Removal of Acetic Acid from Spent Sulfite Liquor Using Anion Exchange Resin for Effective Xylose Fermentation with *Pichia stipitis*

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Acetic acid is one of the major inhibitors of spent sulfite liquor (SSL) fermentation for ethanol production. The objective of this study was to remove acetic acid from hardwood SSL using anion exchange resin in order to achieve effective fermentation with *Pichia stipitis* CBS6054. Lignosulfonate, as well as sulfate and sulfite ions in the SSL hindered the removal of acetic acid by anion exchange resins. CaO treatment was an effective method for removing these materials from SSL, which facilitated the removal of acetic acid in the subsequent ion exchange resin treatment. A two-stage strong base ion exchange resin (OH^- form) treatment removed approximately 90% of the acetic acid from CaO-treated SSL, which decreased the acetic acid concentration to less than 1 g/L. The combined treatment of CaO and ion exchange resin treatments in a relatively short time achieved the selective removal of acetic acid from SSL and significantly increased the ethanol production from SSL.

Keywords: Spent sulfite liquor; Xylose fermentation; Acetic acid removal; Anion exchange resin

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INTRODUCTION

Sulfite cooking is an established process in the pulp and paper industry. The resultant spent sulfite liquor (SSL) from the sulfite cooking contains monosaccharides, oligosaccharides, and lignosulfonate; several sulfite pulp mills have utilized SSL for ethanol production (Rødsrud *et al*. 2012). In softwood SSL, there is a high amount of hexoses which can be fermented by baker's yeast (*Saccharomyces cerevisiae*). However, this yeast cannot ferment pentoses, such as xylose. Hardwood SSL has a higher xylose concentration than softwood SSL. *Pichia stipitis* is a well-known yeast that can assimilate xylose (Agbogbo and Coward-Kelly 2008). SSL also contains furfural, acetic acid, and sulfate ions (Sixta 2006), which inhibit the bioconversion of monosugars to ethanol (Agbogbo and Coward-Kelly 2008). Unfortunately, *P. stipitis* is more sensitive to inhibitory compounds than *S. cerevisiae* (Delgenes *et al*. 1996; Palmqvist and Hahn-Hagerdal 2000). Therefore, the removal of these inhibitory compounds is necessary in order to effectively produce ethanol using *P. stipitis*.

In a previous study, simultaneous detoxification and fermentation (*in situ* detoxification) of SSL using adsorbent was studied (Takahashi *et al.* 2012). Activated carbon removed higher amounts of furfural and acetic acid from the model softwood SSL than did polymeric resin and precipitated calcium carbonate. In that study, ethanol production by *S. cerevisiae* was greater in fermentation in the presence of activated carbon. However, the maximum removal of acetic acid was 50% by the activated carbon treatment, thus 7 g/L of acetic acid still remained in the SSL. A greater acetic acid removal is required in order to use *P. stipitis* more effectively for fermentation.

CaO treatment has been applied to SSL for the recovery of lignosulfonate in pulp mills (Howard 1932). The sulfate and sulfite ions could be removed from SSL by the CaO treatment (Howard 1932; Kuroishi 1983; Nigam 2001). In the current study, the combined treatments of CaO and amine-based ion exchange resin were proposed. It was expected that such a process could enhance the removal of inhibitory compounds from SSL.

Anion exchange resin has an amine group that can selectively adsorb anions. Furthermore, one of the advantages of ion exchange resins is their ability to be regenerated. In a commercial application, sugar mills use an ion exchange resin to purify the sugar. The salts and ash in the sugar solution are removed by cation and anion exchange resins (Alexandratos 2009). Therefore, it was expected that using an ion exchange resin for the removal of acetic acid from SSL could be a feasible method.

A weak base anion exchange resin can adsorb anions under only acidic and neutral pH conditions. By contrast, a strong base ion exchange resin can work within a wide pH range. However, the OH^- form of a strong base anion exchange resin can adsorb monosugars and consequently decompose them (Koizumi and Okada 1980; Phillips and Pollard 1953; Turton and Pacsu 1955). It was reported that the order of the selectivity of anions on the strong base anion exchange resin was as follows: $OH^- < CH_3COO^- < Cl^- < HSO_3^- < HSO_4^-$ (Wang *et al.* 2006). The Cl^- form of a strong base ion exchange resin cannot adsorb acetic acid.

