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# Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw

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**Abstract**

The effect of process conditions used for wheat straw pretreatments on the liquor- and residue-composition was studied. Hereto, the pretreatment conditions were expressed in a 'combined severity $R'_0$-factor'. The higher the combined severity factor ($R'_0$) the more xylan was released from the wheat straw, but the more xylan decomposed and furfural formation occurred. The percentage of residual xylan present after pretreatment appeared to be a good indicator concerning cellulose degradability or bio-ethanol production. Namely, cellulose degradation by using commercial enzymes was higher at higher severities corresponding to a lower amount of residual xylan. The xylan release and degradation was studied in more detail by using HPSEC and MALDI-TOF mass spectrometry. The more severe the treatment the more (acetylated) xylose oligomers with a DP lower than nine were analysed. The presence of (acetylated) xylans with a DP of 9–25 increased slightly from low to medium severity. The quantification of the DP-distribution of the (acetylated) xylans released proved to be a good tool to predict cellulose degradability.


*Keywords:* Wheat straw; Bioethanol; Severity; Heat treatment; Cellulases

## 1. Introduction

The production of fuel bio-ethanol from cellulosic biomass is studied worldwide in order to substitute the use of petrochemical fuels and products by $CO_2$-neutral alternatives derived from biomass. Such a process generally involves a certain type of biomass-pretreatment, which often makes use of heat and acid (Harris et al., 1945; Garrote et al., 1999; Shimizu et al., 1998; Parajo and Santos, 1995; Papatheofanous et al., 1995; Lynd, 1996; Lynd et al., 1991).

Biomass-pretreatment is needed to make the cellulose present in plant cell walls accessible for enzymatic hydrolysis (Lynd et al., 2002; Lynd, 1996). Both the pretreatment and the enzymatic hydrolysis, yielding fermentable sugars like glucose, precede the final fermentation step in which bio-ethanol is obtained. However, the yield of fermentable sugars by using enzymatic hydrolysis highly depends on the type and severity of the biomass-pretreatment (Chang and Holzapple, 2000).

Extensive work has been performed on pretreatments at temperatures above 150 °C in water or in dilute-$H_2SO_4$. The use of different biomasses such as hard woods and corn stover has been reported (Nunes and Pourquie, 1996; Puls et al., 1985; Garrote et al., 2002; Schell et al., 2003). Relatively little work has been described for wheat straw, which is used in the present study. Wheat straw is an agricultural by-product, which is mainly composed of cellulose (34–43%), hemicellulose (26–35%) and lignin (14–21%) (Szczodrak, 1987; Sidiras, 1998; Sun et al., 2004).

In case of hydrothermal processing as a biomass-pretreatment, the severity of the process is often expressed by a 'severity factor' (Overend and Chornet, 1987; Garrote

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et al., 1999). The 'severity factor' $R_0(R_0 = t * \exp((T - 100)/14.75))$ combines the experimental effects of temperature ($T$; in °C) and reaction time ($t$; in minutes) to enable an easy comparison of results and to facilitate process control. However, in order to compare processing conditions, which involve different acid conditions the severity factor is not representative. Therefore, several studies described extension of the severity factor to include the effect of acid catalysts as $R'_0 = [H^+] \cdot R_0$ (Abatzoglou et al., 1992; Chum et al., 1990). The $R_0$ or $R'_0$ can be used to determine the best set of experimental parameters necessary to release hemicelluloses from the biomass enabling the further use of the remaining cellulose and lignin (Garrote et al., 1999). But, despite that the use of $R_0$ or $R'_0$ should facilitate comparison of data, often a lack of description of pH-values, differences in substrate handling or process parameters like heating profiles makes comparison difficult. Therefore, in the present paper not only the pretreatment of wheat straw is studied by determining the relation between $R'_0$ and the composition of the fractions obtained after pretreatment, but also the amount of residual xylan after pretreatment is discussed as an indicator for susceptibility towards cellulose enzymatic hydrolysis. The xylan removal and degradation is analysed in detail by using HPSEC in combination with MALDI-TOF mass spectrometry. Finally, the relation between $R'_0$, residual and the DP-distribution of released xylan is evaluated and reflected against the enzymatic degradability of the residues after pretreatment.

## 2. Experimental

### 2.1. Feedstock materials

Wheat straw, harvest 2003, was provided by A&F (Agrotechnology and Food Innovations, Wageningen, The Netherlands). The wheat straw was pre-milled with a hammer mill to a particle size of about 1 cm. The end-milling of the wheat straw to a particle size of 0.5–1.0 mm was performed with a Comitrol Processor Model 1700 of Urschel laboratories (Valparaiso, USA). Because of the severity of this milling, release of heat of friction, it was necessary to feed the mill with wet pre-milled wheat straw (20% w/w dry matter) to prevent burning of the wheat straw.

The wheat straw used contained 58% w/w of total sugar (glucan 31% w/w, xylan 20% w/w, arabinose side-groups 2.5% w/w and acetyl esters 1.7% w/w) and 25% w/w of lignin.

