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Steam Pretreatment of *Salix* with and without SO_2 Impregnation for Production of Bioethanol

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Abstract

In the wood-to-ethanol process, pretreatment of the material is necessary prior to enzymatic hydrolysis to obtain high overall yields of sugar and ethanol. Steam pretreatment of fast-growing *Salix* either with or without SO_2 impregnation has been investigated by varying different parameters. Overall glucose yields of above 90% and overall xylose yields higher than 80% were obtained both with and without impregnation. However, the most favorable pretreatment conditions for the separate yields of glucose and xylose differed to a lower degree using SO_2-impregnated wood chips, resulting in higher total sugar yield than that obtained with non-impregnated wood chips.

Index Entries: Steam pretreatment; *Salix*; willow; ethanol production; enzymatic hydrolysis.

Introduction

Ethanol can be produced from lignocellulosic materials by enzymatic hydrolysis and fermentation, and has been suggested as an alternative to fossil fuels. For ethanol to be commercially competitive with fossil fuels, reduction in the production cost is necessary. Today, the raw material and the enzyme production are two of the main contributors to the overall cost *(1,2)*. High utilization of the raw material is thus important, and the overall yield, i.e., the number of liters of ethanol that can be produced per kilogram of raw material, has been found to be one of the most important parameters in attaining a lower production cost *(3)*.

In woody materials the lignocellulosic matrix, which consists of cellulose closely associated with hemicellulose and lignin, is resistant to enzymatic attack. Pretreatment of the material prior to enzymatic hydrolysis is therefore necessary to remove the various physical and chemical obstacles that hinder the accessibility of the substrate to cellulolytic

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enzymes. For pretreatment to be successful, other requirements must also be fulfilled, such as avoiding the degradation of carbohydrates as well as avoiding the formation of by-products inhibitory to the subsequent hydrolysis and fermentation steps *(4)*. Furthermore, in order to improve the economy of the process, the pretreatment method must also result in high recoveries of hemicelluloses and lignin for further utilization as either chemical feedstock or solid fuel *(5)*. The hemicellulose fraction could, for instance, also be fermented to ethanol while the lignin could be burnt to produce heat and power.

Steam explosion is claimed to be one the most successful methods of pretreating lignocellulosic materials *(4–8)*. Chipped biomass is treated with high-pressure steam and the pressure is then rapidly reduced. The steam initiates an autohydrolysis reaction, in which organic acids, initially formed from the acetyl groups present in the biomass, act as catalysts in the breakdown of hemicellulose *(9)*. Factors that affect steam explosion pretreatment are *residence time*, which can be varied from a few seconds to several minutes, *temperature*, *chip size*, and *moisture content (9,10)*. Impregnation of the wood chips with sulfuric acid or sulfur dioxide prior to pretreatment has been shown in previous studies to improve enzymatic hydrolysis and decrease the production of inhibitory compounds *(4,9–11)*. Drawbacks or limitations associated with the method are degradation of part of the hemicellulose fraction, incomplete separation of lignin and cellulose, and the production of compounds inhibitory to microorganisms *(4,9)*.

The idea of utilizing short-rotation forest (fast-growing willow) for bioenergy, i.e., for the production of heat and power, was proposed in Sweden in the 1970s. Money was appropriated for a large research project, and this became the starting point for *Salix*-related research, both in Sweden and in other countries. In North America, for instance, fast-growing willow as well as poplar species are utilized in the production of a dedicated woody biomass feedstock *(12)*. Concerning *Salix* and its cultivation, Sweden still has a very high level of competence in a global perspective *(13)*.

Research is being performed to improve the ability and efficiency of converting the biomass into energy via thermochemical and biochemical processes. Through selection, breeding, and plantation management, *Salix* varieties with considerably higher productivities [a 70% increase in yield has been attained over the past decade *(14)*], and frost tolerance in addition to a better resistance to pests have been achieved. This, together with improved cultivation techniques, has helped to improve the economic viability of *Salix (12,14,15)*.

Today, the major use of *Salix* is as a solid biomass fuel, but there are other fields of application. *Salix* is known to accumulate heavy metals *(16)*, and bioremediation of sewage is presently being used at a number of locations in Sweden. This method has actually proved to be cheaper than conventional sewage treatment *(17)*. There are also plantations for purification

of landfill leachate. As a prospective alternative it has been suggested that the carbohydrate fraction (cellulose, hemicellulose) of *Salix* be used for the production of bioethanol, i.e., transportation fuel, and that the solid residue, which consists mainly of lignin, be used as a solid fuel.

The present study was focused on the optimization of the steam pretreatment step for the production of bioethanol from *Salix*. The primary purpose was to investigate which pretreatment conditions gave the highest sugar yields after enzymatic hydrolysis, using a one-step steam pretreatment procedure. Both glucose and xylose, the two most abundant sugars in the raw material, were taken into consideration.

Ethanolic fermentation of pentoses is necessary if such sugars are to be included in ethanol production, and for the process to be more cost-effective. Although no suitable xylose-fermenting microorganism is yet in industrial use *(18)*, the assumption has been made that one will be in the near future.

Pretreatment experiments were performed both with and without SO_2 impregnation prior to pretreatment. The effects of residence time, temperature, and the dry matter content of the wood chips were investigated.

Material and Methods

Material

The raw material used in the pretreatment experiments was wood chips, 2–10 mm in size, derived from 3-yr-old stems of a hybrid called Tora (*Salix schwerinii x Salix viminalis)*, which were chopped and gathered at the plantations of Agrobränsle AB in Svalöv, Sweden. Tora is a variety known for its high yield and resistance to disease and pests *(19)*.

The fresh raw material was stored in plastic bags at 4°C. The contents of sugars and lignin were determined according to the standardized methods of NREL (*National Renewable Energy Laboratory, USA*) *(20–23)*. A finely ground sample is treated with 72% (w/w) H_2SO_4 for 2 h at 30°C, and then with 4% (w/w) H_2SO_4 for 1 h at 120°C. The sugar content is analyzed with high-performance liquid chromatography (HPLC), acid-insoluble lignin is dried at 105°C and weighed, and acid-soluble lignin is analyzed with spectrophotometry at a wavelength of 205 nm. The ash content was determined by placing a dried sample in a crucible, which was slowly heated to 575°C and then maintained at this temperature for at least 3 h or until the sample weight was constant.

