Study on the deacetylation of hemicelluloses during the hydrothermal processing of *Eucalyptus* wood

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Eucalyptus globulus wood samples were subjected to hydrothermal treatments at a liquor to wood ratio in the range 6–10 g/g and temperatures from 145 up to 190 °C. The effects caused by hydrothermolysis included extractive removal, hemicellulose degradation and deacetylation of both hemicelluloses and acetylated oligosaccharides. An analytical procedure based on the determination of the xylose and acetic acid contained in liquors before and after a quantitative posthydrolysis allowed the determination of acetyl groups bound to residual xylan and oligosaccharides. Since hydronium ions (the catalytic species involved in the degradation of the polymeric fractions of biomass) are mainly generated from acetic acid, special attention was paid to interpretate the time course of acetyl groups hydrolysis from both xylan and xylan-degradation products, and their interrelationship with the concentration of acetic acid is established.

Über die Deacetylierung von Hemicellulosen während des hydrothermalen Aufschlusses von Eukalyptusholz

Eukalyptusholz wurde einem hydrothermalen Aufschluß unterworfen. Das Verhältnis Lauge zu Holz variierte zwischen 6 g/g und 10 g/g, die Temperatur zwischen 145 °C und 190 °C. Die beobachteten Effekte waren: Entfernen von Extraktstoffen, Abbau von Hemicellulosen sowie Deacetylierung sowohl der Hemicellulosen als auch der acetylierten Oligosaccharide. Messen des Gehalts an Xylose und Essigsäure in der Lauge, und zwar vor und nach einer quantitativen Nachhydrolyse erlaubte die Bestimmung des Acetylierungsgrades des restlichen Xylans und der Oligosaccharide. Das die Hydronium-Ionen (die als Katalysator beim Abbau der polymeren Biomasse wirken) überwiegend von der Essigsäure stammen, wurde besonders auf den Verlauf der Deacetylierung des Xylans und seiner Abbauprodukte geachtet und deren Abhängigkeit von der Konzentration der Essigsäure nachgewiesen.

$\%Acl^{RM}$	acetyl group content of the raw material (g acetyl group/100 g oven-dry wood)
$\%Gn^{RM}$	glucan content of the raw material (g glucan/100 g oven-dry wood)
$\%Lg^{RM}$	lignin content of the raw material (g lignin/100 g oven-dry wood)
$\%Xn^{RM}$	xylan content of the raw material (g xylan/100 g oven-dry wood)
AcHC	acetic acid concentration in liquors (g acetic acid/kg of liquor)
AcH^{EP}	percent of initial acetyl groups converted into free acetic acid
Acl^{EP}	percent of initial acetyl groups remaining in the solid residue
AcO^{EP}	percent of initial acetyl groups linked to oligomers
AcOH	increase in acetic acid concentration in liquors after posthydrolysis (g acetic acid/kg of liquor)
LSR	liquor to solid ratio (g water/g oven-dry solid)
OEP	percent of initial xylan converted into oligomers after treatments
PRXn	percent of xylan remaining in solid residue after treatments
SY	solid yield (g solids recovered/100 g raw material, oven-dry wood)
T	temperature (°C)
t	time (hours)

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1
Introduction

The current commercial technologies for the production of chemical pulp (sulphite and sulphate processes) involve the utilization of sulphur-containing chemicals able to degrade both lignin and hemicelluloses, which are wasted or utilized for power generation. Alternatively, the "biomass refinery" concept (Myerly et al. 1981) is based on treatments leading to the separation of the lignocellulosic raw materials into several "fractions", each of them being useful for given end-product applications. Following this philosophy, a sequence of treatments may start with a mild hydrothermal stage, in which extractives are removed, hemicelluloses are solubilized, and cellulose is almost quantitatively retained in solid phase with limited lignin alteration. A review on the hydrothermal processing of biomass has recently been reported (Garrote et al. 1999a).

Hydrothermolysis (or water prehydrolysis) is an environmental-friendly technology, in which water is the only reagent necessary for processing the raw material. In comparison to prehydrolysis, corrosion is not important, and the handling of sludges coming from neutralization is avoided, leading to economic advantages (Kubikova et al. 1996). On the other hand, the structural alteration of the solid residues from treatments makes them more susceptible towards the enzymatic hydrolysis of cellulose (Lamptey et al. 1985; Ropars et al. 1992; Weil et al. 1997) or towards organosolv delignification (Lora and Wayman 1978; Biermann et al. 1984; Lawther et al. 1996). Water prehydrolysis is commercially carried out as a previous stage in the prehydrolysis-Kraft technology (Kubikova et al. 1996), leading to pulps with improved susceptibility to bleaching (Pekarovicová et al. 1993).

