Comparison of SO$_2$ and H$_2$SO$_4$ Impregnation of Softwood Prior to Steam Pretreatment on Ethanol Production

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ABSTRACT

The pretreatment of softwood with sulfuric acid impregnation in the production of ethanol, based on enzymatic hydrolysis, has been investigated. The parameters investigated were: H$_2$SO$_4$ concentration (0.5–4.4% w/w liquid), temperature (180 – 240°C), and residence time (1–20 minutes). The combined severity (log Ro-pH) was used to combine the parameters into a single reaction ordinate. The highest yields of fermentable sugars, i.e., glucose and mannose, were obtained at a combined severity of 3. At this severity, however, the fermentability declined and the ethanol yield decreased. In a comparison with previous results, SO$_2$ impregnation was found to be preferable, since it resulted in approximately the same sugar yields, but better fermentability.

Index Entries: Softwood, H$_2$SO$_4$; SO$_2$; steam pretreatment, ethanol production

INTRODUCTION

Several countries, including Sweden, have decided to reduce or to allow no increase in the present level of carbon dioxide discharge to the atmosphere. Interest in fuel ethanol produced from renewable resources has therefore increased during recent years, since ethanol produced from biomass results in no net contribution of carbon dioxide to the atmosphere.

In Sweden, the most abundant raw materials for ethanol production are softwoods in the form of logging waste and waste from the forest industry. In contrast to hardwoods and agricultural residues, which have

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been thoroughly investigated *(1–3)*, softwoods are not easily converted to sugars *(4–6)*.

The enzymatic digestibility of wood is improved when it is impregnated with sulfuric acid or sulfur dioxide prior to steam pretreatment *(5,7–11)*. Acid-catalyzed steam pretreatment of softwood has been utilized to increase sugar yields, but pretreatment conditions resulting in high sugar yields also result in the formation of degradation products *(12)*. When the pretreatment conditions for SO_2-impregnated steam-pretreated softwood were optimized regarding ethanol yield, it was observed that the ethanol production rate was also affected by the pretreatment conditions *(13)*.

One of the most important factors for the economic outcome of ethanol production from lignocellulose is the overall ethanol yield, which requires a high degree of utilization of the raw material *(14)*. In the present study on steam pretreatment of H_2SO_4-impregnated spruce, the effects of the following parameters were investigated: H_2SO_4 concentration (0.5–4.4% w/w liquid), pretreatment temperature (180–240°C), and residence time (1–20 min). To compare the different pretreatment conditions in a normalized way, a single reaction ordinate, the combined severity factor, was used. The combined severity factor is an extension of the severity factor, which has been used in various studies of the pretreatment conditions of lignocellulose *(3,15–18)*. The effects of pretreatment were assessed by the sugar yields after pretreatment and after enzymatic hydrolysis of the fibrous material, and the ethanol yield following fermentation of the sugar solution after pretreatment. The effect on the ethanol productivity was also determined. The results were compared with the results obtained in a previous study using SO_2-impregnated mixed softwoods *(13)*.

MATERIALS AND METHODS

Raw Material

Fresh-chipped spruce, free from bark, was provided by a sawmill, Höörsågen AB (Höör, Sweden). The chip size was less than 30 mm, the material had a dry matter (DM) content of 43%, and was stored in plastic bags at 4°C. The composition, analyzed by STORA AB (Säffle, Sweden), is given in Table 1.

Pretreatment

The following parameters were investigated: sulfuric acid concentrations of 0.5, 2.4, and 4.4% w/w liquid; temperatures of 180, 200, 210, 225, and 240°C, and times of 1, 5, 10, 15, and 20 min (Table 2). Pretreatment was performed in equipment previously described *(13)*. Wood chips corresponding to 200 g DM were mixed, in a plastic bag, with 100 mL dilute

Table 1
Composition of Raw Material

Component	% of DM
Extractives	1.0
Galactan	1.8
Glucan	43.4
Mannan	12.0
Arabinan	1.1
Xylan	4.9
Lignin	28.1

sulfuric acid, and stored at room temperature overnight. The pretreatment vessel (volume 2 L) was preheated with steam prior to loading of the impregnated wood chips. The wood chips were heated by steam to the desired temperature and, when the preset pretreatment time (the heating-up time, less than 10 s, excluded) had elapsed, the material was discharged into a flash drum. The material was then separated, by filtration, into a solid fraction and a filtrate. The solid fraction was washed and the yield of fibrous material determined. The filtrate was analyzed for solubilized glucose, mannose, arabinose, galactose, and xylose, and the byproducts acetic acid, 5-hydroxy-methyl-2-furaldehyde (HMF), and furfural.

