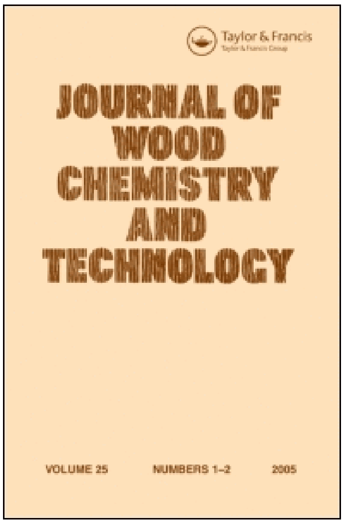

Journal of Wood Chemistry and Technology

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Söderström, Johanna , Galbe, Mats and Zacchi, Guido(2005) 'Separate versus Simultaneous Saccharification and Fermentation of Two-Step Steam Pretreated Softwood for Ethanol Production', Journal of Wood Chemistry and Technology, 25: 3, 187 — 202

To link to this Article: DOI: 10.1080/02773810500191807

URL: http://dx.doi.org/10.1080/02773810500191807

Journal of Wood Chemistry and Technology, 25: 187–202, 2005
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ISSN 0277-3813 print/1532-2319 online
DOI: 10.1080/02773810500191807

Separate versus Simultaneous Saccharification and Fermentation of Two-Step Steam Pretreated Softwood for Ethanol Production

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Abstract: In two previous studies, optimal conditions were identified for two-step steam pretreatment of SO_2- and H_2SO_4-impregnated softwood. In the present study the yield of sugar and ethanol was determined in a process development unit where pretreatment was performed in a 10-L reactor and simultaneous saccharification and fermentation (SSF) or enzymatic hydrolysis (EH) were performed in 30-L reactors. The study showed that a steam pretreatment reactor should be larger than 2 L to yield acceptable results. Two pretreatment combinations were studied. In the H_2SO_4 case, the first pretreatment step was at 180°C for 10 min with 0.5% H_2SO_4 and the second step at 210°C for 2 min with 1% H_2SO_4. In the SO_2 case, first step was at 190°C for 2 min followed by a second step at 210°C for 5 min. The concentration of SO_2 was 3% in both steps. EH and SSF were performed on the whole slurry after the second pretreatment step to determine the yield of sugars and ethanol. The liquid after the first pretreatment step was also analyzed and fermented. When SSF and EH were performed at the same dry matter content and enzymatic activity, the ethanol yield in SSF exceeded the yield obtained with EH in both pretreatment cases, even when 100% yield in the fermentation step was assumed. Thus SSF is a better process if yield is the main priority. Comparison of the yields with the two acid catalysts showed higher yields with SO_2 in both SSF and EH. The overall ethanol yield following SSF of SO_2-impregnated and pretreated wood reached 81% of the theoretical, that is, 357 liters per metric ton of dry raw material.

Keywords: Spruce, ethanol, saccharification, fermentation, enzymatic hydrolysis, sulfur dioxide, sulfuric acid

We gratefully acknowledge the Swedish Energy Agency for financial support.

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INTRODUCTION

Interest in producing biofuels, such as ethanol, has intensified since 1997, when the Kyoto protocol was ratified limiting the net global emission of carbon dioxide. The EU has decided that 12% of its total energy consumption should be derived from renewable sources by 2010. One way of reducing environmental effects and the dependence on fossil fuels is to use ethanol produced from renewable domestic resources. In Sweden 55% of the land is covered with forest, of which 85% is softwood.[1] Being such an abundant feedstock, softwood is of great interest for the production of bioethanol.

Softwood, as all lignocellulosic materials, has three main components: cellulose, hemicellulose, and lignin, the first two being polymers of sugar molecules. These polymers can be hydrolyzed to produce monomeric sugars, which can be fermented to ethanol. Because the cellulose is protected by the matrix of hemicellulose and lignin as well as its partially crystalline nature, pretreatment is necessary to achieve efficient enzymatic hydrolysis.[2–4]

