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Steam Pretreatment of Acid-Sprayed and Acid-Soaked Barley Straw for Production of Ethanol

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Abstract

Barley is an abundant crop in Europe, which makes its straw residues an interesting cellulose source for ethanol production. Steam pretreatment of the straw followed by enzymatic hydrolysis converts the cellulose to fermentable sugars. Prior to pretreatment the material is impregnated with a catalyst, for example, H_2SO_4, to enhance enzymatic digestibility of the pretreated straw. Different impregnation techniques can be applied. In this study, soaking and spraying were investigated and compared at the same pretreatment condition in terms of overall yield of glucose and xylose. The overall yield includes the soluble sugars in the liquid from pretreatment, including soluble oligomers, and monomer sugars obtained in the enzymatic hydrolysis. The yields obtained differed for the impregnation techniques. Acid-soaked barley straw gave the highest overall yield of glucose, regardless of impregnation time (10 or 30 min) or acid concentration (0.2 or 1.0 wt%). For xylose, soaking gave the highest overall yield at 0.2 wt% H_2SO_4. An increase in acid concentration resulted in a decrease in xylose yield for both acid-soaked and acid-sprayed barley straw. Optimization of the pretreatment conditions for acid-sprayed barley straw was performed to obtain yields using spraying that were as high as those with soaking. For acid-sprayed barley straw the optimum pretreatment condition for glucose, 1.0 wt% H_2SO_4 and 220°C for 5 min, gave an overall glucose yield of 92% of theoretical based on the composition of the raw material. Pretreatment with 0.2 wt% H_2SO_4 at 190°C for 5 min resulted in the highest overall xylose yield, 67% of theoretical based on the composition of the raw material.

Index Entries: Barley straw; pretreatment; enzymatic hydrolysis; H_2SO_4; ethanol.

Introduction

Interest in alternative, renewable fuels is increasing world-wide owing to alarming reports on environmental issues as well as the limited oil resources. Within the EU, the goal is to replace 5.75%, calculated on the basis of energy content, of all petrol and diesel for transport purposes with

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biofuels by the year 2010 *(1)*. Transport fuels alone account for 32% of total EU energy consumption *(2)*. Replacing fossil-based transport fuels with biofuels is therefore an essential step in decreasing fossil energy consumption and the emission of greenhouse gases. One alternative fuel that has been found to be well suited to this purpose is ethanol produced from biomass. It can be produced from various lignocellulosic materials, is easy to introduce in the present infrastructure, and results in low net contribution of carbon dioxide to the atmosphere *(3)*. Several varieties of biomass, such as agricultural residues and wood residues *(3–5)* have been evaluated for ethanol production. The choice of biomass in a specific region depends on its availability. In Europe, barley is an abundant crop, which makes barley straw a potential lignocellulosic source for ethanol production *(6,7)*.

The main constituents of straw are cellulose, hemicellulose, and lignin. Cellulose is a linear, polymeric chain of glucose units *(8,9)*. Hemicellulose consists mainly of a polymeric chain of xylose that acts as a backbone with glucuronic acid, arabinose, and acetyl side groups *(10)*. By pretreating the straw at high temperature and pressure and then subjecting it to enzymatic hydrolysis, the polymeric chains are hydrolyzed into monomeric sugar units *(3,11)*.

Addition of sulphuric acid prior to the pretreatment step has been shown to increase the enzymatic digestibility of biomass *(12,13)*. However, the technique of adding the acid to the biomass can vary. A technique common at the laboratory scale is to immerse the material in a large volume of dilute acid and press the wet straw to a desired dry-matter (DM) content. This technique is referred to as soaking. A previous study on pretreatment of acid-soaked barley straw showed that the highest overall yield of glucose, 38.3 g/100 g raw material, was obtained under the condition of 170°C with 1 wt% H_2SO_4 for 5 min. However, use of soaking in an industrial plant is not realistic as it requires large volumes of impregnation liquid and consumes more chemicals. To avoid this, spraying the acid onto the raw material is considered a more feasible alternative. In this study the impregnation techniques of soaking and spraying have been evaluated comparatively to see if the overall yield of fermentable sugars differs.

For ethanol to become more attractive as an alternative fuel, its production cost must be competitive with that of gasoline. The most important factor for the economic outcome of the bio-ethanol process is the overall ethanol yield *(14,15)*. 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 this study pretreatment of acid-sprayed barley straw was optimized in terms of overall yields of glucose and xylose in order to attain the same as for pretreatment of acid-soaked barley straw.

Another factor affecting the production cost is the substrate loading in enzymatic hydrolysis *(11,15,16)*. Increased substrate loading results in decreased flow rate of streams for downstream processing, and thus,

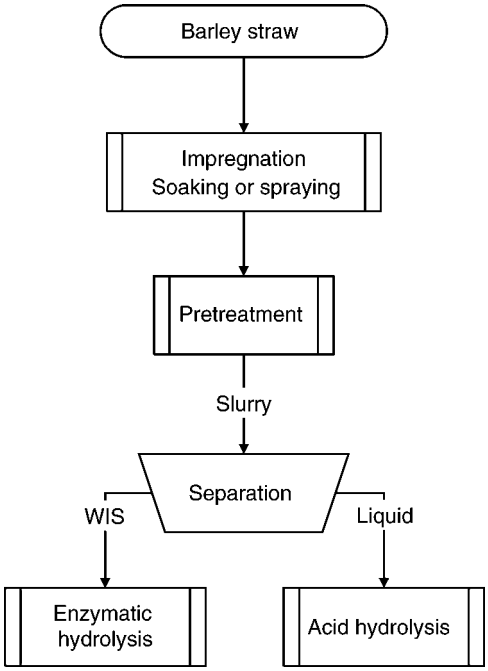

Fig. 1. Experimental setup for assessment of the pretreatment.

reduced energy demand and capital cost in the distillation and the evaporation steps. To achieve this, the amount of DM in the pretreatment has to be increased. The effect of increased substrate loading in the pretreatment step was therefore also investigated.