Enzymatic Hydrolysis

The washed fibrous material was enzymatically hydrolyzed to determine the maximum-obtainable sugar yield. Hydrolysis was performed at 2% wDM/w in a 0.1 M sodium acetate buffer (pH 4.8), supplemented with 0.2 g Celluclast 2L and 0.05 g Novozym per g dry substrate. The activity of Celluclast was 75 FPU/g *(19)*. The β-glucosidase activity in Celluclast was 12 IU/g *(20)*, and in Novozym it was 392 IU/g. The enzyme preparations were gifts from Novo Industri A/S (Bagsvaerd, Denmark). The hydrolysis vessel, containing a total amount of 500 g material, was maintained at 40°C, and the hydrolysis time was 96 h. Samples withdrawn after 0, 6, 24, 48, 72, and 96 h were analyzed for glucose and mannose content.

Fermentation

The filtrates, i.e., the liquid fractions after pretreatment, were fermented using compressed baker's yeast, *Saccharomyces cerevisiae*, Jästbolaget AB (Rotebro, Sweden). The pH was adjusted to 5.5 with 20% w/w Ca(OH)₂, the filtrates were then centrifuged for 10 min at 5000*g*, and the supernatants were fermented. Fermentation was carried out in 25-mL glass flasks containing a total volume of 20 mL (18.5 mL filtrate, 0.5 mL nutrients, 1 mL inoculum), and sealed with rubber stoppers with cannulas for the

Table 2
Experimental Design of Pretreatment Stage and Dry Matter Content
After Pretreatment

Run Id	Combined severity (log Ro-pH)	Temperature (°C)	Time (min)	H_2SO_4 (% w/w Liquid)	DM content after pretreatment (%)
1	1.4	180	1	0.5	17.3
2	2.0	200	1	0.5	11.9
3	2.1	180	5	0.5	14.5
4	2.3	210	1	0.5	10.3
5	2.3	180	1	4.5	14.4
6	2.6	200	1	2.2	10.6
7	2.7	200	5	0.5	10.8
8	2.7	180	20	0.5	10.8
9	2.7	225	1	0.5	9.5
10	2.9	200	1	4.4	11.0
11	2.9	210	1	2.4	8.5
12	3.0	210	5	0.5	8.9
13	3.0	200	10	0.5	9.0
14	3.0	180	5	4.5	10.2
15	3.1	180	10	2.4	10.3
16	3.1	240	1	0.5	6.9
17	3.3	210	10	0.5	7.5
18	3.3	200	20	0.5	7.9
19	3.3	200	5	2.3	6.5
20	3.4	225	1	2.4	6.0
21	3.4	225	5	0.5	7.8
22	3.6	200	10	2.3	5.7
23	3.6	180	20	4.4	8.7
24	3.6	210	5	2.4	6.4
25	3.9	210	10	2.4	7.5
26	3.9	210	10	2.4	7.3
27	3.9	210	10	2.4	6.5
28	4.0	210	10	2.5	7.5
29	4.0	225	20	0.5	7.0
30	4.1	240	1	4.3	5.8
31	4.1	225	5	2.5	4.2
32	4.2	210	10	4.3	6.6
33	4.2	210	20	2.4	7.0
34	4.3	240	15	0.5	8.6
35	4.5	240	20	0.5	6.5
36	4.8	240	10	2.4	6.0
37	4.9	225	20	4.3	5.4
38	5.3	240	15	4.3	4.1
39	5.4	240	20	4.6	3.1