The degradation and/or structural alteration of the polymeric components of lignocellulose observed during the hydrothermal treatments are reached by means of hydronium-catalyzed reactions. In the first stages of hydrothermolysis, the hydronium ions are generated from water by autohydrolysis, but the generation of acetic acid (by acetyl groups hydrolysis) and its further ionization provides most of the catalytic species involved in the subsequent polymer degradation (Dekker and Wallis 1983; Conner 1984).

According to the above ideas, it can be inferred that the susceptibility of a given lignocellulosic raw material towards hydrothermolysis depends on its ability to generate acetic acid in the reaction media. However, it must be considered that the neutralizing ability of the feedstock also plays a role in the presence of hydronium ions.

As the prehydrolysis of lignocellulose is also a hydronium-catalyzed reaction, kinetic studies on both prehydrolysis (Mehlberg and Tsao 1979; Harris et al. 1984; Parajó et al. 1994) and hydrothermolysis (Conner 1984; Carrasco et al. 1987; Garrote et al. 1999b) show a common theoretical background. Some reported hypotheses on the kinetics of these reactions are: i) owing to structural reasons, two xylan fractions with different susceptibility towards hydrolysis exist in feedstocks (Conner 1984; Maloney et al. 1985; Carrasco and Roy 1992); ii) sugar oligomers appear as reaction intermediates, being readily degraded in prehydrolysis (Ranganathan et al. 1985) and accounting for a substantial part of the initial xylan in hydrothermolysis (Conner and Lorenz 1986); iii) oligomers are hydrolyzed to sugars and pentoses are dehydrated to furfural under harsh conditions. The reaction rates of all the three above steps depend on the concentration of hydronium ions, which is controlled by the amount of acetic acid generated from acetyl groups.

Even if acetic acid has been reported to be a byproduct from hydrothermolysis (Muzzy et al. 1983; Schmidt and Thomsen 1998), scarce information exists on the time course of acetyl groups during the chemical processing of lignocellulosic materials. Some studies have been reported in related fields, including the influence of acetyl groups in ethanol-water delignification (Gosselink et al. 1995), in alkaline treatments (Zanutinni and Marzocchi 1997;

Zanutinni et al. 1998) and in prehydrolysis (Maloney et al. 1985).

This work deals with the time course of acetyl groups during the hydrothermal treatments of *Eucalyptus* wood. The main kinetic trends of the hydrolysis of the acetyl groups linked either to the xylan remaining in solid phase or to xylooligomers was established, and the generation of acetic acid from acetyl groups was correlated with the concentrations of xylan and xylan-degradation products.

2
Materials and methods

2.1
Raw material
Eucalyptus globulus wood samples from a local pulp factory were milled to pass a 8 mm screen, air-dried, and homogenized in a single lot to avoid compositional differences and stored.

2.2
Analysis of wood
Aliquots from the homogenized wood lot were subjected to moisture determination and to quantitative acid hydrolysis with 72% sulfuric acid following standard methods (Browning 1967). The solid residue after hydrolysis was considered as Klason lignin. The monosaccharides and acetic acid contained in hydrolyzates were determined by HPLC as reported elsewhere (Garrote et al. 1999b).

2.3
Hydrothermal processing of wood samples
Wood chips and water were mixed in the desired proportions and reacted in a 4563 M Parr reactor with 600 mL total volume (450 mL working volume) fitted with a two six-blade turbine impeller. The vessel was heated with an external fabric mantle, and cooled by an internal stainless steel loop. Temperature was monitored using an inner thermocouple, and controlled by a PID module. The moisture of wood was considered as water in the material balances. The reaction media were heated to the desired temperature (heating time, 15–20 min), and then the time zero was set and a sample was taken. It can be noted that a part of the substrate may have reacted at this moment, and that the data corresponding to the isothermal part of the reaction were the only ones used in this work. Further experiments were carried out to test selected reaction times. The operational variables involved in this part of the study were: reaction time (t, hours), temperature of isothermal operation (T, °C) and liquor to solid ratio (LSR, g water/g oven-dry solid).

2.4
Analysis of solid residues from hydrothermal treatments
At the end of the treatments, the solid residues were recovered by filtration, washed twice with distilled water, air-dried to a moisture content <15%, milled in an impact mill to ensure total conversion in the quantitative acid hydrolysis step, hydrolyzed and analyzed by HPLC using the same methods as for raw wood analysis.

