PURIFICATION OF HARDWOOD-DERIVED AUTOHYDROLYSATES

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Carbohydrate-containing hydrolysates (1.1 to 14.9% of wood dry matter) obtained from autohydrolysis (at 130 to 150°C for 30 to 120 minutes) of birch (*Betula pendula)* chips prior to pulping were purified with respect to non-carbohydrate materials, without carbohydrate losses, either by ethyl acetate extraction or XAD-4 resin treatment. In the former case, about 50% of lignin and practically all the furanoic compounds (2-furaldehyde and 5-(hydroxymethyl)furfural) could be removed, whereas in the latter case, the corresponding amounts were about 30% and 50 to 90%, respectively. A partial recovery of various unsaturated impurities is of importance, because they may act as inhibitors when biochemically converting carbohydrates in hydrolysates into value-added products.

Keywords: Autohydrolysis; Hydrolysate; Purification; Biorefining; Biomass; XAD-4; Ethyl acetate

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INTRODUCTION

During the past two decades, conversion of renewable biomass into fuel energy and chemicals has received growing global attention as a means of replacing energy, chemicals, and materials currently derived from fossil fuels (Kamm and Kamm 2004; Kamm et al. 2006; Carvalheiro et al. 2008; Alén 2011). One attractive possibility is to integrate this production with the existing industry with well-established products. In this respect, chemical pulp mills (Sixta and Schild 2009; Huang et al. 2010) can be considered as conventional integrated biorefineries that have the potential to enhance the efficient utilization of feedstock materials via their proper modification (Kamm and Kamm 2004; Kamm et al. 2006; Huang et al. 2010; Walton et al. 2010; Alén 2011).

In many recent biorefinery concepts, one of the most common and effective options is to introduce a pressurized hot-water pre-treatment (PHWT) stage (autohydrolysis) into the kraft pulping process (Sixta et al. 2006; Yoon and van Heiningen 2008; Amidon and Liu 2009; Sixta and Schild 2009), mainly serving the purpose of recovering hemicelluloses (the removal of cellulose is negligible) and their degradation products from wood chips prior to delignification. These carbohydrates can then be converted further, for example, after total hydrolysis, to fermentable sugars, or to other value-added products.

In autohydrolysis, water is the only reagent, making it an environmentally friendly and inexpensive process (Wiboonsirikul and Adachi 2008). In addition, it is non-corrosive and generally has only a minor negative influence on the strength properties of pulp. However, the effluents (hydrolysates, pH 3.0 to 3.5) are chemically rather complex.

They contain a mixture of various carbohydrates (Garrote et al. 1999; Carvalheiro et al. 2008; Teo et al. 2010) and a minor amount of other organics, including aliphatic carboxylic acids ("volatile acids", i.e., acetic and formic acids) (Tunc and van Heiningen 2011), furans (2-furaldehyde or furfural and 5-(hydroxymethyl)furfural (HMF)), as well as heterogeneous fractions of lignin- and extractives-derived materials. All of these non-carbohydrate materials may be harmful substances, especially when considering the biochemical utilization of hydrolysate-based carbohydrates (Delgenes et al. 1996; Olsson and Hahn-Hägerdal 1996; Palmqvist and Hahn-Hägerdal 2000; Schwartz and Lawoko 2010). For these reasons, the removal of these inhibitors, at least partial if not complete, would be of benefit.

The main aim of this study was to remove lignin and furanoic materials from the PHWT hydrolysates made under varying autohydrolysis conditions. The straightforward purification methods selected for this purpose were extraction (with ethyl acetate) and resin treatment (with XAD-4 resin). In each case, the hydrolysates were analyzed before and after the purification stage in terms of carbohydrates, volatile acids, lignin, and furans.

EXPERIMENTAL

Autohydrolyses

Birch (*Betula pendula*) chips were screened (maximum thickness 7 mm, maximum width 13 mm, and minimum width 7 mm) and dried at room temperature. Autohydrolysis was performed in an oil-heated rotating batch digester (2000 mL). In each treatment, chips (135 g) were heated at two temperatures (130°C and 150°C), with four reaction times (30, 60, 90, and 120 minutes; in each case a heating period of 30 minutes was added to these times), and with ultra-high-quality (UHQ) water (internal resistance ≥ 18.2 MΩcm at 25°C) obtained from a Milli-Q Plus water system (Millipore, Bedford). The liquid-to-wood ratio was 5 L/kg. Pretreatments covered the P-factor (Tunc and van Heiningen 2009) range from 10 to 238. Two separate autohydrolysates were prepared for purification experiments (ethyl acetate extraction and XAD treatments) (Tables 1 and 2).

