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Selection of Anion Exchangers for Detoxification of Dilute-Acid Hydrolysates from Spruce

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Abstract

Six anion-exchange resins with different properties were compared with respect to detoxification of a dilute-acid hydrolysate of spruce prior to ethanolic fermentation with *Saccharomyces cerevisiae*. The six resins encompassed strong and weak functional groups as well as styrene-, phenol-, and acrylic-based matrices. In an analytical experimental series, fractions from columns packed with the different resins were analyzed regarding pH, glucose, furfural, hydroxymethylfurfural, phenolic compounds, levulinic acid, acetic acid, formic acid, and sulfate. An initial adsorption of glucose occurred in the strong alkaline environment and led to glucose accumulation at a later stage. Acetic and levulinic acid passed through the column before formic acid, whereas sulfate had the strongest affinity. In a preparative experimental series, one fraction from each of six columns packed with the different resins was collected for assay of the fermentability and analysis of glucose, mannose, and fermentation inhibitors. The fractions collected from strong anion-exchange resins with styrene-based matrices displayed the best fermentability: a sevenfold enhancement of ethanol productivity compared with untreated hydrolysate. Fractions from a strong anion exchanger with acrylic-based matrix and a weak exchanger with phenol-based resin dis-

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played an intermediate improvement in fermentability, a four- to fivefold increase in ethanol productivity. The fractions from two weak exchangers with styrene- and acrylic-based matrices displayed a twofold increase in ethanol productivity. Phenolic compounds were more efficiently removed by resins with styrene- and phenol-based matrices than by resins with acrylic-based matrices.

Index Entries: *Saccharomyces cerevisiae*; anion-exchange resins; hydrolysate; fermentability.

Introduction

Dilute-acid hydrolysis is the oldest technology for converting lignocellulose-based biomass to ethanol *(1)*. After many years of research and development, the dilute-acid process has evolved into a general concept in which hydrolysis occurs in two stages to maximize sugar yields from the hemicellulose and cellulose fractions of the biomass *(2,3)*. The liquid hydrolysates from each stage are recovered and then fermented to ethanol. Because of the complex structure of lignocellulose, high temperature and/or addition of acid is used to liberate the monosaccharides. Under these conditions, other compounds will be formed in addition to the fermentable sugars. Several of these byproducts are known to inhibit the following fermentation step.

The fermentation inhibitors include furan aldehydes, aliphatic acids, and phenolic compounds. The furan aldehydes, furfural, and hydroxymethyl furfural (HMF), are formed from pentoses and hexoses, respectively *(4,5)*. Several studies indicate that furfural inhibits *Saccharomyces cerevisiae*, at least when present in high concentrations *(6–10)*. HMF has a similar effect *(11,12)*.

The breakdown of furan aldehydes leads to the formation of formic and levulinic acid. Moreover, acetic acid is formed during the degradation of hemicellulose. Partial breakdown of lignin can generate a variety of phenolic compounds *(13)*, which also inhibit *S. cerevisiae (14,15)*. In contrast to furan aldehydes and aliphatic acids, the toxic effect of specific phenolic compounds is highly variable *(15)*. Different raw materials and different approaches to prepare lignocellulose hydrolysates will result in different concentrations of the fermentation inhibitors *(16,17)*.

The most powerful approach to improve the fermentability of a hydrolysate is to perform a detoxification prior to ethanolic fermentation. Chemical, biologic, and physical methods have been used to increase fermentability *(18,19)*. In a previous study, 12 detoxification methods were compared with respect to the effect on the chemical composition of a spruce hydrolysate and the fermentability using *S. cerevisiae (20)*. The results showed that treatment with anion-exchange resin was one of the most efficient detoxification methods. Furthermore, a comparison of three different resins—an anion exchanger, a cation exchanger, and a resin without charged groups *(21)*—showed that treatment of a spruce hydrolysate with anion exchanger increased the fermentability most efficiently. The mechanisms of detoxification were found to be complex and dependent on both the functional groups and the properties of the polymer matrix.

