DETAILED MASS BALANCES OF THE AUTOHYDROLYSIS OF *EUCALYPTUS GLOBULUS* AT 170°C

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Autohydrolysis of *Eucalyptus globulus* was conducted at three different intensity levels typical for prehydrolysis kraft pulping as utilized for manufacturing dissolving pulp grades. The objective was to establish for the autohydrolysis process a detailed mass balance comprising the chemical composition of all three phases: the autohydrolysate, the released gas, and the solid residue. Carbohydrate determination involved both acid methanolysis combined with gas chromatography (GC) and sulfuric acid total hydrolysis with high performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD); this allowed reliable quantification of neutral as well as acidic sugar units in cellulosic and non-cellulosic polysaccharides. Uronic acids present in the *Eucalyptus globulus* wood were progressively degraded through decarboxylation, leading to substantial carbon dioxide formation. The degree of acetylation of xylan remaining in the wood residue was clearly reduced, while the amount of bound acetyl groups in dissolved xylo-oligosaccharides (XOS) stayed relatively constant as a function of autohydrolysis intensity. The bulk of the lignin that was dissolved during autohydrolysis could be attributed to the acid-soluble lignin content of the wood. Only small amounts of Klason lignin were dissolved.

Keywords: Prehydrolysis kraft; Dissolving pulp; Biorefinery; Uronic acid; CO_2; Decarboxylation; Xylan; Xylooligosaccharides

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INTRODUCTION

Fractionation of biomass is a research topic of great current interest. Auto-hydrolysis is one possibility to selectively separate hemicelluloses from lignin and cellulose during biomass pretreatment. Autohydrolysis is applied on an industrial scale for the production of dissolving pulps based on the prehydrolysis kraft process (PHK). Presently only steam prehydrolysis is practiced, whereby the hemicellulose degradation products are neutralized in a subsequent alkaline extraction step and burned together with the removed lignin. Problems in the processability of the autohydrolysis liquor related to hard-to-control precipitates of lignin have prevented its commercialization so far (Annergren et al. 1965; Leschinsky et al. 2007; Leschinsky et al. 2008b; Sixta 2006; Stanciu 1987). If these problems could be solved by innovative process management, the prehydrolyzate would represent an interesting and available source for by-product

generation. Subsequent to prehydrolysis, soda anthraquinone (AQ) pulping can replace kraft pulping without any impairment of the pulp quality (Lin 1979). Thus water prehydrolysis followed by soda AQ pulping represents a realistic sulfur-free biorefinery concept.

Autohydrolysis Yield

The yield of hemicellulose degradation products obtained from autohydrolysis pretreatment of biomass greatly determines the efficiency of the process. The additional expenses for the by-product separation must be justified by the amount and value of the products recovered from the autohydrolysate.

The yield of hemicellulose degradation products during autohydrolysis predominantly depends on the wood species and the intensity of the treatment. The latter is determined by time and temperature and can be expressed as a single variable, the prehydrolysis factor (P-factor), which allows the comparison of autohydrolysis at different temperatures (Sixta 2006). With the raising of the P-factor, increased amounts of hemicelluloses are removed from the wood. The xylan removal from hardwoods can be kinetically modeled on the assumption of two types of xylan that differ in their reactivity towards hydrolysis, one being more and one less reactive; the two types of xylan become hydrolyzed in parallel pseudo-first order reactions: one fast and one slow (Conner 1984). The xylan is dissolved into the autohydrolysis liquor in the form of oligosaccharides, which are then further degraded to xylose and furfural, as well as to other unknown degradation products in consecutive reactions (Conner and Lorenz 1986). When material balances of the process are established, the total amount of analytically detectable xylan degradation products in the hydrolyzate reaches a maximum before the degradation to unknown products results in yield losses. The maximum yield obtainable and the reaction time at which the formation of unknown products becomes significant are dependent on the wood species and on the reaction conditions. High temperatures and short reactions are favorable for attaining high yields of dissolved xylan (Conner and Lorenz 1986; Sixta et al. 1992). For beech wood prehydrolysis at 170°C, a maximum concentration of dissolved xylan was attained at a P-factor 1000 (Sixta et al. 1992). The relative proportions of xylo-oligosaccharides (XOS), xylose, and furfural in the hydrolyzate also depend on the reaction conditions. Since the activation energies of the complex consecutive xylan degradation reactions differ, highly complex kinetic models are necessary to predict the composition of the hydrolyzate (Garrote et al. 1999; Nabarlatz et al. 2004).

The yield of hemicellulose degradation products are, in addition, influenced by the liquor-to-wood ratio (L/W) and by the particle size of the biomass. Whether the L/W ratio has an influence on the xylan degradation rate or not is controversially discussed in the literature (Carrasco and Roy 1992). A significant decrease in the xylan dissolution rate during autohydrolysis of *Eucalyptus saligna* wood chips at 170°C was observed by Schild (1994) when reducing the L/W ratio from 5:1 to 3.5:1 and 2:1. During dilute sulfuric acid hydrolysis, variation of the L/W ratio between 3.6:1 and 1.3:1 influenced the xylan removal only when using large wood particles of 2.45 mm thickness, while no effect was found when using wood particles of 0.38 mm thickness (Springer 1985). Garrote et al. concluded from autohydrolysis studies with *E. globulus* chips passing