removal of carbon dioxide. The concentration of fermentable sugars was adjusted to 60 g/L with glucose. This addition was performed to obtain fermentation results that were as comparable as possible. The final concentrations of nutrients were yeast extract (1 g/L), $(NH_4)_2HPO_4$ (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.025 g/L), and NaH_2PO_4 (0.1 M). The filtrates were inoculated with compressed baker's yeast to a cell mass of 10 g DM/L, incubated at 30°C, and stirred with a magnetic stirrer. Two reference fermentations containing 60 g/L glucose and nutrients were included at every fermentation occasion. Samples were collected at the start, before yeast addition, and after 2, 4, 6, 8, 12, and 24 h of fermentation. The sampling frequency was slightly adjusted, depending on the fermentability of the hydrolysates. The samples were analyzed for glucose, ethanol, lactic acid, acetic acid, and glycerol. Fermentation was continued until a glucose stick (Boehringer Mannheim, Mannheim, Germany) was negative, or for a maximum of 24 h. To estimate the influence of pH on the fermentability of the filtrates, fermentation was performed at pH 5.5 and 5.7.

Analyses

Filtrates and samples from hydrolysis and fermentation were analyzed on an HPLC (Shimadzu, Kyoto, Japan) with refractive index detection (Shimadzu). The samples were filtered (0.20 μm) prior to HPLC analysis. Glucose, mannose, arabinose, galactose, and xylose were separated on an Aminex HPX-87P column (Bio-Rad, Hercules, CA) at 80°C, using ultrapure water as eluent, at a flow rate of 0.5 mL/min. Glucose, ethanol, lactic acid, acetic acid, glycerol, HMF, and furfural were separated on the same equipment, at 65°C, using 5 mM H_2SO_4 as eluent, at a flow rate of 0.5 mL/min. The amount of glucose obtained in the pretreatment step was determined from the HPX-87H chromatograms because of background interference in the chromatograms on the HPX-87P column, whereas glucose from the enzymatic hydrolysis was determined from the HPX-87P chromatograms.

RESULTS

The severity of different pretreatment conditions was compared by calculating a severity parameter, in which the temperature and residence time variables were combined into a single reaction ordinate. The severity factor, log Ro, is defined by

$$Ro = t \cdot \exp[(T_r - T_b)/14.75] \qquad (1)$$

where t is the reaction time (min), T_r the reaction temperature (°C) and T_b a reference temperature, which was set to 100°C *(15)*. Because the pretreatment was performed under acidic conditions, the effect of pH was taken into consideration by the combined severity *(16)* defined as
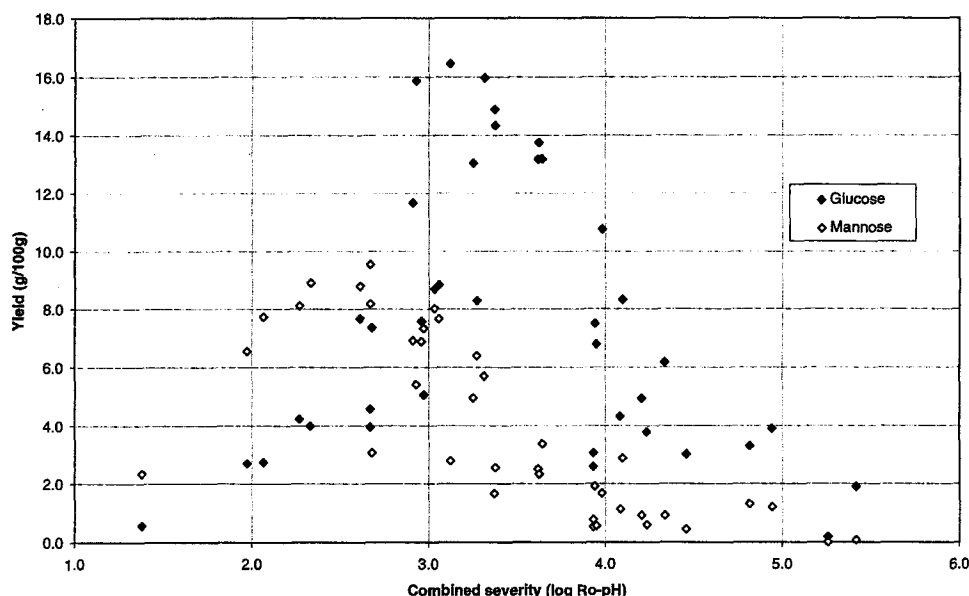
Fig. 1. The yield of glucose and mannose after pretreatment as a function of the combined severity.

$$\text{Combined severity} = \log Ro - pH \qquad (2)$$

The pH was calculated from the amount of sulfuric acid added, and the combined severity parameter was calculated for each set of pretreatment conditions (Table 2).