Therefore, the objectives of this study were: 1) to remove acetic acid from hardwood SSL *via* a combined treatment of CaO and amine-based ion exchange resin and 2) to determine the effect of such a pretreatment on the production of ethanol with *P. stipitis*. First, the effect of acetic acid on ethanol production by *P. stipitis* was studied. Second, the removal of acetic acid from hardwood SSL with the ion exchange resins was evaluated. Third, the effect of ion exchange resin treatment of hardwood SSL on the fermentability of *P. stipitis* was evaluated.

EXPERIMENTAL

SSL Sample

The hardwood SSL was obtained from a pulp mill in eastern Canada. The SSL was collected from the bottom of the cooking digester before pulp washing (*i.e.* diluting the SSL). The cooking was conducted at a temperature range of 135 to 150 °C, pH 1.5, and 7% SO_2. The SSL sample was kept at 4 °C for our analysis in this work. The chemical composition of hardwood SSL was as follows: glucose 4.3 g/L, xylose 18.9 g/L, mannose 10.3 g/L, arabinose 0.6 g/L, galactose 1.8 g/L, acetic acid 11.2 g/L,

lignosulfonate 119.5 g/L, sulfite ion 5.5 g/L, and sulfate ion 18.4 g/L. The pH of this SSL was 3.5.

Ion Exchange Resins and Activated Carbon

Strong base (Diaion PA408 Cl^- form, obtained from Mitsubishi Chemical) and weak base (A103S and Diaion WA30, obtained from Purolite and Mitsubishi Chemical, respectively) ion exchange resins were used in this research. The OH^- form of PA408 was prepared as follows: Cl^- form resin was soaked in 2 N NaOH for 30 min and then filtered. This treatment was repeated five times, and subsequently the resin was washed with distilled water five times. The OH^- form of PA408 was kept in distilled water to prevent oxidation and denaturation. The activated carbon (powder form) was purchased from Sigma Aldrich (cat# C272-500).

Ion Exchange Resin Treatment

The untreated SSL was treated with various ion exchange resins or activated carbon for 24 h at 30 °C at 150 rpm. The resin or activated carbon dosage was 20 wt% on SSL samples in all experiments.

In another set of experiments, the ion exchange resin treatments of acetic acid solution were conducted in the presence of lignosulfonate or sulfite and sulfate ions. At first, 10 g/L of acetic acid solution (AS), AS with 120 g/L of lignosulfonate, and AS with sulfite and sulfate ions were treated with the OH^- form of PA408 for 24 h at 30 °C and 150 rpm. The adsorption tests were performed with 20 mL of sample in a 125 mL Erlenmeyer flask. After treatment, supernatants were separated by filtration and collected for further analysis.

Combined CaO and Ion Exchange Resin Treatments

The CaO treatment of SSL was conducted as follows: The pH of hardwood SSL was adjusted to 10.5 with 100 g/L of CaO slurry and then treated at 70 °C for 15 min in a water bath (Howard 1932; Kuroishi 1983). The mixture was separated by filtration and the filtrate was used for further resin treatments.

Then, the CaO-treated SSL was treated with the OH^- form of PA408 for 24 h at 30 °C and 150 rpm. In another set of experiments, the CaO-treated SSL was neutralized with CO_2 to pH 6.7 and then treated with the OH^- form of PA408, WA30, and A103S for 24 h. After the resin treatment, the mixture was separated by filtration for analysis.

Combined CaO and Two-stage Strong Base Ion Exchange Resin Treatments

One-stage resin treatment: The alkaline (pH 10) and neutralized (pH 6.7) CaO-treated SSLs were treated with the OH- form of PA408 for up to 10 min at 30 °C and 150 rpm. The resin dosage was 20 wt% based on SSL. In another set of experiments, the CaO-treated SSL was neutralized with CO_2 to pH 6.7 and then treated with the OH- form of PA408 for up to 10 min (one stage resin treatment). The supernatants were collected for analysis after treatment.

Two-stage resin treatment: After the first (2 min) stage resin treatment, the supernatant was collected and then neutralized to pH 6.7 with CO_2. Subsequently, it was treated with the OH- form of PA408 resin for the second time up to 10 min. The conditions of the second resin treatment were the same as the first one.