through an 8 mm screen that variation of the L/W ratio between 10:1 and 6:1 had a negligible influence. One possible reason for the contradictory results might originate from the experimental setup; whether or not the wood residue is extensively washed plays an important role. The strong impact of the L/W ratio on xylan dissolution found by Schild (1994) might be explained by the high amounts of hydrolysate remaining in the wood pores because washing was omitted. On an industrial scale, however, washing of the wood residue before kraft pulping would be a costly operation. A significant influence of the particle size on the yield of sulfuric acid catalyzed prehydrolysis was found by Springer (1985), which was explained by the differences in the impregnation of the wood with the catalyst. Recently, Song et al. (2008) found a drastic influence of the particle size on the yield of autohydrolysis of spruce wood. The yield of dissolved products was reduced by 40-50% when using wood chips instead of 1 mm screened ground wood. Diffusion effects are a possible explanation for the particle size influence.

Product Composition

The degree of polymerization (DP) of the released hemicellulose degradation products is important for their potential usage. The extraction of hemicellulose polysaccharides using autohydrolysis processes is difficult. Although hemicelluloses with a high molecular weight of up to 35 kDa were found in a spruce hydrolyzate at extremely low autohydrolysis intensities, they were degraded to monosaccharides and oligo-saccharides as soon as higher yields were obtained by raising the autohydrolysis intensity (Song et al. 2008). Glasser et al. (2000) concluded from investigations on steam explosion that autohydrolysis processes are not appropriate for the isolation of hemicellulose polysaccharides and proposed alkaline extraction of biomass as an alternative. Kabel (2002) reported a maximum DP of the released XOS in the autohydrolysate of *E. globulus* of 25 as determined by HPSEC calibrated with pullulan standards and of 12 as determined by MALDI-TOF.

As expected, the molar composition of the 4-*O*-methylglucuronoxylan in both the wood residue and in the autohydrolysate changes during the course of autohydrolysis. The degree of substitution (DS) of native hardwood-xylan with 4-*O*-methylglucuronic acid (MeGlcA) is dependent on the wood species (Fengel and Wegener 1983; Springer and Zoch 1968), however, within the same species, strong variation of the DS can occur. Values between 1 and 2.1 MeGlcA groups per 10 xylose units have been reported for *E. globulus* (Evtuguin et al. 2003; Fengel and Wegener 1983; Wallis et al. 1996; Willför et al. 2005). Recently, Tunc and VanHeiningen (2008a) reported a decrease in the uronic acid (UA) to xylose ratio in the wood residue from 2.2 to 1 UA per 10 xylose units during the course of hardwood autohydrolysis at 150°C, which is in contradiction to the results of Bernardin (1958), who could not find a significant variation of the xylan to UA ratio over the course of prehydrolysis of black gumwood at 160°C. In mass balances of hardwood autohydrolysis at various temperatures the amount of UA detected in the hydrolyzate was lower than the amount of UA removed from the wood (Tunc and VanHeiningen 2008b). It was concluded that degradation of uronic acids occurred at autohydrolysis for 100 minutes at temperatures ≥ 150°C. The same conclusion was made by Korte et al. (1991), who reported decreasing concentrations of MeGlcA in hemicelluloses extracted from steamed birch wood while increasing the steaming

temperature. Decarboxylation of uronic acids under autohydrolysis conditions is a probable explanation for their degradation. Decarboxylation of uronic acids in HCl can be used as analytical technique for pectin determination (Nanji et al. 1925). Complete degradation of galacturonic acid occurred within 80 hours when refluxing pectic materials with water (Anderson et al. 1961). The degree of MeGlcA substitution of xylan is of particular interest, since a correlation to the xylan removal rate during autohydrolysis was reported. MeGlcA groups should stabilize the xylan chain against hydrolytic attack, thus lowering the rate of xylan degradation (Conner 1984; Springer and Zoch 1968).

In most studies, the uronic acids in wood and hemicelluloses have been quantified by colorimetric methods (Scott 1979) or decarboxylation methods (Browning 1949; Conner 1984; Springer and Zoch 1968; Tunc and VanHeiningen 2008a; Tunc and VanHeiningen 2008b). With these methods, however, a distinction of the different uronic acids present in wood, namely MeGlcA, galacturonic acid (GalA), and glucuronic acid (GlcA) is not possible (Jayme and Hahn 1960; Li et al. 2007). In contrast, acid methanolysis allows distinct quantification of the different uronic acid types (Sundberg et al. 1996).

The XOS released during autohydrolysis of hardwoods are acetylated to a great extent. Only part of the acetyl groups present in the original wood is released as free acetic acid in the hydrolyzate. The degree of acetylation of the xylan remaining in the wood residue is reduced only slightly in the course of autohydrolysis (Garrote et al. 2001; Tunc and VanHeiningen 2008a). The amount of acetyl groups attached to xylan and XOS present in wood and autohydrolysate, respectively, is generally determined by HPLC subsequent to sulfuric acid hydrolysis, but the results have never been compared to an alternative method.

In the present study, detailed mass balances were established from three autohydrolysis experiments conducted at conditions typical for the PHK process comprising P-factors of 300, 600, and 1500 at a maximum temperature of 170°C and a L/W ratio of 5:1. Particular attention was paid to the analytical techniques: Carbohydrate analysis was performed using both sulfuric acid total hydrolysis (TH) combined with HPAEC-PAD, and acid methanolysis combined with GC, allowing quantification of neutral sugars and uronic acids. Acetyl groups were analyzed using two different methods: HPLC separation subsequent to TH and oxalic acid hydrolysis with GC. In addition, carbon dioxide (CO_2) formation was quantified in the gas phase released during the reaction. The present study is related to an investigation of the effect of autohydrolysis on the lignin structure under the same reaction conditions (Leschinsky et al. 2008a,b).