Experimental Design

The experimental procedure employed for assessment of the steam pretreatment step is shown schematically in Fig. 1. In experiments with SO_2-impregnated material a small amount of gaseous SO_2 was added to the wood chips in plastic bags at room temperature, at least 20 min prior to pretreatment. The SO_2 uptake was approx 2%, measured by weighing,

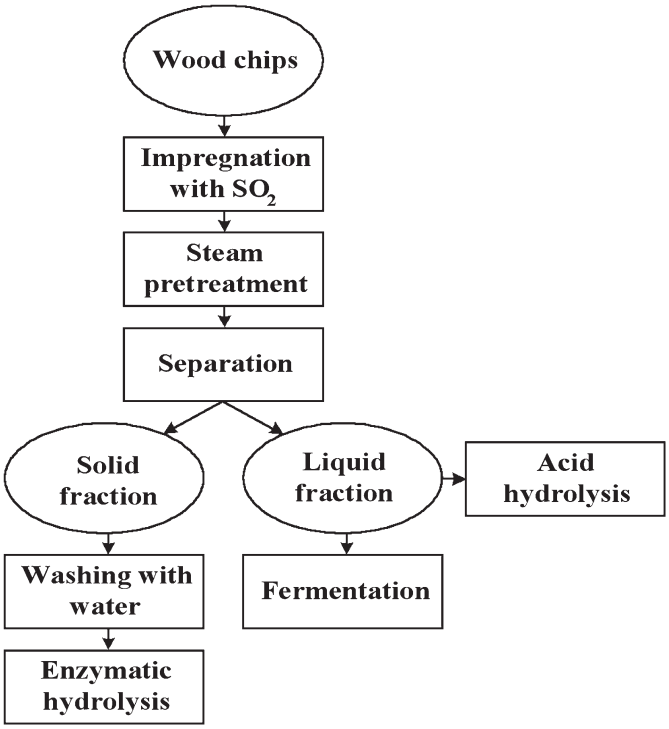

Fig. 1. Schematic procedure for assessment of the steam pretreatment step.

based on the liquid content in the raw material. The pretreated slurry was separated by filtration into a solid residue and a liquid. The liquid fraction was analyzed regarding soluble sugars and degradation products. Acid hydrolysis was performed according to NREL LAP-014 *(24)* to determine the oligomeric sugar content. In this method the sample is treated with 4% (w/w) H_2SO_4 for 1 h at 120°C, and then analyzed with HPLC. Furthermore, fermentation experiments were carried out on each filtrate in a standardized manner to investigate the fermentability of the liquid fraction.

The solid material was thoroughly washed with water to remove all soluble substances, and the composition was determined in accordance with the NREL laboratory procedures *(20–23)*. The material was then enzymatically hydrolyzed using cellulolytic enzymes under standardized conditions.

Pretreatment

Initially, two series of experiments were carried out in which the temperature (180–210°C) and the residence time (4–14 min) in pretreatment either with or without SO_2 impregnation were varied. The dry matter content of the freshly chopped wood chips was 56%. To investigate the effect of the moisture content of the material, a series of pretreatment experiments was performed using air-dried wood chips which were pre-steamed at atmospheric pressure for approx 30 min to obtain dry matter contents of just below 40%.

The investigations mentioned above were all carried out in a 2.4-L reactor, which must be considered small. A total of four pretreatment experiments, two with and two without SO_2 impregnation, was performed in a 10-L reactor *(25)* at conditions that in the small reactor resulted in high yields of glucose and xylose. In these experiments fresh, not air-dried, material was used. When steam pretreatment was carried out in the 2.4-L reactor, the material load was 200 g dry matter (DM). The corresponding amount used in the 10-L reactor was 750 g DM, based on previous studies of softwood that also resulted in higher sugar yields using a larger reactor *(26)*.

Enzymatic Hydrolysis

A commercial cellulase mixture, Celluclast 1.5 L (65 FPU/g and 17 β-glucosidase IU/g) supplemented with the β-glucosidase preparation Novozym 188 (376 β-glucosidase IU/g) from Novozymes A/S (Bagsværd, Denmark) was used in the enzymatic hydrolysis. The cellulase activity was measured according to the method of NREL, using the guidelines of the IUPAC *(27)*, while the β-glucosidase activity was determined by the procedure of Berghem *(28)*.

Enzymatic hydrolysis was performed on washed material at a dry matter content of 2% (w/w) to avoid end product inhibition. The purpose was not to optimize the hydrolysis itself, but to investigate the effect of pretreatment and to determine the potential sugar yield.

All hydrolysis experiments, performed in duplicate, were carried out at 40°C for 96 h in 1-L flasks with mechanical agitation. In each flask 10g DM of pretreated material, 2.32 g Celluclast 1.5 L, 0.52 g Novozym 188, and 0.1 mol/L sodium acetate buffer (pH 4.8) were added to a total weight of 500 g. Samples were withdrawn after 0, 2, 4, 8, 24, 48, 72, and 96 h and analyzed regarding sugar content.