Unless otherwise stated, the yields following pretreatment and hydrolysis are expressed as g/100 g of original dry wood. The yields following fermentation, on the other hand, are expressed as the amount of ethanol divided by the amount of total fermentable sugars, i.e., glucose and mannose, in the fermentation broth.

Arabinose and xylose are not fermentable with S. cerevisiae. Galactose was present at very low concentrations in the filtrates, and was not fermented by the strain of S. cerevisiae used in the present investigation. Therefore, only glucose and mannose, the predominant sugars in softwoods, were used for the evaluation of the yields. The maximum yields of mannose and glucose following pretreatment were achieved in different ranges of combined severity: 2.3–2.7 and 2.9–3.4, respectively (Fig. 1). The highest glucose yield, about 16 g/100 g, was obtained for runs 11, 16, and 19 (Table 2); the highest mannose yield, about 9 g/100 g, was achieved for runs 5, 6, and 8.

The maximum glucose yield in the enzymatic hydrolysis, about 19 g/100 g, was obtained in the same range of combined severity (2.9–3.4) as for the maximum glucose yield in the pretreatment (Fig. 2). One single run deviates from the general trend, resulting in a glucose yield of
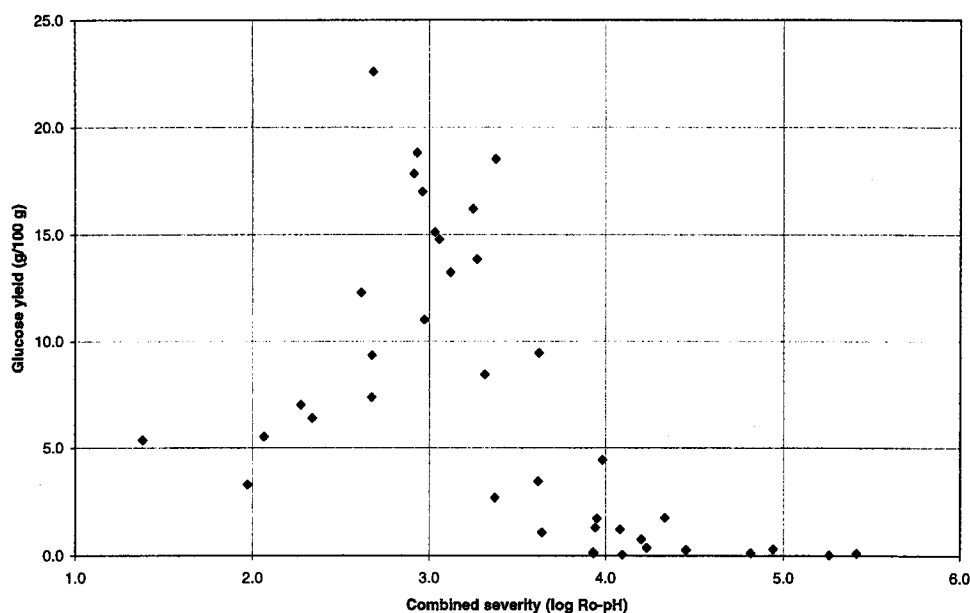
Fig. 2. The yield of glucose following enzymatic hydrolysis as a function of the combined severity.

23 g/100 g at a combined severity of 2.7 (corresponding to 225°C, 1 min, and 0.5% H_2SO_4). For all acid concentrations, the highest yield following hydrolysis was obtained at a residence time of 1 min, but at different temperatures. For 2.4% H_2SO_4, the maximum yield, 19 g/100 g, was obtained at 210°C, and for 4.4% H_2SO_4 the maximum yield of 18 g/100 g was obtained at 200°C.