Methods

The experimental procedure is outlined in Fig. 1. The barley straw was impregnated, either by soaking or spraying, before steam pretreatment. Each impregnation was performed in duplicate. The water-insoluble solids (WIS) in the slurry from pretreatment were separated from the liquid by filtration. The liquid was analyzed for its content of monomeric sugars and acid hydrolysis was performed to determine the content of soluble oligomeric sugars. The filter cake was washed with deionized water to remove all water-soluble solids (WS), from the WIS and then filtrated. An enzymatic hydrolysis was performed on the WIS to assay the pretreatment.

Raw Material

Three batches of barley straw were kindly donated by Abengoa Bioenergy, Spain, in 2004. They were delivered as pieces of approx 50–100 mm and had a DM content of 92%. The straw was cut into smaller sections in a hammer mill, sieved to pieces of 10–30 mm, and stored at room temperature.

Impregnation

Prior to pretreatment the raw material was impregnated with H_2SO_4 solution. Two impregnation techniques were used: either soaking or spraying. Each impregnation was performed in duplicate at room temperature. When evaluating the two different impregnation techniques, the impregnation times were either 10 or 30 min and the acid concentrations in the liquid after impregnation were 0.2 or 1.0 wt%. After comparing the impregnation techniques the pretreatment of acid-sprayed barley straw was optimized and the impregnation time was then 60 min and the acid concentrations in the liquid were 0.2, 0.5, 1.0, or 2.0 wt%.

Soaking

The barley straw was immersed in a liquid containing H_2SO_4 (20 g liquid/g dry straw) and stored in a sealed bucket. After the impregnation time was reached, the wet straw was pressed in a 3-L cylindrical container with a sieve plate in the bottom, which let the liquid pass through but retained the straw. The pressing cylinder was attached to a long screw stem, and was screwed down with manual power onto the wet straw. The straw was pressed to a DM content of 40 wt% and then directly steam pretreated.

Spraying

Sulphuric acid was sprayed over the barley straw through a nozzle creating a mist. The straw was agitated in a cement mixer lined with stainless steel whereas being sprayed to ensure an even layer of acid. When the acid addition techniques were compared, the DM content of the straw after impregnation was 40 wt%. In the optimization of the pretreatment step of the acid-sprayed barley straw the DM content of the straw after impregnation was 60 wt%. Spraying took approx 5 min to complete. The acid-sprayed straw was stored in a sealed bucket to prevent evaporation until reaching the desired impregnation time and was then directly steam pretreated.

Pretreatment

Barley straw was pretreated in a steam pretreatment unit, comprising a 10-L reactor, which has been described elsewhere *(17)*. The temperature was maintained using saturated steam. After the desired pretreatment time of 5 min was reached, the pressure was released and the material collected in a flash tank. The WIS in the slurry from the pretreatment were separated from the liquid by filtration.

The substrate loading into the pretreatment unit was 400 g of DM when the impregnation techniques were compared. The pretreatment temperature was set to 200°C as this temperature gave a high yield of glucose when soaking was optimized in a previous study. For the optimization of the pretreatment of acid-sprayed barley straw the substrate loading was 500 g of DM. The pretreatment conditions are presented in Table 1.

Table 1
Conditions in the Pretreatment Step for the Optimization of Pretreatment of Acid-Sprayed Barley Straw

Time (min)	5													
H_2SO_4 (wt%)	0.2				0.5				1.0				2.0	
Temperature (°C)	190	200	210	220	190	200	210	220	190	200	210	220	190	200

Acid Hydrolysis for Oligosaccharide Determination

The liquid part of the slurry of pretreated material was analyzed for its content of oligosaccharides using the National Renewable Energy Laboratory (NREL) dilute acid hydrolysis procedure for determination of total sugars in the liquid fraction of process samples LAP-014 *(18)*. The oligosaccharide concentration was determined as the difference in monomer sugar concentration before and after acid hydrolysis of the oligosaccharides to monomeric sugars.

Enzymatic Hydrolysis

The effects of the impregnation and the pretreatment conditions on the digestibility were assessed by enzymatic hydrolysis of washed WIS from the pretreatment. The WIS were washed with deionized water to remove all WS from the WIS and then filtrated. All filtrations were performed through a Munktell filter paper, grade 5. Enzymatic hydrolysis was performed in 500-g batches with 2 wt% WIS in 0.1 *M* sodium acetate buffer at a temperature of 40°C for 96 h. The following enzymes were added: 2.52-g *Celluclast 1.5L* (65 FPU/g and 17 β-glucosidase IU/g) and 0.52-g *Novozyme 188* (376 β-glucosidase IU/g), both kindly donated by Novozymes A/S (Bagsværd, Denmark). Samples were withdrawn after 0, 2, 4, 6, 8, 24, 48, 72, and 96 h to monitor hydrolysis. Duplicates were run for each sample.