Microorganism

P. stipitis CBS6054 was obtained from the USDA (Madison, Wisconsin). Stock cultures were kept on YPDX agar plates, which contained 10 g/L yeast extract, 20 g/L peptone, 10 g/L D-glucose, 20 g/L D-xylose, and 10 g/L 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 3.5 g/L peptone, 3 g/L yeast extract, 2 g/L KH_2PO_4, 1 g/L $MgSO_4$, 1 g/L $(NH_4)_2SO_4$, and 80 g/L xylose with pH 5.0 in a 125 mL Erlenmeyer flask. Incubation was conducted at 30 °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

The model fermentation of monosugar solutions was prepared by mixing 17.2 g/L xylose, 3.9 g/L glucose, and 9.4 g/L mannose in the presence of various acetic acid concentrations (0.01, 0.1, 1, 5, and 10 g/L). The fermentation was conducted in test tubes using 10 mL of sample.

In the fermentation of hardwood SSL, the untreated, CaO-treated, and one stage (2 min treatment) and two-stage ion exchange resin-treated (2 min in the 1st stage and 4 min in 2nd stage) SSLs were used. These SSL samples were concentrated *via* evaporation so that the total sugar concentration was 30 g/L. Fermentation was conducted in test tubes using 5 mL of sample.

The peptone, yeast extract, and ions were also added to samples in the same amounts as those used for the inoculum preparation. About 1 g/L dry cell weight was used. The pH of the samples was adjusted to 5.0 with H_2SO_4 or NaOH. All of the fermentation experiments were conducted at 30 °C and 150 rpm. An aliquot of 1 mL was withdrawn to determine the ethanol and sugar concentrations during the fermentation experiment. After fermentation, the samples were centrifuged and supernatant was collected for analysis.

Analytical Methods

Monosugar concentrations were measured using an ion chromatography unit equipped with a CarboPac® PA1 column (Dionex-300, Dionex Corporation, USA) and a pulsed amperometric detector (PAD) (Shen *et al.* 2011). The total monosugar concentration was calculated by the sum of each monosugar concentration.

The furfural and acetic acid concentrations were determined by the ^{1}H-NMR method as described in the literature (Saeed *et al.* 2011).

The lignosulfonate concentration was determined by the absorbance at 205 nm with UV spectrophotometry (Browning 1967). A calibration curve was prepared using commercial lignosulfonate.

Sulfite and sulfate ion analysis was conducted, following TAPPI test method T699 om-87.

The ethanol concentration in the fermented samples was determined by an HPLC device equipped with RI detector (Shimadzu). Separations were performed on a Rezex ROA-organic Acid H^+ column (Phenomenex) at 65 °C. The injection volume was 20 μL,

the mobile phase was 5 mM H_2SO_4, and the flow rate was 0.6 mL/min. This analysis was conducted in duplicates.

RESULTS AND DISCUSSION

Effect of Acetic Acid and Sulfite Ion on Ethanol production with *P. stipitis*

The effect of acetic acid concentration on fermentation with *P. stipitis* is shown in Fig. 1. The maximum ethanol production in the solution that had no acetic acid (control) was 11.2 g/L. *P. stipitis* was able to consume monosugars in the presence of 5 g/L of acetic acid, but ethanol production was marginal. In the presence of 1 g/L acetic acid in solution, the ethanol production was approximately half of that produced from the control sample after 48 h of fermentation. However, 9.6 g/L of ethanol was finally produced after 72 h of fermentation. These results may suggest that the acetic acid concentration should be reduced to less than 1 g/L to effectively produce ethanol from hardwood SSL using *P. stipitis*.

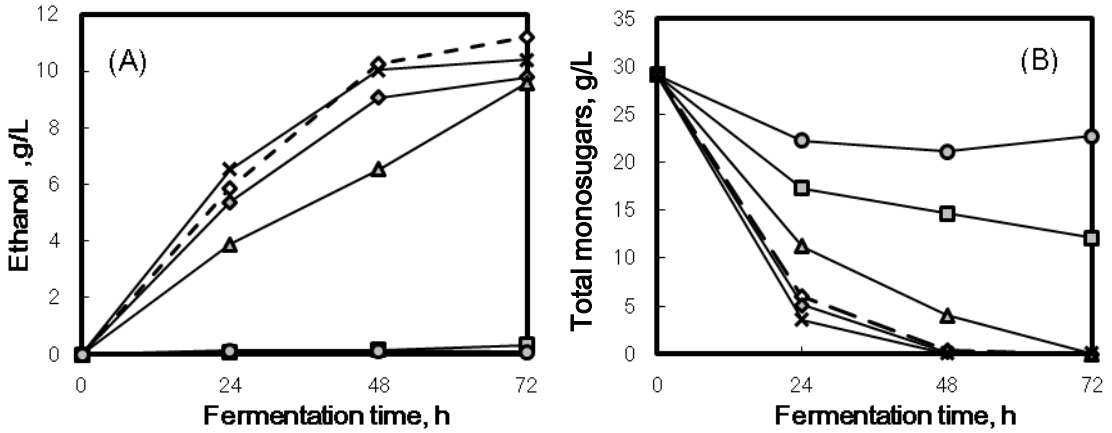

Fig. 1. Effect of acetic acid concentration on fermentation with *P. stipitis* using model solution; (A) ethanol concentration and (B) monosugar concentration
Legend: control (◇), 0.01 g/L (×), 0.1 g/L (◈), 1 g/L (△), 5 g/L (□), 10 g/L (○)