2.5
Analysis of liquors from hydrothermal treatments

A sample of liquors was filtered through 0.45 μm membranes and used for direct HPLC determination of monosaccharides and acetic acid and other reaction by-products. A second sample of liquors was subjected to quantitative posthydrolysis (with 4% sulphuric acid at 121 °C during 60 min) before HPLC analysis. The increase in monosaccharide concentration caused by posthydrolysis measured the oligomer concentration. Figure 1 summarizes the analytical processing of samples.

3
Results and discussion

3.1
Composition of the raw material

The average values of six replicate determinations of the wood contents in glucan, xylan, lignin and acetyl groups (denoted $\%Gn^{RM}$, $\%Xn^{RM}$, Lg^{RM} and $\%Acl^{RM}$) were 46.3%, 16.6%, 22.9% and 3.56%, respectively (oven-dry wood basis). Xylan was the main hemicellulosic polymer, and its degree of acetylation was 0.659 acetyl groups per xylose unit. Other non-determined fractions include extractives, uronic acids, soluble lignin and ashes.

3.2
Definition of variables employed to study the time course of deacetylation

In order to quantify the amount of solid and liquid phases under given operational condition treatments, it must be considered that a part of the raw material is solubilized in treatments. Because of this, the yield in solid residue (denoted SY, and defined as the percent of weight of the solid residue respect to the initial wood, oven-dry basis) was determined in each experiment. For simplification, the time courses of both acetyl groups contained in the several fractions resulting from xylan degradation and acetic acid (after stoichiometric correction) are measured as percent of the initial acetyl groups contained in the feedstock.

The percent of acetyl groups remaining in the solid residue (denoted Acl^{EP}) was calculated from the weight percent of acetyl groups in the solid residue (%Acl). This parameter was obtained from the acetic acid content of liquors coming from the quantitative acid hydrolysis of the solid residues. On the basis of the above definitions, it can be inferred that:

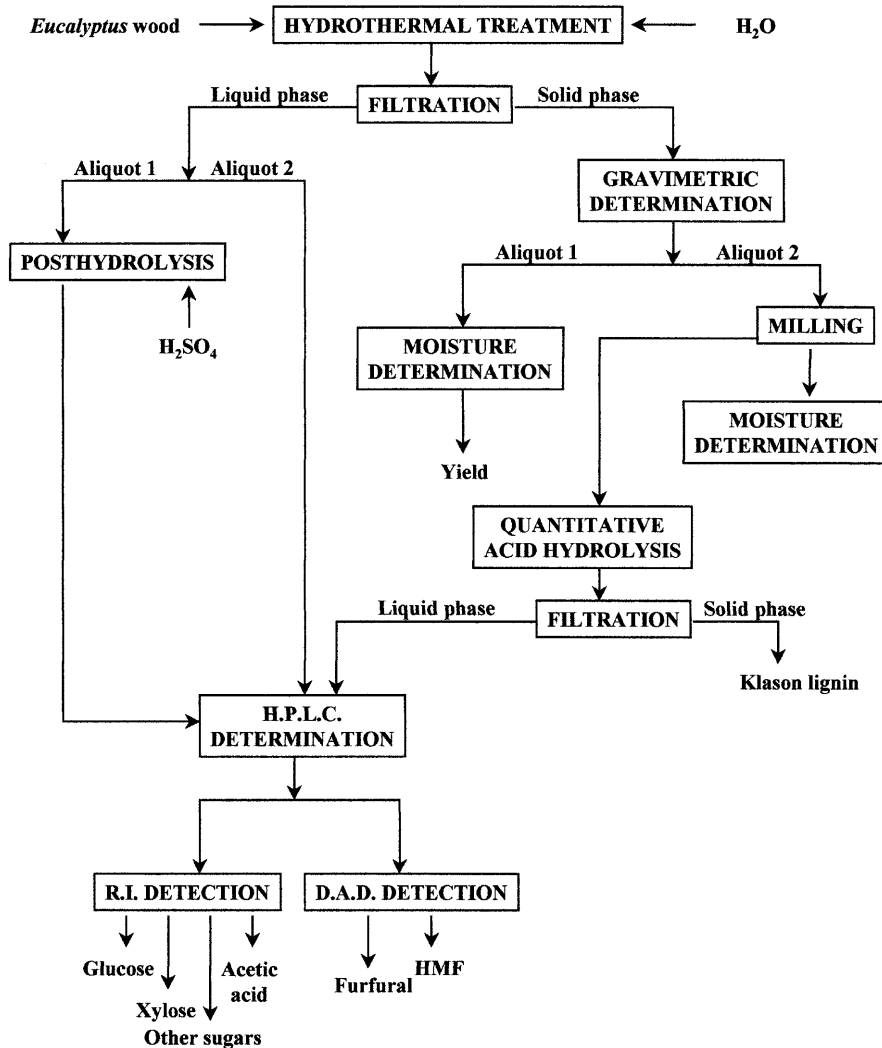$$Acl^{EP} = \frac{\%Acl \cdot SY}{\%Acl^{RM}} \qquad (1)$$