Fermentation

Fermentability tests were performed on the liquids using ordinary compressed baker's yeast, *Saccharomyces cerevisiae* (Jästbolaget AB, Rotebro, Sweden), which ferments hexoses, e.g., glucose and mannose, but not pentoses, e.g., xylose. The pH was adjusted to 5.5 with 20% (w/w) $Ca(OH)_2$. Fermentation was performed in 25-mL glass flasks with a working volume of 20 mL, consisting of 18.5 mL filtrate, 0.5 mL nutrients, and 1 mL inoculum (an aqueous solution of yeast). Yeast was used at a concentration of 5 g dry matter/L, while the final concentration of nutrients was 0.5 g/L $(NH_4)_2HPO_4$, 0.025 g/L $MgSO_4 \cdot 7H_2O$, 0.1 *M* NaH_2PO_4, and 1 g/L yeast extract. The flasks were sealed with rubber stoppers through which hypodermic needles had been inserted for removal of the CO_2 produced, as well as for withdrawal of samples. The concentration of fermentable sugars was adjusted by the addition of glucose to a total concentration of 50 g/L to avoid the influence of variation in sugar concentration between different filtrates. A reference solution was prepared with 50 g/L glucose to serve as a control fermentation. The flasks were

Table 1
Composition of *Salix* Expressed as Percentage of Dry Raw Material

Carbohydrates	
Glucan	41.5 %
Xylan	15.0 %
Galactan	2.1 %
Arabinan	1.8 %
Mannan	3.0 %
Lignin	
Acid-insoluble	23.3 %
Acid-soluble	1.9 %
Ash	1.4 %

incubated at 30°C for 24 h, and the withdrawn samples were analyzed for ethanol, sugars, and sugar degradation products.

Analysis

The liquid fraction from each pretreatment experiment, and all samples from the acid and enzymatic hydrolysis, as well as fermented samples, were analyzed with HPLC using a chromatograph equipped with a refractive index detector. Cellobiose, glucose, xylose, mannose, arabinose, and galactose were analyzed with a Shimadzu LC-10AD chromatograph (Kyoto, Japan) using a Biorad HPX-87P column at 85°C. Water was used as eluent at a flow rate of 0.5 mL/min. Lactic acid, glycerol, acetic acid, ethanol, 5-hydroxymethylfurfural (HMF), and furfural were analyzed with a Shimadzu LC-10AT chromatograph using a Biorad HPX-87H column at 65°C. The eluent in this case was 5 mmol/L H_2SO_4 at a flow rate of 0.5 mL/min.

Results and Discussion

The content of carbohydrates, lignin, and ash in the raw material is presented in Table 1. These figures were used in the sugar yield calculations.

The overall sugar yield was defined according to the equation given below. In the equation, *sugar* stands for either glucose or xylose. The oligomeric sugars present in the liquid fraction are included in the filtrate sugar content, expressed as monomer equivalents:

$$\text{Yield}_{\text{sugar}} = \frac{\left(\text{mass}_{\text{sugar}} \text{ in filtrate}\right) + \left(\text{mass}_{\text{sugar}} \text{ after enzymatic hydrolysis}\right)}{\text{mass}_{\text{sugar}} \text{ in raw material}} \times 100\%$$

Another way that has been used to express the sugar yield is as g per 100 g raw material. This gives a good idea on the potential amount of sugar that can be obtained.

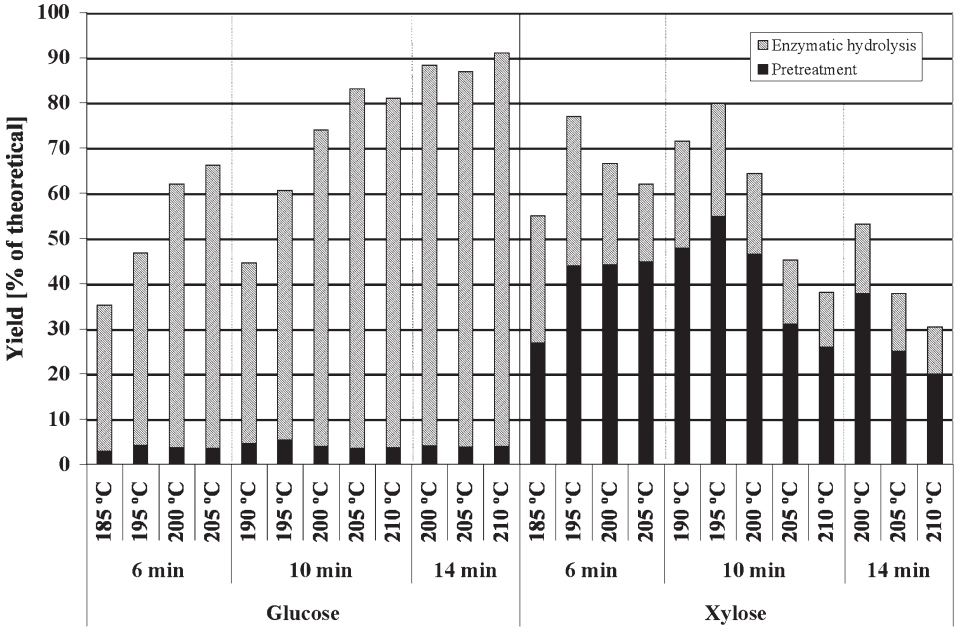

Fig. 2. Overall yields of glucose and xylose, as percentage of the theoretical, obtained after enzymatic hydrolysis of pretreated non-impregnated *Salix* chips with a DM of 56%.

Pretreatment without Impregnation

Figure 2 shows the overall yields of glucose and xylose after pretreatment, as well as after enzymatic hydrolysis, as a percentage of the theoretical (based on the raw material analysis, Table 1), following pretreatment without the addition of an acid catalyst. The experiments were carried out using "fresh" wood chips with a DM of 56 %. It can be seen clearly in the figure that when the wood chips are not impregnated prior to pretreatment, i.e., when pretreatment is performed as autohydrolysis, relatively long residence times are required to obtain high glucose yields. However, at these severe conditions the xylose is degraded to a rather large extent. Milder pretreatment conditions are thus necessary for high xylose yields. The highest glucose yield was 91%, obtained following pretreatment at 210°C for 14 min, while the optimal xylose yield was 80%, following pretreatment at 195°C for 10 min. The pretreatment conditions resulting in the highest total yield of sugar (glucose + xylose), 49.8 g per 100 g dry raw material, were 200°C and 14 min.