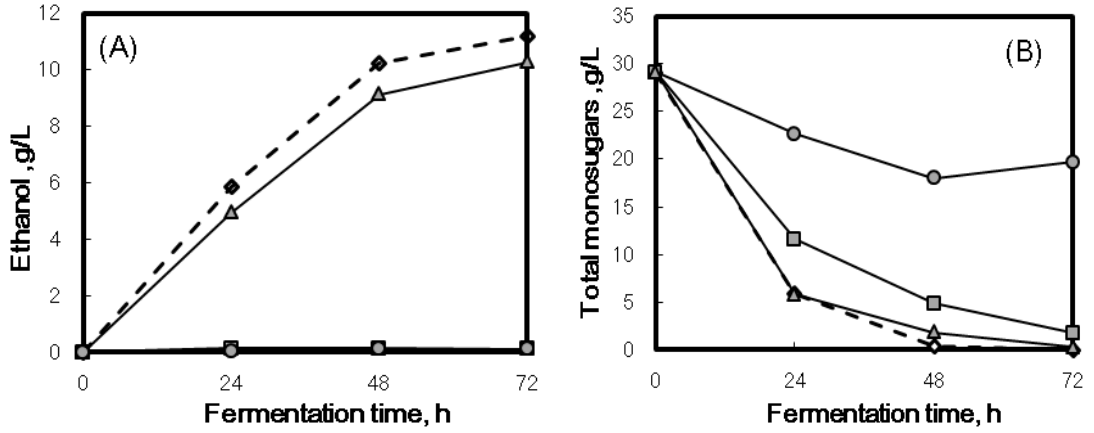

Fig. 2. Effect of sulfite ion concentration on fermentation with *P. stipitis* using model solution; (A) ethanol concentration and (B) monosugar concentration
Legend: control (◇), 0.3 g/L (△), 5 g/L (□), 20 g/L (○)

Figure 2 shows the effect of sulfite ion concentration on fermentation with *P. stipitis*. As can be seen, ethanol was not produced by *P. stipitis* in the presence of 5 g/L of sulfite ion. The original hardwood SSL contained 5.5 g/L of sulfite ion. Therefore, the sulfite ion needs to be removed from SSL for an effective fermentation process.

Ion Exchange Resin Treatment of Hardwood SSL

Table 1 lists the acetic acid and monosugar concentrations in SSL treated with various ion exchange resins. It can be seen that both the weak and strong base resin treatments could not remove acetic acid from untreated hardwood SSL. It seems that the coexisting of substances, *e.g.* lignosulfonate and ions, in SSL hinders the removal of acetic acid from the solution.

It can also be seen in Table 1 that the treatment with PA408 caused a slight decrease in monosugar concentration. This is because PA408 is a strong base ion exchange resin. Other resins (WA30 an AS) are weak base ion exchangers. Monosugars are decomposed by the strong base anion exchange resin in its OH^- form. It has been reported that monosugars are epimerized (Koizumi and Okada 1980; Turton and Pacsu 1955) or converted to organic acids, such as lactic or glycolic acids (Phillips and Pollard 1953) with strong base ion exchange resin treatments.

Table 1. Results of Various Ion Exchange Resin Treatments of Untreated Hardwood SSL

Treatment	Acetic acid (g/L)	Total monosugars (g/L)	Lignosulfonate (g/L)
Untreated	11.2	35.9	119.5
PA408 (OH^- form) treatment	11.2	31.5	78.5
WA30 treatment	10.1	35.9	70.6
A103S treatment	11.2	35.9	-

Effect of the Presence of Lignosulfonate and Ions on Removal of Acetic Acid with Ion Exchange Resin

Figure 3 shows the results of the strong base ion exchange resin (OH^- form PA408) treatment of 10 g/L of various acetic acid aqueous solutions. Apparently, 98% of acetic acid removal was achieved by the strong base resin treatment. In the presence of 120 g/L of lignosulfonate, 30% of the acetic acid was removed from the ion exchange resin treatment. These results supported the conclusion that the presence of lignosulfonate retarded the removal of acetic acid by the ion exchange resin because the sulfonic group in lignosulfonate could be adsorbed to the active site of the resin. Furthermore, the ion exchange treatment of the aqueous solution containing 5 g/L of sulfite ion and 15 g/L of sulfate ion, which are at the same concentrations as the original SSL, showed only 10% acetic acid removal.