Solvent Extractions

Birch hydrolysates (aliquots of 50 mL) were extracted three times with 50 mL of ethyl acetate (Sigma-Aldrich) in a separation funnel. For detailed analyses, the ethyl acetate extracts, as well as the aqueous phases obtained from each sample, were stored in a freezer for less than two weeks.

Resin Treatments

Hydrolysates were treated with an Amberlite XAD-4 resin (Fluka), which was a styrene-divinyl benzene hydrophobic interaction resin with a mean pore diameter of 50 Å and a specific surface area of 750 m^2/g. The resin was regenerated with NH_4OH. Two sets of resin treatment were conducted. The first experiment was conducted with a resin that was washed to neutral pH after regeneration, and the second experiment was

conducted using a resin that was not washed after regeneration. These tests were carried out in a column containing 3.5 g of resin and 25 mL of hydrolysate. Hydrolysates were allowed to flow through the resin bed and were collected in volumetric bottles.

Chemical Analyses

The monosaccharide and total carbohydrate contents (i.e., the monosaccharides obtained after the total sulfuric acid hydrolysis) (Anon. 2000) in the hydrolysates were determined before and after solvent extractions and resin treatments. Carbohydrates were determined with a Dionex high performance liquid chromatography-pulse amperometric detection (HPLC-PAD) equipped with an AS50 autosampler, a LC25 chromatography oven, a GS50 gradient pump, a CarboPac PA-1 column, and an ED50 detector with carbohydrate pulsing. Samples were eluted (UHQ water with NaOH gradient) at a flow rate of 0.3 mL/min. Post-column alkali (NaOH) addition was performed at a flow rate of 0.1 mL/min with and IC25 isocratic pump to enhance the performance of PAD. Data were stored and processed using a Dionex Chromeleon (6.50) data system. The peak identification was based on the model monosaccharides arabinose, galactose, mannose, glucose, and xylose (all from Fluka). Fucose (Sigma) was used as an internal standard.

Volatile acids in the hydrolysates were determined with a previously described method (Käkölä et al. 2008) using a Dionex chromatography system equipped with an AS50 autosampler, a LC25 chromatography oven, an EG40 eluent generator, and an IC25 ion chromatograph. The system was equipped with an anion trap column (IonPac ATC-1), and the separation column was an IonPac AS 11-HC analytical column combined with an AG11-HC guard column (Dionex). Samples were injected via a 25 µL-loop and were eluted (KOH/UHQ water) at a flow rate of 1.0 mL/min. Data were stored and processed using a Dionex Chromeleon (6.50) data system. The identification of the chromatographic peaks was based on the model substances sodium acetate (J. T. Baker) and sodium formiate (Riedel-de Haën). Due to the partial hydrolysis of ethyl acetate to ethanol and acetic acid, the hydrolysates extracted with ethyl acetate were not analyzed in terms of volatile acids.

Dissolved lignin was measured from the hydrolysates before and after purification experiments. The samples were diluted to concentrations that gave absorbance values in the range of 0.3 to 0.8. The measurements were performed with a Beckmann DU 640 UV/Vis-spectrophotometer at 205 nm. UHQ water was used as a blank sample. The content of dissolved lignin was calculated according to Eq. 1,

$$c = \frac{A}{a*b}\,, \qquad (1)$$

where c is the lignin concentration (g/L), A is absorbance, a is absorptivity (110 L/(gcm) for birch lignin (Swan 1965)), and b is the light path (cm).

Furanoic compounds were determined from the hydrolysates using an HPLC equipment containing 510 pumps (Waters), a 717 injection system, a 996 diode-array detector (DAD), and a Phenomenex Gemini C18 column. The injection volume was 30 µL, and the detection wavelength was 280 nm (UV-region). UHQ-water/acetonitrile (ACN) (in a volume ratio of 9:1) and pure ACN were used as eluents. The eluent flow

rate was 1.0 mL/min. The identification of the chromatographic peaks was based on the model substances 2-furaldehyde and HMF (Aldrich).