EXPERIMENTAL

Wood Chips

E. globulus wood chips from plantations in Uruguay, supplied by ENCE, were ground with a Retsch-type mill and fractionated. The fractionated particles which measured between 2.50 and 3.55mm were collected.

Autohydrolysis

Autohydrolysis was conducted in a lab-scale Parr reactor station equipped with mechanical stirring and a reactor volume of 450 ml. 50 grams of wood was placed inside the reactor together with enough deionized water to achieve a L/W ratio of 5:1 (w/w). After heating the reactor to 170° C within 20 minutes, the temperature was maintained until the desired prehydrolysis factor (P-factor) was reached. The P-factor was calculated from the recorded temperature/time data using an activation energy of 125.6 kJ/mol for the xylan removal (Sixta 2006). One hour of isothermal autohydrolysis reaction at 170°C corresponds to a P-factor of 603.7.

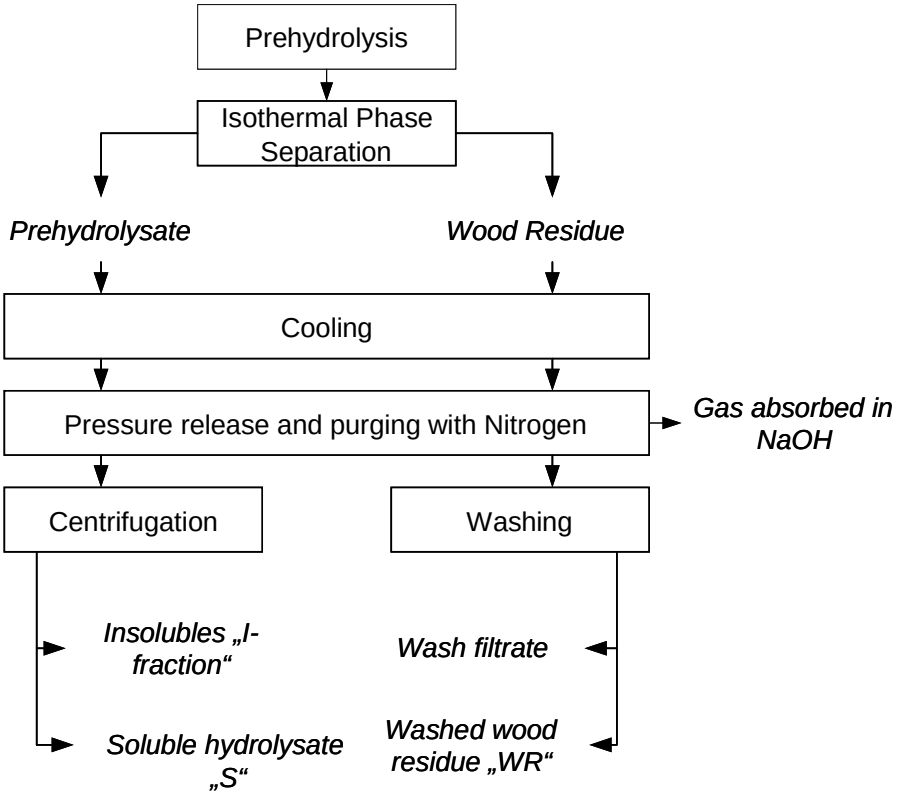

Fig. 1. Diagram of the fractionation subsequent to prehydrolysis

Subsequently, the autohydrolyzate was separated from the wood residue by displacement with nitrogen gas to a preheated, second reactor containing saturated steam with a temperature of 170°C. During this separation step, no temperature or pressure drop occurred. The aim of this isothermal phase separation was to recover all compounds dissolved in the 170°C autohydrolyzate, particularly those that were insoluble at lower temperatures. Both reactors were immediately cooled to below 30°C with ice water after the phase separation step. The pressure of the cooled reactors was then released, and the gas which emerged was absorbed in a caustic soda solution. The system was purged with N_2 gas in order to capture the entire gas phase present in the reactors. Contained within the cold crude autohydrolyzate was an insoluble fraction, causing turbidity. This insoluble fraction (I) was separated from the soluble part of the autohydrolyzate (S) by centrifugation for 15 minutes at 5000 revolutions per minute (rpm). The wood residue

was then washed with 2 liters of 70°C deionized water. An overview of the fractionation procedure is depicted in Fig. 1. The fractions gas (G), soluble hydrolyzate (S), insolubles within the hydrolyzate (I), washed wood residue (WR), and the wash filtrate (WF) were quantitatively collected and analyzed. Material balances were established from the obtained results.