In the present study, six different anion-exchange resins with different properties were selected for detoxification of a dilute-acid hydrolysate from spruce. The anion-exchange resins tested have styrene-, phenol-, and acrylic-based matrices and strong as well as weak functional groups. In previous studies *(20,21)*, detoxification was performed using a batch procedure, which is useful when the method is studied from an analytical point of view. However, although the batch approach is simple, its applicability to practical separation is limited owing to inconvenience, inefficient use of the resin, and the large amounts of regeneration agents required. In our study, a column treatment was used instead. In an analytical experiment, fractions of a spruce dilute-acid hydrolysate were collected and analyzed for fermentable sugars and inhibitors. A preparative treatment was also performed, and the effect on the fermentability was assayed using *S. cerevisiae*. The aim was to select the most efficient anion exchanger for detoxification.

Materials and Methods

Dilute-Acid Hydrolysate from Spruce

Chipped Norway spruce, *Picea abies*, was used in a two-step hydrolysis process in which H_2SO_4 was used as catalyst. The conditions and procedures were as previously reported *(22)*. The solid fraction was removed by filtration after hydrolysis, and the liquid fraction, hereafter referred to as the hydrolysate, had a pH of 1.9.

The hydrolysate contained: 15.7 g/L of glucose, 12.8 g/L of mannose, 1.4 g/L of HMF, 0.4 g/L of furfural, 1.4 g/L of levulinic acid, 3.2 g/L of acetic acid, 1.1 g/L of formic acid, and 2.4 g/L of phenolic compounds, (based on Folin and Ciocalteu's reagent [Sigma, Steinheim, Germany] and vanillin as the standard).

Detoxification by Anion-Exchange Resins

Six different anion-exchange resins were used representing both strong (Dowex 1x4 [Carl Roth KG, Chemische Fabrik, Karlsruhe], Dowex 2x8 [J.T. Baker, Phillipsburg, NJ], and Amberlite IRA 458 [Polyscience, Warrington, PA]) and weak (Amberlite IRA 67 [Fluka Chemie Gmbh, Buchs, Switzerland], Amberlite IRA 92 [Supelco, Bellefonte, PA], and Duolite A7 [Sigma-Aldrich]) anion exchangers (Table 1).

Five grams of dry anion exchanger were used. The amount of each anion exchanger was calculated using the dry content (Table 1). All anion exchangers were swollen in a saturated aqueous solution of NaCl, then converted to OH form with a 1 *M* solution of NaOH, and finally washed with Milli-Q® water (Millipore, Billerica, MA) until the pH was neutral.

All column experiments were performed with 1-cm id columns. The pumping rate was 0.5 mL/min, corresponding to a velocity of 0.4 m/h.

Table 1
Anion-Exchange Resins Used for Detoxification

Resin	Matrix	Type	Physical structure	Capacity (meq/mL)	Dry wt (%)[a]
Dowex 1x4	Styrene-DVB[b]	Strong base, type I	Gel	1.0	67
Dowex 2x8	Styrene-DVB	Strong base, type II	Gel	1.4	71
IRA 458	Acrylic-DVB	Strong base	Gel	1.25	53
IRA 67	Acrylic-DVB	Weak base	Gel	1.6	58
IRA 92	Styrene-DVB	Weak base	Macroporous	1.6	75
Duolite A7	Phenolic	Weak anion	Macroporous	2.1	58

[a] The dry weight was determined by using an MA-40 moisture analyzer (Sartorius AG, Geottingen, Germany).

[b] DVB, divinylbenzene.

In an analytical experimental series (Fig. 1), the effluent was collected in fractions. These fractions were analyzed with respect to pH, fermentable sugars, furan aldehydes, phenolic compounds, aliphatic acids, sulfate, and ultraviolet (UV) absorption at 280 nm.

In a preparative experimental series (Table 2, Fig. 2), 100-mL fractions were collected from columns with each of the different anion exchangers starting from the appearance of glucose in the effluent. These six fractions, obtained with different resins, were used for the fermentation experiments and analyzed as just described.