Steam explosion with an acid catalyst such as SO_2 or H_2SO_4 is an effective method of enhancing both the hemicellulose recovery and the subsequent enzymatic hydrolysis of the cellulose.[5–10] During steam pretreatment, the pentoses and hexoses formed from hydrolyzed hemicellulose and cellulose may be further degraded to furfural and 5-hydroxymethylfurfural (HMF), levulinic acid, and formic acid. These substances, together with lignin degradation products and released organic acids, act as inhibitors in the fermentation step.[5,6,11,12] More severe pretreatment conditions will cause greater degradation of hemicellulosic sugars with loss of yield and possible inhibition as a consequence.[5,11,13] However, a high degree of severity is required to enhance the enzymatic digestibility of the cellulose.[6,14]

The most important factor for the economic outcome of the wood-to-ethanol process is the overall ethanol yield.[15] As a consequence it is important to maximize the overall sugar yield in the process, that is, obtain high yields of both glucose and hemicellulosic sugars. In the present study the focus was on glucose and mannose, because these are the sugars fermented by the yeast used, *Saccharomyces cerevisiae.* An increased yield can be achieved with a two-step steam pretreatment method, in which the first step is performed at low severity to hydrolyze the hemicellulose. In the second pretreatment step the remaining solid material from the first step is treated at more severe conditions to disrupt the physical structure of the cellulose. By separating the solid and the liquid phases after the first pretreatment step it is possible to minimize reaction of the hemicellulosic sugars to form furfural and HMF. Thus, a two-step steam pretreatment process has been proposed in the literature.[9,14,16–21] In this study two different combinations of the two pretreatment steps that were previously determined to give the highest yield were further investigated.[19,20] The choice of acid catalyst, that is, SO_2 or H_2SO_4, influences the result of the pretreatment. Sulfuric acid (H_2SO_4) is known to improve hemicellulose hydrolysis but also to produce more inhibitory substances that cause poor yields in the subsequent fermentation stage. However, SO_2 affects hemicellulose hydrolysis to a lesser extent, and the treated material contains fewer inhibitory substances, thus providing better fermentation.[22]

The two steps after pretreatment, that is, hydrolysis and fermentation, can either be performed as separate hydrolysis and fermentation (SHF) or as simultaneous saccharification and fermentation (SSF). The advantage of SHF is that each step can be performed under optimal conditions, whereas in SSF a compromise must be made. The major drawback of SHF is that the sugars released inhibit the enzymes during hydrolysis: end-product inhibition of β-glucosidase occurs. In SSF, the sugars are immediately consumed by the yeast and converted to ethanol.

Previous studies on one-step steam pretreatment have shown that SSF gives higher yields than SHF when performed under the same conditions.[23,24] In previous screening studies of two-step steam pretreatment SHF proved to give higher yields. However, only washed material was used in SHF with a water-insoluble material content of 2% dry matter (DM), whereas the whole slurry was used for SSF at 5% DM.[19,20]

In the present study, a larger steam vessel was used for the first pretreatment than previously. The reactor volume of 10 L is larger than that used by most researchers studying steam pretreatment, where a typical reactor size is 2–4 L.[17,25–27] In addition, both SSF and SHF were performed using the whole slurry with 5% DM in identical fermentors. Previously, SHF was performed using only 2% DM and in a different kind of fermentor. Thus, in this study, SSF and SHF have been compared under equivalent conditions.

MATERIALS AND METHODS

Figure 1 illustrates the experimental procedure used in this study. The softwood was impregnated with either H_2SO_4 or SO_2 and then treated with steam in the first step. The resulting slurry was separated into a solid residue and a liquid. The liquid was analyzed for sugars and then fermented. The solid material was washed with water and then impregnated again with H_2SO_4 or SO_2 and again treated with steam in the second step. Enzymatic hydrolysis and SSF were performed on the whole slurry. Two different pretreatment combinations were investigated, one in which SO_2 was used for impregnation in both steps, and the other in which the material was impregnated with dilute H_2SO_4 in both steps.