Titration

The buffering capacity of barley straw was investigated by titration; 50 g of unwashed barley straw in pieces of 10–30 mm was immersed in 1000-g deionized water at a temperature of 80°C for 30 min and then filtered to remove the straw. The resulting barley water was titrated with 0.01 *M* H_2SO_4 (0.1 wt% H_2SO_4) with a GP Titrino 736 from Metrohm Ion analysis (Switzerland). Deionized water was used as reference.

Increasing Substrate Loadings

The effect of increased substrate loading on the concentration of WIS in the slurry from the pretreatment step was investigated. In the study on soaking vs spraying, 1000 g of impregnated straw with a DM content of 40 wt%

was pretreated in a 10-L reactor at 200°C for 5 min with 0.2 wt% H_2SO_4. The acid concentration corresponds to 0.30-g H_2SO_4/100-g dry straw. This pretreatment condition was compared with one performed in the optimization of the pretreatment step, which resulted in approximately the same acid : dry straw ratio but with a different substrate loading. The loading of acid-sprayed barley straw was then 833 g of impregnated straw with a DM content of 60 wt% and pretreated in the 10-L reactor at 200°C for 5 min with 0.5 wt% H_2SO_4. The pretreatment condition corresponded to an acid : dry straw ratio of 0.33-g H_2SO_4/100-g dry straw.

Analysis

DM contents were determined by drying samples in an oven at 105°C until constant weight was obtained. The composition of barley straw in batches 1 and 2, as well as the WIS after pretreatment, was determined according to LAP-002, LAP-003, LAP-004, and LAP-005 from NREL *(19–22)*. The composition of barley straw in batch 3 was analyzed after removing all starch present by hydrolyzing to monomeric sugar using *Termamyl 120L* and *AMG 300L*, kindly donated by Novozymes A/S (Bagsværd, Denmark). The straw to water ratio was 1:10, and the starch-removal procedure was divided into two steps. In the first, the starch was liquefied by thermostable α-amylases (24-mL termamyl 120 L/kg dry straw) for 4 h at 80°C and pH 6.0 to catalyze the hydrolysis of α-1,4 linkages. In the second step, saccharification of the liquefied starch was performed with amyloglucosidase (72 mL AMG 300 L/kg straw) at 55°C and pH 5.0 for 48 h *(23)*. The composition of batch 3 and the starch-free straw, as well as the WIS after pretreatment of batch 3, was determined according to the NREL procedure for determination of structural carbohydrates and lignin in biomass *(24)*.

The liquids from the determination of carbohydrates in biomass, the filtrate from the pretreated material, and the liquids after acid hydrolysis for oligosaccharide determination were analyzed for their content of monomeric sugars using HPLC (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (Shimadzu). The column used was an Aminex HPX-87P (Bio-Rad, Hercules, CA) at 85°C with an eluent flow rate of 0.5 mL/min for separation of glucose, xylose, galactose, arabinose, and mannose. The resulting liquids from the filtration of the pretreated materials were also analyzed for their content of byproducts, HMF, and furfural, using an Aminex HPX-87H column (Bio-Rad, Hercules, CA) at 65°C, with 5 m*M* H_2SO_4 as eluent, at a flow rate of 0.5 mL/min. All samples were filtered through a 0.2-μm filter before analysis to remove particles.

Results and Discussion

All yields are expressed as g/100 g raw material unless otherwise stated. The overall yield includes the soluble sugars in the liquid from

Table 2
Composition of Barley Straw

	Batch 1	Batch 2	Batch 3	
Percentage	B	B	B	A
Glucan	37.1	38.2	39.6	40.5
Starch	n.d.[a]	n.d.[a]	n.d.[a]	2.4
Xylan	21.4	21.7	16.4	24.2
Galactan	BDL[b]	BDL[b]	1.1	0.4
Arabinan	3.1	2.6	3.9	3.0
Lignin	19.5	19.6	23.9	19.8
Ash	2.7	2.6	7.2	3.8
Others	16.2	15.3	7.9	5.9

[a]Not determined.
[b]Below detectable level.
"A" and "B" are determined with and without starch removal, respectively.

pretreatment, including soluble oligomers, and monomer sugars obtained in the enzymatic hydrolysis. The recovery after pretreatment is defined as the sum of monomeric, oligomeric, and polymeric glucose or xylose in the liquid and WIS as the percentage of the theoretical value for either sugar, based on the raw material.

Soaking Vs Spraying

The comparison of the impregnation techniques was performed for two different concentrations of sulphuric acid: 0.2 and 1 wt%. Raw materials were from batches 1 and 2, respectively, *see* Table 2. The starch content was not measured or removed before the analysis of these batches. In batch 3 the composition was determined with and without starch removal, and the result differed between the analyses, *see* Table 2. The starch did not only have an effect on the glucose content but also on measurement of xylose, probably owing to interference from starch in the raw material analysis. Batches 1 and 2 resulted in overall sugar yields above what is theoretically possible. This is most probably owing to the presence of starch in these batches as well but the starch content was not analyzed in these raw materials as all material was consumed before the problem was realized. However, it is still possible to compare the impregnation techniques when the yield is expressed in g/100 g raw material, as this is not affected by the determination of the composition of the raw material.

