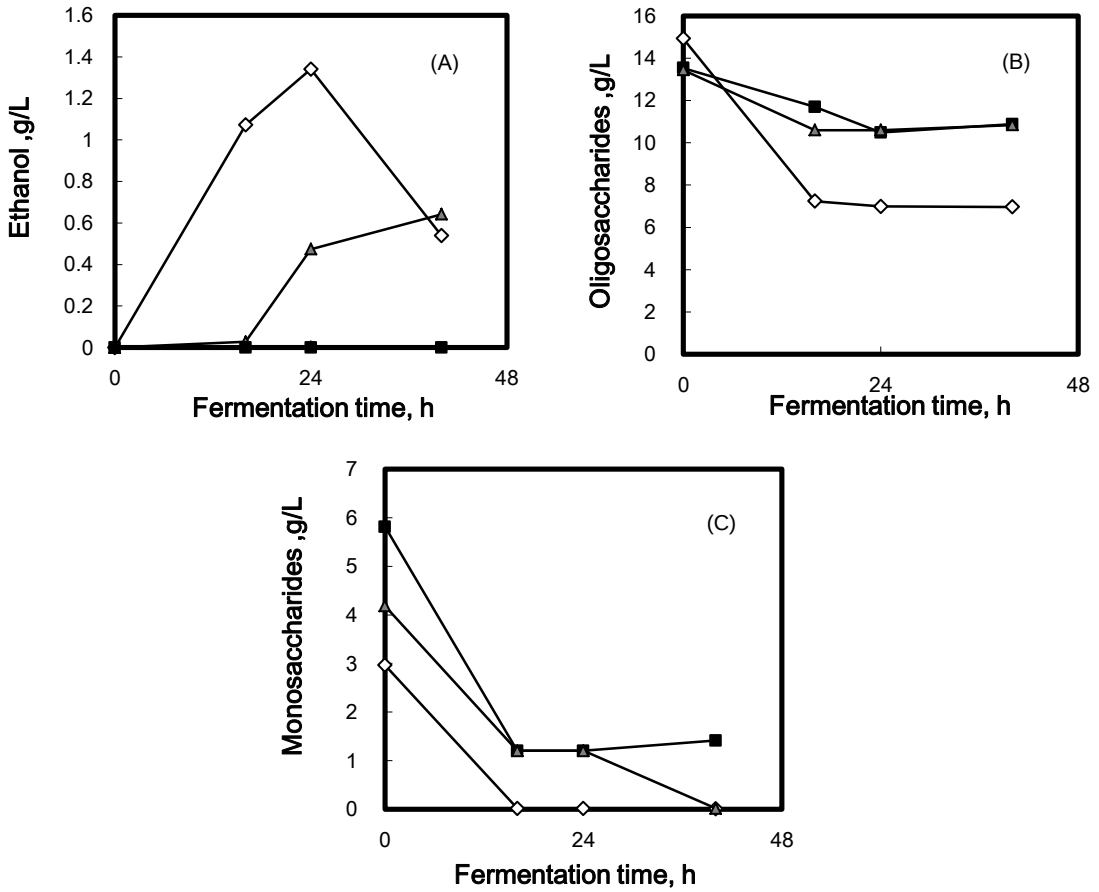

Fig. 5. Effect of ion exchange resin treatment on ethanol production from oligosaccharides in softwood SSL with *P. stipitis*: (A) ethanol; (B) total oligosaccharides; (C) total monosaccharides. Legends: (◇) CaO and ion exchange resin treated; (▲) CaO treated; (■) untreated

The ethanol concentration from the CaO-treated SSL after 40 h fermentation was 0.5 g/L, and no ethanol was obtained from the untreated SSL. Based on these results, it can be concluded that the combined CaO and ion exchange treatments of SSL are effective for improving ethanol production from oligosaccharides in softwood SSL.