Raw Material

Freshly chipped softwood, *Picea abies*, free from bark, was kindly provided by a sawmill (Harry Nilsson, Hästveda, Sweden). The wood chips were rechipped and fractionated to a chip size between 2.2 and 10 mm. The compo-

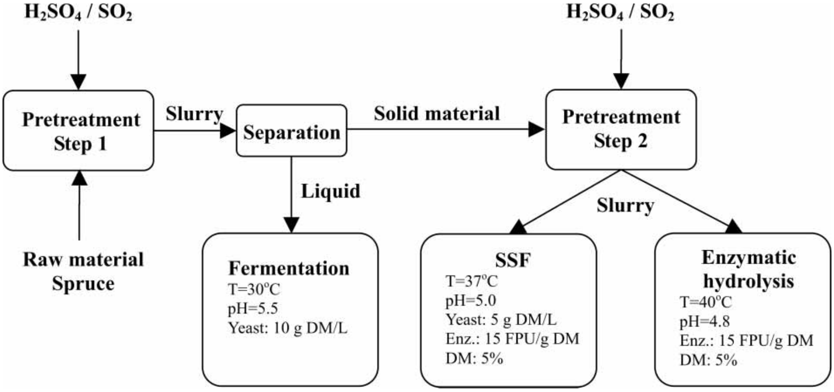

Figure 1. Experimental procedure.

sition of the material is presented in Table 1. The material had a dry matter content of 42% and was stored in plastic bags at 4°C.

Pretreatment

Steam pretreatment was carried out in a 10-L pressure vessel with a flash tank to collect the pretreated material.[28] Several batches of pretreated material were produced for each pretreatment step before emptying the flash tank. The material was well mixed before use in the subsequent process steps.

H_2SO_4 and SO_2 Impregnation

Impregnation with sulfuric acid was carried out by leaving the wood chips immersed in dilute H_2SO_4 in plastic buckets overnight prior to steam pretreat-

Table 1. Composition of the raw material, and the material after the first and second pretreatment steps

	% Dry matter SO_2 impregnation			% Dry matter H_2SO_4 impregnation		
Compound	Raw material	Step 1	Step 2	Raw material	Step 1	Step 2
Glucan	48.7	57.6	56.8	47.1	51.4	48.2
Mannan	12.9	3.2	2.4	12.4	2.8	2.3
Arabinan	1.8	0	0	2.3	0.2	0.1
Galactan	2.2	0.9	1.0	2.3	2.4	0
Xylan	4.6	1.7	0	4.4	1.8	0
Lignin	28.4	36.7	39.8	27.5	41.4	52.0

ment. A concentration of 0.5% acid (w/w) based on the total water content was used for the first pretreatment step and a concentration of 1% in the second pretreatment step. When using SO_2 the concentration of gaseous SO_2 was 3% (w/w) based on the water content in the wood for both steps. Impregnation was performed in plastic bags for 20 min at room temperature.

First Pretreatment Step

The material impregnated with H_2SO_4 was pretreated at 180°C for 10 min and the material impregnated with SO_2 was pretreated at 190°C for 2 min. The material collected after pretreatment was separated by filtration into a solid residue and a liquid. The liquid was analyzed for soluble sugars and degradation products. The loss of solid material in the reactor after pretreatment was determined by rinsing the reactor and the flash tank with water and measuring the amount of solid material collected.

Washing between Pretreatment Steps

The solid material after the first pretreatment step was thoroughly washed with water to remove all soluble substances. The solid material was washed in a surplus of water three times. The dry matter content after washing was 28% for the H_2SO_4-impregnated material and 30% for the SO_2-impregnated material. The material was stored at 4°C.

Second Pretreatment Step

The washed solid material was re-impregnated with either H_2SO_4 or SO_2 as described earlier. It was then steam pretreated at 210°C for 2 min (H_2SO_4-impregnation) or 210°C for 5 min (SO_2-impregnation). The resulting slurry was analyzed with regard to soluble sugars and degradation products. The composition of the solid fraction was also determined. The slurry was used for SSF and enzymatic hydrolysis.

Determination of Oligosaccharides by Acid Hydrolysis

The amount of oligomers in the liquid after the first pretreatment step was determined by acid hydrolysis. To a 2 mL sample of the liquid 10.6 mL of H_2O and 1.4 mL of 1.0 M H_2SO_4 were added. The flasks were autoclaved at 121°C for 4 h. The increased amount of monomers in the liquid after acid hydrolysis originates from the oligomers.