RESULTS AND DISCUSSION

Ethyl Acetate Extraction

Chemical compositions of the hydrolysates used for the extractions with ethyl acetate are presented in Table 1. The chemical composition of the original wood was 68.8% carbohydrates (Ara 0.7%, Gal 0.7%, Glu 42.4%, Xyl 23.3%, and Man 1.7%), 24.2% lignin (Klason lignin 18.4% plus acid soluble lignin 5.8%), and 2.7% extractives. Since some extractives and other compounds of a low concentration were also removed during hydrolysis, the total mass loss was in the range of 1.1 to 14.9% (Tables 1 and 2).

Table 1. Chemical Composition of the Hydrolysates used for Ethyl Acetate Extractions

$T_{(max)}$ (°C)	Time at $T_{(max)}$ (min)	P-factor	pH	Mass loss (% of o.d. wood)	Sugar moieties in hydrolysate (% of o.d.wood)					Lignin in hydrolysate (% of o.d. wood)
					Ara	Gal	Glu	Xyl	Man	
130	30	10	4.2	1.1	0.1	<0.1	0.1	0.1	<0.1	0.5
130	60	20	4.0	1.4	0.1	0.1	0.2	0.1	0.1	0.6
130	90	30	3.9	1.9	0.2	0.1	0.2	0.3	0.1	0.8
130	120	41	3.8	2.4	0.2	0.1	0.2	0.4	0.1	0.9
150	30	60	3.5	4.3	0.2	0.2	0.2	1.6	0.2	1.3
150	60	119	3.2	8.9	0.3	0.3	0.3	4.6	0.3	2.2
150	90	179	3.1	12.7	0.3	0.5	0.4	7.3	0.3	2.3
150	120	238	3.1	14.9	0.3	0.6	0.4	8.9	0.4	2.4
Ara=arabinose, Gal=galactose, Glu=glucose, Xyl=xylose, Man=mannose										

The free monosaccharide (MSs) and total carbohydrates (i.e., MSs together with oligo- (OSs) and polysaccharides (PSs)) contents in the hydrolysates (the pre-treatments corresponded to the P-factors 10 to 238) before and after the ethyl acetate extractions are shown in Figs. 1 and 2, respectively. The results indicated that the ethyl acetate extraction had no significant effect on the concentration of the free MSs or the total carbohydrates (MSs+OSs+PSs). In addition, no degradation of OSs or PSs to MSs could be observed, since, in each case, the ratio (OSs+PSs/MSs) remained rather constant.