### 2.2. Pretreatment of wheat straw

A mild acid/heat pretreatment was performed with the milled wheat straw (wheat straw/demineralised water in a ratio of 1/10 (g/g)). For this pretreatment a 1000 ml Premex (Lengnau, Switzerland) HPM-T-1000 high pressure autoclave was used set at a temperature between 160 °C and 200 °C with corresponding water vapour pressure. Concentrated $H_2SO_4$ (98%) was added to the wheat straw slurry in a range of 0–2.5 g per 100 g of wheat straw. The exact conditions of all pretreatments performed are reported in Table 1.

Table 1
Pretreatment conditions of the pretreatments of wheat straw, calculated $R'_0(R'_0 = [10^{-pH}] * (t * \exp((T - 100)/14.75)$; calculated from pH of liquors obtained), and residual xylan (%)

| Experiment | Temperature (°C) | Process time (min) | $H_2SO_4$ (% w/w) | pH | Log $R'_0$ | Residual xylan (%) |
|---|---|---|---|---|---|---|
| 1 | 180 | 2 | 0 | 1.95 | −1.7 | 93 |
| 2 | 190 | 2 | 0.1 | 4.64 | −1.6 | 85 |
| 3 | 190 | 2 | 0.5 | 4.54 | −1.2 | 78 |
| 4 | 190 | 2 | 1 | 4.2 | −0.8 | 63 |
| 5 | 190 | 1 | 1 | 3.8 | −1.2 | 65 |
| 6 | 190 | 10 | 1 | 3.88 | −0.1 | 43 |
| 7 | 190 | 2 | 1 | 3.7 | −1.4 | 82 |
| 8 | 170 | 2 | 1 | 3.79 | −1.2 | 80 |
| 9 | 180 | 2 | 1 | 3.83 | −0.4 | 31 |
| 10 | 200 | 20 | 0 | 3.64 | −0.1 | 35 |
| 11 | 190 | 30 | 0 | 4.1 | −0.5 | 52 |
| 12 | 180 | 15 | 2.5 | 4.32 | 0.9 | 19 |
| 13 | 160 | 15 | 2.5 | 2 | 1.2 | 20 |
| 14 | 170 | 15 | 2.5 | 2 | 1.5 | 8 |
| 15 | 180 | 15 | 1.4 | 2 | 0.5 | 24 |
| 16 | 180 | 15 | 0.7 | 3 | −0.5 | 34 |
| 17 | 180 | 5 | 2.5 | 4 | 1.1 | 21 |
| 18 | 180 | 10 | 2.5 | 2 | 1.4 | 19 |
| 19 | 180 | 25 | 0.7 | 2 | −0.2 | 26 |
| 20 | 180 | 35 | 0.7 | 4 | −0.1 | 20 |
| 21 | 180 | 30 | 0 | 4 | −0.8 | 23 |

The autoclave had a typical heating rate of about 10 °C/min, depending on the loading of the autoclave. The wall of the autoclave was equipped with internal wall cooling and ensures rapid cooling of the reaction product. Typical maximum cooling rates were 15 °C/min.

The autoclave was loaded with the mildly acidic slurry, closed and made oxygen free through flushing the gas phase of the autoclave with nitrogen gas. The autoclave was heated to the desired temperature and held at this temperature during the desired reaction time. After reaching the reaction time, rapid cooling of the reaction product was ensured by switching on the water-cooling of the autoclave.

Pretreated samples were centrifuged and both the residue and liquor were collected. Residues were washed three times with distilled water, and the washing water was added to the liquors. Both residues and liquors were freeze dried before analysis.

### 2.3. Enzymatic degradation of the pretreated wheat straw residues

A solution of dried residue (100 mg in 25 mM sodium acetate buffer pH 5 (10 ml)) was incubated with the commercially available cellulase preparations Cellubrix (Novozymes (Bagsvaerd, Danmark)) and GC220

(Genencor-Danisco (Rochester, USA)) (Kabel et al., 2006). Per incubation 100 μl of the commercial cellulase was used and incubations were performed for 24 h at 40 °C. After inactivation of the enzyme (5 min, 100 °C) the digests were analysed for their glucose and xylose content by HPAEC.

### 2.4. Neutral sugar composition

The neutral sugar composition of the dried residues and liquors was determined by gas chromatography according to Englyst & Cummings (Englyst and Cummings, 1984), using inositol as an internal standard. The samples were treated with 72% w/w $H_2SO_4$ (1 h, 30 °C) followed by hydrolysis with 1 M $H_2SO_4$ for 3 h at 100 °C and the constituent sugars released were analysed as their alditol acetates.

### 2.5. Lignin

Wheat straw and residues were analysed for acid insoluble (Klason) lignin. To each sample of 300 mg (dry weight) 3 ml of 72% $H_2SO_4$ was added and samples were hydrolysed for 1 h at 30 °C. After this pre-hydrolysis, 37 ml of distilled water was added to each sample and samples were put in a boiling water bath for 3 h. Each half hour samples were shaken. Samples were filtered over G4 glass filters. The residual part was washed until it was free of acid and dried overnight at 105 °C. The weight of the dried residual part is a measure for the acid insoluble lignin content.