Throughout the series, less than 5% of the initial amount of glucan was recovered as soluble sugars, which were mainly in oligomeric form, after pretreatment. The xylan present in the raw material was hydrolyzed to a greater extent. At higher pretreatment severities, less remained unhydrolyzed, but the degree of degradation increased significantly, resulting

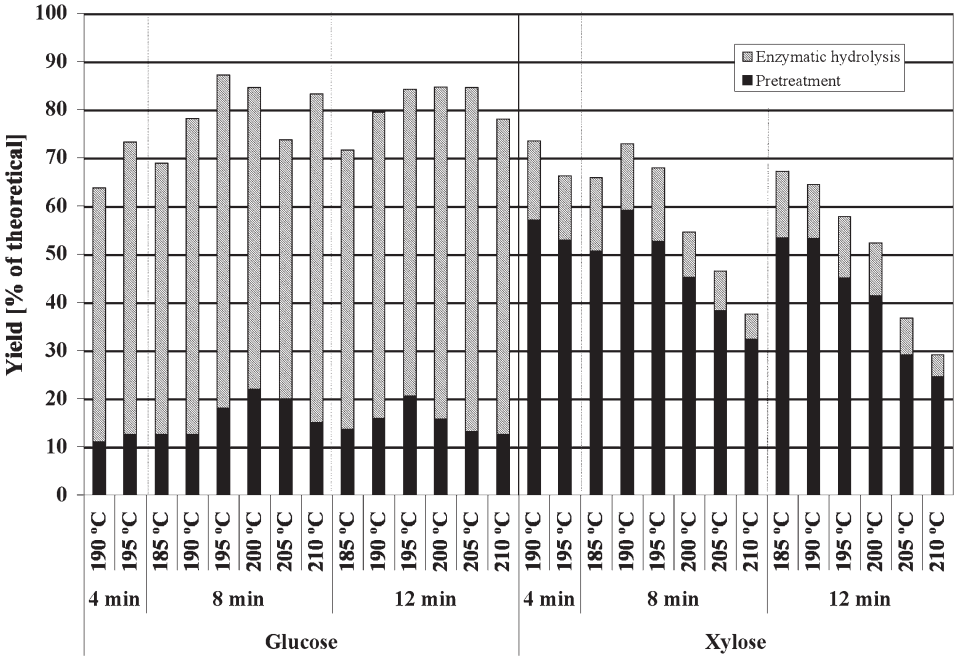

Fig. 3. Overall yields of glucose and xylose, as percentage of the theoretical, obtained after enzymatic hydrolysis of pretreated SO_2-impregnated *Salix* chips with a DM of 56%.

in greater losses and lower overall yields. Fifty-five percent of the initial amount of xylan was recovered in the liquid, at most, and then almost exclusively in the form of xylo-oligomers.

Pretreatment with SO_2 Impregnation

Figure 3 shows the overall yields of glucose and xylose after pretreatment and after enzymatic hydrolysis, when the wood chips had been impregnated with SO_2. The highest yield of glucose was 87%, obtained following pretreatment at 195°C for 8 min, while the highest xylose yield was 73%, obtained at 190°C for 4 min. The maximum glucose yield was slightly lower than pretreatment without impregnation. This was mainly owing to the fact that when pretreatment was performed without an acid catalyst, a larger amount of the starting material remained solid, and was thus not subjected to further degradation, and that almost all the glucose was obtained in the subsequent enzymatic hydrolysis. However, with SO_2-impregnation there is a significantly wider range of pretreatment conditions in which high yields of glucose were obtained.

When an acid catalyst was used, the carbohydrates were hydrolyzed to a greater extent during pretreatment, i.e., more sugar was present in the liquid after pretreatment with a considerably higher monomeric fraction than that obtained following pretreatment without impregnation.

The highest amount of xylan recovered as xylose or soluble xylo-oligomers in the liquid fraction was 59% of the initial xylan content of the raw material. The corresponding value for glucan was just above 20%. On the other hand, when pretreatment was performed without impregnation, a larger amount of xylose was obtained in the succeeding enzymatic hydrolysis. This resulted in more or less equal maximum xylose yields for the two pretreatment procedures, with and without impregnation. In both cases, but especially in the latter, xylose was affected to a higher degree by the pretreatment conditions, i.e., it was more sensitive to changes in temperature.

The pretreatment conditions resulting in the highest total yield of sugar (glucose + xylose), 51.8 g per 100 g dry raw material, were 195°C and 8 min. The most favorable pretreatment conditions regarding the yields of glucose and xylose separately, differed less when pretreatment was performed on SO_2-impregnated wood chips, resulting in higher total sugar yield than that obtained with non-impregnated wood chips.

Effect of the Dry Matter Content in Raw Material

When pretreatment was not preceded by SO_2 impregnation, the major effect of higher moisture content in the untreated wood chips was that more condensate was generated during pretreatment. This led to a lower concentration of the acetyl groups released, and resulted in less severe pretreatment conditions. Owing to the milder conditions, the enzymatic digestibility of the pretreated material decreased significantly, resulting in lower overall yields of glucose in comparison with experiments with less moist wood chips pretreated at identical temperatures and residence times (data not shown). On the other hand, a larger fraction of the initial glucan was recovered as soluble sugar in the liquid using more moist wood chips.

For xylose, a higher liquid content in the pretreated slurry seemed to prevent further degradation, resulting in the highest yield using the moistest wood chips. However, this effect was most obvious at pretreatment conditions that were beneficial for the glucose yield, and thus resulted in rather low xylose yields in experiments with drier wood chips at the same temperatures and residence times. Thus, in pretreatment without impregnation, the effect of a lower dry matter content of the raw material was mainly negative. Pretreatment at a higher temperature could perhaps compensate for this, but this requires further investigation.

Regarding pretreatment on SO_2-impregnated wood chips, the main effect of a higher moisture content in the untreated wood chips, obtained by pre-steaming, was that a larger amount of SO_2 was incorporated into the material, as the amount was based on the liquid content. However, the SO_2 concentration was still around 2% (w/w liquid).