CONCLUSIONS

1. From the result of model experiments using cellobiose solution, 2.6 g/L of ethanol was obtained with 1 g/L of acetic acid present, while 2.9 g/L of ethanol was produced from the control with no acetic acid present. The acetic acid concentration was decreased during the *P. stipitis* fermentation; thus, the inhibitory effect of acetic acid was lowered in the late stage of fermentation.
2. It was found that inhibitory compounds such as acetic acid, furfural, and sulfite ions in the softwood spent sulfite liquor (SSL) and the hardwood waste liquor can be removed by the combined CaO and ion exchange resin treatments with minimum effect on the oligosaccharides. The acetic acid concentration in the softwood SSL decreased from 5.2 g/L to 0.9 g/L by the combined method.
3. The combined method was effective for improving ethanol production from oligosaccharides in softwood SSL with *P. stipitis*. 38% of total oligosaccharides (initial concentration; 14.8 g/L) were consumed and 1.3 g/L of ethanol was obtained from the SSL treated by the combined method, while no ethanol was obtained from the untreated SSL.

ACKNOWLEDGMENTS

One of the authors, Keishi Tanifuji, was supported by JSPS Program for Young Researcher Overseas Visits.

REFERENCES CITED

Agbogbo, F. K., and Coward-Kelly, G. (2008). "Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*," *Biotechnol. Lett.* 30(9), 1515-1524.

Amartey, S., and Jeffries, T. W. (1994). "Comparison of corn steep liquor with other nutrients in the fermentation of D-xylose by *Pichia stipitis* CBS 6054," *Biotechnol. Lett.* 16(2), 211-214.

Browning, B. L. (1967). *Methods of Wood Chemistry, Vol. II*, Interscience, New York.

Delgenes, J. P., Moletta, R., and Navarro, J. M. (1996). "Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*," *Enzyme Microb. Technol.* 19(3), 220-225.

Howard, G. C. (1932). "Process of treating waste sulfite liquor," *U.S. Patent* 1,856,558, May 3.

Jeffries, T. W. (2006). "Engineering yeasts for xylose metabolism," *Curr. Opin. Biotechnol.* 17(3), 320-326.

Jeffries, T. W., Grigoriev, I. V., Grimwood, J., Laplaza, J. M., Aerts, A., Salamov, A., Schmutz, J., Lindquist, E., Dehal, P., Shapiro, H., Jin, Y., Passoth, V., and Richardson, P. M. (2007). "Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast *Pichia stipitis*," *Nat. Biotechnol.* 25(3), 319-326.

Koizumi, K., and Okada, Y. (1980). "Transformation of sugars with strongly basic anion-exchange resin. I," *Yakugaku Zasshi* 100(2), 183-191.

Kuroishi, H. (1983). *Sulfite Pulp Dissolving Pulp*, Japan TAPPI, Tokyo, Japan.

Lee, H., Biely, P., Latta, R. K., Barbosa, M. F. S., and Schneider, H. (1986). "Utilization of xylan by yeasts and its conversion to ethanol by *Pichia stipitis* strains," *Appl. Environ. Microbiol.* 52(2), 320-324.

Liu, X., Fatehi, P., and Ni, Y. (2011). "Adsorption of lignocelluloses dissolved in prehydrolysis liquor of kraft-based dissolving pulp process on oxidized activated carbons," *Ind. Eng. Chem. Res.* 50(20), 11706-11711.

Liu, X., Fatehi, P., and Ni, Y. (2012). "Removal of inhibitors from pre-hydrolysis liquor of kraft-based dissolving pulp production process using adsorption and flocculation processes," *Bioresource Technology* 116, 492-496.

Nigam, J. N. (2001). "Ethanol production from hardwood spent sulfite liquor using an adapted strain of *Pichia stipitis*," *J. Ind. Microbiol. Biotechnol.* 26(3), 145-150.

Palmqvist, E., and Hahn-Hagerdal, B. (2000). "Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification," *Bioresour. Technol.* 74(1), 17-24.

