0273-2289/01/91–93/0035/$13.75

Detoxification of Lignocellulose Hydrolysates with Ion-Exchange Resins

Nils-Olof Nilvebrant,[1] Anders Reimann,[1] Simona Larsson,[2] and Leif J. Jönsson*,[2]

[1]STFI, Swedish Pulp and Paper Research Institute, PO Box 5604, SE-114 86 Stockholm, Sweden; and [2]Applied Microbiology, Lund University/Lund Institute of Technology, PO Box 124, SE-221 00 Lund, Sweden, E-mail: leif.jonsson@tmb.lth.se

Abstract

Lignocellulose hydrolysates contain fermentation inhibitors causing decreased ethanol production. The inhibitors include phenolic compounds, furan aldehydes, and aliphatic acids. One of the most efficient methods for removing inhibiting compounds prior to fermentation is treatment of the hydrolysate with ion-exchange resins. The performance and detoxification mechanism of three different resins were examined: an anion exchanger, a cation exchanger, and a resin without charged groups (XAD-8). A dilute acid hydrolysate of spruce was treated with the resins at pH 5.5 and 10.0 prior to ethanolic fermentation with *Saccharomyces cerevisiae*. In addition to the experiments with hydrolysate, the effect of the resins on selected model compounds, three phenolics (vanillin, guaiacol, and coniferyl aldehyde) and two furan aldehydes (furfural and hydroxymethyl furfural), was determined. The cation exchanger increased ethanol production, but to a lesser extent than XAD-8, which in turn was less effective than the anion exchanger. Treatment at pH 10.0 was more effective than at pH 5.5. At pH 10.0, the anion exchanger efficiently removed both anionic and uncharged inhibitors, the latter by hydrophobic interactions. The importance of hydrophobic interactions was further indicated by a substantial decrease in the concentration of model compounds, such as guaiacol and furfural, after treatment with XAD-8.

Index Entries: Detoxification; inhibition; ethanol production; *Saccharomyces cerevisiae*; softwood; ion exchange.

Introduction

Acid hydrolysates of lignocellulosic materials contain inhibitors that cause decreased productivity in ethanolic fermentations by microbes such

*Author to whom all correspondence and reprint requests should be addressed.

as baker's yeast. In the production of fuel ethanol from lignocellulose, the problem with fermentation inhibition can be overcome by detoxification of the hydrolysate prior to fermentation. In a comparison of different methods for detoxification of a dilute-acid hydrolysate of spruce *(1)*, treatment with anion-exchange resin was one of the most efficient. The chemical effect of the different detoxification methods on the hydrolysate was analyzed, and it was concluded that the anion exchanger affected all the different types of inhibitors measured (phenolics, furan aldehydes, and aliphatic acids), as well as the concentration of fermentable sugars in the hydrolysate. The poor specificity observed for the anion-exchange treatment was tentatively explained by the occurrence of hydrophobic interactions with the matrix of the used anion-exchange resin.

Ion-exchange resins have been employed previously in the detoxification of lignocellulose hydrolysates (reviewed in ref. *2*). A birch wood hemicellulose hydrolysate was detoxified using a cation exchanger prior to fermentation with *Gluconobacter oxydans (3)*. A mixed-bed ion resin was used in combination with overliming for improving the fermentability of a hemicellulose hydrolysate of red oak with the yeast *Pichia stipitis (4)*. A bagasse hydrolysate was treated by a combination of anion and cation exchangers prior to fermentation with *Pachysolen tannophilus (5)*. Treatment with ion-exchange resin was also found to decrease the toxicity of a waste paper hydrolysate *(6)*, although the mechanism of the detoxification was not further elucidated. Anion exchange was used in combination with active carbon and evaporation to improve the fermentability of a dilute acid hemicellulose hydrolysate from corncobs *(7)*. The alternating use of anion, cation, and mixed resins to treat the lignocellulose hydrolysates in these studies indicates a lack of knowledge of the nature of the target compounds for the detoxification treatment. This stresses the importance of a systematic approach to determine the type of ion-exchange resin that most efficiently provides the desired effect.

The aims of the present study was to investigate whether anion- or cation-exchange resins provide the best detoxification effect, to elucidate whether interactions between the fermentation inhibitors and the matrix material have to be considered in addition to the interactions with the charged groups, and to examine the effects of pH and the amounts of ion-exchange resin. We used three different resins (an anion exchanger, a cation exchanger, and a resin without charged groups) under different conditions for the detoxification of a spruce dilute-acid hydrolysate. In addition, we explored the interactions between the three resins and selected model compounds representing fermentation inhibitors and fermentable sugars. Three phenolic model compounds with different pK_a values were selected (guaiacol, vanillin, and coniferyl aldehyde) together with two furan aldehydes (furfural and hydroxymethyl furfural [HMF]) generally present in lignocellulose hydrolysates in substantial amounts (Fig. 1).