### 2.6. Furfural

The liquors were analysed for their furfural content by using a Waters (Milford, USA) 600 controller, a Waters 717 autosampler, and a Waters 616 pump equipped with a guard column (Biorad H cartridge) and an Aminex HPX-87 N column (Bio-Rad (Hercules, USA); 300 × 7.8 mm). The columns were heated in a CH30 column heater (Eppendorf (Hamburg, Germany)) at 80 °C. Elution took place at 80 °C with 0.01 M $Na_2HPO_4$ at 0.6 ml $min^{-1}$. The eluate was monitored using a refractive index (RI) detector (Waters (Milford, USA)).

### 2.7. HPSEC

Dried liquors were dissolved in pure water (4 mg/ml) and subjected (20 μl) to high-performance size-exclusion chromatography (HPSEC). HPSEC was performed on a Thermo Separation Products (San Jose, USA) HPLC system equipped with a membrane solvent-degasser, three TSKgel columns (4.8 mm ID × 30 cm per column) in series (G4000 PWXL, G3000 PWXL, G2500 PWXL; Tosohaas (Stuttgart, Germany)), in combination with a PWXL-guard column (Tosohaas). Elution took place at 30 °C with 0.2 M sodium nitrate at 0.8 ml $min^{-1}$. The eluate was monitored using a refractive index (RI) detector (Shodex (Kawasaki, Japan) RI-71).

Additionally, from the liquor of Exp. 3 (Table 1; 20 mg/ml and 100 μl injection) HPSEC was performed as described, but after RI-detection 8 fractions were collected manually. Fractions 1 to 8 corresponded with material eluting at 26–28 min, 28–30.5 min, 30.5–32 min, 32–33.2 min, 33.2–34.5 min, 34.5–35.5 min, 35.5–36.5 min and 36.5–37.5 min respectively. The fractions were directly subjected to MALDI-TOF mass spectrometry.

### 2.8. HPAEC (pH 12)

High-performance anion-exchange was performed on a Thermo Separation Products (San Jose, USA) system equipped with a Dionex (Sunnyvale, USA) CarboPac PA-20 column (3 mm ID × 150 mm) in combination with a Dionex CarboPac PA guard column (3 mm × 25 mm) and PAD-detection (Dionex). For the analysis of xylose and glucose in the hydrolysates, an isocratic elution (0.5 ml $min^{-1}$) of 20 min was carried out with 18 mM NaOH. Each elution was followed by a washing (5 min 300 mM NaOAc) and two equilibration steps (5 min 100 mM NaOH and 15 min 18 mM NaOH).

### 2.9. MALDI-TOF mass spectrometry

MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight) mass spectrometry was performed using an Ultraflex instrument (Bruker D altonics (Bremen, Germany)) equipped with a nitrogen laser of 337-nm and operated in the positive mode. After a delayed extraction time of 200 ns, the ions were accelerated to a kinetic energy of 12 000 V. Hereafter, the ions were detected using the reflector mode. The lowest laser power required to obtain good spectra was used and at least 100 spectra were collected. The mass spectrometer was calibrated with a mixture of maltodextrins (mass range 365–2309).

The samples were mixed with a matrix solution (1 μl of sample in 1 μl of matrix), after desalting the samples with resin (AG 50 W-X8 Resin; Bio-Rad (Hercules, USA)). The matrix solution was prepared by dissolving 9 mg of 2,5-dihydroxybenzoic acid (Bruker Daltonics (Bremen, Germany)) in a 1-ml mixture of acetonitrile:water (300 μl:700 μl). Of the prepared (sample + matrix) solutions 1 μl was put on a MALDI-TOF-plate (Bruker Daltonics (Bremen, Germany)) and allowed to dry under a constant stream of air.

## 3. Results and discussion

### 3.1. Effect of pretreatment on the composition of the residues and liquors obtained

The effects of pretreatment conditions (Table 1) on the composition of the residues and of the liquors obtained from wheat straw were studied. Fig. 1A presents the per-