The slurry after pretreatment contains mainly WIS and oligomeric and monomeric sugars from hydrolyzed hemicellulose and cellulose. Figure 2 shows the yield, including oligomers, of glucose and xylose in the liquid from the slurry of the pretreated material after pretreatment. There is no noticeable difference in glucose yield between the impregnation techniques at an acid concentration 0.2 wt%. At the higher acid concentration,

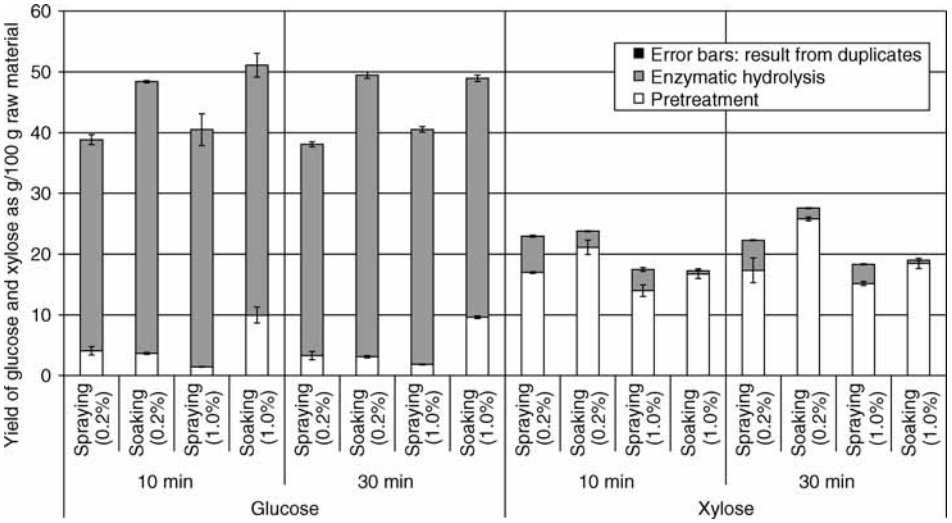

Fig. 2. Yield of glucose and xylose in pretreatment, enzymatic hydrolysis, and overall for various impregnation and pretreatment conditions. The maximum yield of glucose is 40.8 and 42 g/100 g straw for 0.2% and 1.0% H_2SO_4, respectively. The maximum yield of xylose is 37.1 and 38.2 g/100 g straw for 0.2% and 1.0% H_2SO_4, respectively.

1.0 wt%, there was a considerable difference in glucose yield between the impregnation techniques in which soaking resulted in the highest yield, 9.6 and 10.0 g/100 g raw material at 10 and 30 min impregnation time, respectively. Spraying resulted in glucose yields below 1.4 and 1.9 g/100 g raw material, respectively. The xylose yield after pretreatment was higher for soaking at both acid concentrations. Soaking using 0.2 wt% H_2SO_4 for 30 min resulted in the highest xylose yield after pretreatment, 25.8 g/100 g raw material. The highest xylose yield after the pretreatment for acid-sprayed barley straw, 17.3 g/100 g raw material, was also obtained using 0.2 wt% H_2SO_4.

There was a decrease in glucose yield over the pretreatment step for acid-sprayed barley straw when the acid concentration was increased from 0.2 to 1.0 wt%. If the glucose in the liquid were solely a product of hydrolyzed cellulose, one would expect an increase in glucose yield when the severity was increased. However, the drop in glucose amount supports the assumption that the material contained starch. The decrease of glucose can then be explained if starch was released at 0.2 wt% H_2SO_4 and further degraded to HMF at 1.0 wt% H_2SO_4, consistent with the observed increase in HMF production shown in Fig. 3. The production of degradation products, HMF and furfural, increased when the acid concentration increased owing to higher severity in the pretreatment step *(17,25–27)*. The production of degradation products was also higher for the acid-soaked material than the acid-sprayed material. This shows that spraying resulted in less severe conditions in pretreatment.

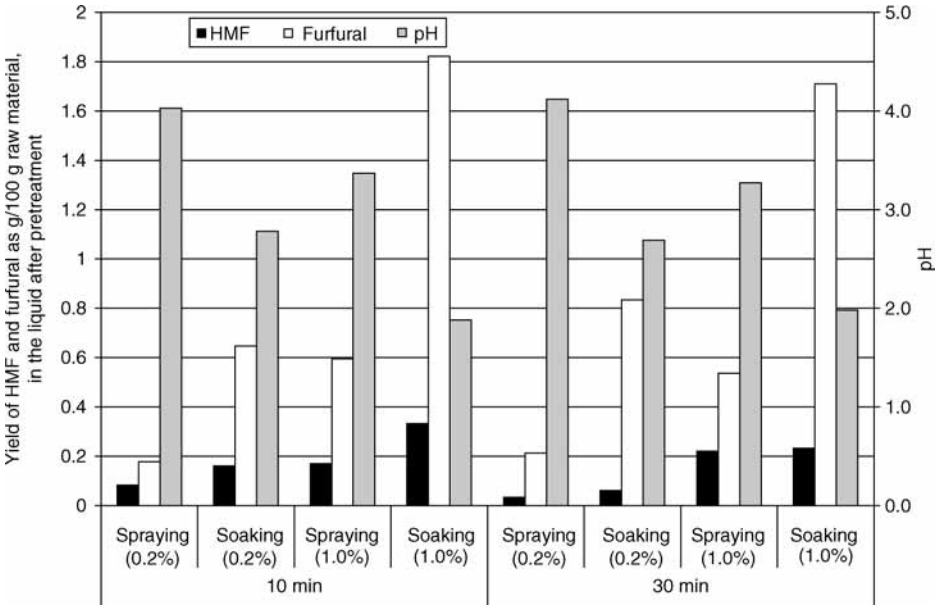

Fig. 3. Yield of HMF and furfural as g/100 g raw material, and pH, in the liquid after pretreatment.