Figure 4 shows the overall yields of glucose and xylose after pretreatment and after enzymatic hydrolysis, as a percentage of the theoretical, for

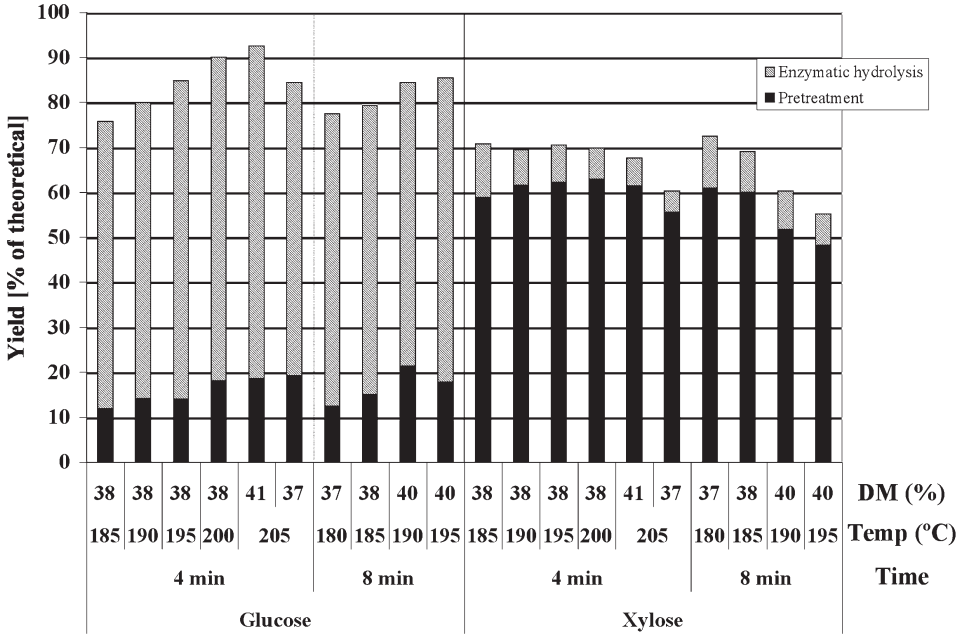

Fig. 4. Overall yields of glucose and xylose as percentage of the theoretical obtained after enzymatic hydrolysis of SO_2-impregnated *Salix* chips with a DM of approx 40%.

a series of experiments performed with SO_2-impregnated wood chips with a DM of approx 40%. Two pretreatment experiments were performed at 205°C and 4 min. In this case the dry matter content of the material after pre-steaming was 37%. This material was then pressed to remove the water to obtain 41% DM. The reason for employing this procedure was to determine the amount of water that could be pressed out of the material, and to study how this affected the pretreatment step. After pressing, the wood chips were easier to handle, as they showed less tendency to stick together, indicating that water had been removed from the particle surfaces. Analysis of the water removed showed negligible amounts of cellobiose and glucose.

The glucose yield increased with increasing pretreatment temperature within the temperature range investigated. The highest yield of glucose was 93% obtained at 205°C and 4 min, using wood chips with a DM of 41%. These pretreatment conditions also resulted in the highest total yield of sugar (glucose + xylose), 54.2 g per 100 g dry raw material. The somewhat lower sugar yields in the experiment at 205°C using wood chips with a DM of 37%, may be explained by the fact that, in this specific experiment, an unusually high amount of liquid was obtained in the pretreated slurry after pretreatment. This was probably the reason for the slightly poorer enzymatic digestibility of the pretreated material.

For xylose the best yield was 73%, obtained following pretreatment at 180°C for 8 min. At higher pretreatment temperatures more of the initial

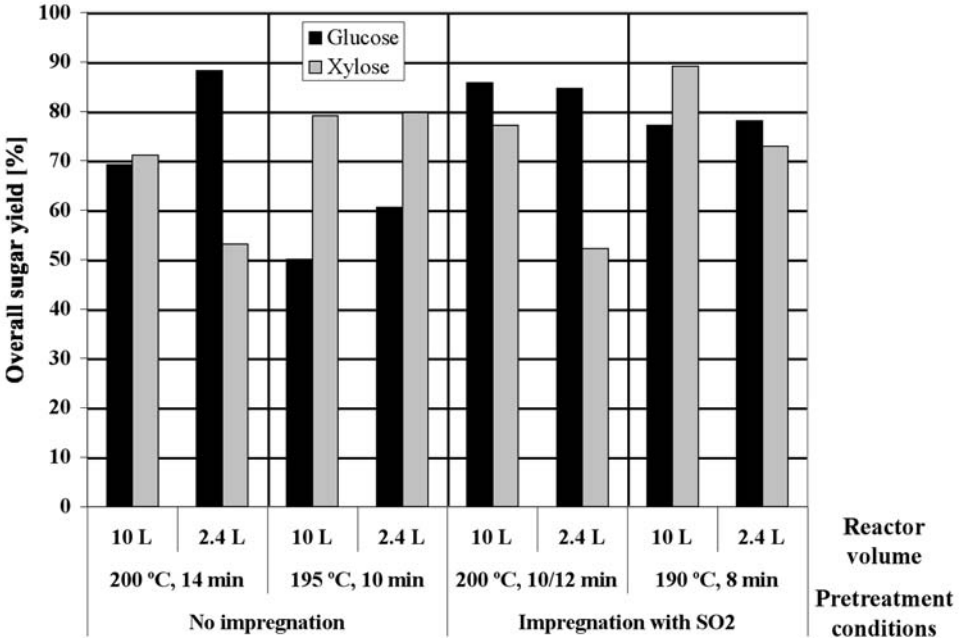

Fig. 5. Yields of glucose and xylose as percentage of the theoretical in a comparison between reactor sizes.

xylan was hydrolyzed and recovered in the liquid fraction, but it was also subjected to a higher degree of degradation. Therefore, less xylan was left in the solid fraction compared with material pretreated at lower temperatures, resulting in less xylose in the succeeding enzymatic hydrolysis. The result of this was that the overall xylose yields were more or less the same throughout the experiments.

The effect of a lower dry matter content was that more condensate was generated during pretreatment. Owing to the lower concentration, this may have prevented the hydrolyzed sugars, especially xylose, from degradation to a greater extent. For glucose, however, this seemed to be of minor importance when using SO_2-impregnated wood chips.