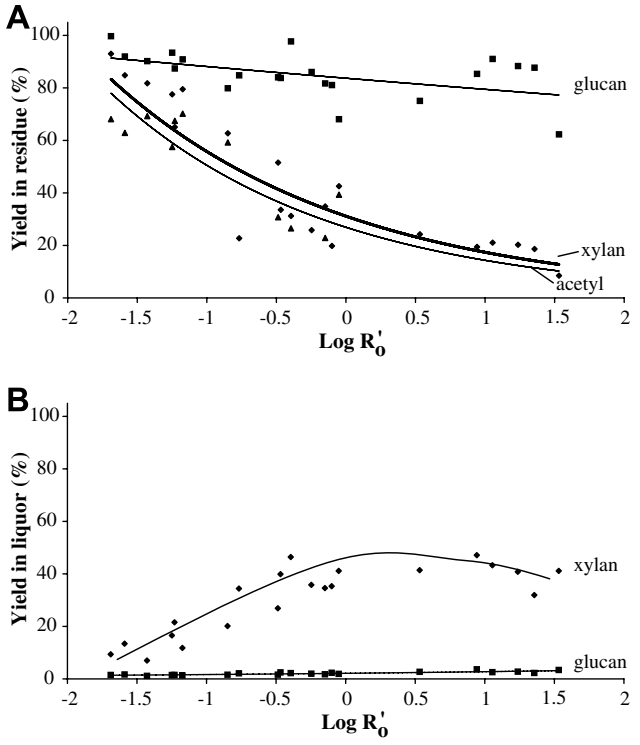


Fig. 1. The yield of xylan, glucan and acetyl esters in the residues (A) and the yield of xylan and glucan in the liquors (B) obtained after pretreatment expressed in % of the amounts originally present in the wheat straw as a function of the combined severity $R'_0$ ($R'_0 = [10^{-\mathrm{pH}}] * (t * \exp((T-100)/14.75))$). The lines show only trends.

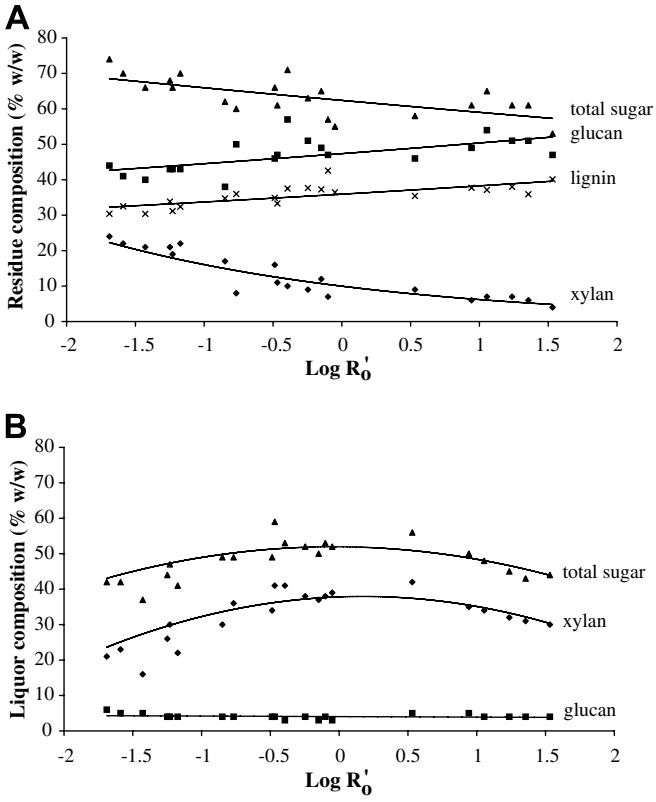


Fig. 2. The composition (% w/w) of the residues (A) and liquors (B) obtained after pretreatment of wheat straw as a function of the combined severity $R'_0$ ($R'_0 = [10^{-\mathrm{pH}}] * (t * \exp((T-100)/14.75))$). The lines show only trends.

centage of glucan, xylan and acetyl esters still present in the insoluble residue after pretreatment calculated from the amount originally present in the wheat straw as a function of the combined severity $R'_0$ ($R'_0 = [10^{-\mathrm{pH}}] * (t * \exp((T-100)/14.75))$). Fig. 1B presents similar results for glucan and xylan obtained in the liquors.

Fig. 1A shows that the higher the $R'_0$ the less xylan remained in the residues. At lower $R'_0$ most of the released xylan was accumulated in the liquors. However, at higher $R'_0$, the yield of xylan in the liquors was not covering the amount of xylan released from the residues (Fig. 1). Also, some of the cellulose was released at higher $R'_0$. These are common observations made for several raw materials treated in a similar way (Lloyd and Wyman, 2003; Bouchard et al., 1991; Garrote et al., 2002, 2001a; Lynd, 1996). Furthermore, the release of acetyl esters from the residues plotted against the $R'_0$ followed a similar pattern as the xylan release, which was seen in other studies using *Populus deltoides* or *Eucalyptus globulus* wood (Garrote et al., 2001b; Bouchard et al., 1991). According to Garrote and Parajo (2002) part of the acetyl esters are removed as acetic acid, catalysing the xylan depolymerization. Another part of the acetyl esters will remain to be covalently linked to the xylan backbone and are released from the residue together with the xylan as esterified xylo-oligosaccharides (Kabel et al., 2002b).

Fig. 2 presents the composition of the residues and liquors obtained after treatment as a function of $R'_0$. Cellulose was slightly degraded at higher $R'_0$ (Fig. 1A), but the cellulose level in the residues increased at higher $R'_0$ (Fig. 2A). This was mainly due to the increased release of xylan at higher $R'_0$, leaving mainly cellulose and lignin in the residues. As was already mentioned, at higher $R'_0$ the yield of xylan in the liquors was not covered by the amount of xylan released from the residues (Fig. 1). Most likely, part of the released xylose was converted to furfural.