Analysis of Composition of Hydrolysate

The glucose, mannose, and xylose concentrations were determined by high-performance anion-exchange chromatography (HPAEC) using a DX 500 high-performance liquid chromatography (HPLC) system (Dionex, Sunnyvale, CA) equipped with a CarboPac PA-1 column (Dionex). The column was initially activated with a mixture of 200 mM NaOH and 170 mM sodium acetate for 5 min. The eluent was then changed to Milli-Q water (1 mL/min), and after 7 min the sample was injected. The injection was followed by an isocratic elution (1 mL/min) with Milli-Q water and a postcolumn addition of 300 mM NaOH. The sugar concentrations were determined by external calibration using a pulsed amperometric detector (Dionex ED 40).

The furan aldehydes HMF and furfural were determined by HPLC using a Waters 2690 separation module, with a binary pump, an autoinjector, and a diode array detector at 282 nm. The furan aldehydes were separated on a YMC ODS-AL column (50×3 mm, 120 Å, and 5-µm particles) (Waters, Milford, MA). The flow rate was 0.8 mL/min. Elution was performed with a gradient composed of Milli-Q water and acetonitrile containing 0.016% (v/v) trifluoroacetic acid. The gradient was formed in four steps over 17 min. In the first step, 10% acetonitrile was added for 3 min.