Even though soaking and spraying were performed at the same acid concentrations, soaking produced a liquid after pretreatment with a lower pH than spraying, *see* Fig. 3. At an acid concentration of 0.2 wt%, soaking gave a pH of approx 2.7, whereas spraying gave a pH of 4.0. At an acid concentration of 1.0 wt%, the pH was 1.9 and 3.3 for soaking and spraying, respectively, i.e., 1.0 wt% H_2SO_4-sprayed barley straw had a higher pH than 0.2 wt% H_2SO_4-soaked barley straw. This too shows that soaking resulted in more severe conditions for barley straw than spraying.

The difference in pH is probably owing to the buffering capacity of barley straw, which was overcome by the large volume of impregnation liquid when soaking was applied, thus increasing the severity of the pretreatment. Figure 4 shows the titration curve of the barley water and of deionized water. A decrease in pH from 6.0 to 3.0 in pure water resulted in consumption of approx 3 mL, 0.01 *M* H_2SO_4, whereas the barley water consumed approx 35 mL, 0.01 *M* H_2SO_4. This shows that soaking of barley straw in water extracts compounds that buffer, for example, ash components *(28)*. As impregnation with soaking is performed with an excess of dilute acid, the buffering is overcome, whereas this is not the case for spraying.

Enzymatic hydrolysis was performed to assess the effect of the pretreatment on digestibility, with Fig. 2 showing the yields of glucose and xylose for enzymatic hydrolysis. Soaking gave a higher yield of glucose than spraying for both 0.2 and 1.0 wt% H_2SO_4 in the enzymatic hydrolysis, but the difference in yield between the impregnation techniques decreased at 1.0 wt%. This was owing not only to an increase in yield for the sprayed

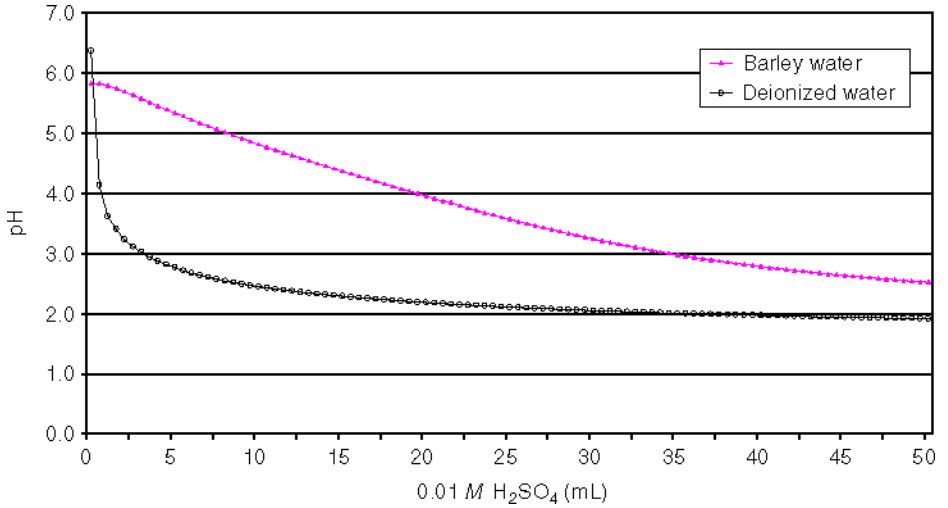

Fig. 4. Titration curves with 0.01 *M* H_2SO_4 (0.1 wt%) for water from a barley-straw and water slurry and deionized water.

material but also to a decrease in yield for the soaked material caused by an increase in released glucose in the pretreatment at 1.0 wt% H_2SO_4.

The xylose yield for enzymatic hydrolysis was lower for the soaked material, which is owing to the fact that almost all the xylan was already hydrolyzed in the pretreatment step. The impregnation time had a negligible effect on both the glucose and xylose yield in the enzymatic hydrolysis.

Soaking resulted in a higher overall sugar yield than spraying when compared at the same acid concentration and pretreatment condition, *see* Fig. 2. Soaking in 1.0 wt% sulphuric acid for 10 min gave the highest overall yield of glucose, 51.1 g/100 g raw material (122% of theoretical). Increased impregnation time did not have any effect on the overall yield, i.e., it was not possible to make up for the lower glucose yield obtained with spraying by increasing the impregnation time from 10 to 30 min. The increase in acid concentration from 0.2 to 1.0 wt% H_2SO_4 using spraying resulted in a lower glucose yield than with soaking at 0.2 wt%. The overall glucose yield when soaking at 0.2 wt% was 48.9 g/100 g raw material, whereas spraying at 1.0 wt% only reached an overall glucose yield of 40.5 g/100 g raw material in spite of the fact that the acid concentration was five times higher.

The highest overall yield of xylose, 27.6 g/100 g raw material (115% of theoretical), was obtained for soaking in 0.2 wt% H_2SO_4 for 30 min. The higher acid concentration, 1.0 wt%, had a negative effect on the overall yield of xylose for both impregnation techniques. For acid-soaked barley straw, the xylose yield in pretreatment was higher than for the acid-sprayed barley straw. In the enzymatic hydrolysis step it was the opposite; i.e., acid-sprayed barley straw had a higher xylose yield in the enzymatic hydrolysis step than acid-soaked barley straw. The overall xylose yield therefore did not markedly differ between the impregnation techniques.