It was reported that the order of affinity among various ions and the strong base resin was $CH_3COO^- < Cl^- < HSO_3^- < HSO_4^-$ (Wang *et al.* 2006). Therefore, sulfite and sulfate ions were adsorbed to the resin more preferentially than the acetate ion, thus acetic acid removal could be compromised if sulfite and sulfate are present in solutions. The results in Fig. 3 also imply that the presence of sulfite and sulfate at their actual concentrations in industrially produced SSL (*i.e.* 5 g/L and 15 g/L, respectively) had

more negative impacts on the removal of acetic acid than lignosulfonate (120 g/L). Therefore, it is essential to decrease the sulfite and sulfate ions and lignosulfonate concentrations in order to remove acetic acid from SSL using ion exchange resin.

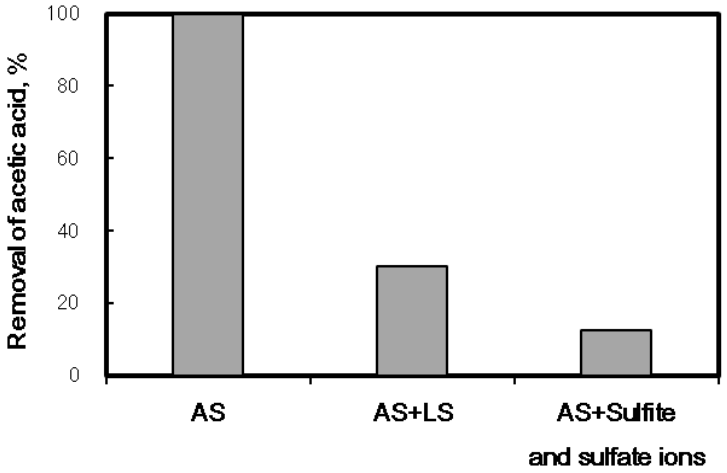

Fig. 3. Effect of the presence of lignosulfonate, sulfite, and sulfate ions on the removal of acetic acid with strong base ion exchange resin (PA408) from an aqueous solution
Legend; AS; acetic acid solution, LS; lignosulfonate

CaO and Activated Carbon Treatments of Hardwood SSL

The chemical compositions of CaO-treated and activated carbon-treated hardwood SSL are listed in Table 2. The lignosulfonate content in SSL decreased from 119.5 g/L to 68.4 g/L *via* the CaO treatment. The sulfite ion was reduced from 5.5 g/L to 0.7 g/L, and sulfate ion was totally removed by CaO treatment. The CaO treatment did not affect the removal of acetic acid and monosugars.

In a previous study, activated carbon treatment of model softwood SSL removed 100% and 50% of furfural and acetic acid, respectively, when the dosage of activated carbon was 20% and the ethanol production with *S. cerevisiae* increased 10-fold, compared to the untreated sample (Takahashi *et al*. 2012). In this study, 50% of the acetic acid was removed by activated carbon treatment of hardwood SSL, but the remaining acetic acid and sulfite ion in SSL prevented ethanol production by *P. stipitis*. The activated carbon treatment decreased lignosulfonate marginally and did not affect the removal of sulfite and sulfate ions. In other words, these compounds inhibited acetic acid removal by ion exchange resin (Fig. 2).

The activated carbon use did not improve acetic acid removal by ion exchange resin, and thus was ineffective in the detoxification of SSL for producing ethanol *via* fermentation with *P. stipitis*. The CaO treatment was effective in removing lignosulfonate, sulfite, and sulfate ions from SSL. Thus, this process along with ion exchange resin facilitated the detoxification of SSL in producing ethanol *via* fermenting monosugars with *P. stipitis* and was selected for further analysis. As can be seen in Table 2, the acetic acid concentration was increased with CaO treatment. It was reported that the cleavage of acetyl groups that are associated with hemicelluloses at a high pH and temperature would result in acetic acid formation (Shen *et al.* 2012).