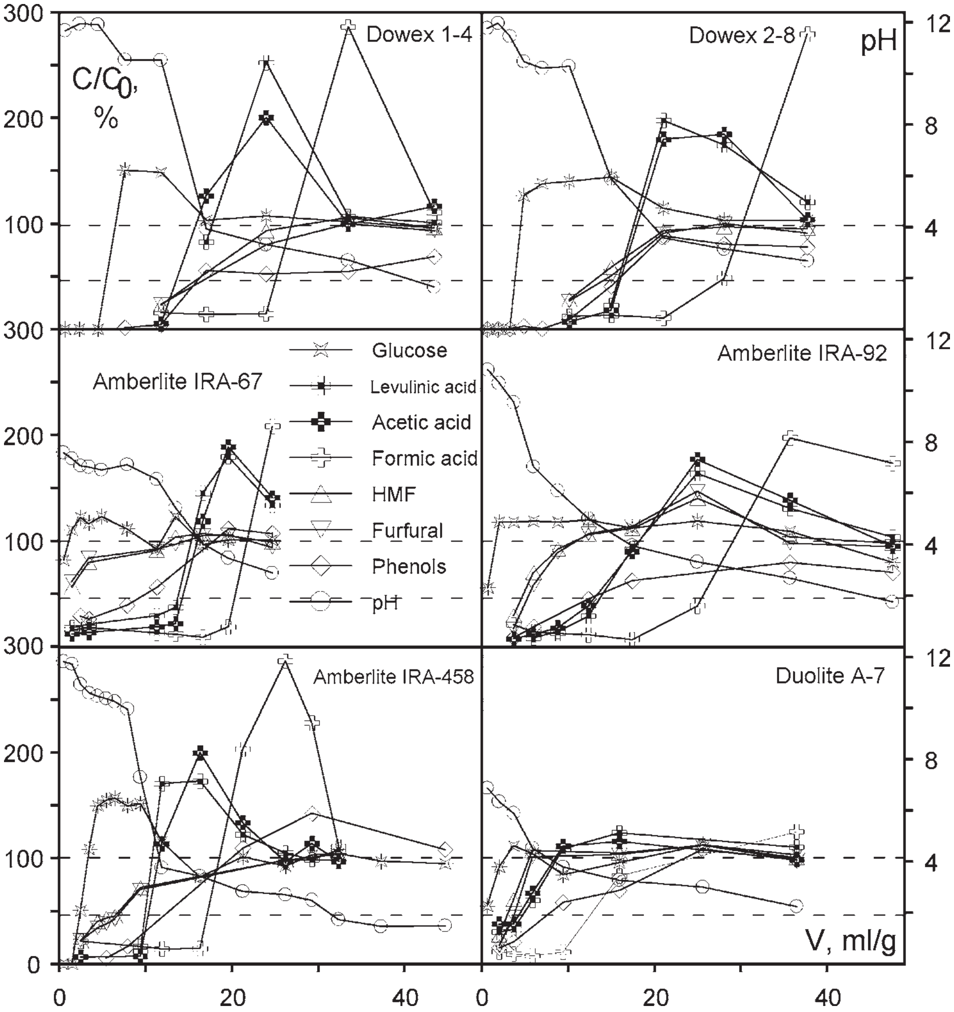

Fig. 1. Relative concentrations of glucose, levulinic acid, acetic acid, formic acid, HMF, furfural, and phenolic compounds compared with the initial concentration (100%, upper dashed lines) in fractions obtained from dilute-acid hydrolysate of spruce allowed to pass through columns with six different anion-exchange resins. The sulfate concentration was also determined but was zero in the fractions shown. The right axis shows the pH (the initial pH, 1.9, is indicated by the lower dashed lines).

Then, the gradient of acetonitrile was increased linearly to 100% in 7 min, was maintained for 5 min, and finally was decreased to 10% in 2 min. For quantification, syringic acid was used as an internal standard. The UV absorbance at 280 nm was also determined for all samples. The values followed approximately the concentrations of furfural and HMF, but the phenols also contributed to the absorbance. As a result, the UV measurements were used only as a convenient way to monitor the process.

Table 2
Effect of Anion-Exchange Treatments
on Concentrations of Fermentable Sugars and Inhibitors[a]

	Fermentable sugars							Total phenolics	
Method	Glucose	Mannose	Levulinic acid	Acetic acid	Formic acid	Furfural	HMF	Folin	HPLC[b]
Hydrolysate	100	100	100	100	100	100	100	100	100
Dowex 1x4	107	111	10	28	27	68	71	7	8
Dowex 2x8	101	102	98	96	28	62	71	40	24
IRA 458	103	106	124	108	33	91	94	65	79
IRA 67	93	92	18	93	23	117	136	57	39
IRA 92	99	100	51	50	26	109	137	21	17
Duolite A7	98	100	65	68	21	117	129	31	24

[a] The concentrations are given in percent of initial values.
[b] See ref. *21*.

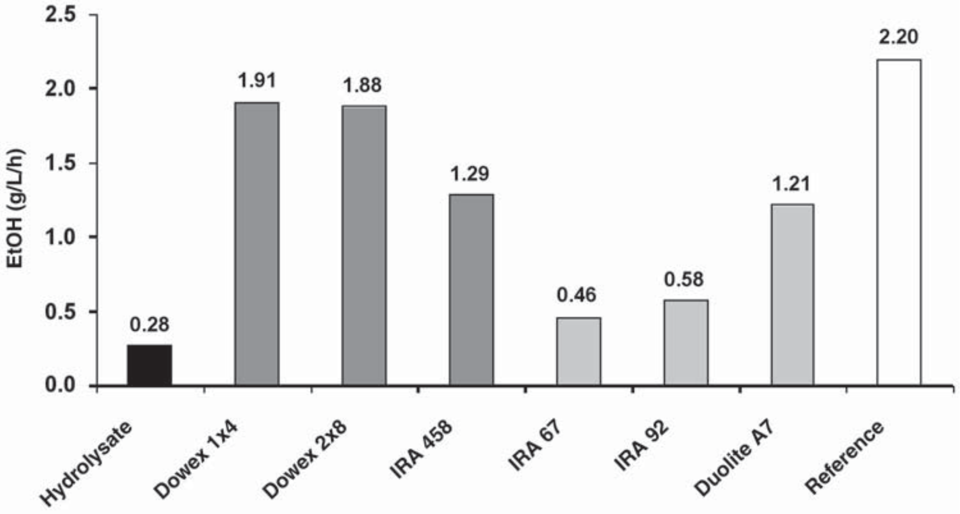

Fig. 2. Ethanol productivity for untreated hydrolysate (black bar), fractions from strong anion-exchange resins (dark gray bars), fractions from weak anion-exchange resins (light gray bars), and reference fermentation (glucose added but no hydrolysate) (white bar). The maximum mean volumetric productivity of ethanol (Q_{EtOH}) in g/(L·h) is indicated above each bar.