Effect of Reactor Size

A scale-up of the pretreatment step from a 2.4-L reactor to one with a volume of 10 L resulted in a visibly more homogeneously pretreated material, as so-called wall effects, which lead to non-uniform temperature distribution, were reduced. Pretreatment in the small reactor occasionally resulted in a material in which some wood chips appeared to be almost unaffected by the pretreatment process. This occurred especially at lower severities.

Figure 5 shows a comparison of the overall yields of glucose and xylose after enzymatic hydrolysis with the two reactors for four combinations of

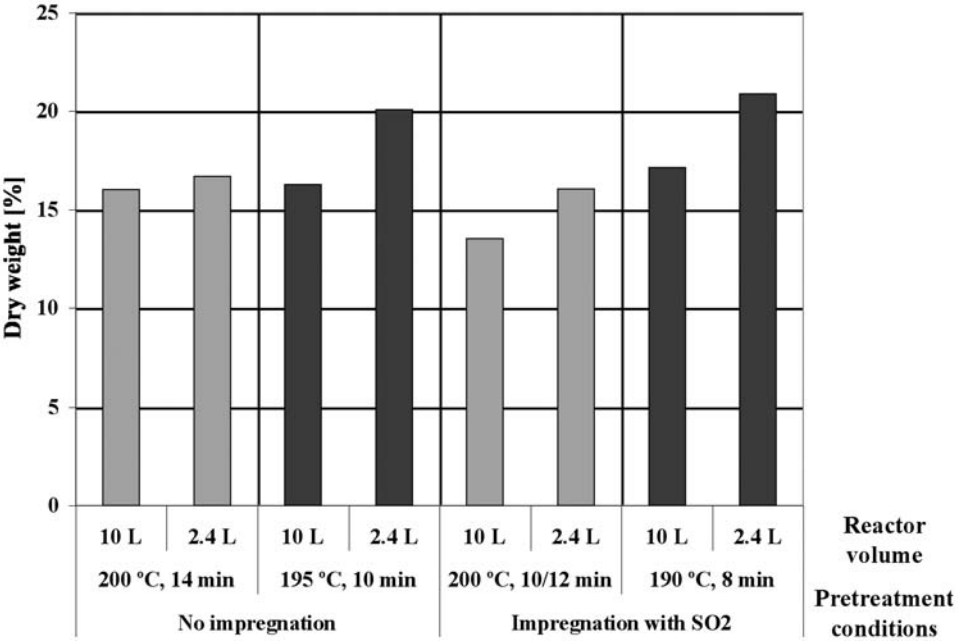

Fig. 6. Dry matter content of material after pretreatment.

pretreatment conditions. Pretreatment with SO_2-impregnated wood chips resulted in very similar glucose yields. For pretreatment without impregnation the outcome was quite the opposite, with significantly lower glucose yields in the large reactor. The dry matter content of the resulting slurry, which is shown in Fig. 6, was lower when using the larger reactor, i.e., the amount of condensate generated during pretreatment relative to the amount of solid material was higher. This could be one reason for the lower glucose yields, compared with those obtained in the smaller reactor, in the experiments performed without impregnation prior to pretreatment. Owing to the lower dry matter content of the pretreated slurry, the concentration of the acetyl groups released was lower. The amount of condensate generated during pretreatment relative to the amount of material is dependent not only on the pretreatment conditions, but also on the quantity of wood chips used. The material loadings were based on previous studies of softwood *(26)*.

The xylose yields, at best 89% of the theoretical, were enhanced in all experiments except one when pretreatment was performed in the 10-L reactor. The increase was significantly greater when a catalyst was used. This is probably due to the more even temperature distribution in the large reactor, as well as the higher liquid content of the pretreated slurry, both of which seem to prevent xylose from further degradation. An overall total yield of 52.8 g glucose + xylose per 100 g dry raw material was achieved following pretreatment for 10 min at 200°C using SO_2-impregnated wood chips.

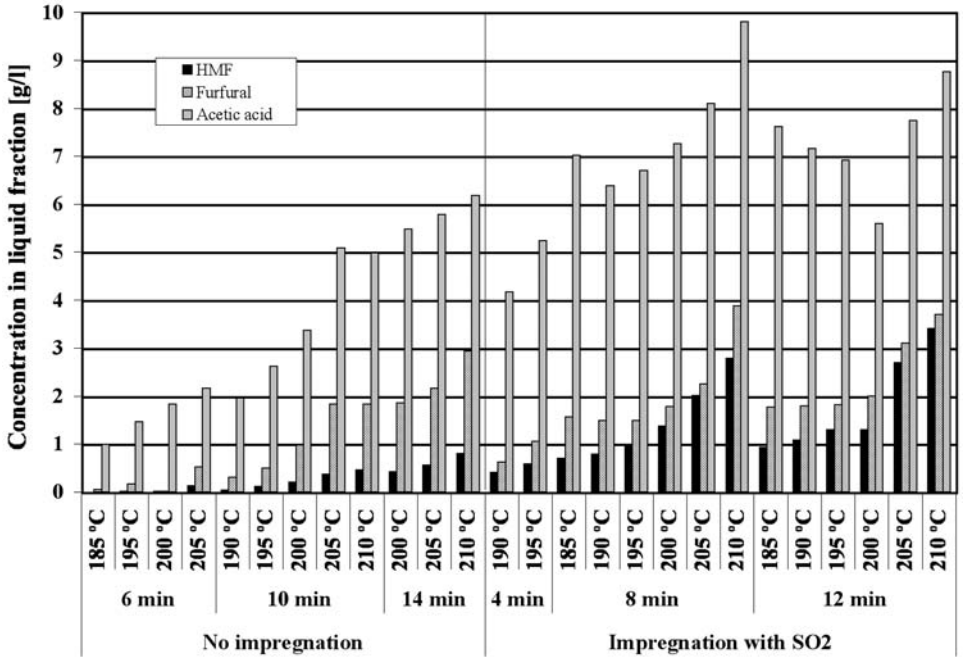

Fig. 7. Concentration of HMF, furfural, and acetic acid in the liquid fraction after pretreatment.