Simultaneous Saccharification and Fermentation

SSF of the slurry from the second pretreatment step was used to assess the efficiency of the steam pretreatment. It was performed in a 30-L Laboratory Fermentor (NLF 22, Bioengineering AG, Wald, Switzerland), with a working volume of 22 L. SSF was performed under non-sterile conditions. The slurry was diluted with water to a final water-insoluble solids concentration of 5% DM. A commercial cellulase mixture, Celluclast 1.5 L (65 FPU/g and 17 β-glucosidase IU/g), was used, supplemented with the β-glucosidase preparation Novozyme 188 (376 β-glucosidase IU/g), both kindly donated by Novozymes A/S (Bagsværd, Denmark). The initial cellulase activity in the reactor was 15 FPU/g DM and the β-glucosidase activity was 23 IU/g DM. Compressed baker's yeast, *Saccharomyces cerevisiae*, (Jästbolaget AB, Rotebro, Sweden) was used at an initial concentration of 5 g DM/L. SSF was performed at 37°C and pH 5.0 for 72 h. The pH was controlled by adding 30% (w/w) NaOH and the foam level in the reactor was controlled by the addition of an anti-foaming agent. Samples were withdrawn throughout the process. For comparison, the SSF experiments were also performed in 1-L fermentors (Belach AB, Stockholm, Sweden) using a total weight of 600 g, while maintaining the same conditions as those used for the 30-L fermentor.

Enzymatic Hydrolysis

Enzymatic hydrolysis of the slurry from the second pretreatment step was performed in the same kind of 30-L fermentor as described earlier. The slurry was diluted with water to a final water-insoluble solids concentration of 5% DM. The cellulase and the β-glucosidase activity were the same as in the SSF experiment. Enzymatic hydrolysis was performed at 40°C and pH 4.8 for 96 h. The pH and foam level were controlled by the addition of 30% (w/w) NaOH and an anti-foaming agent, as described earlier.

Fermentation

Fermentation of the liquid was performed after the first pretreatment step to investigate the fermentability and the extent of inhibition. The liquids were diluted $1+0$, $1+1$, $1+2$, $1+3$, or $1+4$ with water, and glucose was added to the liquids to adjust the concentration of fermentable sugar to 50 g/L. A reference solution containing 30 g/L glucose and 20 g/L mannose was also fermented. *S. cerevisiae* was used at a concentration of 10 g DM/L. Fermentation was performed at 30°C and pH 5.5 for 24 h. The fermentation experiments were performed in duplicate.

Analysis

Dry weights were determined by oven drying at 105°C to constant weight. The composition of the solid material was determined according to the National Renewable Energy Laboratory (NREL) standard method for determination of carbohydrates in biomass, LAP-002.[29] Soluble and insoluble lignin were determined with the NREL method for the analysis of acid-insoluble and acid-soluble lignin in biomass, LAP-003 and LAP-004.[30,31] The material before and after each pretreatment step, as well as after enzymatic hydrolysis and SSF was analyzed (although the SSF material contained yeast). The liquids obtained after the pretreatment steps and all samples from the acid and the enzymatic hydrolysis, fermentation, and SSF were analyzed with HPLC (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (Shimadzu). Glucose, mannose, arabinose, galactose, and xylose were separated using an Aminex HPX-87P column (Bio-Rad, Hercules, USA) at 80°C, using water as eluent, at a flow rate of 0.5 mL/min. Cellobiose, glucose, arabinose, lactic acid, glycerol, acetic acid, ethanol, HMF, and furfural were separated on an Aminex HPX-87H column (Bio-Rad) at 65°C using 5 mmol/L H_2SO_4 as the eluent, at a flow rate of 0.5 mL/min. All samples were filtered through a 0.20 μm filter before HPLC analysis. Samples from the enzymatic hydrolysis and the liquid phases after the pretreatment steps were analyzed on the HPX-87P column. However, because of interference between ethanol and mannose on this column, samples from SSF and fermentation were analyzed on the HPX-87H column. The analysis of glucose in the liquid phase after pretreatment was also carried out on the HPX-87H column.

RESULTS AND DISCUSSION

First Pretreatment Step

The overall yields of fermentable sugars and ethanol are based on the amount of glucose and mannose in the raw material. Following impregnation with SO_2 the total recovery of fermentable sugars in the solid and in the liquid (monomers + oligomers) was 92% of the glucan and 84% of the mannan. Ten percent of the recovered glucan and 56% of the recovered mannan were found in the liquid. The distributions of oligomeric and monomeric sugars are presented in Table 2.