The concentrations of phenolic compounds were determined by HPLC (Waters) using a method described previously by Nilvebrant et al. *(21)*. The total concentration of phenols was also estimated by a spectrophotometric method *(23)* based on the Folin and Ciocalteu's reagent (Sigma).

Sulfate was determined using the DX 500-series ion chromatograph (Dionex) equipped with a conductivity detector (ED-40). An IonPac AS4A-SC anion-exchange column (250 × 4 mm id), equipped with an AG4A-SC guard column, was used for separation. A membrane suppressor (ASRS-I) was used to convert the eluent and the separated anions to their respective acid forms and to lower the conductivity of the eluent relative to that of the ions of interest. The eluent used consisted of a mixture of 1.7 m*M* $NaHCO_3$ and 1.8 m*M* Na_2CO_3. For all analyses, a flow rate of 2.0 mL/min was used.

The analysis of aliphatic acids was performed using a P/ACE MDQ capillary electrophoresis instrument equipped with a 60 cm × 50 µm id fused silica capillary (Beckman Coulter, Fullerton, CA). The samples were filtered through a 0.45-µm cellulose acetate filter (Whatman, Maidstone, UK) prior to hydrodynamic injection at 15 psi for 4 s. The voltage was set to 20 kV at reversed polarity. The electrolyte, composed of 5.0 m*M* trimellitic acid, 50 m*M* tris(hydroxymethyl)-aminomethane, 1.0 m*M* tetradecyl-trimethylammoniumbromide, and 0.5 m*M* calcium chloride, had a pH of 9.8. Before use, it was filtered through a 0.2-µm cellulose nitrate filter and degassed with helium. Detection was performed by indirect UV absorption at 220 nm. Succinic acid was used as internal standard.

Yeast Strain and Growth Conditions

The fermenting microorganism was baker's yeast, *S. cerevisiae*, kindly provided by Jästbolaget AB (Rotebro, Sweden). Agar plates with YEPD medium (2% yeast extract, 1% peptone, 2% D-glucose, 2% agar) were used to maintain the strain. Cultures for inoculum were grown aerobically in 2000-mL cotton-plugged conical flasks with 500 mL of YEPD medium at 30°C using a rotary shaker. Cells were harvested in the exponential growth phase by centrifugating at 1500*g* for 5 min at 4°C and washed using a solution of NaCl (9 g/L).

Fermentations

Prior to the fermentations, the pH of the different hydrolysate samples was adjusted to 5.5 with NaOH (5 *M*). All fermentations were carried out under oxygen-limited conditions in 55-mL glass vessels containing 50 mL of medium of which 47.5 mL was hydrolysate (or, alternatively, an aqueous glucose solution for reference fermentations). The vessels were sealed with rubber stoppers and equipped with cannulas for outlet of CO_2. The hydrolysates were supplemented with nutrients as previously described *(20)*. Fermentations of 35 g/L of glucose and nutrients but no hydrolysate were used for reference. The flasks were inoculated to an initial cell mass concentration of 2.0 g/L dry wt and incubated at 30°C with stirring. The fermentations were run for 36 h. Samples of 200 µL were taken after 0, 2, 4, 6, 8, 10, 24, and 36 h.

Analysis of Fermentation Products

Samples taken from the different fermentations were diluted and filtered with 0.45-µm GHP Acrodisc syringe filters (Pall Gelman Laboratory, Ann Arbor, MI) prior to analysis.

The glucose and mannose concentrations were determined by HPAEC as described before. The ethanol concentration was measured using an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a BP-20 column with a film thickness of 1.0 µm (SGE, Austin, TX) and a flame ionization detector. The temperature was maintained at 30°C for 5 min followed by heating to 180°C with a heating rate of 15°C/min. Acetonitrile was used as internal standard.

The biomass concentration was determined from dry wt measurements. Three-milliliter samples were taken in duplicate at the beginning and the end of the fermentations. The samples were then centrifuged (1500*g*, 5 min), and the pellets were washed once with distilled water and dried at 103°C for 24 h.