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Fig. 1. Analytical processing of samples
Bild 1. Analytische Verfolgung der Proben

The percent of acetyl groups converted into acetic acid (AcH^{EP}) can be calculated from the concentration of acetic acid in the reaction media before posthydrolysis (AcHC, g/kg liquor) using the equation:

$$AcI^{EP} = \frac{AcHC \cdot \left(LSR + 1 - \frac{SY}{100}\right) \cdot 10}{\%AcI^{RM}} \cdot \frac{43}{60} \qquad (2)$$

where LSR is the initial ratio liquor/solid (o. d. basis) and the coefficient (43/60) gives the corresponding stoichiometric correction.

After the posthydrolysis shown in Fig. 1, oligomers are saccharified to sugars and the acetyl groups linked to oligomers or free sugars are converted into acetic acid. Considering the increase in acetic acid concentration of liquors observed after posthydrolysis (variable denoted AcOC, g/kg), the percent of acetyl groups linked to oligomers (AcO^{EP}) can be calculated as:

$$AcO^{EP} = \frac{AcOC \cdot \left(LSR + 1 - \frac{SY}{100}\right) \cdot 10}{\%AcI^{RM}} \cdot \frac{43}{60} \qquad (3)$$

3.3
Time course of deacetylation

Table 1 shows the operational conditions under which the hydrothermal treatments were carried out, and Fig. 2 shows the time course of the percents of acetyl groups linked to xylan (AcI^{EP}), bound to oligomers (AcO^{EP}) or converted into free acetic acid (AcH^{EP}).

In experiments 1 to 5, the rate of xylan deacetylation (measured by the slope of curves AcI^{EP}/time) increased during the first stages of reaction, and then declined progressively. This behaviour can be understood on the basis of the concentration of hydronium ions in the reaction media: at the beginning of the reaction, the availability of catalyst was the rate-limiting factor, and the generation of acetic acid from acetyl groups increased the hydronium concentration (and so, the reaction rate). In further reaction stages, the reaction rate was mainly governed by the concentration of the substrate, and the decreased concentration of acetylated xylan justified the main kinetic trends. In experiment 6 (performed at high temperature), about 20% of the initial acetyl groups were converted into acetic acid during the non-isothermal stage of reaction, and deacetylation followed the pattern corresponding to media with non-limiting catalyst concentrations. The experimental data show that a substantial part

of the initial acetyl groups (up to 90%) can be removed from the substrate by means of hydrothermal treatments.

The acetyl groups bound to oligomers (measured by variable AcO^{EP}) followed a behaviour typical of a reaction intermediate. The most important finding is that a substantial part of the initial acetyl groups (up to 66%) is associated to oligomers even under conditions leading to a substantial xylan removal from the raw material. This finding is important when autohydrolysis is considered as the first stage of a sequence of treatments for fractionation of *Eucalyptus* wood, since in this case the primary objectives are the solubilization of hemicelluloses and the production of marketable chemicals from this fraction. Kabel et al. (1999) reported the presence of acetylated oligomers in hydrothermal liquors from *Eucalyptus globulus* wood, including compounds such as X_4Ac, X_4Ac_2 and X_4Ac_3 (X = xylose, Ac = acetyl substituent).

The experimental data of Fig. 2 concerning experiments 3, 4 and 5 proved that the liquor to solid ratio (see Table 1) caused limited effects on deacetylation. Material balances showed that acetic acid did not react significantly under the operational conditions considered in this study. This finding is in agreement with the data reported by Muzzy et al. (1983) for the continuous steam hydrolysis, who found that the acetic acid generated from the feedstock accounted for 4.7 weight percent within a range of operational conditions. In harsh treatments (temperatures of 260 °C), losses of acetic acid have been reported (Weil et al. 1997).