In the case of H_2SO_4 impregnation the total recovery of glucan and mannan was 93% and 75%, respectively. Sixteen percent of the recovered glucan and 44% of the recovered mannan was found in the liquid. The fractions of oligomeric and monomeric sugars in the liquid are presented in Table 2.

The total recovery of glucan was slightly lower than that obtained in our previous studies,[19,20] using the same conditions. The total recovery of mannan

Table 2. Recovery of glucose and mannose in liquid and solid after first pretreatment step

Sugar recovery (%) of theoretical yield	SO_2	H_2SO_4	a	b	c	d	e
Glucose							
Total	91.5	92.6	97	93	—	—	—
Solid	81.1	75.4	88	81	—	—	—
Liquid	10.4	17.2	9	12	2	—	16
As oligomers (%)	7.9	7.4	6	13	5	21	12
As monomers (%)	92.1	92.6	94	87	95	79	88
Mannose							
Total	83.8	74.7	97	100	—	—	—
Solid	16.9	15.6	9	12	—	—	—
Liquid	66.9	59.2	88	88	63	—	87
As oligomers (%)	10.3	6.1	17	12	11	25	21
As monomers (%)	89.7	93.9	83	88	89	75	79

a: 190°C, 2 min, 3% SO_2(19);
b: 180°C, 10 min, 0.5% H_2SO_4 (20);
c: 212°C, 105 s, 0.35% H_2SO_4 (5);
d: 170°C, 7.5 min, 4.5% SO_2(7);
e: 190°C, 3 min, 0.7% H_2SO_4 (27).

was considerably lower than that obtained previously. These differences are probably due to the fact that different batches of raw material were used, and in the case of H_2SO_4 impregnation, the pretreatment was performed in another type of equipment.

The amount of oligomeric sugars in the liquid was generally slightly lower than that obtained previously. Compared with the amounts obtained by Nguyen et al.[27] and Wu et al.[7] the amount of oligomers was considerably lower, but the pretreatment severity employed in the present study was higher, and thus a higher degree of hydrolysis of the oligomers would be expected.

The concentrations of furfural and HMF in the liquid from pretreatment of both the H_2SO_4- and the SO_2-impregnated material were very low, indicating that it would be easy to ferment the liquid. However, fermentation did not succeed unless the liquid was diluted, despite the fact that high yeast concentrations (10 g DM/L) and long residence times (48 h) were used. A dilution of 1 to 1 with water was required to obtain an adequate ethanol yield. After dilution the fermentation yield was above 95%, which was higher than that for the reference solution. The presence of small amounts of organic acids, such as acetic acid, may increase the fermentation yield.[12] A possible explanation of the low fermentation yield in the non-diluted liquid is the presence of phenolic compounds resulting from lignin degradation. However, quantification of the total amount of phenolic compounds did not reveal a high concentration. Only a small amount of the lignin was solubilized, 3% and

6% for the H_2SO_4- and the SO_2-impregnated material, respectively. The poor fermentability could thus be caused by very low concentrations of phenolic compounds released from the lignin. Extractives that were released during pretreatment could have the same effect on the fermentability.

Second Pretreatment Step

The material after the first pretreatment step was washed thoroughly to remove all soluble substances and then used in a second pretreatment step. This material contained mainly glucose and lignin (Table 1). After impregnation with 3% SO_2 or 1% H_2SO_4 the material was steam pretreated in the second step at 210°C for 5 min or 2 min, respectively. The total yield of glucose in the second pretreatment step was almost 100% (Figure 2), and the total yield of mannose in the second step was 83% and 74% for the SO_2-impregnated and the H_2SO_4-impregnated material, respectively. The overall carbohydrate yield in the second pretreatment step was about 43 g/100 g raw material, where most of the sugars were still in the form of glucan and mannan in the solid material; 88% and 75% in the SO_2-impregnated and the H_2SO_4-impregnated material, respectively (Figure 3). The overall carbohydrate yield was higher than that achieved in previous studies with the same conditions, where the yield was only about 35 g/100 g raw material (Figure 3).[19,20] The main reason proposed for the higher yield is that a larger steam pretreatment reactor (10 L) was used in the present study, whereas in the previous study a reactor volume of only 2 L was used for the second pretreatment step. This indicates that the reactor volume used is too small for the material to be treated homogeneously, due to the influence of the reactor walls. In the present study several batches of material were steam-pretreated before emptying the collecting vessel, which reduced losses due to handling to only 0.5% of the dry weight and also facilitated higher yields.