Fermentability Tests

At higher pretreatment severities, a larger proportion of the material is hydrolyzed, resulting in a higher degree of sugar degradation, i.e., the formation of products such as HMF, furfural, levullinic acid and formic acid. These compounds may inhibit the succeeding steps. Also, other aliphatic acids and furan derivatives, as well as phenolic compounds derived from degraded soluble lignin, may cause inhibition in the fermentation step *(29)*.

The filtrates from each experiment were analyzed with regard to HMF and furfural as a measure of the quantity of by-products produced. Figure 7 shows the concentrations of these components, together with the concentration of acetic acid, in the liquid fraction in the two experimental series covering pretreatment of fresh wood chips with and without SO_2 impregnation. More severe pretreatment conditions, i.e., higher temperatures and longer residence times, resulted in higher concentrations of potential inhibitors, and thus these liquids would be expected to have reduced fermentability, which was also the case (see Fig. 8). When pretreatment was performed at temperatures above 200°C, the fermentability of the liquid decreased dramatically, especially for the SO_2-impregnated wood chips, but also for wood chips without impregnation when residence times of 14 min were applied. The ethanol

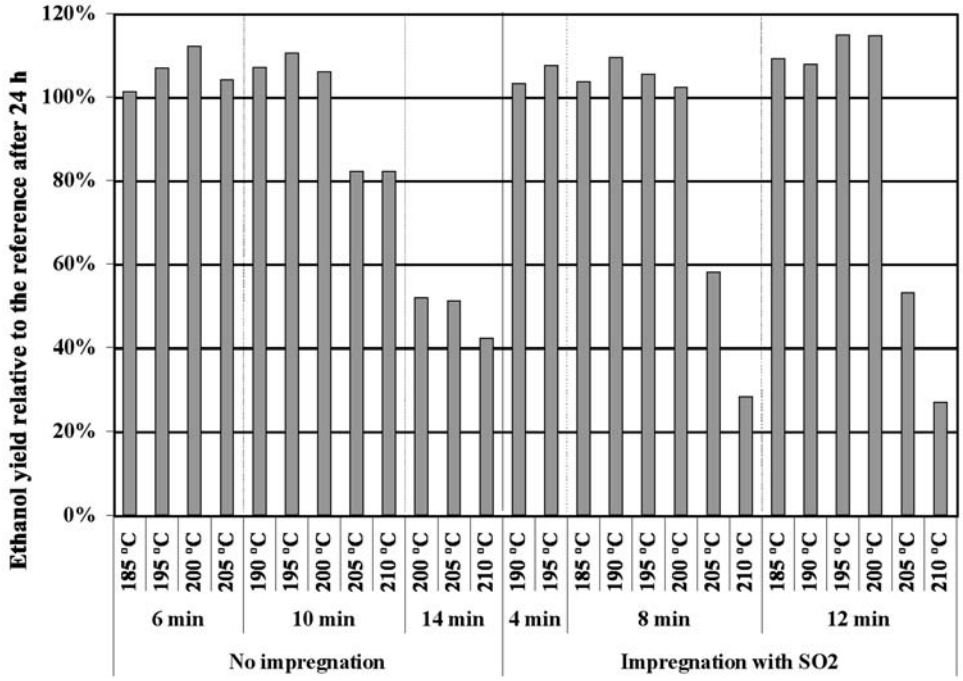

Fig. 8. Ethanol yields of hydrolysates relative to that for the reference after 24 h of fermentation.

produced after 24 h of fermentation was as low as 27% of that produced in the reference solution. This inhibition could probably be alleviated by performing the fermentation as a fed batch, as has been observed in previous studies *(30)*. The fact that the ethanol yield is somewhat higher than the reference solution in many hydrolysates may, to some extent, be explained by the presence of acetic acid, which at low concentrations favors ethanol production *(31)*.

The decrease in fermentability of the liquids does not completely correspond to the increase in the concentrations of HMF and furfural, indicating that other inhibitors, probably derived from soluble lignin, are present. It is also likely that different inhibiting compounds are found in the liquid depending on whether the wood chips were SO_2-impregnated prior to pretreatment or not.

The liquid fractions after pretreatment using SO_2-impregnated wood chips with a higher moisture content were shown to be less toxic to the yeast, i.e., they were in general easily fermented (results not presented). The higher moisture content of the raw material led to larger quantities of liquid, which resulted in lower concentrations of potential inhibitors, as well as a reduction in the formation of inhibitory components. The outcome of the latter was higher sugar recoveries.

Conclusion

The consequence of adding an acid catalyst to wood chips prior to pretreatment was that a pH of around 2 was obtained in the pretreatment hydrolysate, compared with a value of 3.5 obtained with non-impregnated wood chips. The more acidic conditions result in a higher demand for neutralization in the downstream processing and more expensive process equipment. The results of the studies presented here show similar maximum yields for both glucose and xylose following pretreatment with and without SO_2 impregnation. However, the major difference was that the best pretreatment conditions for glucose and xylose, separately, were closer when the wood chips were impregnated with SO_2 prior to pretreatment, showing that this procedure is beneficial for the total sugar yield (glucose + xylose). For pretreatment without impregnation, the longer residence times required to obtain good yields of glucose resulted in a high degree of xylose degradation and thus in a decrease in fermentability. This was not observed for the pretreatment conditions that resulted in the highest sugar yields with SO_2-impregnated wood chips. Furthermore, the results from the larger-scale experiments as well as those performed with a lower dry matter content of the raw material, also indicate that the sugar yields after enzymatic hydrolysis following pretreatment without impregnation are more sensitive to changes in the pretreatment conditions.

To achieve better utilization of the raw material, the losses of pentose sugars during pretreatment must be reduced. In a previous study with *Salix caprea*, which is closely related to the material used in this study, impregnation with dilute H_2SO_4 resulted in higher yields of xylose than with impregnation with SO_2 *(32)*. In an attempt to increase the overall total sugar yield, Söderström et al. have thoroughly investigated a two-step steam-pretreatment procedure with softwood *(33–35)*. The first step was performed under conditions at which mainly the hemicellulose fraction was hydrolyzed. The remaining material was then washed and pretreated at a somewhat higher severity to hydrolyze a fraction of the cellulose, or at least make it more susceptible to enzymatic attack in the subsequent enzymatic hydrolysis step. Similar experiments, although not as comprehensive, have also been carried out with willow *(36)*. Based on the results presented here, it is mainly when pretreatment is performed without the addition of a catalyst that the optimal pretreatment conditions differ between glucose and xylose to such an extent that a two-step procedure could be considered. The question is whether the cost of an additional pretreatment step would be covered by an increase in the xylose yield.