Analysis

Carbohydrates

Sugar monomers in the S-fraction were determined by direct application of a high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (Sixta et al. 2001). The total carbohydrate composition of S, WR, and the original wood were investigated using two different methods, allowing for quantification of neutral sugars and uronic acids: Acid methanolysis with GC-FID detection according to Sundberg et al. (1996) and sulfuric acid total hydrolysis (TH) with HPAEC-PAD. A two-stage sulfuric acid hydrolysis was used for the solid samples (WR and original wood), whereas the S-fraction was hydrolyzed with 4% H_2SO_4 at 105°C for 50 minutes before HPAEC analysis to avoid extensive carbohydrate degradation. Furfural and hydroxymethylfurfural (HMF), which are formed during TH from pentoses and hexoses, were quantified and added to the results for xylose and glucose, respectively. Determinations by methanolysis/GC-FID and TH/HPAEC-PAD were performed at least thrice and twice, respectively.

The amount of sugar oligomers contained in S was calculated by subtracting the monomer content from the results determined after methanolysis or TH.

Neutral XOS were quantified by HPAEC-PAD up to a DP of 5 (Griebl et al. 2006). XOS with a DP higher than 5 could not be quantified due to a lack of commercially available standards.

Furfural and hydroxymethylfurfural (HMF) in S and in the acid hydrolyzates were measured by HPLC on a Hypersil ODS column with UV detection (277 nm) using 14% (v/v) acetonitrile as eluent at a temperature of 65°C.

Size exclusion chromatography

Size-exclusion chromatography (SEC) of the S-fractions was performed as described by Sixta et al. (2001) on two PSS MCX columns (1000 Å, 300*8 mm) with 0.5 M NaOH as mobile phase at a flow rate of 1 ml/minute, refractive index (RI) and viscosimetric detection, allowing universal calibration. Calibration was carried out with a set of cello-oligomers and pullulan standards.

Acetic acid and acetyl groups

The concentration of acetic acid in S was determined by HPLC on a Rezex ROA column with RI detection using 0.005M H_2SO_4 as eluent at a temperature of 65°C. The acetyl groups in S, WR, and in the native wood were determined with two different methods: Acetic acid was measured by HPLC subsequent to TH. In addition, acetic acid was quantified by GC-FID subsequent to oxalic acid hydrolysis (OxA) according to Solar et al. (1987).

Carbon analysis

Total organic carbon (TOC) analyses of S and of WF were performed according to EN 1484. The concentration of the wood degradation products in WF was calculated from the ratio of the TOC values of S and WF, suggesting an identical composition at different concentration levels of the two liquors. Total inorganic carbon (TIC) analysis according to EN 1484 was used to determine the amount of CO_2 in the gas phase that was absorbed in 25 g/l NaOH during pressure release and N_2-purging of the reactors.

Lignin

Klason lignin and acid soluble lignin (ASL) were determined according to TAPPI T222 om 98 and TAPPI UM 250, respectively, in the original wood and in the WR. The WR was not extracted prior to the Klason determination due to the increased solubility of lignin in organic solvents after prehydrolysis. The lignin concentration in S was estimated similar to that of ASL from the UV/VIS spectra, which was recorded in a 3% H_2SO_4 solution using an absorption coefficient of 110 l/(g*cm) at 205 nm (TAPPI 1991).

RESULTS AND DISCUSSION

Comparison of Analytical Techniques

Comparison of the results for carbohydrate content and composition in the solid samples obtained by TH and methanolysis, as depicted in Table 1, revealed that the latter technique gave lower results for all neutral sugars as compared to TH, except for galactose, arabinose, and rhamnose. It is well known that crystalline cellulose is not degraded by methanolysis (Sundberg et al. 1996). Xylan, the major hemicellulose in the samples, was obviously not fully cleaved by methanolysis. Sundberg et al. (1996) also found a slightly higher xylan content in spruce fibers when using TH compared to methanolysis. The highest sugar contents obtained from either TH or methanolysis were used for establishing the mass balances.

In contrast to the solid samples, methanolysis revealed higher concentrations for all sugars present in the liquid samples as compared to the results obtained after acid hydrolysis (Table 2). Although a mild one-step hydrolysis was chosen for the S-fractions and furfural formation was considered, degradation reactions led to an analytical error of 10% for XOS determination. Thus, only the methanolysis results of S were used for the material balances. The uronic acids in all samples were determined by methanolysis both in liquid and solid samples.

Determination of acetyl groups in wood by sulfuric acid TH is believed to give excessive values, due to the formation of acetic acid from sugar degradation (Roudier and Nick 1962; Solar et al. 1987). In the present study, the acetyl group contents determined after TH were confirmed using oxalic acid hydrolysis. The two methods gave comparable results for solid as well as for liquid samples (Tables 1 and 2).

Table 1. Comparison of Carbohydrate and Acetyl Group Contents of Solid Samples Obtained with Different Analysis Techniques

% oven dry weight	*Method*	Glc	Xyl	Man	Ara	Gal	Rha	GalA	MeGlcA	GlcA	*Method*	OAc
Original Wood	*TH*	41.7	15.3	0.9	0.4	1.2	0.3				*TH/HPLC*	3.3
	*ME**	3.2	13.5	0.6	0.5	1.3	0.4	1.7	2.2	0.4	*OxA/GC*	3.2
WR P 300	*TH*	53.4	7.8	0.8	0.1	0.3	0.1				*TH/HPLC*	1.5
	*ME**	5.7	6.2	0.5	0.0	0.5	0.1	0.6	0.8	0.1	*OxA/GC*	1.6
WR P 600	*TH*	55.0	5.7	0.8	0.1	0.1	0.0				*TH/HPLC*	1.0
	*ME**	5.9	4.1	0.4	0.1	0.4	0.1	0.4	0.4	0.1	*OxA/GC*	1.0
WR P 1500	*TH*	55.9	4.1	0.6	0.0	0.1	0.0				*TH/HPLC*	0.6
	*ME**	5.6	2.7	0.3	0.0	0.2	0.0	0.2	0.2	0.0	*OxA/GC*	0.7
All results expressed as anhydrosugars or anhydro-acetic acid. ME = methanolysis												

Besides the carbohydrates and acetyl groups, the original wood contained 22.9% of Klason lignin, 4.8% of acid soluble lignin, 1.7% of Toluol/EtOH and EtOH extractives and 0.4% of ash. In total 97.5% of the wood substance could be specified.