Parekh, S., and Wayman, M. (1986). "Fermentation of cellobiose and wood sugars to ethanol by *Candida shehatae* and *Pichia stipitis*," *Biotechnol. Lett.* 8(8), 597-600.

Parekh, S. R., Parekh, R. S., and Wayman, M. (1988). "Fermentation of xylose and cellobiose by *Pichia stipitis* and *Brettanomyces clausenii*," *Appl. Biochem. Biotechnol.* 18, 325-338.

Phillips, J. D., and Pollard, A. (1953). "Degradation of sugars on ion-exchange columns of Amberlite IRA 400 (OH^-)," *Nature (London, U. K.)* 171, 41-42.

Purwadi, R., Niklasson, C., and Taherzadeh, M. J. (2004). "Kinetic study of detoxification of dilute-acid hydrolyzates by $Ca(OH)_2$," *J. Biotechnol.* 114(1-2), 187-198.

Rødsrud, G., Lersch, M., and Sjöde, A. (2012). "History and future of world's most advanced biorefinery in operation," *Biomass and Bioenergy*, 46, 46-59.

Saeed, A., Fatehi, P., Ni, Y., and van Heiningen, A. (2011). "Impact of furfural on the sugar analysis of pre-hydrolysis liquor of kraft-based dissolving pulp production process using the HPAEC technique," *BioResources* 6(2), 1707-1718.

Shen, J., Fatehi, P., Soleimani, P., and Ni, Y. (2011). "Recovery of lignocelluloses from pre-hydrolysis liquor in the lime kiln of kraft-based dissolving pulp production process by adsorption to lime mud," *Bioresour. Technol.* 102(21), 10035-10039.

Shen, J., Fatehi, P., Soleimani, P., and Ni, Y. (2012). "Lime treatment of prehydrolysis liquor from the kraft-based dissolving pulp production process," *Ind. Eng. Chem. Res.* 51(2), 662-667.

Shi, H., Fatehi, P., Xiao, H., and Ni, Y. (2011). "A combined acidification/PEO flocculation process to improve the lignin removal from the pre-hydrolysis liquor of kraft-based dissolving pulp production process," *Bioresour Technol*. 102(8), 5177-5182.

Shi, H., Fatehi, P., Xiao, H., and Ni, Y. (2012). "Optimizing the poly ethylene oxide flocculation process for isolating lignin of prehydrolysis liquor of a kraft-based dissolving pulp production process," *Ind. Eng. Chem. Res.* 51(14), 5330-5335.

Sixta, H. (2006). *Handbook of Pulp, Volume 1*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Takahashi, S., Shiell, K., Fatehi, P., and Ni, Y. (2012). "Ethanol production via in-situ detoxification of spent sulfite liquor," *Journal of Bioprocess Engineering and Biorefinery* 1(1), 105-112.

Takahashi, S., Tanifuji, K., Nakagawa-Izumi, A., Ohi, H., and Nakamata, K. (2010). "Estimation of kraft and acid sulfite cooking methods as processes of bioethanol production," *Japan TAPPI Journal* 64(4), 420-436.

Takahashi, S., Tanifuji, K., Shiell, K., Jahan, M. S., Ohi, H., and Ni, Y. (2013). "Removal of acetic acid from spent sulfite liquor using anion exchange resin for effective xylose fermentation with *Pichia stipitis*," *BioResources* 8(2) , 2417-2418.

Tanifuji, K., Takahashi, S., Kajiyama, M., Ohi, H., and Nakamata, K. (2011). "Advantage of acid sulfite cooking as processes of bioethanol production," *Japan TAPPI Journal* 65(5), 494-505.

Turton, C. N., and Pacsu, E. (1955). "The effect of anion-exchange resins on reducing sugars," *J. Am. Chem. Soc.* 77, 1059-1061.

Article submitted: January 14, 2013; Peer review completed: March 24, 2013; Revised version received: May 17, 2013; Accepted: May 30, 2013; Published: June 4, 2013.