Further assessment on deacetylation can be carried out considering a kinetic model for xylan degradation recently reported by our research group (Garrote et al. 1999b), which allows the calculation of the concentrations of both xylan and xylan-degradation products in hydrothermolysis carried out under given operational conditions. On the basis of both the calculated concentration profiles and the experimental results shown in Fig. 2, the degree of acetylation of both residual xylan and xylooligomers can be calculated. The acetylation degree of xylan decreased with the reaction time, particularly when more than 40% of the initial xylan was removed (i. e., when the reaction became slower owing to substrate consumption), but the acetylation degree of the xylan remaining in wood after prolonged treatments accounted for more than 80% of the acetylation degree corresponding to the raw wood. On the contrary, the acetylation degree of oligomers increased with the reaction time. The oligomers generated in the final reaction stages had an acetylation degree 50% higher than the oligomers produced at the beginning of the reaction.

In order to provide a deeper understanding on the catalytic effects involved in the overall hemicellulose degradation, we tried to correlate the concentration of acetic acid in the reaction media with the concentrations of xylan and xylan-degradation products. A first attempt dealing with this idea is shown in Fig. 3. Figure 3a shows that the acetic acid concentration increased with the percent of xylan removed, but the experimental data clearly revealed different experimental trends for assays carried out at different temperatures. However, the fact that the acetic

Table 1. Operational conditions employed in this work
Tabelle 1. Verwendete Prozeßbedingungen

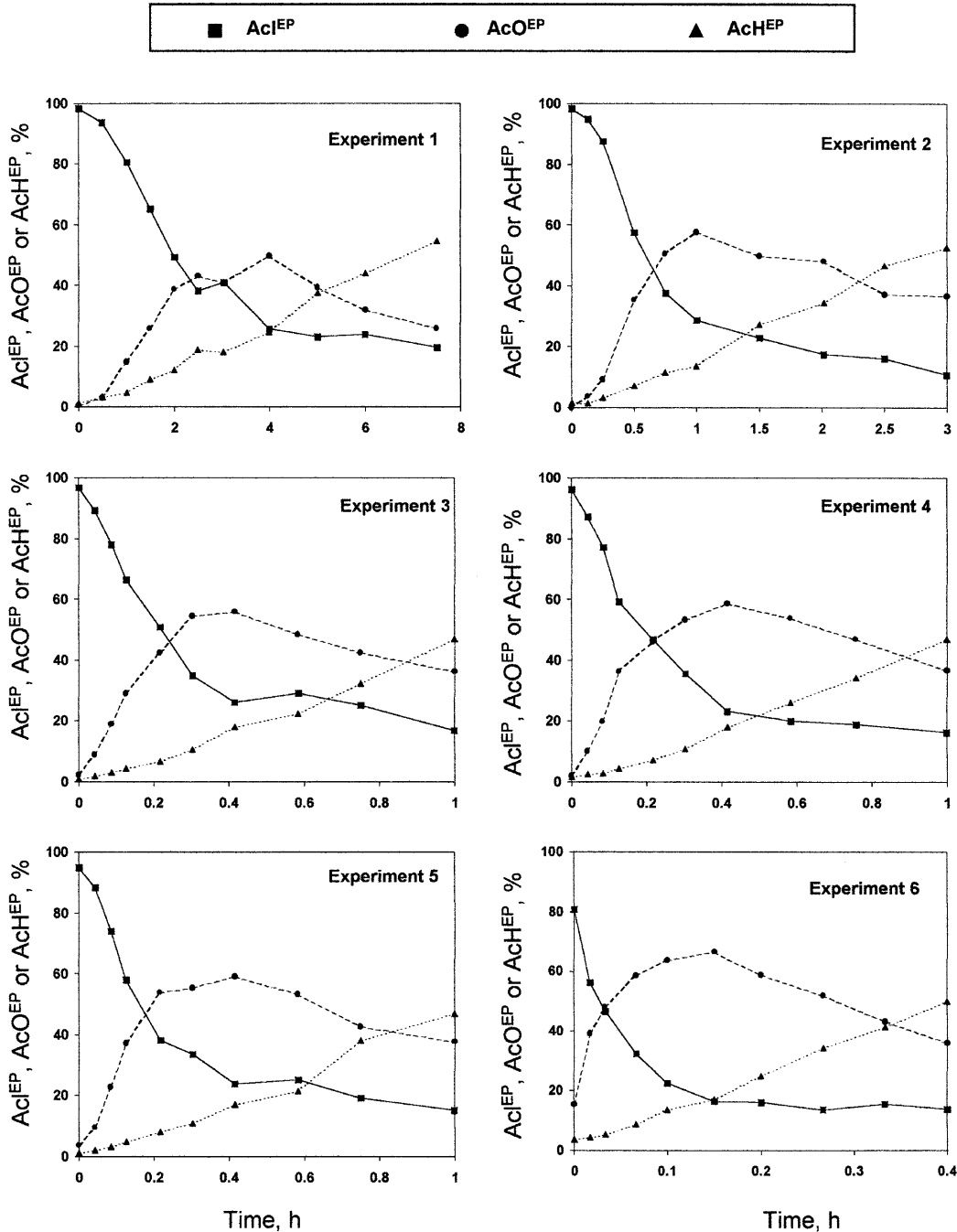| Experiment	Temperature (T, °C)	Time (t, hours)	Liquor/solid ratio (LSR, kg/kg)
1	145	0–7.5	8
2	160	0–3	8
3	175	0–1	6
4	175	0–1	8
5	175	0–1	10
6	190	0–0.4	8