Table 2. Comparison of Total Sugar and Acetic Acid Contents of Liquid Samples Obtained with Different Analysis Techniques

mg/kg of hydrolyzate	*Method*	Xyl	Glc	Man	Ara	Gal	Rha	GalA	MeGlcA	GlcA	*Method*	AcOH
S-fraction P 300	*TH*	17770	527	502	703	1706	351				*TH/HPLC*	5318
	*ME**	19471	912	676	738	1936	654	489	1925	40	*OxA/GC*	5270
S-fraction P 600	*TH*	20622	665	591	567	1922	444				*TH/HPLC*	6467
	*ME**	22276	1154	843	700	2132	724	290	1131	15	*OxA/GC*	6391
S-fraction P 1500	*TH*	17391	675	725	450	1700	425				*TH/HPLC*	6821
	*ME**	18026	1181	989	607	1856	589	115	141	0	*OxA/GC*	6629
All results expressed as concentrations of the monomers. ME = methanolysis												

Mass Balances

Dissolution and degradation of xylan

All xylan contained in the untreated wood could be detected in the various fractions in the form of xylan, XOS, xylose, and furfural at P-factors of 300 and 600 (Fig. 2). XOS were the dominant products dissolved at these P-factors. They were gradually degraded to xylose and furfural with increasing P-factors, as demonstrated for P-factor 1500. Under these conditions, losses of 5% of the xylan contained in the untreated wood occurred in the mass balance due to degradation to unknown products. This coincides with the findings of Tunc and VanHeiningen (2008b), who detected an imbalance between the amount of carbohydrates removed from the wood and the amount determined in the liquid phase when exposing southern mixed hardwoods to a temperature of 170°C for 100 minutes.

Table 3. Yields of Xylan Degradation Products Based on the Total Xylan Content in the Wood

P-factor	Furfural	X1	X2	X3	X4	X5	XOS > DP 5 + acidic XOS (estimation by difference to total)	Total
300	1.2%	4.8%	3.7%	3.4%	4.0%	3.3%	43.8%	64.4%
600	4.2%	14.8%	10.6%	8.8%	8.8%	6.2%	22.5%	75.9%
1500	15.6%	37.0%	11.9%	6.0%	3.7%	1.7%	-0.1%	75.7%
Yield of products calculated as anhydroxylose based on the xylan content in the original wood								

The dissolved neutral XOS could be quantified up to a DP of 5 (Table 3). XOS that were substituted with MeGlcA (acidic XOS) and XOS with a DP higher than 5, could only be estimated by the difference to the total amount of XOS. At the lowest P-factor, the majority of the dissolved xylan was in the form of acidic XOS and XOS > DP 5. At P-factor 1500, acidic XOS and XOS with DP > 5 could not be detected in the hydrolyzate by the difference. However, HPAEC chromatographs revealed the presence of XOS up to a DP of 7 in extremely low concentrations. The determination of MeGlcA by methanolysis also revealed the presence of small amounts of acidic XOS at P-factor 1500 (Table 2). The estimation of acidic XOS and XOS > DP 5 by the difference to total was not completely precise due to the summation of analytical errors.

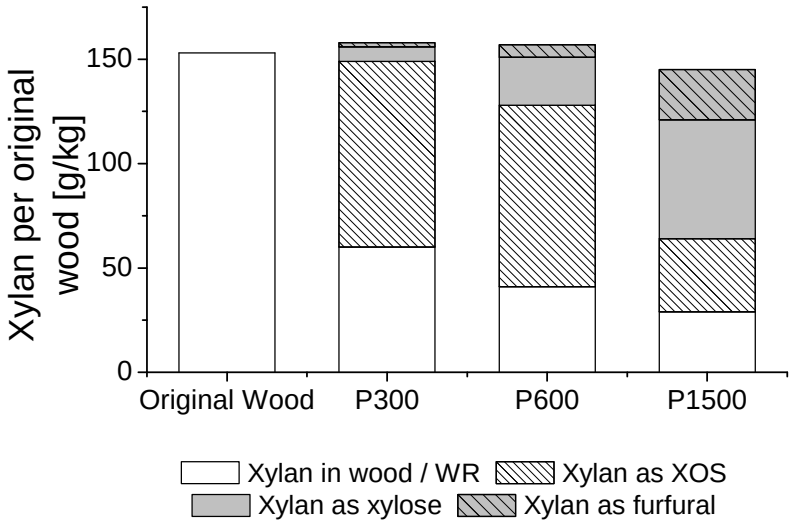

Fig. 2. Mass balances of xylan and its degradation products as a function of autohydrolysis intensities (P-factor)

The SEC chromatograms of the S-fractions depicted in Fig. 3 revealed broad multimodal distributions for all fractions. The low molecular weight peak around 200 g/mol could be assigned to sugar monomers. The weight-average molecular weights (Mw) were calculated from the RI-signal, suggesting that XOS built of anhydroxylose are the main constituent of the S-fractions. Average DPs of 11, 6, and 3 could be calculated for the P-factors 300, 600, and 1500, respectively. These values are, however, only

approximations because all constituents other than anhydroxylose are included in chromatograms. Nevertheless, the results concur with those from the XOS determination by HPAEC given in Table 3.