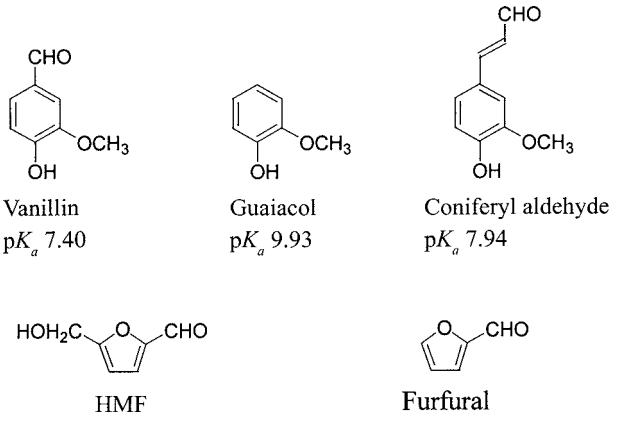

Fig. 1. Model compounds used and the dissociation constants of the phenols *(12)*.

Materials and Methods

Hydrolysate

The raw material, its composition, and the conditions for hydrolysis were as previously reported *(1)*. Chipped Norway spruce, *Picea abies*, was impregnated with 0.5% (w/w) sulfuric acid prior to treatment with saturated steam at 222°C (23 bar) for 7 min. The solid fraction was removed by filtration and the liquid fraction, referred to as the hydrolysate, had a pH of 1.9.

Model Compounds

Vanillin, guaiacol, and coniferyl aldehyde were chosen as representatives for phenols with inhibiting properties. The furan aldehydes were HMF and furfural (see Fig. 1). All model compounds were obtained from Sigma-Aldrich (St. Louis, MO). A solution of the five compounds (0.2 m*M* each) was prepared in double-deionized water (Millipore, Bedford, MA). The model experiments were performed under a slow stream of helium to prevent oxidation by air. In addition to the model experiments with inhibitors, a solution of 139 m*M* glucose (corresponding to 25 g/L, the approximate glucose concentration in the hydrolysate) was used with 8.0 g of the resin at room temperature for 1 h to determine sugar losses under different conditions. The influence of sulfate ions was studied by the addition of sodium sulfate (23 m*M*) corresponding to the concentration of sulfate ions determined in the hydrolysate (unpublished data).

Ion-Exchange Resins

The anion exchanger (AG 1-X8, 20–50 mesh, 3.2 meq/g [dry]) and the cation exchanger (AG 50W-X8, 20–50 mesh, 5.1 meq/g [dry]) were obtained from Bio-Rad (Richmond, CA). AG 1-X8, a strong anion-exchange resin, was used in its HO^- form. The cation exchanger was changed to sodium

form prior to use. XAD-8 (40–60 mesh) was obtained from Supelco (Supelco Park, Bellefonte, PA). The different resins were carefully washed with distilled water. The well-washed resins contained approx 50% water. The concentrations determined in the treated hydrolysate were adjusted for the dilution. The pH was adjusted with NaOH or H_2SO_4.

Detoxification with Ion-Exchange Resins

The mixture of model compounds and the hydrolysate were treated at room temperature for 1 h. The hydrolysate was stirred with the different resins for 1 h and then filtered. The ion exchangers were used in two different quantities, 8.0 or 3.6 g in 50 mL of hydrolysate. The amounts chosen were based on the amount of anion exchanger needed to adjust the pH of the hydrolysate to 10.0 and 5.5, respectively.

Analysis of Hydrolysate Composition

Formic, acetic, lactic, and oxalic acid were determined using a DX 500 high-performance liquid chromatography (HPLC) system (Dionex, Sunnyvale, CA) and an AS11-HC (Dionex) column, eluted with 80% (v/v) water and 20% (v/v) of a mixture consisting of 0.4 m*M* NaOH and methanol (50% [v/v]) at a flow rate of 1.4 mL/min. Levulinic acid was determined using an AS15 column (Dionex) and isocratic elution with 25 m*M* NaOH. The samples were directly injected after dilution with water and filtration through a 0.2-µm MFS-25 filter (Advantec MFS, Pleasanton, CA). The amounts of aliphatic acids were determined from external calibration curves using a conductivity detector. The analyzed acids were collectively reported as aliphatic acids.

The furan aldehydes, HMF and furfural, were determined by HPLC using a Gynkotek system 480 equipped with a UVD 340S diode array detector (Gynkotek, Germering, Germany). The furan aldehydes were separated on an ODS-AA column (50 × 4.6 mm, 5-µm particles) from YMC (Waters, Milford, MA). The flow rate was 0.8 mL/min. Elution was performed with a gradient consisting of water containing 5–25% (v/v) acetonitrile and 0.025% (v/v) trifluoroacetic (TFA) acid for 10 min. For quantification, syringic acid was used as an internal standard.