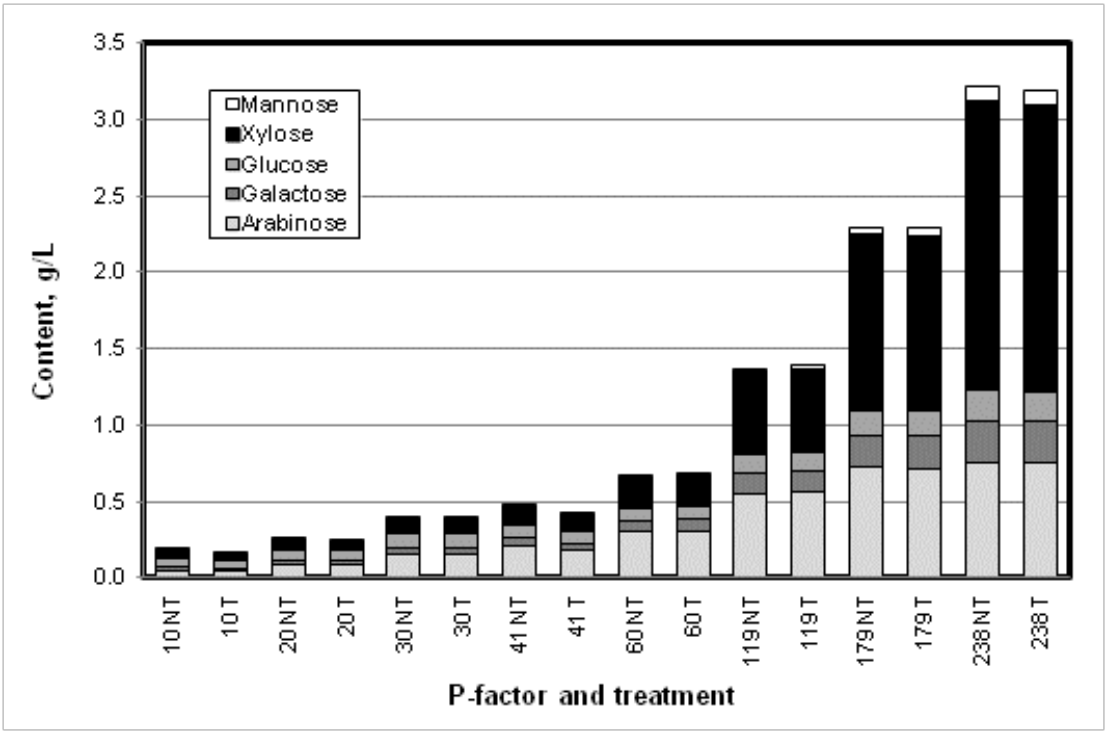

Fig. 1. Content of the free monosaccharides in different hydrolysates before ("not treated", NT) and after ("treated", T) ethyl acetate extraction. The numbers on the horizontal axis labels refer to the P-factor (10–238).

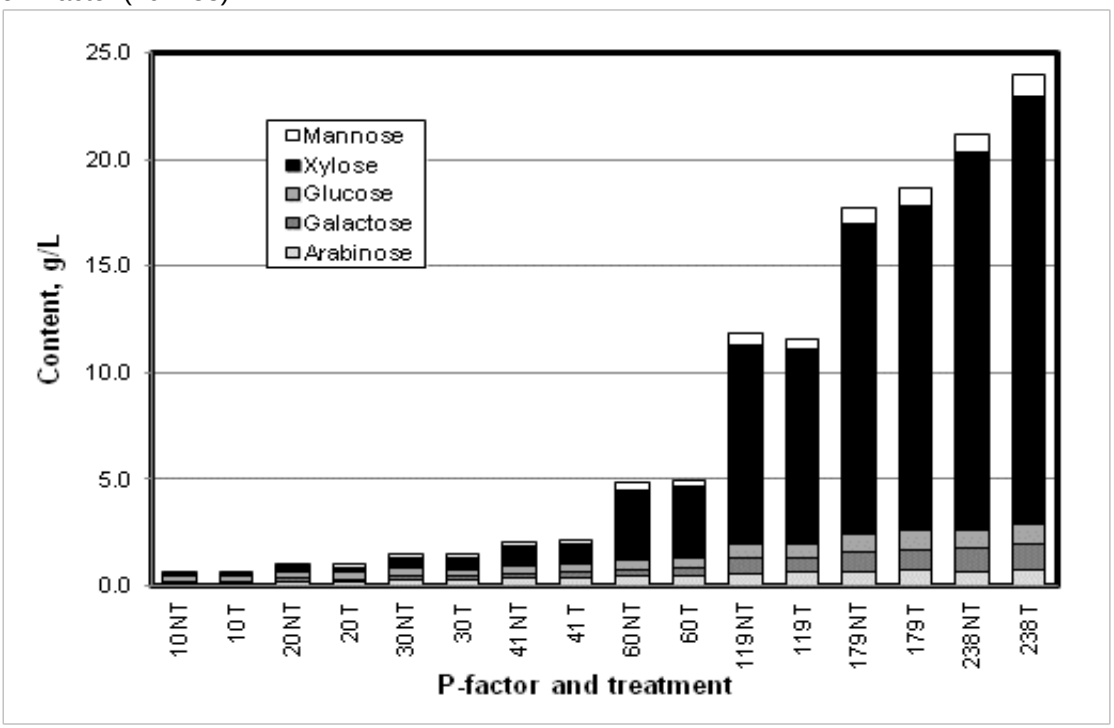

Fig. 2. Content of the total carbohydrates in different hydrolysates not treated (NT) and treated (T) with the ethyl acetate extraction. The numbers on the horizontal axis labels refer to the P-factor (10–238).

It was noticed that, in each case, roughly half of the dissolved lignin was removed by this simple extraction procedure (Fig. 3). In addition, the furanoic compounds were effectively removed, and no detectable amounts of them were found after extractions. In contrast, due to the high water solubility of volatile acids, they were probably not removed to the organic phase.