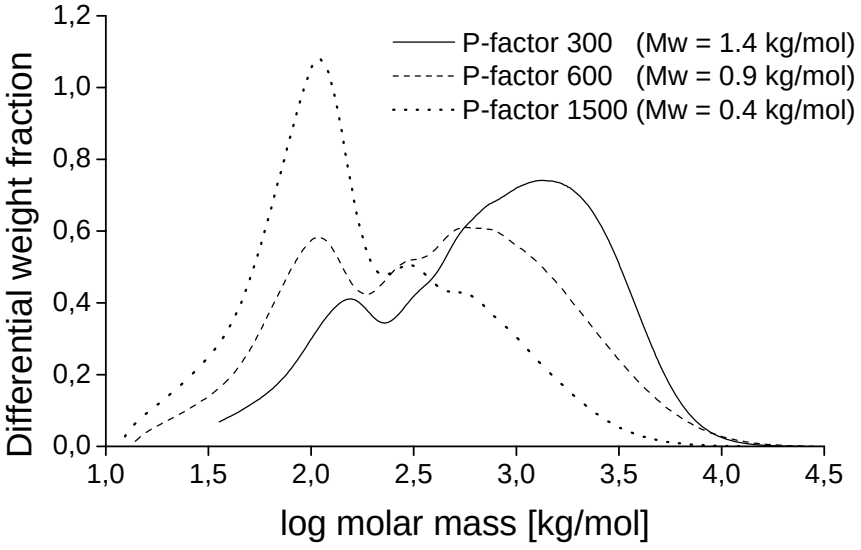

Fig. 3. Size-exclusion chromatograms of S-fractions

Although significant amounts of xylan were removed from the wood when increasing the P-factor from 600 to 1500 (Fig. 2), the total yield of detectable dissolved xylan degradation products was slightly reduced. The maximum yield of dissolved xylan was obtained at P-factor 600, representing 75.9% of the xylan originally contained in the wood. The yields obtained in the present study can be described as theoretical yields because they include the wash filtrate. Extensive washing of the wood residue would be unrealistic in an industrial process, and consequently significantly lower effective yields can be expected. Reduction of the L/W ratio, which is required for industrial PHK autohydrolysis, further reduces effective yields of hydrolyzates. XOS and xylose are the main products that could be recovered in practice.

Degradation of acetyl groups

Acetyl groups, which are bound to the xylan in the native wood, were removed to a similar extent as the xylan from the WR in the course of autohydrolysis (compare Figs. 2 and 4). At P-factor 300, 64% of the acetyl groups and 61% of the xylan were removed from the wood, at P-factor 600, 77% acetyl groups and 73% xylan, and at P-factor 1500, 86% acetyl groups and 81% xylan were removed. Acetyl groups can be removed from the wood by two different reaction pathways: by direct hydrolysis of acetyl groups from the solid xylan backbone and by dissolution of acetylated XOS (Maloney et al. 1985). The molar ratio of acetyl groups per 10 xylose units was clearly reduced in the wood residue during the course of autohydrolysis, indicating that deacetylation proceeds somewhat faster than xylan dissolution at 170°C (Table 4). It can be concluded that direct hydrolysis of acetyl groups plays a significant role at this temperature. In a kinetic study of Garrote et al. (2001), the degree of acetylation of the xylan in the *E. globulus* wood

residue was reduced by maximum 20% even after prolonged autohydrolysis at various temperatures. In the present study the degree of acetylation of the xylan remaining in the wood at P-factor 1500 accounted for 73% of its original value. In contrast, Tunc and VanHeiningen (2008b) could not observe any decrease of the acetylation degree of the residual wood xylan when applying autohydrolysis of mixed southern hardwoods for 100 minutes at 170°C. However, slight deacetylation of the residual wood xlyan was reported by the same authors at an autohydrolysis temperature of 150°C (Tunc and VanHeiningen 2008a).

The free acetic acid in the hydrolyzate originates either from direct deacetylation of the xylan in the wood or from deacetylation of XOS dissolved in the hydrolyzate. Garrote et al. (2001) reported that the amount of free acetic acid in the hydrolyzate depends on the amounts of residual xylan in the wood and xylan converted into high and low molecular weight XOS as well as into xylose. The amount of acetic acid in the hydrolyzate can thus be predicted, considering all these factors, by using multiple regression analysis. The mass balances depicted in Fig. 4 show that only small amounts of free acetic acid were released into the hydrolyzate in the beginning of the reaction. The major part of the dissolved acetyl groups was attached to the XOS. Larger amounts of free acetic acid were formed at P-factor 1500, when most of the XOS were degraded to xylose and furfural. The amount of free acetic acid is, thus, related to the number of cleavage of glycosidic bonds in XOS. This relationship is depicted in Fig. 4, where the amount of free acetic acid is correlated to 1/Mw of the S-fractions.