The concentrations of phenolic compounds were estimated by HPLC. The separation was performed with an ODS-AQ column (150 × 3 mm, 3-µm particles) from YMC (Waters). The flow rate was 0.4 mL/min. Elution was performed with a gradient composed of Eluent A and B. Eluent A consisted of water containing 1% (v/v) acetonitrile and 0.025% (v/v) TFA. Eluent B consisted of acetonitrile containing 1% (v/v) water and 0.025% (v/v) TFA. The gradient was formed in three steps for 45 min by going from (1) 100% Eluent A to 86% A and 14% B in 20 min, (2) 86% A and 14% B to 75% A and 25% B in 20 min, and (3) 75% A and 25% B to 100% B in 5 min. The areas for all peaks eluting after furfural (from 15.5 to 45 min) and detected at 280 nm were given as the sum of low molecular weight phenols. The quantifications were made relative to the untreated hydrolysate.

The total concentrations of phenols were also estimated with a spectrophotometric method based on the Folin & Ciocalteaus reagent *(8)*. The samples were diluted 400 times with distilled water and 14 mL was mixed with the Folin & Ciocalteaus reagent (Sigma, Steinheim, Germany), and 5 mL of saturated Na_2CO_3 was added. The absorbance at 725 nm was determined after 30 min at room temperature. The amounts of phenols were determined from a calibration curve based on vanillin, since it was the most abundant phenol in the hydrolysate.

Analyses of Model Compound Experiments

Furan aldehydes, vanillin, guaiacol, and coniferyl aldehyde were separated by HPLC using a reversed-phase Nucleosil 100-5 C18 column (Macherey-Nagel, Düren, Germany). The model compounds were eluted with a gradient of methanol and water, both containing TFA at a concentration of 0.025%. The methanol concentration was increased from 5 to 100% over 35 min at a flow rate of 0.8 mL/min. The amounts were calculated using syringic acid as internal standard.

The concentration of glucose was determined by high-performance anion-exchange chromatography with a Dionex DX 500 chromatography system coupled with pulsed amperometric detection (Dionex ED 40) and using a CarboPac PA-1 column (all from Dionex). The column was first equilibrated with a mixture of 200 m*M* NaOH and 70 m*M* sodium acetate for about 5 min. After sample injection, an isocratic elution with pure water and postcolumn addition of 300 m*M* NaOH was applied. L-Fucose was used as an internal standard.

The concentrations obtained after treatment with the resins were compensated for the dilution caused by the water in the resins. The changes in concentrations are reported as percentages.

Fermentation

Compressed baker's yeast, *Saccharomyces cerevisiae* (Jästbolaget AB, Rotebro, Sweden), was used in all fermentations. The pH of the fermentations was 5.5. The hydrolysate was supplemented with nutrients as previously described *(1)*. Fermentations of a solution with only the nutrients, 35 g/L of glucose, and no hydrolysate were performed for every fermentation and are hereafter referred to as the reference fermentations. All fermentations were carried out with equipment and conditions as described elsewhere *(1)*. Each sample was fermented on at least two separate occasions.

The fermentability was evaluated by comparing the total ethanol yield, calculated as produced ethanol divided by the amount of fermentable sugars (glucose and mannose) present in the hydrolysate (Y_{tot} [g/g]); the ethanol yield on the amount of consumed fermentable sugars (Y_{cons} [g/g]); and the maximum mean volumetric productivity, hereafter referred to as the volumetric productivity, calculated as ethanol produced within the first 6 h of fermentation divided by 6 (Q_{6h} [$g \cdot L^{-1} \cdot h^{-1}$]), because the maximum

mean volumetric productivity was obtained after 6 h in the reference fermentations. Anaerobic growth yield, hereafter referred to as biomass yield (Y_X [g/g]), was calculated as the produced biomass in 24 h (dry wt) divided by the consumed amount of fermentable sugars.

HPLC and Dry Weight Analyses of Fermentations

In all fermentation samples glucose, ethanol, lactic acid, and glycerol were separated with a high-performance liquid chromatograph (Waters) equipped with a refractive index detector (RID-6A; Shimadzu, Kyoto, Japan), using an Aminex HPX-87H column (Bio-Rad) at 45°C with 5 m*M* H_2SO_4 at a flow rate of 0.6 mL/min as the mobile phase. The system was equipped with a Cation-H Refill Cartridge (Bio-Rad) prior to using the HPX-87H column. Samples were diluted and filtered through 0.2-µm membrane filters (Advantec MFS) prior to analysis. Dry weight was determined at the beginning and the end of the fermentation as described previously *(1)*.