After the second pretreatment step the dry matter content was 16% and 12%, in the SO_2-impregnated and the H_2SO_4-impregnated material, respectively. The concentrations of HMF and furfural were very low in both slurries, less than 2 g/L, and did lead to inhibition in the subsequent process steps, that is, SSF and enzymatic hydrolysis (EH), where the slurry was even further diluted to concentrations lower than have been shown to be inhibiting.[12]

SSF and Enzymatic Hydrolysis

Simultaneous Saccharification and Fermentation

Both SSF and EH were performed with 5% DM in the fermentor. The ethanol yield in SSF proved to be very high, 91% of the theoretical value, for the SO_2--

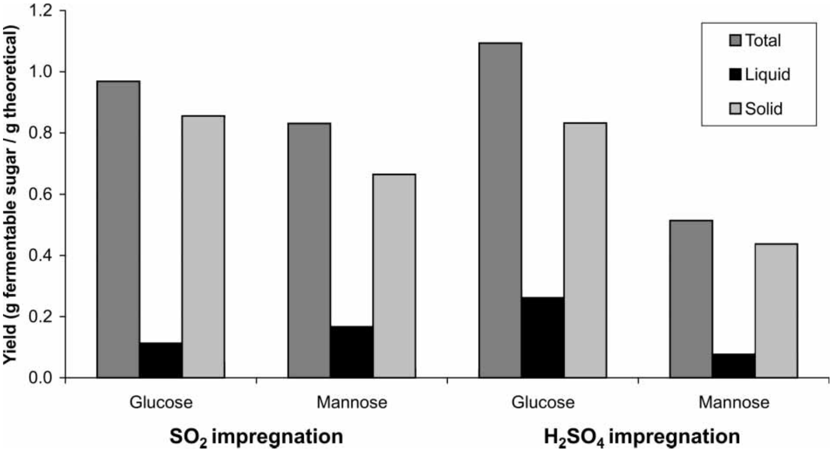

Figure 2. Yield of fermentable sugars (glucose and mannose) in the second pretreatment step following SO_2 impregnation and H_2SO_4 impregnation.

impregnated material, when using the 30-L fermentor. The use of 1-L fermentors resulted in an ethanol yield of 90% of the theoretical value in that step. This shows that the pretreatment was successful.

The H_2SO_4-impregnated material resulted in an ethanol yield of only 71% in SSF using a 30-L fermentor, whereas the yield obtained in the 1-L fermentor was 75%. However, in the large fermentor, significant production of lactic acid was observed after 20 h. Lactic acid is produced by lactic acid bacteria present in commercial baker's yeast. When the amount of lactic acid was recalculated

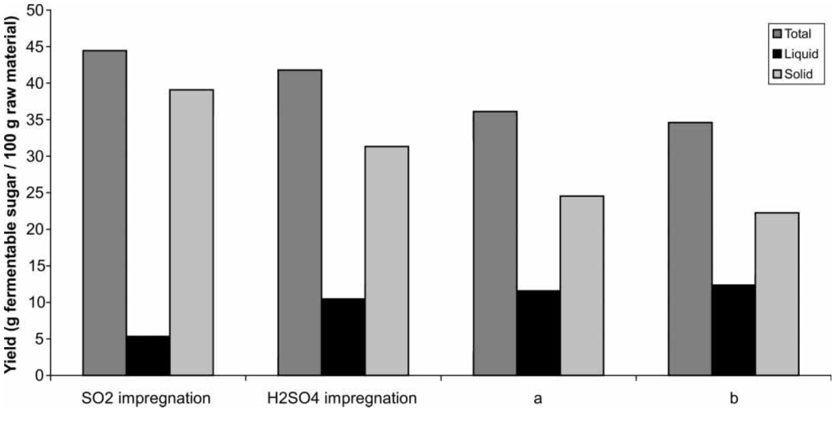

Figure 3. The overall yield of glucose and mannose in the second pretreatment step expressed as g/100 g raw material. (a) Söderström et al.[20] and (b) Söderström et al.[21] steam pretreatment in a 2-L reactor.