Table 2. Results of CaO and Activated Carbon Treatments of SSL

Treatment	Acetic acid (g/L)	Total monosugars (g/L)	Lignosulfonate (g/L)	Sulfite ion (g/L)	Sulfate ion (g/L)
Untreated	11.2	35.9	119.5	5.5	18.4
CaO	13.3	34.5	68.4	0.7	-
Activated carbon	5.6	33.8	95.1	6.2	22.7

Combined CaO and Ion Exchange Resin Treatments of Hardwood SSL

Table 3 lists the concentrations of acetic acid, monosugars, and lignosulfonate of hardwood SSL after treatment with various ion exchange resins. Apparently, the acetic acid concentration in CaO-treated SSLs was decreased by 48% *via* strong base ion exchange resin treatment.

This treatment caused a significant monosugar removal as well. However, when the CaO-treated SSL was neutralized by CO_2 to pH 6.7, the subsequent ion exchange resin treatment resulted in the acetic acid and total monosugars concentrations of 4.9 g/L and 9.7 g/L, respectively. Furthermore, this process resulted in a higher monosugar concentration (9.7 g/L).

Table 3. Results of Various Anion Exchange Resin Treatments of CaO-treated SSL for 24 Hours

Treatment Sequence	Acetic acid (g/L)	Total monosugars (g/L)	Lignosulfonate (g/L)
CaO→PA408	6.7	3.3	12.3
CaO→CO_2→PA408	4.9	9.7	27.9
CaO→CO_2→WA30	13.3	34.5	22.6
CaO→CO_2→A103S	13.3	34.5	52.8

Legend: CaO→PA408: the SSL was treated with CaO and then PA408. CaO→CO_2→PA40: the SSL was treated with CaO, neutralized with CO_2 and then treated with PA408. CaO→CO_2→WA30: the SSL was treated with CaO, neutralized with CO_2 and then treated with WA30; CaO→CO_2→A103S: the SSL was treated with CaO, neutralized with CO_2, and then treated with A103S.

It is well known that monosugars are decomposed by the strong base anion exchange resin in its OH^- form. As explained earlier, monosugars have been epimerized (Koizumi and Okada 1980; Turton and Pacsu 1955) or converted to organic acids, such as lactic or glycolic acids (Phillips and Pollard 1953) with strong base ion exchange resin treatments.

Koizumi and Okada (1980) also reported that sugars were not released from the strong base resin by organic solvent extraction; rather they were released by soaking with 10% NaCl solution or aerating with CO_2 gas. These results indicated that sugars and strong ion exchange resin would bind together by ionic bonds. However, by the neutralization of SSL with CO_2 gas, carbonate ion would be produced in the SSL. The carbonate ion could be adsorbed to resin surface more preferentially than sugars during ion exchange resin treatment, which would leave the monosugars in SSL for the downstream fermentation process.

On the other hand, the weak base resin treatment of CaO-treated SSL after neutralization with CO_2 gas did not affect the removal of sugars and acetic acid (Table 3).

In this case, carbonate ions could be more easily adsorbed to the weak base resin, compared with monosugars and acetate ion. Weak base resins cannot adsorb ions under alkaline conditions (Wang *et al.* 2006). Generally, the order of uptake ions for weak base resin is $CH_3COO^- < Cl^- < HSO_3^- < HSO_4^- < OH^-$ (Wang *et al.* 2006). Therefore, it is expected that after neutralization of hardwood SSL by mineral acids, such as sulfuric acid or hydrochloric acid, these ions were adsorbed to the weak base resin more preferentially than acetate ion.

Optimizing Ion Exchange Resin Treatment of Hardwood SSL

As can be seen in Table 3, the total monosugars were significantly decreased by the strong base resin treatment after 24 h. The resin treatment process of SSL should be optimized in order to minimize the decomposition of monosugars. Figure 4 shows the acetic acid and monosugar concentrations of SSL after various pretreatment steps. As can be seen, the resin treatment for 2 min removed 6.6 g/L acetic acid from SSL. A further increase in the treatment time did not lead to a significant increase in acetic acid removal, but the monosugar concentration decreased significantly. Additionally, the removal of acetic acid from non-neutralized SSL was less than the neutralized SSL. These results confirmed that the neutralization of CaO-treated SSL with CO_2 prior to ion exchange resin was effective in maximizing the removal of acetic acid and in minimizing sugar decomposition. Additionally, 2 min treatment of CaO- and CO_2-treated SSL with ion exchange resin was sufficient to minimize the decomposition of monosugars. Thus, 2 min resin treatment was used for the subsequent experiments. Meanwhile, the ion exchange resin had a similar performance when adsorbing different monosugars.