Results

Nine different detoxification treatments were performed (Table 1, methods 2–10). The ethanol yield on consumed sugar (Y_{cons}) ranged from 0.42 to 0.47 g/g and did not deviate much from either the untreated hydrolysate (Y_{cons} = 0.42 ± 0.03 g/g) or the reference fermentation (Y_{cons} = 0.42 ± 0.01 g/g). The highest Y_{cons} was for the sample treated with 3.6 g of XAD-8 at pH 5.5, which, on the other hand, did not give so high a total ethanol yield (Y_{tot}) on fermentable sugars.

The total ethanol yield (Table 1) was 0.06 ± 0.02 for the untreated hydrolysate and 0.42 ± 0.02 for the reference. The highest total ethanol yield was achieved after the anion-exchange treatment at pH 10.0, using either 8.0 or 3.6 g of resin, and after the XAD-8 treatment at pH 10.0. Even though the corresponding treatments at pH 5.5 (Table 1, methods 8 and 6) resulted in considerable improvement in fermentability compared with the control (Table 1, method 1), they could not compare with the treatments performed at pH 10.0. The cation exchanger was the only resin that at pH 10.0 did not result in a total ethanol yield comparable or better than the reference. An increased amount of anion-exchange resin at pH 10.0 did not greatly improve yield, since the yield was high already with 3.6 g, whereas an increased amount of XAD-8 at pH 5.5 raised the Y_{tot} from 0.19 to 0.28 g/g.

The highest biomass yield (Table 1) was obtained after anion exchange with 8.0 g at pH 10.0, and the yield was the same as that of the reference (0.05 ± 0.01 g/g). The anion-exchange treatment with 3.6 g at pH 10.0 resulted in lower biomass yield (0.040 g/g), and treatment with XAD-8 at pH 10.0, which also provided high Y_{tot}, showed even lower biomass yield.

The volumetric ethanol productivity, Q_{6h}, of the untreated hydrolysate was 0.21 ± 0.04 g/(L·h). Treatment at pH 10.0 without any resin present raised the productivity to 0.34 g/(L·h) (Fig. 2). The productivity was for all treatments lower than in the reference fermentation (0.92 ± 0.17 g/[L·h]),

Table 1
Ethanol and Biomass Yield After Detoxification

Detoxification method	Y_{cons} (g/g)	Y_{tot} (g/g)	Y_x (g/g)
1. None (NaOH, pH 5.5)	0.42	0.06	0.000
2. NaOH, pH 10.0	0.45	0.19	0.000
3. Cation exchanger (8.0 g), pH 5.5	0.45	0.17	0.000
4. Cation exchanger (8.0 g), pH 10.0	0.45	0.22	0.000
5. XAD-8 (3.6 g), pH 5.5	0.47	0.19	0.000
6. XAD-8 (8.0 g), pH 5.5	0.46	0.28	0.001
7. XAD-8 (8.0 g), pH 10.0	0.46	0.45	0.004
8. Anion exchanger (3.6 g), pH 5.5	0.42	0.20	0.004
9. Anion exchanger (3.6 g), pH 10.0	0.45	0.45	0.040
10. Anion exchanger (8.0 g), pH 10.0	0.46	0.46	0.050
Reference	0.42	0.42	0.050

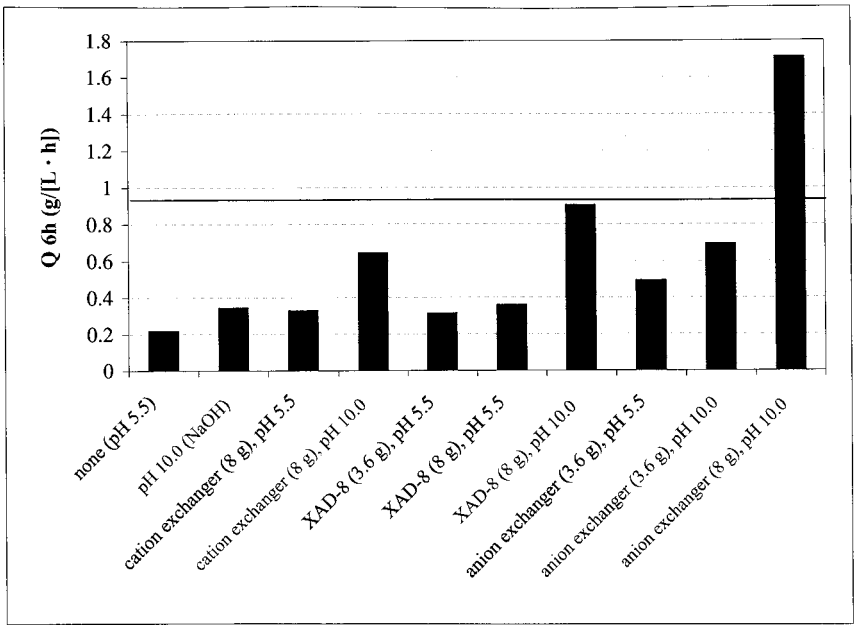

Fig. 2. Volumetric ethanol productivity in a hydrolysate treated with different types of resins. Solid line indicates the productivity in the reference fermentations.