to equivalent amounts of ethanol (stoichiometrically) this correspond to an ethanol yield of 100%. No lactic acid formation took place in the 1-L fermentor and yet the yield did not increase very much. One reason for the lactic acid production might be the very low concentrations of furfural and HMF, substances that are known to suppress lactic acid production.[32] In an attempt to avoid lactic acid production, SSF was performed with a water-insoluble solids concentration of 8% DM in a 7-L fermentor (L 1523, Bioengineering, AG, Wald, Switzerland) with a working volume of 4 L. The higher concentration of DM also means a higher concentration of the degradation products as less fresh water is added to the slurry from the pretreatment. However, a small amount of lactic acid was formed after 40 h and the overall ethanol yield obtained was only 68%, which increased to 77% when the produced lactic acid was recalculated to the corresponding amount of ethanol.

Enzymatic Hydrolysis

The sugar yield in enzymatic hydrolysis was measured after 96 h of enzymatic hydrolysis. In the SO_2-impregnated material a sugar yield of 81% was obtained in the 30-L vessel. The sugar yield obtained for the H_2SO_4-impregnated material was 57% in the 30-L vessel. For verification of this poor result the EH was performed in a 1-L fermentor (Belach AB, Stockholm, Sweden) where a yield of 52% was achieved. The yield in EH is much lower than that in SSF of the same material, with the same enzyme activity and DM content. Thus the potential ethanol yield after EH was lower than the actual ethanol yield obtained in SSF. One reason for the lower yield in EH compared with SSF is the end-product inhibition of β-glucosidase by glucose, which may occur in EH preventing more cellulose from being hydrolyzed. One reason for the poor yield from the H_2SO_4-impregnated material could be the presence of non-analyzed inhibitory substances in the slurry, which prevented the efficient action of the enzymes. In SSF these substances may be metabolized by the yeast allowing the enzymes to work properly.[33]

Overall Yield

The formation of glucose and mannose, expressed as g/g theoretical in the raw material, took place in different steps. Mannose was mainly formed during the first pretreatment step, whereas glucose was formed in the second pretreatment step as well as during enzymatic hydrolysis. The H_2SO_4-impregnated material resulted in higher sugar yields in the liquid from the second pretreatment step than the SO_2-impregnated material. However, the significantly higher glucose yield in EH of the SO_2-impregnated material outweighed any advantage of the second pretreatment step. The overall sugar yield from the SO_2-impregnated material was 75% and from the H_2SO_4-impregnated

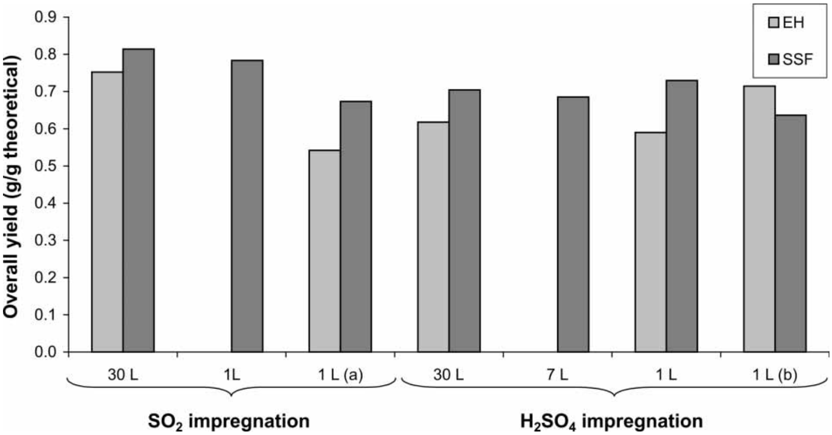

Figure 4. Overall yield of fermentable sugars and ethanol following EH and SSF of SO_2- and H_2SO_4-impregnated material in vessels of different sizes (30 L, 7 L, and 1 L). 5% DM was used in all experiments except in the 7-L vessel (8% DM). (a) Söderström et al.[20] and (b) Söderström et al.[21] steam pretreatment in a 2-L reactor, using 1-L fermentor for SSF (5% DM) and 500-mL vessel for EH (2% DM).

material 62% (Figure 4). The yields previously attained with the same pretreatment conditions are of the same magnitude as the present ones.[19,20] However, comparison is difficult for two main reasons. The previous results were obtained using small steam pretreatment equipment, which resulted in lower yields in the second pretreatment step. Furthermore, in the earlier studies only washed solid material was used in EH with a DM of 2%, which resulted in higher sugar yields in the EH step.