The overall ethanol yield from wood chips for the most favorable pretreatment conditions (SO_2-impregnated wood chips at 205°C for 4 min) regarding the total sugar yield was estimated to be 315 L ethanol per metric ton dry raw material. This was obtained based on the assumptions

that both hexoses and pentoses were fermented and that the yield in the fermentation step was 90% of the theoretical. Other sugars, such as galactose, arabinose and mannose, which would make a minor contribution to the amount of sugar obtained, were not taken into consideration.

Acknowledgment

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References

1. Gregg, D. J., Boussaid, A., and Saddler, J. N. (1998), *Bioresour. Technol*. **63**, 7–12.
2. Wingren, A., Galbe, M., and Zacchi, G. (2003), *Biotech. Prog*. **19**, 1109–1117.
3. von Sivers, M. and Zacchi, G. (1996), *Bioresour. Technol.* **56**, 131–140.
4. Sun, Y. and Cheng, J. (2002), *Bioresour. Technol***. 83**, 1–11.
5. Nguyen, Q. A. and Saddler, J. N. (1991), *Bioresour. Technol.* **35**, 282.
6. Ramos, L. P., Breuil, C., and Saddler, J. N. (1992), *Appl. Biochem. Biotech.* **34-35,** 37–48.
7. Glasser, W. G. and Wright, R. S. (1998), *Biomass. Bioenergy.* **14**, 219–235.
8. Excoffier, G., Toussaint, B., and Vignon, M. R. (1992), *Biomass. Energy Ind. Environ*. 6th conf, pp. 418–424.
9. Duff, S. J. B. and Murray, W. D. (1996), *Bioresour. Technol*, **55**, 1–33.
10. Saddler, J. N., Ramos, L. P., and Breuil, C. (1993), *Biotech. Agric. Ser.* **9**, 73–91.
11. Grethlein, H. E., Allen, D. C., and Converse, A. O. (1984), *Biotechnol. Bioeng***. 26**, 1498–1505.
12. Tharakan, P. J., Volk, T. A., Abrahamson, L. P., and White, E. H. (2003), *Biomass. Bioenergy*, **25**, 571–580.
13. Lundborg, A. (2003), Växande energi—Bioenergin i Sverige—en marknad i utveckling, *Swedish Energy Agency, Eskilstuna, Sweden.*
14. Larsson, S. (2002), *J. Swedish Seed Association*, **111**, 91–97.
15. Bawelin, F. (2002), *J. Swedish Seed Association* **111**, 69–72.
16. Bertholdsson, N. O. (2002), *J. Swedish Seed Association* **111**, 84–90.
17. Hasselgren, K. (2002), *J. Swedish Seed Association* **111**, 73–83.
18. Kuyper, M., et al. (2003), *FEMS Yeast Research* 69–78.
19. *http://www.agrobransle.se*.
20. Ehrman, T. (1994), Standard Method for Determination of Total Solids in Biomass, LAP-001, US Department of Energy, *USA* :http://www.eere.energy.gov/biomass/analytical_procedures.html.
21. Ruiz, R. and Ehrman, T. (1996), Chemical Analysis and Testing Task Laboratory Procedure, LAP-002 US Department of Energy, USA http://www.eere.energy.gov/biomass/analytical_procedures.html.
22. Templeton, D. and Ehrman, T. (1995), Chemical Analysis and Testing Task, Laboratory Analytical Procedure, LAP-003, US Department of Energy, USA: http://www.eere.energy.gov/biomass/analytical_procedures.html.
23. Ehrman, T. (1996), Chemical Analysis and Testing Task Laboratory Analytical Procedure, LAP-004 US Department of Energy, USA: http://www.eere.energy.gov/biomass/analytical_procedures.html.
24. Ruiz, R. and Ehrman, T. (1996), Dilute Acid Hydrolysis Procedure for Determination of Total Sugars in the Liquid Fraction of Process Samples; LAP-014 *US* Department of Energy, USA: http://www.eere.energy.gov/biomass/analytical_procedures.html.
25. Palmqvist, E., et al. (1996), *Bioresour. Technol***. 58**, 171–179.

26. Söderström, J., Galbe, M., and Zacchi, G. *J Wood Chem Technol*, Manuscript accepted, will be published in 2005.
27. Adney, B. and Baker, J. Measurement of Cellulase Activities; LAP-006 (1996), US Department of Energy, *USA*: http://www.eere.energy.gov/biomass/analytical_procedures.html.
28. Berghem, L. E. R. and Pettersson, L. G. (1974), *Eur. J. Biochem.* **46**, 295–305.
29. Tengborg, C., Galbe, M., and Zacchi, G. (2001), *Enzyme Microb. Technol*. **28**, 835–544.
30. Taherzadeh, M. J., Niklasson, C., and Liden, G. (1999), *Bioresour. Technol*. **69**, 59–66.
31. Larsson, S., et al. (1999) *Enzyme Microb. Technol*. **24**, 151–159.
32. Eklund, R., Galbe, M., and Zacchi, G. (1995) *Bioresour. Technol*. **52**, 225–229.
33. Söderström, J., Pilcher, L., Galbe, M., and Zacchi, G. (2003), *Appl. Biochem. Biotech.* **105–108**, 127–140.
34. Söderström, J., Pilcher, L., Galbe, M., and Zacchi, G. (2002), *Appl. Biochem. Biotech.* **98–100**, 5–21.
35. Söderström, J., Pilcher, L., Galbe, M., and Zacchi, G. (2003), *Biomass. Bioenergy* **24**, 475–486.
36. Eklund, R., Galbe, M., and Zacchi, G. (1988), *J. Wood Chem. Technol.* **8**, 379–395.