with the exception of the anion-exchange treatment at pH 10.0 (Fig. 2), which resulted in an ethanol productivity of 1.71 g/(L·h). When the amount of anion exchanger used was decreased, the volumetric productivity decreased as well, to 0.69 g/(L·h). When the pH of the anion-exchange treatment was decreased to 5.5, the productivity decreased even further, to 0.49 g/(L·h). The treatment with 8.0 g of XAD-8 at pH 10.0 resulted in the next best ethanol productivity, 0.90 g/(L·h), which is comparable with that

of the reference fermentation. At pH 5.5, there was a minor difference in productivity between 3.6 (0.31 g/[L·h]) and 8 g of XAD-8 (0.36 g/[L·h]). Treatment with 8 g of cation exchanger at pH 10.0 gave higher productivity (0.64 g/[L·h]) than at pH 5.5 (0.33 g/[L·h]).

Chemical analyses of the different groups of inhibiting compounds in the hydrolysate were done before and after detoxification with the resins (Table 2). The untreated hydrolysate has been thoroughly analyzed (unpublished data) and the concentrations of monosaccharides, furan aldehydes, aliphatic acids, and 14 phenolic compounds were reported previously *(1)*.

No decrease in fermentable sugars, glucose and mannose, was observed after the different treatments of the hydrolysate (data not shown). Adjustment of the concentrations, to take the dilution into account, is one way in which the experimental approach differs compared to previous detoxification with ion exchange *(1)*. The concentration of aliphatic acids was affected only by the anion exchanger (Table 2). At pH 5.5, almost all the aliphatic acids were removed by the anion exchanger. When the anion exchange was performed at pH 10.0 with the same amount of resin (3.6 g), less than half of the aliphatic acids was removed. However, when the amount of anion exchanger was increased to 8.0 g at pH 10.0, then again the aliphatic acids were efficiently removed. All treatments with resins caused a decrease in the concentrations of HMF and furfural in the hydrolysate (Table 2). The method that most efficiently removed furan aldehydes was anion exchange with 8.0 g at pH 10.0. The cation- and the anion-exchange resin removed roughly equal proportions of HMF and furfural, while the XAD-8 removed a higher percentage of furfural than of HMF.

The total amount of phenols in the hydrolysate was determined spectrophotometrically and by HPLC. More than 50 individual phenols were detected in the hydrolysate by HPLC (unpublished data). The ultraviolet (UV)-absorbing peaks at 280 nm, with retention times and UV spectra making them likely to be phenols, were summarized to a relative value of the content of phenols in the hydrolysate before and after the different treatments. Among the phenols, five were of the Hibbert's ketone type and could be separated and quantified with acceptable certainty. The sum of the Hibbert's ketones was not affected differently from the other phenols when treated with the different resins. At pH 5.5, some of the nonionized phenols were trapped by the cation-exchange resin in spite of its negatively charged groups. The XAD-8 resin with no ionizable groups removed phenols from the hydrolysate and was more efficient at the low pH when few of the phenols were charged. Efficiency increased when the amount of resin was increased. The anion exchanger was most efficient in removing the phenols from the hydrolysate. This ability increased with pH and the amount of anion exchanger used.

The ability of a strong anion exchanger to efficiently extract glucose from a water solution was confirmed in model experiments (Table 3). A solu-

Table 2
Effect of Different Treatments on Chemical Composition of Hydrolysate[a]

Detoxification method	Aliphatic acids[b] (IC) (%)	HMF[b] (HPLC) (%)	Furfural[b] (HPLC) (%)	Phenols[b] (Folin & Ciocalteau) (%)	Phenols[b] (HPLC) (%)	Hibbert's ketones[b] (HPLC) (%)
1. None (NaOH, pH 5.5)	100	100	100	100	100	100
2. NaOH, pH 10.0	100	100	100	100	ND	ND
3. Cation exchanger (8.0 g), pH 5.5	ND	81	76	84	ND	ND
4. Cation exchanger (8.0 g), pH 10.0	100	73	64	97	100	100
5. XAD-8 (3.6 g), pH 5.5	100	93	59	47	ND	ND
6. XAD-8 (8.0 g), pH 5.5	100	80	41	34	ND	ND
7. XAD-8 (8.0 g), pH 10.0	100	58	35	69	82	88
8. Anion exchanger (3.6 g), pH 5.5	9	76	70	43	ND	ND
9. Anion exchanger (3.6 g), pH 10.0	62	70	75	38	36	32
10. Anion exchanger (8.0 g), pH 10.0	4	35	32	21	19	10

[a]Data presented are the percentages remaining in the hydrolysate after each treatment. ND, not determined; IC, ion chromatography; HPLC, high-performance liquid chromatography.