Nguyen et al.[14] obtained an overall sugar yield of 82% using two-step pretreatment of mixed softwood followed by enzymatic hydrolysis. In the enzymatic hydrolysis only washed material was used, with a concentration of 1% (w/v) cellulose and an enzyme activity of 60 FPU/g of cellulose. This result, 82%, is slightly higher than that achieved in the present study. However, in the present study the whole slurry was used with higher cellulose concentrations and much lower enzyme loadings. The cellulose concentration was 2.9% and 2.7% and the enzyme activity in the enzymatic hydrolysis was 26 FPU/g cellulose and 31 FPU/g cellulose for the SO_2-impregnated and the H_2SO_4-impregnated material, respectively.

The overall ethanol yield obtained when applying SSF to the two different impregnation alternatives was 81% and 70% for the SO_2 and H_2SO_4, respectively. When using 8% DM in SSF (H_2SO_4 impregnation) the overall ethanol yield only decreased to 69% (Figure 4). The yields obtained in this study are markedly higher than those achieved in our previous studies.[19–21] When using the same conditions for pretreatment and SSF, but smaller reactors for pretreatment and fermentation, the overall ethanol yield achieved was 67%

and 64% for the SO_2- and H_2SO_4-impregnated material, respectively. The major contribution to the increased yield in both cases was the increased yield in the second pretreatment step, obtained when the reactor size was increased from 2 L to 10 L.

In previous studies using one-step steam pretreatment (10-L reactor) the highest overall ethanol yield achieved with SSF performed with the same enzyme activity and DM content was 68% and the overall glucose yield was 69% (23,24).

Mass Balances

The material losses during the different process steps were also measured. The yield of hexoses in the pretreatment steps has been given earlier. In the first pretreatment step the overall mass yield, including the formation of furfural and HMF was 91% in the SO_2-impregnated material and 97% in the case of H_2SO_4 impregnation. The lignin yield, following SO_2 and H_2SO_4 impregnation, taking into account both the solid and the solubilized lignin, was 92% and 110%, indicating that pseudo-lignin was formed in the latter.[34] In the second pretreatment step the overall mass yield was 97% and the yields of lignin and hexoses, including furfural and HMF, were close to 100% in both cases.

The mass balance calculation for SSF assumes that the yeast is not growing. This gives a somewhat higher value of the yield than if the yeast had been assumed to be growing, although it is a reasonable assumption due to the high initial cell concentration. The ethanol and lactic acid were taken into account as well as solubilized hexoses and remaining glucan. The lignin yield was 101% and 78%, whereas the hexose mass balance was 97% and 113% in the case of SO_2 and H_2SO_4 impregnation, respectively. If a small biomass growth is assumed, the yields will decrease only slightly and thus potential yeast growth is considered of little importance in calculations of the mass yield.

The material impregnated with SO_2 resulted in a lignin yield in the enzymatic hydrolysis step of 93%, and a hexose yield of 100%. The material impregnated with H_2SO_4, gave a lignin yield of 96% and a hexose yield of only 93%.

CONCLUSIONS

This study shows that the steam pretreatment reactor should be larger than 2 L to give acceptable results, as the yields in the pretreatment step improved considerably when the pretreatment reactor volume was increased to 10 L. While using the 10 L steam pretreatment equipment the overall ethanol yield attained was 81% of the theoretical value (357 liters per ton of dry raw material) for

SO_2 impregnation using SSF. H_2SO_4 impregnation resulted in a lower overall ethanol yield, 70% (298 L/ton DM). Lactic acid production and other by-products restricted the ethanol yield, although this is not proposed as the only possible reason.

SSF gave a higher yield than SHF, when both processes were performed at 5% DM with the same enzyme activity. SSF of the H_2SO_4-impregnated material with 8% DM did not result in a significant decrease in the overall ethanol yield (69%) compared with SSF performed on material with 5% DM. A significant reduction in cost of the wood-to-ethanol process could thus be achieved if less water were introduced into the process. Further economic evaluation is needed to determine whether the higher ethanol yield achieved with two-step steam pretreatment outweighs the cost of introducing an extra pretreatment step in the process.

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