Calculations

All peaks obtained in the chemical analysis were integrated and the concentrations were calculated using the EZChrom Elite software (Scientific Software, Pleasanton, CA).

The maximum mean volumetric productivity of ethanol was obtained after 4 h in the reference fermentations. Therefore, the fermentability was evaluated by comparing the volumetric productivity, Q_{EtOH} (g/[L·h]), of the different fermentations. Q_{EtOH} was calculated as the produced ethanol within the first 4 h of the fermentations divided by 4.

The ethanol yield, Y_{EtOH} (g/g), was calculated as the produced ethanol after the depletion of glucose and mannose divided by the amount of consumed fermentable sugar (glucose and mannose). The anaerobic growth yield, Y_x (g/g), hereafter referred to as the biomass yield, was calculated as the produced biomass in 36 h divided by the amount of fermentable sugars initially present.

Results

Six different detoxification treatments were performed using both weak and strong anion-exchange resins (Table 1) in an analytical experiment (Fig. 1) and a preparative experiment (Table 2, Fig. 2), in which the fermentability of the fractions was assayed. Figure 1 shows how the pH and the concentrations of glucose, furan aldehydes, phenols, aliphatic acids, and sulfate varied in hydrolysate fractions collected from columns packed with the six different resins in the analytical anion-exchange experiment.

Considerably higher (~50%) glucose concentrations than in the untreated hydrolysate were observed for Dowex 1x4, Dowex 2x8, and IRA 458 (Fig. 1). The fractions containing the highest concentration of glucose appeared after about 10 mL/g of resin. In addition, the weak anion exchangers, IRA 92, IRA 67, and Duolite A7, gave rise to fractions with higher glucose concentrations than the untreated hydrolysate, although the increase was more modest (~20%).

All resins initially adsorbed furan aldehydes and phenols (Fig. 1). In some cases, a subsequent release of adsorbed furan aldehydes and phenols was observed, resulting in concentrations slightly over the concentrations in untreated hydrolysate (Fig. 1). Levulinic, acetic, and formic acid initially adsorbed well to all resins. The trapped levulinic and acetic acid were released before formic acid.

Sulfate was trapped very efficiently, as expected, by all anion-exchange resins. All sulfate was removed from the hydrolysate fractions used for fermentation. The sulfate ions remained adsorbed to the anion exchangers, displacing previously trapped aliphatic acids.

Chemical analyses of the fermentable sugars and different inhibitory compounds in the preparative anion-exchange experiment were done before and after detoxification (Table 2). Relatively limited decreases (≤8%) or increases (≤11%) in the concentration of the fermentable sugars, glucose and mannose, were observed in the fractions collected for fermentation. As already evident from the analytical anion-exchange experiment, the strong anion exchangers initially trapped the monosaccharides, which were then released when the adsorbed sugars were replaced by compounds with higher affinity toward the resin. This resulted in increases in the concentration of glucose and mannose in the fractions collected from the strong anion exchangers. The increases ranged from 1 to 11% compared with the untreated hydrolysate.

The furan aldehydes were also affected differently by the resins. The fractions from the strong anion exchangers showed decreased concentrations of furan aldehydes, whereas the weak anion exchangers showed increased concentrations of furan aldehydes in the fractions used for fermentation. The lowest concentrations of furan aldehydes were observed in fractions from the strong anion exchangers with styrene-based matrices, Dowex 1x4 and Dowex 2x8, which displayed a decrease in the concentrations of furfural and HMF to approx 60–70%.