Fig. 2. Percent of the initial acetyl groups bound to xylan (AcI^{EP}), to oligomers (AcO^{EP}) or converted into acetic acid (AcH^{EP}) for experiments 1 to 6 of Table 1

Bild 2. Anteil ursprünglicher Acetylgruppen in Xylan (AcI^{EP}), Oligomeren (AcO^{EP}) oder umgewandelt in Essigsäure (AcH^{EP})

acid generated at a given percent of xylan removal decreased with temperature provides qualitative information on the relative values of the activation energies of the reactions involving xylan degradation and acetyl group hydrolysis. The dependence of the acetic acid generated on the percent of xylan converted into xylooligomers or into xylose (Fig. 3b and c) showed similar general trends in all the cases, but temperature-dependent tendencies among experiments can be clearly observed.

On the basis of the interrelationships found between the xylan conversion and the acetylation degrees of residual xylan and oligomers, and considering the linear dependence of the concentrations of acetic acid on the concentration of xylose (see Fig. 3c), an empirical estimate of the acetic acid in liquors could be obtained as the sum of terms linearly dependent on amounts of residual xylan in wood, xylan converted into xylooligomers and xylan converted into xylose. According to the mathematical model used to predict the concentration profile of xylooligomers (Garrote et al. 1999b), and for calculation purposes, oligomers were divided in two fractions, with high and low polymerization degrees:
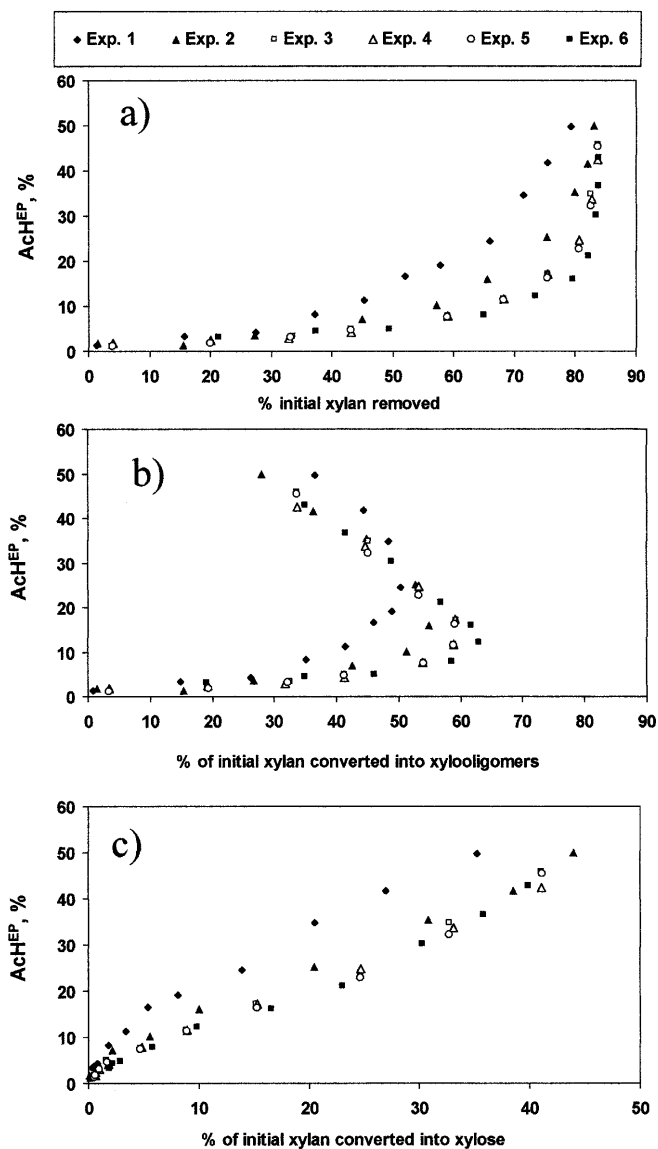
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Fig. 3a–c. Dependence of the percent of acetyl groups converted into acetic acid (AcH^{EP}) on: **a** percent of xylan solubilized from wood, **b** percent of xylan converted into xylooligomers, and **c** percent of initial xylan converted into xylose
Bild 3a–c. Abhängigkeit des Anteils an Acetylgruppen, der umgewandelt wird: **a** an löslichem Xylan; **b** an Xylan, das in Oligomere umgewandelt wird; **c** an Xylan, das in Xylose umgewandelt wird