[b]The value 100% corresponds to 7.97 g/L of aliphatic acids (the sum of formic, acetic, lactic, oxalic, and levulinic acids), 5.10 g/L of HMF, 0.82 g/L of furfural, 3.7 g/L of phenols determined as vanillin equivalents with the Folin & Ciocalteau method, 517 au (arbitrary absorbance units at 280 nm) of phenols (HPLC), and 59 au of Hibbert's ketones (HPLC), respectively.

Table 3
Effect of Treating a Glucose Solution with Different Resins[a]

Treatment	Glucose (%)
Cation exchanger (8.0 g), pH 10.0	100
XAD-8 (8.0 g), pH 5.5	97
XAD-8 (8.0 g), pH 10.0	100
Anion exchanger (8.0 g), pH 5.5	83
Anion exchanger (8.0 g), pH 10.0, 30 min[b]	25
Anion exchanger (8.0 g), pH 10.0	25
Anion exchanger (8.0 g), pH 10.0, added SO_4^{2-}	99

[a]Data presented are the percentages of remaining glucose of the initial concentration.
[b]The other samples were incubated for 1 h.

tion of 25 g/L of glucose treated with 8.0 g of anion exchanger at pH 10.0 showed a loss of glucose of 75% (the mean of two experiments giving 79 and 70%, respectively). The pH was maintained at 10.0 by the addition of carbon dioxide, since the release of hydroxide ions tended to increase the pH. At pH 5.5, the anion exchanger trapped 17% of the glucose. Neither the cation exchanger nor the XAD-8 resin affected the concentration of glucose. The ability of the anion exchanger to trap ionizable monosaccharides could be eliminated by the addition of sulfate ions. No significant loss of glucose was observed when 23 m*M* Na_2SO_4 was added. This result should be compared with the experiment in which the hydrolysate was treated with 8.0 g of anion exchanger at pH 10.0, and no decrease in the concentration of monosaccharides was observed.

The ability of the different resins to remove fermentation-inhibiting furan aldehydes was further investigated in a series of model experiments. The aqueous solution containing 0.2 m*M* each of the five model compounds was treated with 8 or 3.6 g of the different resins at pH 10.0 and 5.5. Table 4 shows the resulting decrease in concentrations. Control experiments were also done with no added resins. It was observed that part (11%) of the volatile furfural was lost. In addition, a negligible amount of the semi-volatile guaiacol was lost by evaporation. No other losses were observed in the control experiments. The furan aldehydes, furfural and HMF, were partly removed by all the resins tested (Table 4). The XAD-8 resin, with no ionized functional groups, efficiently removed almost half of the HMF and two-thirds of the furfural. XAD-8 was most efficient at pH 10.0, but the difference was very small. The anion and the cation exchangers were less efficient than XAD-8 in removing the furan aldehydes (Table 4). The addition of more resin slightly increased the removal of furan aldehydes for both the anion and the cation resin, but mostly for the XAD-8 resin.

The removal of phenolic compounds in a hydrolysate with an anion exchanger was shown at both high pH and a pH far below the pK_a value of the ionizable phenolic group (Table 4). The cation exchanger was much less

Table 4
Experiments with Model Compounds[a]

Detoxification method	HMF (%)	Furfural[b] (%)	Vanillin (%)	Guaiacol (%)	Coniferyl aldehyde (%)
None (NaOH, pH 5.5)	100	100	100	98	100
NaOH, pH 10.0	100	100	100	97	100
Cation exchanger (3.6 g), pH 5.5	96	89	80	85	66
Cation exchanger (8.0 g), pH 5.5	91	81	66	72	54
Cation exchanger (8.0 g), pH 10.0	87	82	100	82	100
XAD-8 (3.6 g), pH 5.5	78	57	12	13	6
XAD-8 (8.0 g), pH 5.5	56	39	4	4	1
XAD-8 (8.0 g), pH 10.0	54	38	96	7	30
Anion exchanger (3.6 g), pH 5.5	96	89	6	47	12
Anion exchanger (8.0 g), pH 5.5	82	84	1	28	2
Anion exchanger (8.0 g), pH 10.0	78	88	0	4	0
Anion exchanger (8.0 g), added SO_4^{2-}, pH 10.0	77	74	5	19	3

[a]Data presented are percentages of the compound remaining after treatment.
[b]The values are compensated for the losses owing to evaporation, estimated to 11%.

efficient, but still significant removal of phenols was obtained, especially at low pH. At high pH all ionized phenols were left unaffected. The fully ionized vanillin and coniferyl aldehyde were completely excluded. Guaiacol, which is ionized to 50% at pH 10.0, was only partly trapped. At pH 5.5, when almost all the phenols are nonionized, the phenols were trapped to a considerable extent, which showed the active participation of the resin matrix. This was confirmed by the use of a resin without ionized groups, XAD-8. At pH 5.5, all the phenols were almost completely trapped by the XAD-8 matrix. By contrast, only the least acidic phenol, guaiacol, was efficiently removed at pH 10.0. The fully ionized vanillin was excluded, while the somewhat less acidic but more lipophilic coniferyl aldehyde was partly removed. The drastic effect of the addition of sulfate to the anion exchanger at pH 10.0, as shown for glucose, was less pronounced for the phenols, although the removal of phenols decreased slightly. The added SO_4^{2-} ions competed for the positive sites of the anion exchanger. The amount of resin

used also influenced the degree of removal, as expected by increased removal with increased amount used.