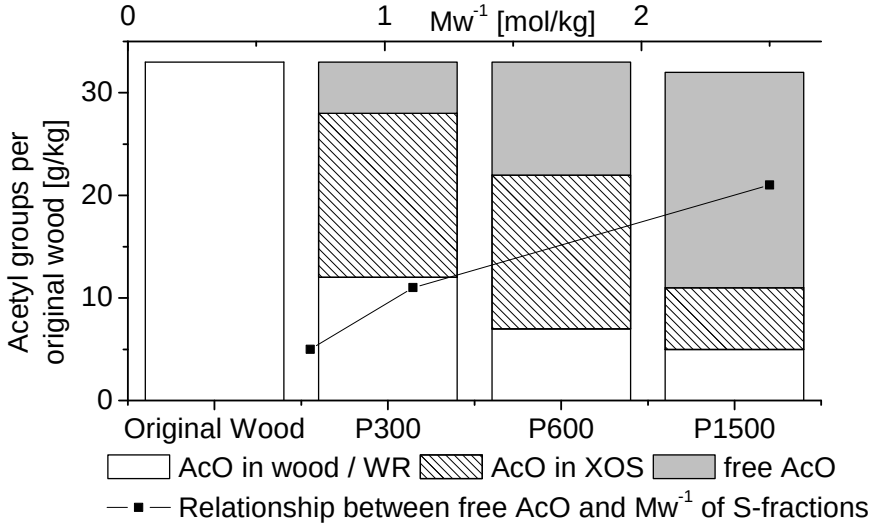

Fig. 4. Mass balances of the acetyl groups and correlation between free AcO and molecular weight of dissolved xylan

The degree of acetylation of the XOS remained relatively constant during the reaction at a slightly lower level than in the native wood (Table 4). In the study of Tunc and VanHeiningen (2008a), the XOS released during autohydrolysis of mixed southern hardwoods at 150°C were initially acetylated to the same extent as the original wood xylan (6 acetyl groups per 10 xylose units) and showed a constant degree of acetylation of 3 acetyl groups per 10 xylose units at reaction times between 100 and 500 minutes. In

contrast, Garrote et al. (2001) found that the degree of acetylation of XOS released during *E. globulus* autohydrolysis increased with the reaction time. Reasons for the considerably different results could not be identified.

Table 4. Molar Amounts of Acetyl Groups per 10 Xylose Units in the Xylan Degradation Products

P-factor	Wood Residue	XOS
0	6.7	-
300	6.0	5.6
600	5.6	5.3
1500	4.9	5.7

Degradation of uronic acid groups

The major uronic acid groups in the wood were MeGlcA, a side group of the xylan backbone, and GalA, which originated from pectins in the wood (Table 1). Both uronic acids were degraded in the course of autohydrolysis (Fig. 5). GlcA was also degraded, but is not depicted in Fig. 5 because of its negligible concentration in the wood. Part of the uronic acids were first dissolved into the hydrolyzate at P-factors 300 and 600 and were almost completely decarboxylated at P-factor 1500. Losses of uronic acids in mass balances of autohydrolyses of a mixture of southern hardwood were reported by Tunc and VanHeiningen (2008b): 100 minutes of autohydrolysis at 170°C corresponding to a P-factor of 1000 resulted in an uronic acid loss of approximately 60%. In our study, the uronic acid losses were even higher, with 67% at P-factor 600 and 90% at P-factor 1500.

In Fig. 5, both the molar amounts of CO_2 formed and the molar amounts of uronic acids remaining in the mass balance are depicted. The amount of CO_2 formed corresponded to the amount of uronic acids degraded, revealing that uronic acid degradation during autohydrolysis occurred through decarboxylation. The amounts of CO_2 formed can disturb the down-flow of prehydrolysis liquor during continuous PHK cooking and result in an increased reactor pressure (Annergren et al. 1965).

Table 5. Molar Amounts of MeGlcA per 10 Xylose Units in the Xylan Degradation Products

P-factor	Wood Residue	XOS
0	1.00	-
300	0.67	0.77
600	0.45	0.46
1500	0.27	0.15

MeGlcA was dissolved into the hydrolyzate while being bound to XOS. Monomeric MeGlcA in the hydrolyzate could not be detected. The molar ratio of MeGlcA per 10 xylose units reduced from one in the original wood to 0.27 in the WR at P-factor 1500 (Table 5). The average degree of substitution of dissolved XOS with MeGlcA was also gradually decreased with increased P-factors.