The total yield of fermentable sugars after both pretreatment and hydrolysis is shown in Fig. 3, together with information on whether or not the filtrates were fermentable. The fermentability was defined as an ethanol yield of more than 50% of the theoretical yield after 24 h. The highest total yield of fermentable sugars, 40 g/100 g, was obtained following pretreatment at 210°C, for 1 min with 2.4% H_2SO_4 (Fig. 3). These conditions also resulted in the highest total glucose yield after pretreatment and hydrolysis, i.e., 35 g/100 g, corresponding to 72% of the theoretical yield. Among the fermentable samples, the highest fermentable sugar yield was 35 g/100 g, obtained for the pretreatment conditions 225°C, 5 min, and 0.5% H_2SO_4.

Only the filtrates after pretreatment were fermented, since no additional byproduct formation occurs in the enzymatic hydrolysis. The ethanol yield showed a sharp decline at a combined severity of about 3, from 0.3–0.35 g/g to 0–0.05 g/g (Fig. 4). The ethanol yield in the reference fermentation was 0.40 ± 0.02 g/g. For the samples obtained with a combined severity of about 3, a small variation in pH, such as 0.2 units, influ-
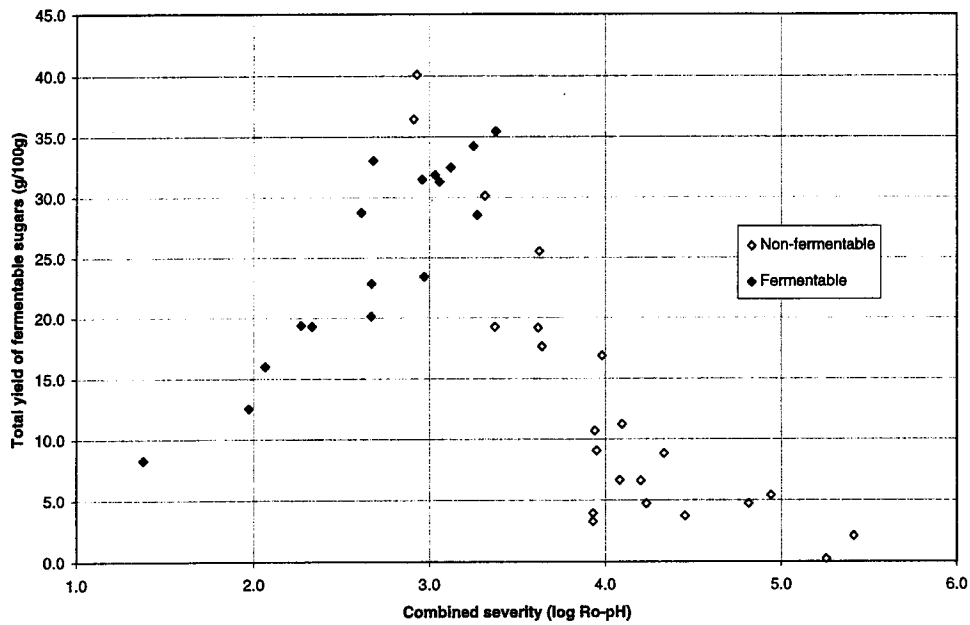
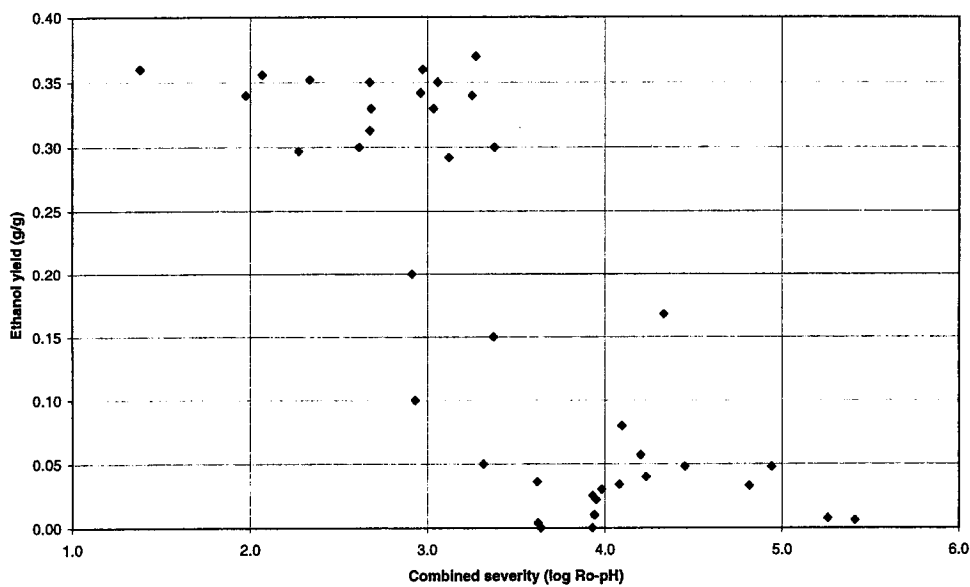Fig. 3. The total yield of fermentable sugars as a function of the combined severity.