Discussion

The use of model compounds facilitated the interpretation of the detoxification effects seen with fermentation experiments on a dilute-acid hydrolysate from softwood when combined with chemical analysis of the remaining inhibiting compounds. Anion exchange at pH 10.0 was the most efficient method for increasing the fermentability, in terms of both ethanol yield and productivity, which agrees well with previous comparisons of different detoxification methods *(1)*.

Loss of any fermentable sugar would have a negative influence on the economy of the detoxification step. The decrease in fermentable sugar that was previously observed when anion exchange was used for detoxification *(1)* was studied in model experiments. The strong alkaline surface of the anion exchanger containing quaternary ammonium groups with hydroxyl ions as counterions was able to ionize and capture the neutral monosaccharide. In the model experiment with glucose, up to 75% could be removed with the anion exchanger. Consequently, the amount of anion exchanger used to achieve detoxification must not be too high. This ability to trap ionizable monosaccharides could be eliminated by the addition of sulfate ions, which have a higher affinity to the quaternary ammonium groups. However, in the hydrolysate the concentrations of ionized aliphatic acids, phenols, and inorganic ions such as sulfate were sufficient to efficiently compete for the sites in the anion-exchange resin. The amount of anion exchanger that can be added without loss of sugars is thus determined by the presence of both inorganic and organic ions at the present pH. Considering the result obtained with the hydrolysate, in which ionized acids, phenolics, and sulfate ions assisted in displacing all the glucose so that no loss occurred, loss of fermentable sugars is unlikely to be a problem in industrial processes.

Even at low pH, the quaternary ammonium groups in the anion exchanger catch the aliphatic acids very efficiently, because the acids occur mostly in their ionized forms at pH 5.5. At the high pH of 10.0, most of the phenolic groups have become ionized, and the phenolates then contribute to that the competition for the cationic sites increases. When the number of compounds competing for the positive sites increased at pH 10.0, the anion exchanger retained less of the aliphatic acids. For the hydrolysate, 3.6 g of the anion exchanger was not enough to fully trap the acids; however, 8.0 g was sufficient.

When comparing results with the reference fermentations, it is noteworthy that the buffer capacity may be quite different compared with that of fermentations with hydrolysate and that the final pH, therefore, also may be different. This is owing to the fact that the hydrolysate contains compounds, e.g., aliphatic acids, that act as buffers at the pH of fermentation. Detoxification of the hydrolysate may also affect the buffering capacity.

The increase in productivity after treatment with the different resins can probably be attributed to the decrease in the concentrations of furan aldehydes. The effect of furfural and HMF on ethanolic fermentation by *S. cerevisiae* has recently been studied *(9–11)*. The furan aldehydes are known to cause a lag phase in ethanol formation, resulting in a substantial impact on the values for volumetric ethanol productivity. The neutral furan aldehydes were removed by the matrixes by another mechanism than ion exchange. The ionized groups of the anion and cation exchangers make them more hydrophilic than the noncharged XAD-8 resin. Consequently, the anion and cation exchangers were less attractive for the furan aldehydes than XAD-8, as shown by the model compound experiments. The interaction between furan aldehydes and the resins was not affected by pH in the model compound experiments. In the experiments with hydrolysate, pH 10.0 was more efficient for removing the furan aldehydes, at least with XAD-8. The high ionic strength in the hydrolysate at high pH is a plausible explanation. Removal also increased when more of the resins was used.