All treatments decreased the concentration of phenolic compounds in the hydrolysate (Table 2). The two different methods used to estimate the phenolic content, based on Folin-Ciocalteu's reagent and HPLC, indicated the same order for the treated hydrolysates regarding the concentration of phenolic compounds: IRA 458 > IRA 67 > Dowex 2x8 ≥ Duolite A7 > IRA 92 > Dowex 1x4. However, the concentrations of remaining phenolic compounds after the treatments did not always agree perfectly when the results from the two different methods for determination of phenols were compared (Table 2). The reason is that HPLC quantification is based on UV absorbance from the individual phenols and the Folin-Ciocalteu's reagent reacts with the phenolic groups. The fraction obtained with the strong anion-exchange resin Dowex 1x4 showed a decrease in the total amount of phenols by as much as 93 to 94%. The anion exchangers with styrene- and phenolic-based matrices (Dowex 1x4, Dowex 2x8, IRA 92, and Duolite A7) gave larger decreases (60–90%) than the acrylic-based matrices (20–60%).

The concentration of aliphatic acids was affected differently depending on the selectivity of the resins used for the treatment (Table 2). The treatment with Dowex 1x4 resulted in a removal of 90% of the levulinic acid and about 70% of the acetic and formic acid. By contrast, the fraction collected from IRA 458 was enriched in acetic and levulinic acid (about 110–120%), while the concentration of formic acid was decreased by two thirds. Acetic and levulinic acid separated only partly, and the relative concentrations in the accumulated fractions thus partly differed depending on when the fractions were collected.

Volumetric productivity and ethanol yield increased after treatment with all six resins. The volumetric productivity in the reference fermentation, 2.20 g/(L·h), was eight times higher than in the untreated hydrolysate (Fig. 2). The volumetric productivity in the anion-exchange-treated samples was two to seven times higher than in the untreated hydrolysate. The lowest ethanol productivities among the anion-exchange-treated samples, 0.46 and 0.58 g/(L·h), about twice as high as that for the untreated hydrolysate (0.28 g/[L·h]), were observed for the fractions from the weak anion exchangers IRA 67 and IRA 92, respectively (Fig. 2). The fractions from Duolite A7 and IRA 458 showed an intermediate improvement in productivity, four to five times higher than for the untreated hydrolysate.

The highest ethanol productivities, seven times higher than the untreated hydrolysate, were achieved using the strong anion exchangers Dowex 1x4 and Dowex 2x8. The fractions from the strong anion exchangers showed a larger improvement in productivity (five to seven times) than the fractions from the weak ones (two to four times).

The ethanol yield was 0.4–0.5 g/g of consumed glucose and mannose for all anion-exchange-treated samples, although the time required to achieve depletion of glucose and mannose differed greatly (8–24 h) owing to the large difference in productivity. At the end of the experiment, only about 20% of the fermentable sugar had been consumed in the untreated hydrolysate. The biomass yield increased (0.05–0.1 g/g) in the samples treated with anion exchangers compared with the untreated hydrolysate (0.015 g/g), which also indicates that a detoxification had been achieved for all anion-exchange-treated samples.

Discussion

Treatment with anion exchangers affected the inhibitors as well as the fermentable sugars in the hydrolysate (Fig. 1). The anion-exchange resins can act by substitution of anions in the hydrolysate for hydroxyl ions leading toward neutralization. Furthermore, the strong alkaline surfaces of the anion exchanger lead to ionization and entrapment of uncharged compounds. The results suggest that monosaccharides as well as furan aldehydes are involved in such interactions, considering the initial decrease in the concentrations of these compounds. This was followed by a relatively rapid increase to higher concentrations than in untreated hydrolysate. The pH profile could be used to monitor the process. For instance, the displacement of glucose from the column occurred simultaneously as a slower decrease in pH in the effluent was observed.

Another type of interaction is represented by hydrophobic interactions between the matrix and some of the components in the hydrolysate. When these interactions occur, the removal of a compound is dependent on the matrix polymer, rather than on the type of the functional groups. The results indicate that styrene- and phenol-based matrices (here represented by Dowex 1x4, Dowex 2x8, IRA 92, and Duolite A7) have an excellent ability to trap phenols, whereas acrylic-based matrices (represented by IRA 67 and IRA 458) have poorer ability. Nevertheless, the concentration of phenolic compounds decreased by more than 20% after all treatments.