In Fig. 3 the xylan loss, which is decomposed xylan, and the furfural formation is presented both as a function of $R'_0$ (Fig. 3A) and as a function of the percentage of residual xylan (Fig. 3B). From these figures, it was seen that the amount of furfural and the xylan loss increased both at higher $R'_0$ and a decreased percentage of residual xylan. However, the graph of furfural plotted against $R'_0$ did not show a very good correlation (Fig. 3A), while the graph of furfural plotted against the residual xylan did present a rather nice line (Fig. 3B). The latter is probably the result of a direct relation between xylan decomposition and furfural formation, which is apparently better than the correlation between the more indirect plot of $R'_0$ versus furfural formation. Therefore, from Fig. 3 it was concluded that to present the severity of pretreatments, besides $R'_0$, also the percentage of residual xylan after pretreatment appeared to be a good indicator.

Degradation of arabinose present as xylan side-chains (results not shown) contributed to the formation

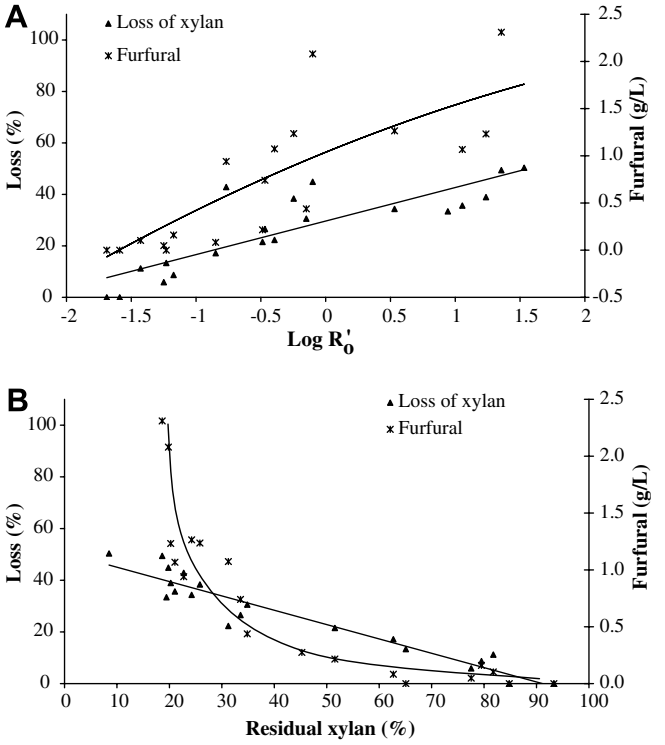


Fig. 3. Decomposed (loss of) xylan (%) and formation of furfural (g/L) after pretreatment as a function of the combined severity $R'_0$ ($R'_0 = [10^{-pH}] * (t * \exp((T-100)/14.75))$) (A) and as a function of residual xylan present after pretreatment (calculated as originally present in wheat straw) (B). The lines show only trends.

of furfural. In the bioethanol process the formation of furfural is undesirable, because in the fermentation step resulting in ethanol production the presence of furfural is toxic for the yeast used. The results correlated rather well with the results described previously for similar pretreatments of *Eucalyptus* wood and brewery's spent grain (Garrote and Parajo, 2002; Carvalheiro et al., 2004). They all mentioned a sharp increase in furfural production at a percentage of residual xylan of about 20% correlating with a rather high $R'_0$, which is comparable to the results observed from Fig. 3.

Using the percentage of residual xylan for predicting the severity has some advantages over using the $R'_0$. Namely, in most studies published so far the amount of residual xylan is presented, while the pH of the liquors obtained after pretreatment is hardly mentioned in $R_0$-studies making the calculation of $R'_0$ from these studies difficult (Garrote et al., 2001a; Lloyd and Wyman, 2003; Nunes and Pourquie, 1996). Additionally, in some $R'_0$-studies the pH is taken from analysis of the liquors after pretreatment (Schell et al., 2003), but in other $R'_0$-studies the pH is calculated from the amount of $H_2SO_4$ added (Larsson et al., 1999; Carvalheiro et al., 2004). This will result in different $R'_0$s under similar process conditions, because the buffering capacity of the substrate used and the severity of the pretreatment performed usually affects the final pH obtained (Bouchard et al., 1991). Furthermore, calculating the $R'_0$ from the amount of acid added is not possible when conditions without addition of acid ($[H^+] = 0$, so $R'_0 = 0$) are tested as well.