$$ACH^{EP} = a + b \cdot PRXn + c \cdot XO_H + d \cdot XO_L + e \cdot XEP$$

(4)

where PRXn is the percent of initial xylan remaining in solid phase, XO$_H$ and XO$_L$ are the percents of initial xylan converted into high- and low-molecular weight oligomers, and XEP is the percent of the initial xylan converted into xylose.

The experimental data of AcH^{EP} and the results calculated from kinetic models for variables PRXn, XO$_H$, XO$_L$ and XEP under the same conditions led to a set of regression coefficients (a = 208.7, b = −2.0735, c = −2.0907, d = −1,0768, e = −1,5738) that allowed the

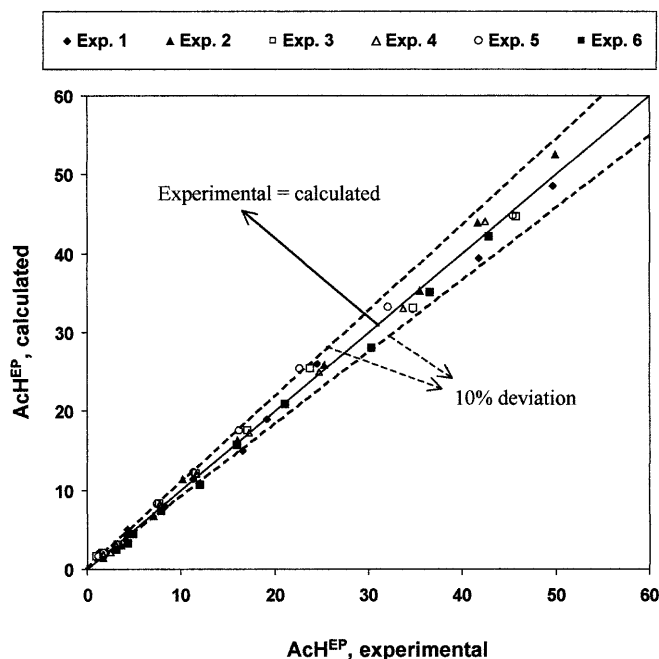**Fig. 4.** Interrelationship between experimental and calculated data of the percent of acetyl groups converted into acetic acid (AcH^{EP})
Bild 4. Beziehung zwischen experimentellen und berechneten Werten an Acetylgruppen, die in Essigsäure umgewandelt werden (AcH^{EP})

close reproduction of experimental results shown in Fig. 4 ($R^2 = 0.9950$) with a distribution of data independent from temperature. This operational procedure provides a bridge between two interconnected phenomena (xylan degradation and deacetylation) having a common physicochemical basis (catalysis by hydronium ions), which is responsible for the major effects caused by hydrothermal treatments in lignocellulosic substrates.

References

Biermann CJ, Schultz TP, McGinnis GD (1984) Rapid steam hydrolysis/extraction of mixed hardwoods as a biomass pretreatment. J Wood Chem Technol 4: 111–128
Browning BL (1967) Methods of wood chemistry. Ed. John Wiley & Sons. New York p. 356
Carrasco F, Chornet E, Overend RP, Heitz M (1987) Fractionnement de deux bois tropicaux (*Eucalyptus* and Wappa) par traitment thermomécanique en phase aqueuse. Partie II: Caractéristiques chimiques des résidus et considérations cinétiques sur la solubilisation des hémicelluloses. Can J Chem Eng 65: 71–77
Carrasco F, Roy C (1992) Kinetic study of dilute-acid prehydrolysis of xylan-containing biomass. Wood Sci Technol 26: 189–208
Conner AH (1984) Kinetic modeling of hardwood prehydrolysis. Part I: Xylan removal by water prehydrolysis. Wood Fiber Sci 16: 268–277
Conner AH, Lorenz LF (1986) Kinetic modeling of hardwood prehydrolysis. Part III: Water and dilute acetic acid prehydrolysis of southern red oak. Wood Fiber Sci 18: 248–263
Dekker RFH, Wallis AFA (1983) Autohydrolysis-explosion as a pretreatment for the enzymic saccharification of sunflower seed hulls. Biotechnol Lett 5: 311–316
Garrote G, Domínguez H, Parajó JC (1999a) Hydrothermal processing of lignocellulosic materials. Holz Roh- Werk 57: 191–202