Table 3
Recovery of Glucose and Xylose in the WIS and in the Liquid After the Pretreatment Step as % of Theoretical Value Based on the Glucan and Xylan Content in the Raw Material

			Glucose (%)			Xylose (%)		
H_2SO_4 (wt%)	°C	WIS (%)[a]	Total	WIS	Liquid	Total	WIS	Liquid
0.2	190	80	102	97	5	86	50	36
	200	64	96	92	4	63	26	38
	210	71	86	84	2	81	39	42
	220	72	92	89	3	57	34	22
0.5	190	70	91	88	3	82	45	37
	200	62	94	90	4	72	24	48
	210	64	101	97	4	48	11	37
	220	58	91	87	4	22	5	17
1	190	68	98	95	3	76	40	36
	200	61	99	94	5	71	23	48
	210	58	95	91	4	41	9	32
	220	62	97	93	4	20	5	15
2	190	63	99	94	5	71	20	51
	200	58	96	90	6	57	9	48

[a]Recovery of WIS after pretreatment.

Optimization of Steam Pretreatment of Acid-Sprayed Barley Straw

In this study with unwashed barley straw, the yield as well as the response to increased acid concentration differed between the impregnation techniques. As spraying is a more feasible impregnation alternative than soaking, in terms of use in an industrial plant, it was of interest to study the possibility of attaining as high a yield with spraying as with soaking. Optimization was therefore performed on the pretreatment step using acid-sprayed barley straw to find the maximum yield of glucose and xylose for acid-sprayed barley straw.

Batch 3, *see* Table 2, was used as raw material when optimizing the pretreatment step for acid-soaked barley straw. The total glucose content was 47.1 g/100 g raw material, of which 2.6 g/100 g raw material was from starch. The xylose content was 27.1 g/100 g raw material.

Table 1 shows the pretreatment conditions, whereas Table 3 shows the recovery of glucose and xylose after pretreatment. When the temperature was increased from 190°C to 220°C, xylose recovery decreased from 86% to 57% for 0.2 wt% H_2SO_4 and from 76% to 20% for 1.0 wt% H_2SO_4. This is owing to degradation of the xylose during the pretreatment, which can be seen as an increase of furfural in the liquid from the pretreatment, *see* Fig. 5. The production of degradation compounds increased with increased temperature and acid concentration. Figures 6 and 7 show the yields including oligomers, of glucose and xylose in the liquid from the slurry of the pretreated

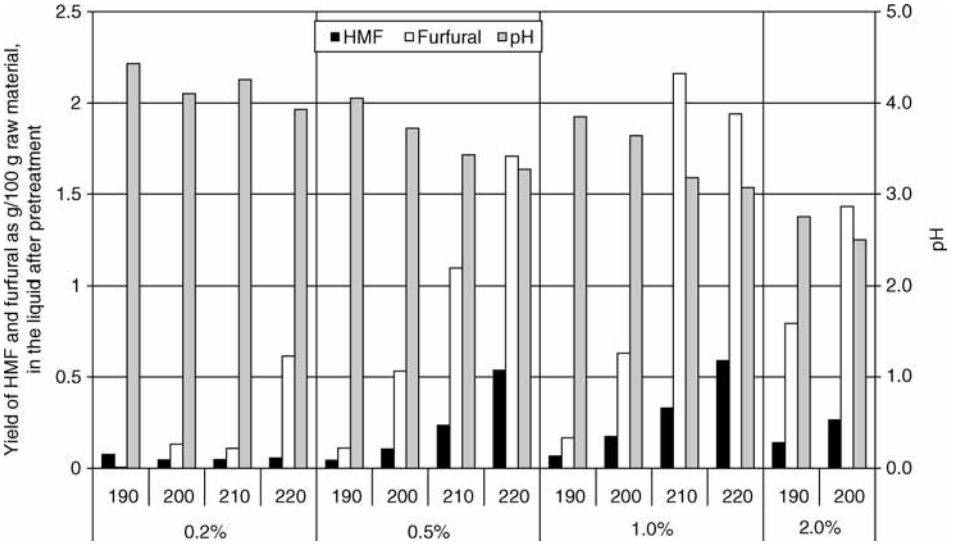

Fig. 5. Yield of HMF and furfural as g/100 g raw material, and pH, in the liquid after pretreatment.