### 3.2. Size distribution of released xylan related to severity of pretreatment

To study the mechanisms involving xylan release and losses during pretreatment the molecular weight ($M_w$) distribution of the xylans in the liquors obtained were analysed by using HPSEC in combination with RI-detection. Generally, the area obtained underneath a RI-graph represents the amount of sugars present. Keeping this in mind, the percentages of areas of three parts (low, medium and high $M_w$) as calculated from the total area in the HPSEC diagram were compared. The low, medium and high $M_w$-parts were set in the HPSEC-diagram from 32.5–37 min, 28–32.5 min and 17–28 min respectively, which is shown in Fig. 4 for the HPSEC-diagram of the liquor obtained in Exp. 3 (Table 1) as an example. Moreover, Fig. 4 presents the distribution of low, medium and high $M_w$ xylans in the liquors after pretreatment as a function of residual xylan present. Actually, in Fig. 4 the $M_w$ distribution is studied as a function of pretreatment-severity as proposed in the preceding text. It was concluded (Fig. 4) that the more severe the treatment (lower levels of residual xylan) the more low $M_w$ xylans and the less high $M_w$ xylans were detected in the liquor. The presence of medium $M_w$ xylans first increased slightly upon a medium severity, but then decreased rapidly at higher severity in favour of low $M_w$ xylans formed. These results confirm unquestionably the previously suggested theory that xylan is dissolved in the reaction first as high $M_w$ material and that upon higher severity more and more bonds between xylose residues are broken (Lloyd and Wyman, 2003). For the production of bioethanol in an economic feasible process both the glucose from cellulose and the xylose from xylan need to be fermented to ethanol. Therefore, in the enzymatic hydrolysis step following the pretreatment not only cellulases but also xylanases are of importance. Relating this to the results shown in Fig. 4, it was concluded that the composition of the enzyme-mixture needed is influenced by the pretreatment performed.

Next, the determination of the range of oligomeric sizes in the liquors is helpful in constructing models for predicting release of hemicellulose from cellulose (Lloyd and Wyman, 2003). Up to now, many publications only distinguish between monomeric xylose and other soluble xylan-material (Lloyd and Wyman, 2003; Garrote and Parajo, 2002; Schell et al., 2003). Therefore, the degree of polymerisation (DP) of the xylans in the defined low, medium and high $M_w$ range of HPSEC was analysed. Fractions were collected from HPSEC (liquor of Exp. 3 (Table 1)) and directly subjected to MALDI-TOF mass spectrometry. An example of MALDI-TOF mass spectra of two HPSEC-fractions, both present in the medium $M_w$-part, is shown in Fig. 5. In Fig. 5 not all peaks were coded to obtain a more presentable figure. However, all peaks pres-

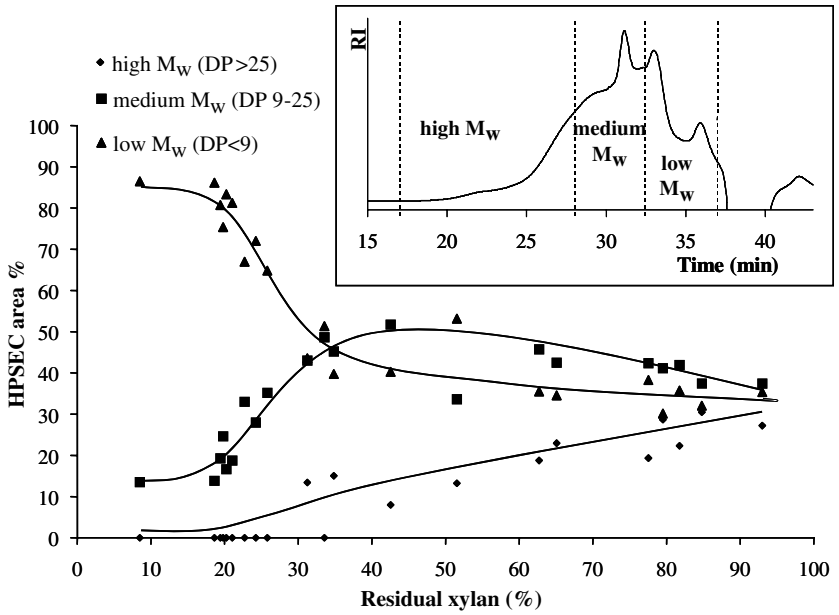


Fig. 4. Distribution of high $M_w$ (DP > 25), medium $M_w$ (DP9-25) and low $M_w$ (DP < 9) xylans present in the liquors after pretreatment, which were analysed by HPSEC, as a function of residual xylan present after pretreatment (calculated as originally present in wheat straw). The lines show only trends. The HPSEC-diagram of the liquor obtained in Exp. 3 (Table 1) is presented as insert.