Fig. 4. The ethanol yield at pH 5.5 as a function of the combined severity.

enced the yield. For example, for run 10 the yield in the fermentation increased from 0.21 g/g at pH 5.5 to 0.35 g/g at pH 5.7. However, the sample that gave the highest yield of fermentable sugars did not ferment. The ethanol yield was 0.1 g/g at pH 5.5, and, when the pH was adjusted
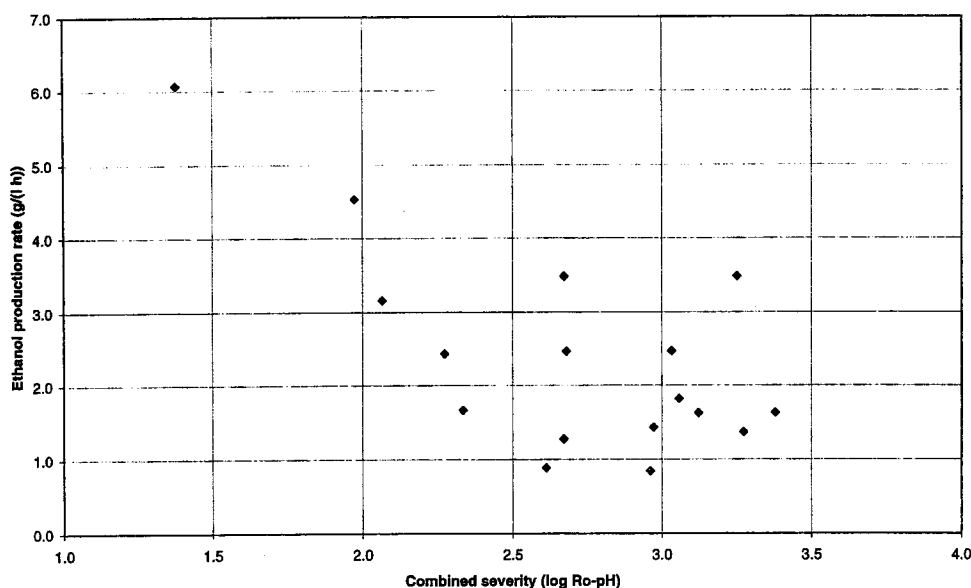
Fig. 5. The average ethanol production rate after 2 h (excluding the lag phase), r_{2h}, as a function of the combined severity.

to 5.7, the yield increased only marginally, to 0.14 g/g. The average ethanol production rate during the first 2 h, r_{2h}, was determined excluding the lag phase, which was less than 2 h. If the lag phase was included in the ethanol production rate, no distinct relationship between the production rate and the combined severity could be seen. For the fermentable filtrates, r_{2h} varied between 0.8 and 6.1 g/(L h), and decreased with increasing combined severity (Fig. 5). The mean value of the ethanol production rate in the reference fermentations was 5.9 g/(L h). The dry wt of the pretreated material varied between 3 and 17% (Table 2). The samples treated under the most severe conditions had the lowest DM contents. A long residence time and high pretreatment temperature increased the heat losses in the pretreatment reactor, which resulted in the formation of more condensate and a reduction in the DM content, since direct steam is used in the pretreatment unit. Therefore, the amount of compounds solubilized in the pretreatment stage were unequally diluted in the liquid after pretreatment. Increased degradation of the material also resulted in a reduced DM content because of an increase in the formation of soluble byproducts. The fermentability was low for the samples with the highest combined severity, despite the higher dilution.