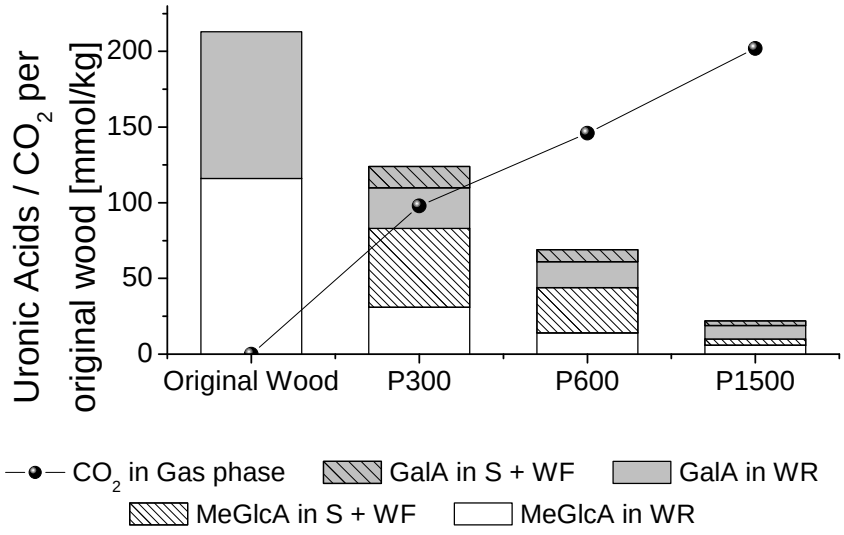

Fig. 5. Mass balances of uronic acid groups and of CO_2

Dissolution of lignin

Lignin is degraded and dissolved to a significant extent during autohydrolysis. Part of the dissolved lignin precipitates during cooling of the hydrolyzate. This lignin fraction was separated from the hydrolyzate by centrifugation and denoted as an I-fraction. The I-fractions formed at different P-factors were investigated in detail in another study (Leschinsky et al. 2008a). The I-fractions turned out to be pure lignin degradation products with low sugar content.

The bulk of the lignin that was dissolved remained in the S-fraction even after centrifugation. Quantification of the dissolved lignin for the purpose of mass balances is difficult because of a lack of reliable analytical methods: UV/Vis spectrometric methods are generally used to quantify lignin in solution using wavelengths of 205 nm (TAPPI UM 250) or 280 nm (Lin 1992). At 280 nm furfural and HMF strongly interfere with the lignin absorption because their specific absorption coefficients are more than tenfold higher than for lignin. At 205 nm, furfural and HMF absorption plays only a minor role; therefore the lignin concentration was measured at this wavelength. Since the lignin structure is heavily altered during autohydrolysis, it is uncertain if the absorption coefficient at any wavelength remains constant. The results of the soluble lignin determination, stated in Table 6, should thus be considered as approximate.

The removal of wood extractives using Soxhlet extraction with toluene and ethanol prior to Klason lignin determination is important to obtain accurate results. However, a substantial part of the lignin contained in WR dissolves in organic solvents after autohydrolysis. The Soxhlet extraction was therefore omitted prior to Klason lignin determination of WR and also of the original wood to allow for comparable results. The lignin contents of the original, extractive free wood, which are stated in Table 6 in parentheses, are, as expected, lower than those of the wood which was not extracted. The presence of extractives was obviously the reason for excessive Klason lignin values, which resulted in higher total amounts of lignin in the mass balance.

With rising autohydrolysis intensity, the amount of ASL in WR was clearly reduced, while the amount of soluble lignin in S and WF was slightly increased. The amount of ASL, which was removed from the wood, corresponded well to the amount of soluble lignin detected in S and WF at all P-factors. It can be concluded that ASL was dissolved into the hydrolyzate during prehydrolysis and remained dissolved after cooling and centrifugation of the hydrolyzate. Klason lignin dissolved in a small amount of 5-6% of the primary lignin content in the wood at P-factors 300 and 600. A slight increase in the amount of Klason lignin at P-factor 1500 was observed, which can probably be explained by condensation reactions of dissolved lignin, sugar degradation products, or polyphenolic extractives. The amount of dissolved Klason lignin corresponded to the amount of insoluble lignin (I-fraction) detected in the hydrolyzate. Hence, the I-fraction originated most likely from dissolved Klason lignin. The I-fraction revealed structural features, which clearly demonstrated its origin as a degradation product of native lignin (Leschinsky et al. 2008a,b).

Table 6. Mass Balances of Lignin

P-factor	Original wood and WR		I-fraction	Soluble lignin in S + WF	Total Lignin
	Klason lignin	ASL			
0	243 (229)*	52 (48)*	-	-	294 (277)*
300	231	19	11	32	293
600	230	15	13	35	292
1500	239	11	11	36	296
All values given in g per kg of the original wood; * Soxhlet extraction prior to analysis					

CONCLUSIONS

1. Combination of methanolysis/GC and total hydrolysis/HPAEC was shown to be useful for establishing detailed mass balances of the autohydrolysis process. Acetyl group determination using total hydrolysis/HPLC and oxalic acid hydrolysis/GC delivered similar results.
2. At low and medium P-factors of 300 and 600, the dissolved main products were XOS with approximate average DPs of 11 and 6, respectively. At P-factor 1500, significant formation of furfural occurred and xylose was the main product. Degradation to unknown products led to a reduced overall xylan yield.
3. Although the major portion of acetyl groups was removed from the wood in the form of acetylated XOS, direct deacetylation of the wood xylan played a role at a temperature of 170°C. The acetylation degree of the xylan in the wood residue was slightly reduced in the course of autohydrolysis.
4. During autohydrolysis of *E. globulus* wood at 170°C, MeGlcA and GalA were progressively degraded with increasing P-factor through decarboxylation. Equimolar amounts of CO_2 were formed as a consequence.
5. At a P-factor of 1500, over 90% of the uronic acids were degraded. Acidic XOS and XOS with a DP > 5 were only contained in insignificant amounts in the hydrolyzate at this P-factor.

6. The soluble lignin, which was dissolved into the hydrolyzate during autohydroysis, corresponded to the acid-soluble lignin of the original wood.
7. The composition of the hydrolyzate was highly dependent on the autohydrolysis intensity. Hence, the further processing of the hydrolyzate must be able to cope with an altering hydrolyzate composition.

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