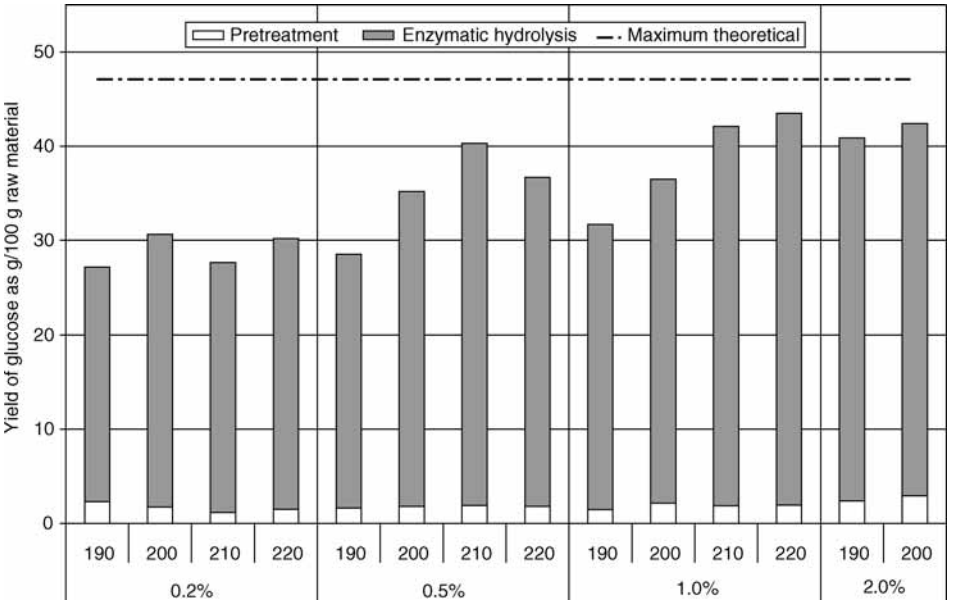

Fig. 6. Yield of glucose in pretreatment, enzymatic hydrolysis, and overall as function of the pretreatment conditions.

material and in the liquid after enzymatic hydrolysis. The maximum yield of xylose in the liquid from the pretreatment step was 14 g/100 g raw material (52% of theoretical) at 190°C with 2.0 wt% H_2SO_4.

Enzymatic hydrolysis was performed on washed WIS after pretreatment to assess the effect of the pretreatment step on the digestibility. Yields

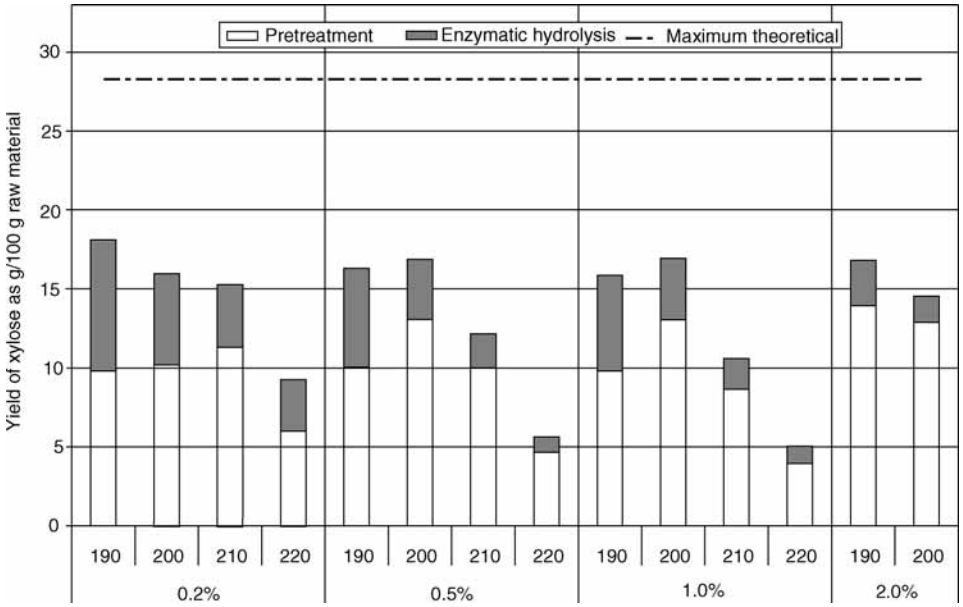

Fig. 7. Yield of xylose in pretreatment, enzymatic hydrolysis, and overall as function of pretreatment conditions.

were calculated from the 96-h sample taken during enzymatic hydrolysis. Figures 6 and 7 show the yield of glucose and xylose, respectively, for enzymatic hydrolysis. The glucose yield in the pretreatment step was low and approximately the same for all pretreatment conditions. Thus, the pretreatment conditions that gave the highest glucose yield in the enzymatic hydrolysis also resulted in the highest overall glucose yield. The highest overall glucose yield was 43.5 g/100 g raw material (which corresponds to a glucose yield of 92% based on the glucose content in the raw material including starch), obtained at the pretreatment condition of 1.0 wt% H_2SO_4 and 220°C for 5 min, *see* Fig. 6.

Severe pretreatment conditions hydrolyze more of the hemicellulose in the straw to monomeric sugars, and the xylose yield in the enzymatic hydrolysis will be low owing to less xylan in the material. However, if the pretreatment conditions are too severe degradation of xylose to furfural will occur in the pretreatment step and result in a low overall xylose yield. An example is the pretreatment conditions, 190°C and 200°C at 0.5 wt% H_2SO_4 (Fig. 7), in which the xylose yield in the pretreatment step was higher at 200°C. However, the xylose yield in the enzymatic hydrolysis was higher for 190°C, resulting in approximately the same overall xylose yield at 190°C as at 200°C, owing to less degradation of xylose to furfural in the pretreatment step (Fig. 5). The maximum overall yield of xylose, 18.1 g/100 g raw material (which corresponds to a xylose yield of 67% based on the xylose content in the raw material), was obtained at 0.2 wt% H_2SO_4 and 190°C for 5 min, *see* Fig. 7.