Both ethanol productivity and ethanol yield were improved after treatment. However, treatment with the strong anion exchangers resulted in a larger improvement than treatment with weak exchangers. The improvement in fermentability was clearly indicated by comparisons of the volumetric ethanol productivity data (Fig. 2), although the yields did not differ dramatically. Comparison of the different resins revealed that the best result was obtained after treatment with Dowex 1x4, which increased the fermentability by nearly seven times, whereas treatment with IRA 67 resulted in an increase of only two times. Treatment with

Dowex 1x4 resulted in the largest decrease of all inhibitors determined, which is in agreement with the high increase in fermentability.

Different types of anion-exchange resins have previously been used for detoxification of lignocellulose hydrolysates. Watson et al. *(24)* used a weak anion-exchange resin for detoxification of a bagasse hemicellulose hydrolysate. Dominguez et al. *(25)* used a weak anion-exchange resin for detoxification of corncob hemicellulose hydrolysates. We have previously used a strong anion-exchange resin with a styrene-based matrix for detoxification of spruce hydrolysates *(20,21)*. The results of the present study suggest that the use of a strong anion exchanger with styrene-based matrix is a good alternative for dilute-acid hydrolysates of softwood.

The concentration of furan aldehydes was relatively low (0.4 g/L of furfural and 1.4 g/L of HMF) in the untreated hydrolysate used in the present study. The concentration of furfural and HMF in spruce hydolysates prepared under different conditions varied between 0.2–1.4 and 1.5–8.4 g/L, respectively *(17)*. Although the concentration of furan aldehydes increased in some fractions in the preparative experiment (Table 2), the fermentability was improved compared with the untreated hydrolysate (Fig. 2). This observation agrees with the low initial concentrations and suggests that furan aldehydes were not important inhibitors in the present hydrolysate. However, it is well known that high concentrations of furan aldehydes will prolong the lag phase of cell growth and ethanol formation *(26)*. The fractions collected from strong anion exchangers generally contained less furan aldehydes, but also other factors could possibly affect the interaction with furan aldehydes.

It is well known that phenolic compounds are important inhibitors in hydrolysates prepared from hardwood *(27)* as well as softwood *(20)*. The hydrophobicity of the matrix is likely to be an important property that contributes to the ability of an ion-exchange resin to remove phenols. Although the knowledge regarding the suitability of different methods to determine phenolics in lignocellulose hydrolysates has increased *(28)*, the quantification of phenolics and the correlation with the inhibitory effect is still a challenging task, because hydrolysates contain a wide variety of phenolic compounds that have very different toxic effects.

Although the resins adsorbed levulinic, acetic, as well as formic acid, formic acid was released later than acetic and levulinic acid, which typically were eluted similarly. This can be explained by the fact that the pK_a of formic acid (3.75) is considerably lower than that of acetic (4.74) and levulinic (4.5) acid. The initial concentrations of levulinic, acetic, and formic acid in untreated hydrolysate were 12, 53, and 24 mM, respectively. The total concentration of the three aliphatic acids, 89 mM, is relatively modest considering that previous results indicate that concentrations below 100 mM lead to an increase rather than a decrease in the ethanol yield *(11)*. The effect of the acids is pH dependent, and under anaerobic conditions, the concentration of the undissociated form of acetic acid in the medium should not exceed 5 g/L (80 mM) for growth to occur *(29)*. The low concentration of aliphatic acids in the untreated hydrolysate in the present study strongly suggests that they do not play any significant role as inhibitors. Notably, the highest ethanol yield (0.48 g/g) was determined for the IRA 458–treated sample, which contained the highest concentration of aliphatic acid among the anion-exchange-treated samples.

In agreement with previous findings *(20)*, it can be concluded that treatment with anion-exchange resin is a very effective way to detoxify dilute-acid hydrolysates of spruce. Treatment with strong anion exchangers leads to higher ethanol productivity than treatment with weak ones. Furthermore, styrene- and phenol-based resins are more efficient than acrylic-based resins in trapping phenolic inhibitors. Finally, detoxification using anion-exchange resins can result in removal of inhibitors as well as in accumulation of sugars in the treated hydrolysate.

Acknowledgments

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