Phenolics are important inhibitors in dilute-acid hydrolysates of spruce. This was shown by enzymatic detoxification of a hydrolysate with laccase, which specifically removed the low molecular weight phenolics and simultaneously caused a major improvement in the fermentability *(1)*. From the experiments with the different resins and the hydrolysate and the model compounds, it can be concluded that the mechanisms for removal of phenols by ion-exchange resins are complex. As expected, the ionized phenols were completely trapped on the anion exchanger and repelled by the cation exchanger, both in the model experiments and in the hydrolysate. At pH 5.5, only a small fraction of the phenols are in their ionized form, but they were still efficiently trapped. The influence of the acidity of the phenolic group was shown: the acidic phenols with conjugated carbonyl groups were completely trapped even at low pH. The dissociation constants for the model compounds are vanillin, 7.40; guaiacol, 9.93; and coniferyl aldehyde, 7.94 *(12)*. The positively charged quaternary ammonium ions form strong ionic bonds with the negatively charged phenols and the negatively charged sulfonic acid groups make the cation exchanger nonattractive for all of the identified inhibiting compounds. When the phenols were ionized, they were completely repelled by the sulfonic acid groups of the cation exchanger. The previously presented detoxification effect of a cation exchanger *(3)* probably can be explained by being performed at a pH allowing lipophilic nonionized phenols to be retained by the resin together with the uncharged furan aldehydes. The importance of the resin matrix was shown by the ability to trap phenols on a cation exchanger and by the most efficient effect on all the model compounds by XAD-8 at low pH. The phenols used in the model experiments were completely removed by the anion exchanger at pH 10.0. With the hydrolysate, the anion exchanger at pH 10.0 was the most efficient way to remove phenols. The group of phenols referred to as Hibbert's ketones, which previously have been suspected to cause inhibition of the ethanolic fermenta-

tion *(13)*, were affected in the same way as the other phenols when treated with the different resins, with the possible exception of the anion exchanger at pH 10.0, which removed them almost completely.

It has previously been shown that a cation exchanger can be useful for removing inhibiting metal ions present in a bagasse hydrolysate prepared in a stainless steel vessel *(5)*. Our comparison of cation and anion exchangers indicates that inhibition by metal ions is not a major problem in the hydrolysate used in our study. The minor improvement in fermentability, which we observed after treatment with the cation exchanger, could possibly be attributed to hydrophobic interactions between the resin and organic inhibitors present in the hydrolysate.

In conclusion, treatment with the anion exchanger at pH 10.0 was the most efficient way to remove fermentation-inhibiting compounds from all three groups measured: phenols, furan aldehydes, and aliphatic acids. Treatment with anion-exchange resin at pH 10.0 could even result in a hydrolysate showing higher productivity than a reference fermentation with comparable amounts of fermentable sugars. The presence of counterions, such as sulfate, was found to be important to prevent unwanted losses of fermentable sugars at high pH with the anion exchanger. All three types of resins at least partly removed all groups of fermentation inhibitors investigated, with the exception of the aliphatic acids, which were only removed by the anion exchanger. Regardless of the type of resin used, treatment at pH 10.0 was always better in terms of improved fermentability than treatment at pH 5.5. The positively charged functional groups of the anion exchanger contributed to the detoxification effect. On the other hand, the negatively charged groups of the cation exchanger resulted in a resin providing only a poor detoxification effect. This was attributed to a repulsion effect of anionic inhibitors present in the hydrolysate. Future work will address optimization of the use of the anion exchanger at high pH for detoxification of dilute-acid hydrolysates.

Acknowledgments

We gratefully acknowledge Christer Larsson and Linda Björklund for technical assistance. This work was supported by a grant from the Swedish National Energy Administration.

References

1. Larsson, S., Reimann, A., Nilvebrant, N.-O., and Jönsson, L. J. (1999), *Appl. Biochem. Biotechnol.* **77–79,** 91–103.
2. Olsson, L. and Hahn-Hägerdal, B. (1996), *Enzyme Microb. Technol.* **18,** 312–331.
3. Buchert, J., Niemelä, K., Puls, J., and Poutanen, K. (1990), *Proc. Biochem.* **25,** 176–180.
4. Tran, A. V. and Chambers, R. P. (1986), *Enzyme Microb. Technol.* **8,** 439–444.
5. Watson, N. E., Prior, B. A., Lategan, P. M., and Lussi, M. (1984), *Enzyme Microb. Technol.* **6,** 451–456.
6. Rivard, C. J., Engel, R. E., Hayward, T. K., Nagle, N. J., Hatzis, C., and Philippidis, G. P. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 183–191.

7. Dominguez, J. M., Cao, N., Gong, C. S., and Tsao, G. T. (1997), *Bioresour. Technol.* **61,** 85–90.
8. Singleton, V. L., Orhofer, R., and Lamuela-Raventos, R. M. (1999), *Methods Enzymol.* **299,** 152–178.
9. Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., and Nilvebrant, N.-O. (1999), *Enzyme Microb. Technol.* **24,** 151–159.
10. Taherzadeh, M. J., Gustafsson, L., Niklasson, C., and Lidén, G. (1999), *J. Biosci. Bioeng.* **87,** 169–174.
11. Palmqvist, E., Almeida, J. S., and Hahn-Hägerdal, B. (1999), *Biotechnol. Bioeng.* **62,** 447–454.
12. Ragnar, M., Lindgren, C. T., and Nilvebrant, N.-O. (2000), *J. Wood Chem. Technol.* **20,** 277–305.
13. Clark, T. A. and Mackie, K. L. (1984), *J. Chem. Tech. Biotechnol.* **34B,** 101–110.