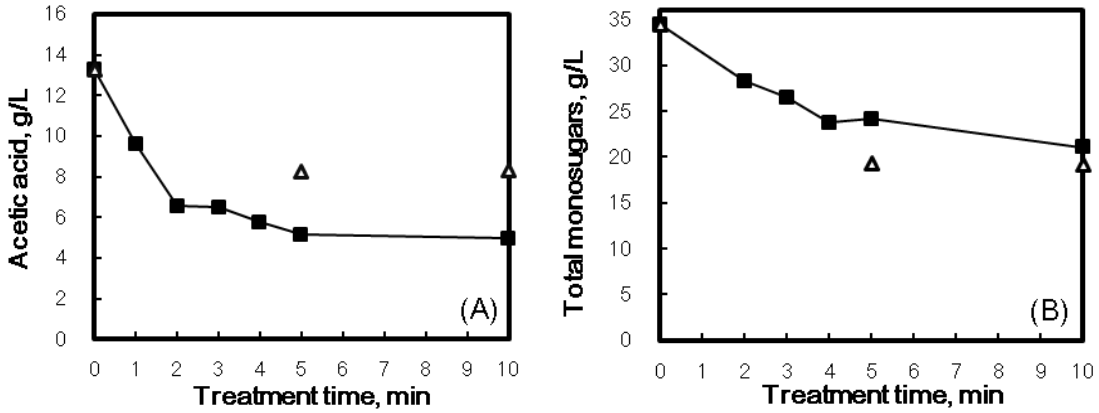

Fig. 4. Removal of acetic acid and sugars from neutralized (with CO_2) or non-neutralized SSLs with strong base ion exchange resin (PA408); (A) acetic acid and (B) total sugars changes
Legend: SSL was treated with CaO, neutralized with CO_2 and then treated with PA408 (■), SSL was treated with CaO and treated with PA408 without neutralization (△)

Combined CaO and Two-stage Strong Base Ion Exchange Resin Treatments of Hardwood SSL

As shown in Fig. 1, the ethanol concentration of prepared solution in the presence of 5 g/L of acetic acid was low (0.3 g/L). Additionally, more than 6 g/L of acetic acid remained in hardwood SSL after the first stage of resin treatment (Fig. 4), which would be still too high to achieve effective ethanol fermentation with *P. stipitis*. Subsequently, a second stage of resin treatment of SSL was conducted after the first stage in order to

remove the remaining acetic acid from the treated SSL. The pH of the solution after the first stage of treatment increased to around 10. Thus, the treated SSL was neutralized by CO_2 before the second stage of the resin treatment. Fig. 5 shows the impact of the second stage resin treatment on acetic acid and sugar removal as a function of treatment time. As can be seen, the second stage of resin treatment (4 min) resulted in 0.9 g/L acetic acid and 15.9 g/L monosugar concentrations. These results confirmed that a combined CaO, CO_2, and two-stage strong base ion exchange resin treatments could achieve a selective removal of acetic acid from the SSL.

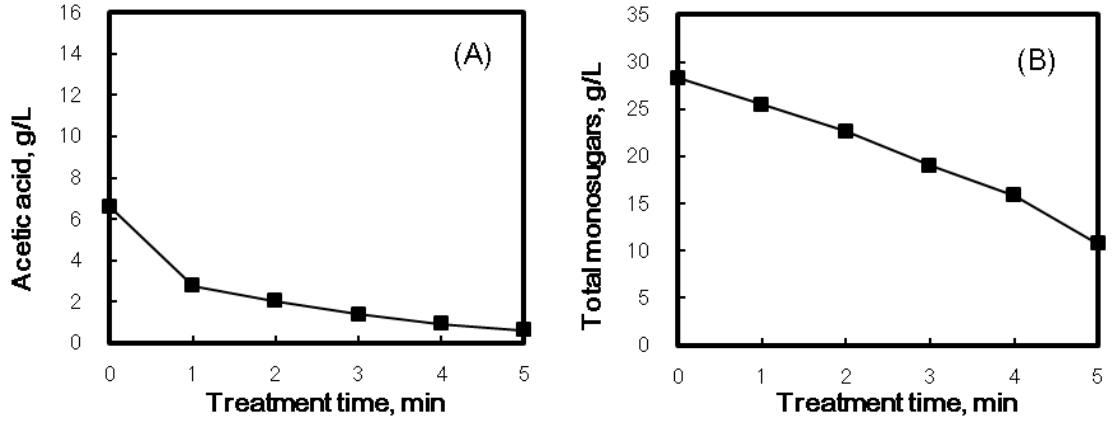

Fig. 5. Removal of acetic acid and monosugars from SSL by two-stage OH^- form of strong base anion exchange resin treatments: (A) acetic acid and (B) total monosugars

Ethanol Production *via* Combined CaO and Two-stage Strong Base Ion Exchange Resin Treatments Using Hardwood SSL

The fermentation of SSL samples treated with CaO and two-stage ion exchange resin was conducted, and the results are shown in Fig. 6.