The maximum concentrations of the byproducts furfural and HMF were found to be approx 2 g/L and 5 g/L, respectively, at a combined severity between 3 and 4 (Fig. 6). These concentrations correspond to a yield of about 1.5 g/100 g and 3 g/100 g, respectively. The concentration
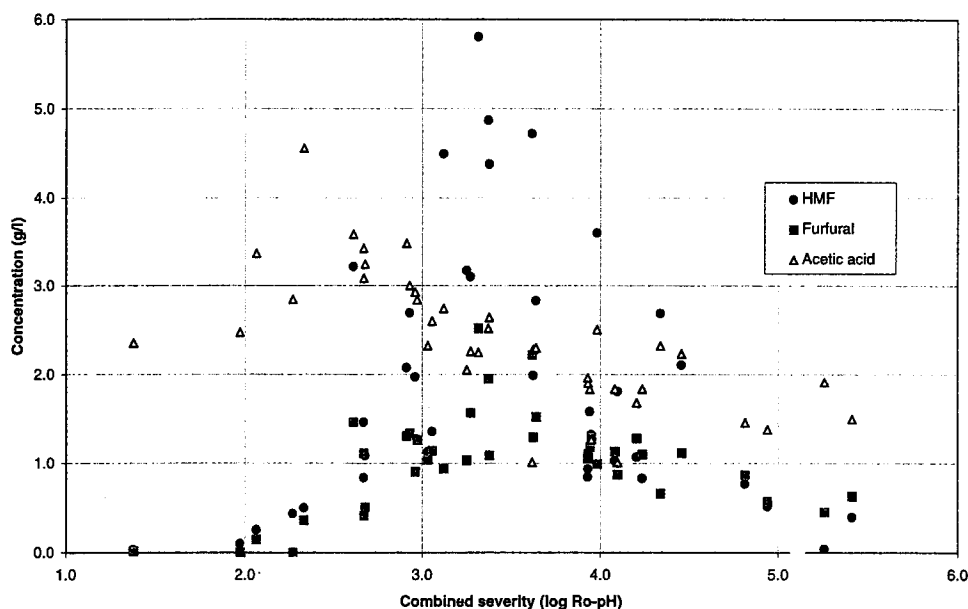
Fig. 6. The concentration of the byproducts HMF, furfural, and acetic acid, as a function of the combined severity.

of acetic acid reached a maximum at a combined severity of 2.3, then the concentration decreased slightly from 3.0 to 1.5 g/L (Fig. 6), corresponding to yields of 2 g/100 g and 1 g/100 g, respectively.

DISCUSSION

Impregnation with SO_2 or H_2SO_4 prior to steam pretreatment and enzymatic hydrolysis has been investigated in several studies (5,7–9). It is difficult to compare the efficiencies of the two acid catalysts, since different equipment and raw materials have been used. However, comparisons between SO_2 and H_2SO_4 have been performed on willow (21) and aspen (7). In the study on willow, the sugar yields after pretreatment and after enzymatic hydrolysis were compared; in the study on aspen, the enzymatic hydrolysis was replaced by combined hydrolysis and fermentation. The latter investigation was based on a limited amount of data. In the present study, a comparison for softwood was made possible by comparing the results obtained for H_2SO_4 impregnation with the results from a previous study on SO_2 impregnation, performed with the same equipment, and using the same procedure (13). In the SO_2 study, the following pretreatment conditions were investigated: temperature (190–230°C) and residence time (2–15 min). The SO_2 concentration absorbed by the wood was less than 1% w/w DM. The sugar yields after pretreatment and after enzymatic hydrolysis, as well as the fermentability, were investigated. To the best of the authors' knowledge, this is the first time both hydrolysis yield and

fermentation yield have been considered in a comparison between SO_2 and H_2SO_4 impregnation of softwood. The most significant differences between H_2SO_4 and SO_2 impregnation were the step in which the sugars were formed (pretreatment or enzymatic hydrolysis) and the difference in fermentability.