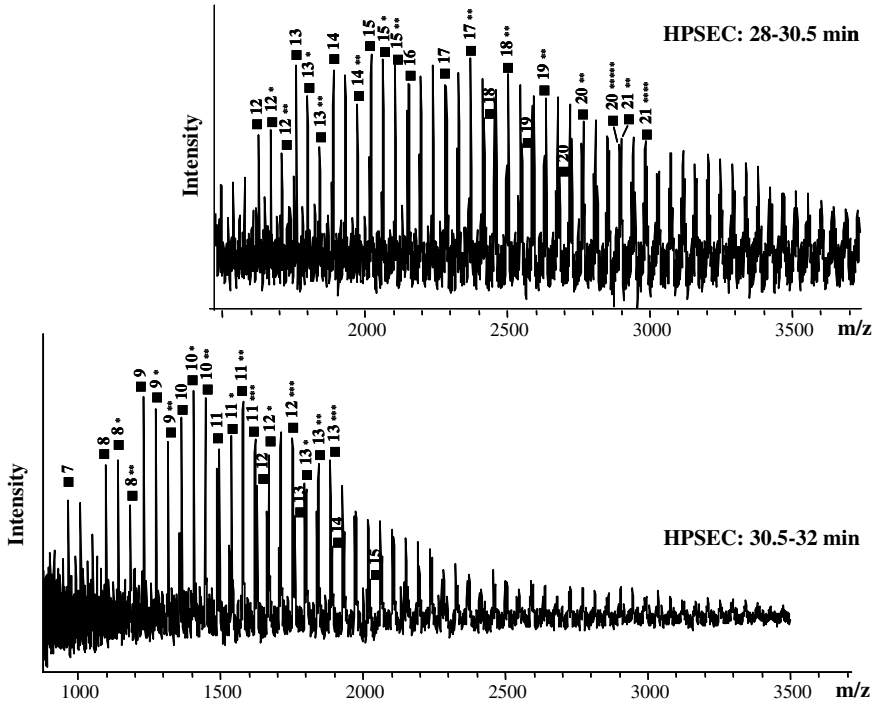


Fig. 5. MALDI-TOF mass spectra of two HPSEC-fractions (28–30.5 min and 30.5–32 min) of liquor from Exp. 3 (Table 1); ■ stands for xylose (with the number of residues present in arabic) and * stands for acetyl group.

ent were identified to correlate with (acetylated) xylo-oligosaccharides, as was also seen in former research for thermally treated *Eucalyptus* wood as well (Kabel et al., 2002b). In summary, the low $M_w$ (acetylated) xylans were constituted of a DP lower than nine, the medium $M_w$ (acetylated) xylans of a DP of 9–25, and the high $M_w$ (acetylated) xylans of a DP higher than 25 (Figs. 4 and 5). Additionally, mainly xylo-oligomers without arabinose side chains were present as analysed by HPAEC (results not shown). This was expected since in wheat straw on average only 11 out of 100 xylose residues were linked to an arabinose. Furthermore, arabinose side-groups are

usually rather unstable during heat treatments (Oosterveld et al., 2003; Kabel et al., 2002a), and therefore will be released from the xylan backbone easily resulting in mainly (acetylated) xylo-oligomers without arabinoses. The presence of acetyls groups will hinder xylanases to be active and hereby will hinder the conversion of xylan to xylose to be fermented to ethanol. The latter problem could be overcome by additive enzyme activities like acetylxylanesterases.

### 3.3. Enzymatic degradability of residues obtained related to the severity of the pretreatment

In view of the ethanol-production not only the release of hemicellulose from the cellulose is of importance, but also the effect of this release on the enzymatic degradability of the cellulose and hemicellulose. Therefore, the degradation of the remaining residues after pretreatment by using an overdose of two commercial cellulase preparations (GC220 and Cellubrix) was studied as a function of the $R'_0$ and of residual xylan (Figs. 6 and 7).

The degradability of the entire residues increased from a $R'_0$ of −1.7 to 0 and stabilised from a $R'_0$ of 0–1.5 by using the commercial cellulases Cellubrix or GC220 (Fig. 6). Furthermore, the GC220 resulted in a higher degradation of the residues than Cellubrix for the complete range of $R'_0$ analysed. The two commercial cellulase preparations used were shown to degrade both cellulose and xylan from plant materials rather well (Kabel et al., 2006). The ratio of the content of cellulose to xylan in the residues differed a lot depending on the $R'_0$ of the pretreatment performed (Fig. 2A). Taking into account the relatively high ratio of cellulose to xylan at a $R'_0$ of 0 or higher, the stabilised degradability of the residues obtained at a $R'_0$ of 0 or higher was most likely due to a better cellulose degradability at higher $R'_0$. The latter was analysed and the results are presented in Fig. 7. It should be noticed that Fig. 6 represents the percentage of degraded solids calculated from the total amount of solids as present in the residues after pretreatment, while Fig. 7 represents the percentage of degraded glucan calculated from the total amount of glucan as present in the same residues. Indeed, an increase in cellulose degradation was seen with increasing process severity, which had already been observed many years ago for similar experiments by using pretreated wood chips (Harris et al., 1945) and more recent described for e.g. pretreated rice hulls (Saha et al., 2005) and corn stover (Yang and Wyman, 2004). The results shown in Fig. 7B were complementary with the results of Schell et al. (2003) presenting enzymatic cellulose degradability of samples in the range of 0–20% of residual xylan. In this range the trend-line of cellulose degradation by using Cellubrix was followed very well by the results of Schell et al. (2003) (Fig. 7B), although they used a different cellulase preparation and corn stover as feedstock material. Furthermore, again the trend-line of degraded cellulose as a function of residual xylan represented the data points obtained slightly better compared to the trend-line of degraded cellulose versus $R'_0$ (Fig. 7A versus B). This suggested that also for prediction of enzymatic degradability after pretreatment the amount of residual xylan still present after pretreatment could be used as an indicator.