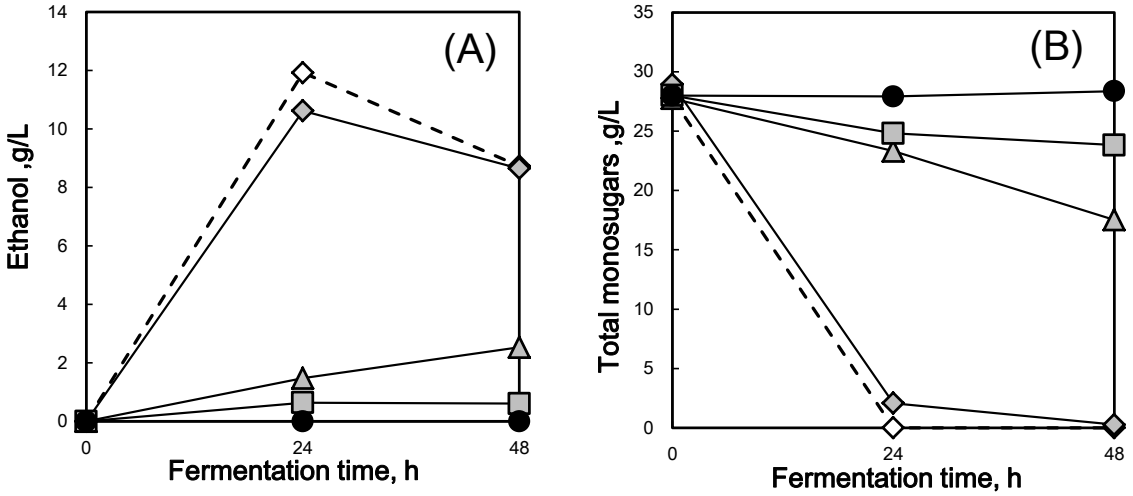

Fig. 6. Effect of ion exchange resin treatment on ethanol production from hardwood SSL with *P. stipites*: (A) ethanol and (B) total monosugars
Legend: control (◇), untreated (●), CaO-treated (□), one-stage resin treatment (△), two-stage resin treatment (◈). Untreated: original SSL, CaO-treated: the SSL was treated with CaO, one-stage resin treatment: The SSL was treated with CaO, neutralized with CO_2, and then treated with PA408 for 2 min, two-stage resin treatment: The SSL was treated with CaO, neutralized with CO_2, treated with PA408 for 2 min, neutralized with CO_2, and finally treated with PA408 for 4 min

The ethanol production from the fermentation was marginal in the untreated and CaO-treated SSL. After the first stage of strong base resin treatment, ethanol with a low concentration (*i.e*. 2.5 g/L) was produced. Ethanol production increased to 10.6 g/L after 24 h of fermentation due to two-stage ion exchange resin treatment of the CaO- and CO_2-treated hardwood SSL. This concentration was almost the same as that of the control (*i.e*. a sample without any acetic acid, but with the same monosugars concentration as was in hardwood SSL). It was revealed that the two-stage ion exchange resin treatments were effective in increasing ethanol production from monosugars in hardwood SSL with *P. stipitis*.

CONCLUSIONS

1. For effective ethanol production from hardwood spent sulfite liquor (SSL) using *P. stipitis*, the acetic acid concentration should be reduced to less than 1 g/L in SSL. Ethanol was not produced in the presence of more than 5 g/L of sulfite ion or acetic acid.
2. The combined treatments of CaO, CO_2, and two-stage strong base ion exchange resin (OH^- form) in a relatively short time was effective in selectively removing acetic acid from hardwood SSL and in improving the ethanol production of *P. stipitis*.
3. The CaO treatment was effective in removing lignosulfonate and sulfite and sulfate ions from SSL, which helped the subsequent strong base resin treatment in removing acetic acid.
4. The neutralization of CaO-treated SSL with CO_2 was effective in maximizing acetic acid removal and minimizing the decomposition of sugars by resin.

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