Garrote G, Domínguez H, Parajó JC (1999b) Mild autohydrolysis: an enviromentally friendly technology for xylooligosaccharide production from wood. J Chem Technol Biotechnol 74: 1101–1109

Gosselink RJA, van Dam and Frans JEG, Zomers FHA (1995) Combined HPLC analysis of organic acids and furans formed during organosolv pulping of fiber hemp. J Wood Chem Technol 15: 1–25

Harris JF, Baker AJ, Zerbe JI (1984) Two-stage, dilute sulfuric acid hydrolysis of hardwood for ethanol production. In: Energy from biomass and wastes VIII: Symp. Papers pp. 1151–1170

Kabel MA, Schols HA, Voragen AGJ (1999) Use of enzymes and Maldi-TOF-MS in the structural characterisation of xylo-oligo-saccharides. In: 3rd Carbohydrathe Bioengineering Meeting. Newcastle Upon Tyne p. 6.13

Kubikova J, Zemann A, Krkoska P, Bobleter O (1996) Hydro-thermal pretreatment of wheat straw for the production of pulp and paper. TAPPI J 79: 163–169

Lamptey J, Robinson CW, Moo-Young M (1985) Enhanced enzymatic hydrolysis of lignocellulosic biomass pretreated by low-pressure steam autohydrolysis. Biotechnol Lett 7: 531–534

Lawther JM, Sun R, Banks WB (1996) Effects of steam treatment on the chemical composition of wheat straw. Holzforschung 50: 365–371

Lora JH, Wayman M (1978) Delignification of hardwoods by autohydrolysis and extraction. TAPPI J 61: 47–50

Maloney MT, Chapman TW, Baker AJ (1985) Dilute acid hydro-lysis of paper birch: kinetic studies of xylan and acetyl-group hydrolysis. Biotechnol Bioeng 27: 355–361

Mehlberg R, Tsao GT (1979) Low liquid hemicellulose hydrolysis of hydrochloric acid. 178th ACS National Meeting., Washington DC

Muzzy JD, Roberts RS, Fieber CA, Faass GS, Mann TM (1983) Pretreatment of hardwood by continuous steam hydrolysis. In: Wood and agricultural residues. Ed. Academic Press. London pp. 351–368

Myerly RC, Nicholson MD, Katzen R, Taylor JM (1981) The forest refinery. Chemtech 11: 186–92

Parajó JC, Vázquez D, Alonso JL, Santos V, Domínguez H (1994) Prehydrolysis of *Eucalyptus* wood with dilute sulphuric acid: operation in autoclave. Holz Roh Werk 52: 102–108

Pekarovicová A, Rybáriková D, Košík M, Fišerová M (1993) Prebleaching kraft pulp xylanases; the effect of water prehydro-lysis. Tappi J 76: 127–130

Ranganathan S, McDonald DG, Bakhshi NN (1985) Kinetic studies of wheat straw hydrolysis using sulphuric acid. Can J Chem Eng 63: 840–844

Ropars M, Marchal R, Pourquié J, Vandecasteele JP (1992) Large-scale enzymatic hydrolysis of agricultural lignocellulosic biomass. Part 1: Pretreatment Procedures. Biores Technol 42: 197–204

Schmidt AS, Thomsen AB (1998) Optimization of wet oxidation pretreatment of wheat straw. Biores Technol 64: 139–151

Weil J, Sarikaya A, Rau S-L, Goetz J, Ladisch CM, Brewer M, Hendrickson R, Ladisch MR (1997) Pretreatment of yellow poplar sawdust by pressure cooking in water. Appl Biochem Biotechnol 68: 21–40

Zanuttinni M, Citroni M, Martínez MJ, Marzocchi V (1998) Chemimechanical pulping of poplar wood: pretreatment at low temperature. Holzforschung 52: 405–409

Zanuttinni M, Marzocchi V (1997) Kinetics of alkaline deacety-lation of poplar wood. Holzforschung 51: 251–256