Table 4
Overall Yield of Glucose and Xylose at Pretreatment Temperature of 200°C for Different Substrate Loadings as g/100 g Raw Material

	1000 g wet straw	833 g wet straw	
	40 wt% DM	60 wt% DM	
	0.2 wt% H_2SO_4	0.2 wt% H_2SO_4	0.5 wt% H_2SO_4
Acid : dry-straw ratio	0.30	0.13	0.33
Glucose (g/100 g)	38.5 (40.8)[a]	30.6 (47.1)	35.2 (47.1)
Xylose (g/100 g)	22.6 (24.0)[a]	15.9 (27.1)	16.8 (27.1)

[a]Starch content not analyzed and removed before raw material analysis.

The values in bracket are the maximum (theoretical) values based on the composition of the raw material.

The two different optima for glucose and xylose are near the two extremes in pretreatment conditions evaluated with acid-sprayed barley straw. The overall yield of xylose is more affected by an increase in temperature from 190°C to 220°C than by an increase in acid concentration from 0.2 to 2.0 wt%. The best total overall yield of glucose and xylose, 57.6 g/100 g raw material (which corresponds to 78% based on the glucose and xylose content in the raw material), was obtained at 2.0 wt% H_2SO_4 and 190°C for 5 min.

One objective of the study was to increase the concentration of WIS in the slurry from the pretreatment step in order to decrease the flow rate of the stream going to the downstream processing and increase the concentration of ethanol *(15)*. The concentration of WIS and total DM (including WS) obtained in the slurry after the pretreatment of spray-impregnated barley straw at 200°C and acid:dry-straw ratio 0.3 was measured for the substrate loadings of 1000 g with 40 wt% DM and 833 g with 60 wt% DM. Concentrations of WIS obtained in the pretreated material were 6.9–8.1 and 13.9 g/100 g slurry for 40 and 60 wt% DM, respectively. DM concentrations obtained in the pretreated material were 10.4–12.5 and 20.4 g/100 g slurry for 40 and 60 wt% DM, respectively. The increase in amount of dry-straw and DM concentration in the pretreatment step increased the WIS and DM content in the pretreated material by approx 100%. This shows that the substrate loading have an evident effect on the WIS and DM content after pretreatment and thus the ethanol concentration.

The overall yield of glucose for spray-impregnated barley straw at pretreatment condition 200°C with 0.2 wt% H_2SO_4 and a substrate loading of 1000 g straw with 40 wt% DM was 38.5 g/100 g raw material, *see* Table 4. For the same conditions but with 833-g straw and 60 wt% DM the overall glucose yield was lower, 30.6 g/100 g raw material. The overall xylose yields with 0.2 wt% H_2SO_4 were 22.6 and 15.9 g/100 g raw material at 40 and 60 wt% DM, respectively. However, the overall yield of glucose

at 60 wt% DM and the same acid:dry-straw ratio as with 40 wt% DM, i.e., 0.33, gave an overall glucose yield of 35.2 g/100 g raw material. Thus, an increase in dry-straw loading and DM concentration in the pretreatment at the same acid : dry-straw ratio still decreased the yield of glucose, although the decrease was not as marked. The overall xylose yield at acid: dry-straw ratio of 0.33 and 60 wt% DM was 16.8 g/100 g. The low overall yield of xylose at an increased dry-straw loading and DM concentration was owing to an increase in xylose concentration in the pretreatment liquid, which probably increased the rate of degradation *(29)*.

Conclusions

Pretreatment of acid-soaked and acid-sprayed barley straw under the same conditions followed by enzymatic hydrolysis resulted in different overall yields of glucose and xylose. Acid-soaked barley straw gave the highest overall yield of glucose, regardless of impregnation time and acid concentration. An increased acid concentration from 0.2 to 1.0 wt% did not increase the yield from the acid-sprayed barley straw to the level of 0.2 wt% acid-soaked barley straw. For xylose, an increase in acid concentration resulted in a decrease in yield for both acid-soaked and acid-sprayed barley straw. In this study, the raw material was unwashed barley straw. A similar study with washed barley straw should be performed. As a result of washing the barley straw, its buffering capacity would probably decrease and the difference in severity in the pretreatment of acid-sprayed and acid-soaked barley straw would diminish thus yielding the same amount of fermentable sugars for the same acid concentration.

For acid-sprayed barley straw the pretreatment optimum for glucose and xylose are close to the two extremes of pretreatment conditions evaluated in this study. Thus, to obtain a high yield of glucose, a severe condition in the pretreatment is preferable when using spraying as an impregnation technique. However, a high yield of xylose is an important aspect for a future process when a pentose-fermenting microorganism is available. This suggests that a two-step steam pretreatment *(30)* where the xylose is released in the first step at a low severity would be a better option. The WIS is then pretreated again under a more severe condition, improving the enzymatic hydrolysis and thus achieving high overall yields of both glucose and xylose.

The concentration of DM and WIS in the pretreated material increased when the dry-straw loading and DM concentration were increased. However, the overall yield of both glucose and xylose decreased and partially counteracted the positive effect of an increase in DM concentration, i.e., an increase in ethanol concentration. The decrease in overall glucose yield, on the other hand, was significantly reduced when the acid:dry-straw ratio was kept the same for the different substrate loadings, whereas the decrease in overall xylose yield remained unchanged.

The pretreatment optimum for acid-soaked barley straw was not the same as the optimum for acid-sprayed barley straw. Studies on acid-soaked barley straw should be used with caution for design of processes utilizing acid-sprayed barley straw, and the buffering capacity of barley straw should not be overlooked as it has a significant effect on the severity in pretreatment.

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