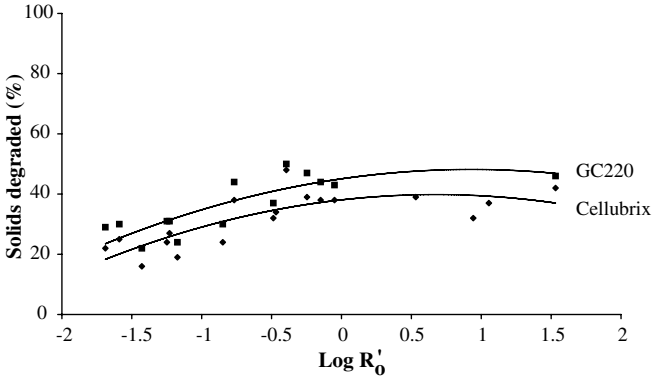


Fig. 6. Enzymatic digestion by using two commercial cellulases (Cellubrix and GC220) of residues obtained after pretreatment; total material degraded (% w/w) as a function of the combined severity $R'_0$ ($R'_0 = [10^{-pH}] * (t * \exp((T - 100)/14.75))$).

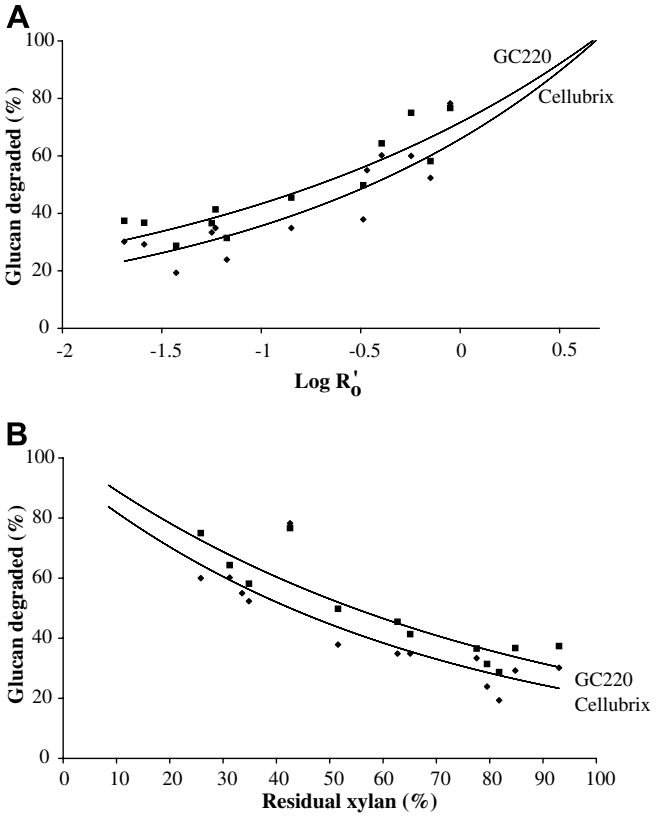


Fig. 7. Enzymatic digestion by using two commercial cellulases (Cellubrix and GC220) of residues obtained after pretreatment; glucan degraded (% w/w) as a function of the combined severity $R'_0(R'_0 = [10^{-pH}] * (t * \exp((T - 100)/14.75)))$ (A) and as a function of residual xylan present after pretreatment (calculated as originally present in wheat straw) (B). The lines show only trends.

It should be noted that the relationship found between xylan removal and cellulose degradability might indirectly be a result of lignin disruption (Yang and Wyman, 2004). But, in our experiments lignin was hardly removed resulting in high lignin containing residues (Fig. 2), which were used to test cellulose digestibility. Still the possibility of lignin disruption and re-precipitation could not be excluded from these data. However, it was shown in our lab that xylan, having a DP of 15 and higher, is adsorbed onto cellulose and hereby is hindering cellulases to act (results not shown). Therefore, to degrade all cellulose surely the presence of xylan should be taken into account.

## 4. Conclusions

The severity of the pretreatments performed in this research could be presented by using the previously defined $R'_0$. The higher the $R'_0$ the more xylan was released from the wheat straw and the higher cellulose conversions by using commercial enzymes were reached. However, also more xylan 'losses' and furfural production was observed. The presence of furfural is undesirable in the bioethanol process, because it may hinder yeast growth in the final fermentation step. Furthermore, the pretreatment severity strongly influenced the size of dissolved xylan as analysed by using HPSEC in combination with MALDI-TOF mass spectrometry. The introduction of these state-of-the-art techniques enabled us to visualize the structural features of degraded and solubilised xylans (e.g. arabinosyl and acetyl substitution), which have remarkable consequences for the subsequent enzymatic degradation and fermentation steps in the bioethanol production process.

In view of the bioethanol process, the overall efficiency of the process is depending on a good balance between a low furfural production and good substrate degradability. The latter could be established by using more specific enzymes to degraded both cellulose and substituted xylans, while choosing a lower severity for the pretreatment.

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