H_2SO_4 impregnation resulted in high yields of both glucose and mannose after the pretreatment step; the glucose yield following enzymatic hydrolysis was low. With SO_2 impregnation, the situation was the opposite: Most of the fermentable sugars were released during hydrolysis. However, SO_2 impregnation resulted in approximately the same total yield of fermentable sugars, based on theoretical yield (66%), as H_2SO_4 impregnation (65%). This was also observed in a similar study on willow, in which H_2SO_4 impregnation resulted in high yields of hemicellulose sugars, but the glucose yield after pretreatment and enzymatic hydrolysis was low in comparison with the yield obtained with SO_2 impregnation *(21)*.

As shown in the present study, there is a relation between the yields of sugars and ethanol, and the combined severity in the region investigated. In consequence, the maximum yield was the same for all acid concentrations, but at different temperatures and residence times. In contrast, an increase in SO_2 concentration in the impregnation step, up to about 3%, increased the yield of glucose *(9,12)*. The optimal sugar yield was achieved at the same temperature and the same residence time for different levels of SO_2. In the latter studies, no fermentation was performed, and it is therefore not possible to conclude whether the overall ethanol yield increased or not. However, another study showed that softwood pretreated with SO_2 at a concentration of 4% w/w DM could be fermented *(22)*.

An obvious difference in fermentability was observed between H_2SO_4 and SO_2 impregnation. The sharp decline in ethanol yield at a combined severity of about 3 for H_2SO_4-impregnated samples was not observed for SO_2 impregnation, in which all samples were fermentable *(13)*. Despite the fact that only the fermentable filtrates were considered, the average ethanol production rate for H_2SO_4-impregnated samples was low compared with SO_2-impregnated samples. The ethanol production rate, r_{2h}, for H_2SO_4-impregnated samples was about 2 g/(L h), while it was about 5 g/(L h) for the SO_2-impregnated samples. In H_2SO_4 impregnation, both the pretreatment temperature and the residence time influenced the ethanol production rate; for SO_2 impregnation the pretreatment temperature had a major influence, but the effect of the residence time was negligible. The difference in fermentability between H_2SO_4- and SO_2-impregnated material indicates that H_2SO_4 impregnation either leads to higher concentrations of byproducts or to the formation of additional inhibitory byproducts.

The sharp decline in ethanol yield for H_2SO_4 impregnation did not correlate with the concentration of the analyzed byproducts HMF, furfural, and acetic acid (Fig. 6), which decreased at combined severities higher than 3. However, the decline in ethanol yield was obtained at the same

combined severity as the maximum concentration of the analyzed byproducts. At higher values of combined severity, the ethanol yield was still low, despite the decrease in the concentration of the analyzed byproducts caused by dilution and further degradation (HMF and furfural). This indicates that the production of byproducts not analyzed increased, e.g., levulinic acid and formic acid, which are known to be secondary degradation products from HMF (23,24), and lignin degradation products (25).

The maximum yield of fermentable sugars and the decline in ethanol yield were observed at the same combined severity, emphasizing the importance of considering the ethanol yield when optimizing the pretreatment conditions. In this region, the fermentability could be improved by a small increase in pH, which indicates that the fermentability was affected by weak acids (26,27). However, other byproducts, such as lignin degradation products, were probably also responsible for the inhibition (25), since an increase in pH did not improve the fermentability for all filtrates.

To recover the maximum amount of sugars from both hemicellulose and cellulose, different pretreatment conditions are required. Two-step, countercurrent pretreatment of hardwood, using dilute sulfuric acid at different temperatures, resulted in high yields of both xylose and glucose (28). The different conditions for maximum mannose yield and maximum glucose yield obtained in the present study indicate that a two-step pretreatment procedure would also be advantageous for softwoods. Another alternative would be to recover the hemicellulose sugars in the first step, using H_2SO_4 under mild conditions, and, in the following step, to increase the reactivity of the cellulose, using SO_2 at a higher temperature. In such a process configuration, the increased consumption of chemicals would have to be evaluated in relation to a potentially higher yield of ethanol.

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