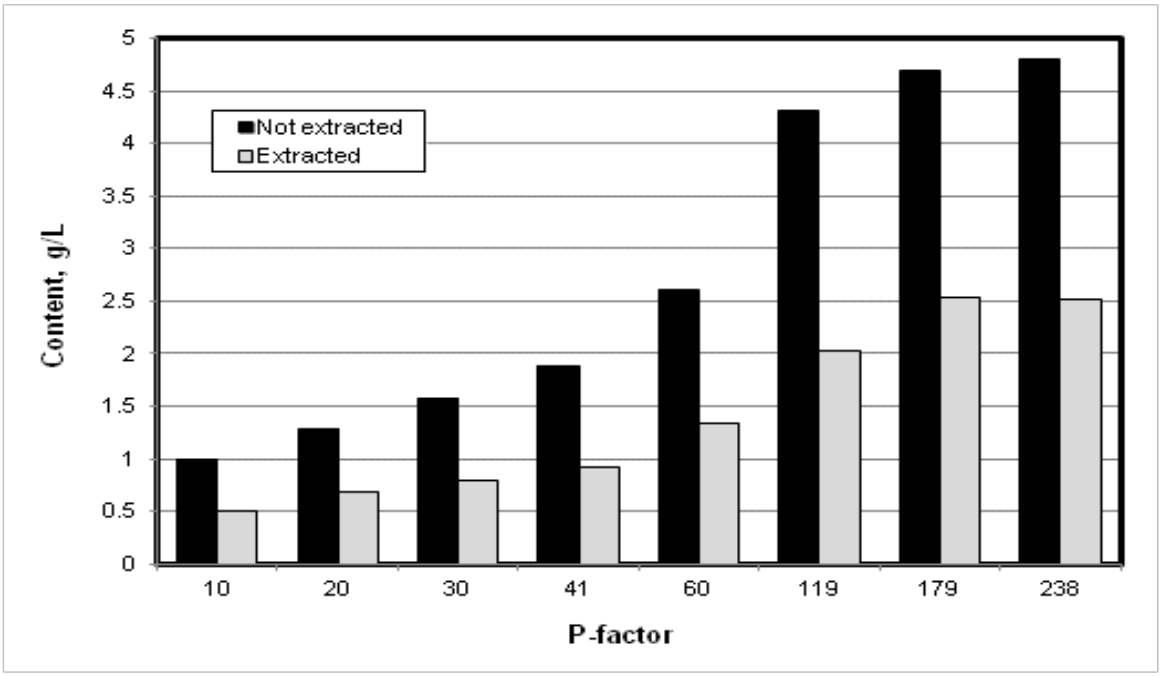

Fig. 3. Content of the dissolved lignin in different hydrolysates before and after ethyl acetate extraction

Treatment with XAD-4 Resin

The chemical composition of the hydrolysates used for the XAD treatments is presented in Table 2.

Table 2. Chemical Composition of the Hydrolysates used for XAD Treatments

$T_{(max)}$ (°C)	Time at $T_{(max)}$ (min)	P-factor	pH	Mass loss (% of o.d. wood)	Sugar moieties in hydrolysate (% of o.d.wood)					Lignin in hydrolysate (% of o.d. wood)
					Ara	Gal	Glu	Xyl	Man	
130	30	10	4.3	1.2	0.1	<0.1	0.2	0.1	0.1	0.6
130	60	20	4.2	1.8	0.2	0.1	0.2	0.2	0.1	0.9
130	90	30	4.1	2.2	0.2	0.1	0.2	0.3	0.1	1.0
130	120	41	4.0	2.9	0.3	0.1	0.2	0.6	0.1	1.3
150	30	60	3.7	3.2	0.2	0.1	0.2	0.7	0.1	1.4
150	60	119	3.5	6.5	0.4	0.3	0.3	2.3	0.2	2.4
150	90	179	3.4	9.4	0.5	0.3	0.4	4.3	0.3	2.6
150	120	238	3.3	11.5	0.5	0.4	0.4	5.8	0.3	2.9
Ara=arabinose, Gal=galactose, Glu=glucose, Xyl=xylose, Man=mannose										

The effects of the different treatments with XAD-4 resin on the contents of various carbohydrates are presented in Fig. 4 (for the free MSs) and Fig. 5 (for the total carbohydrates).

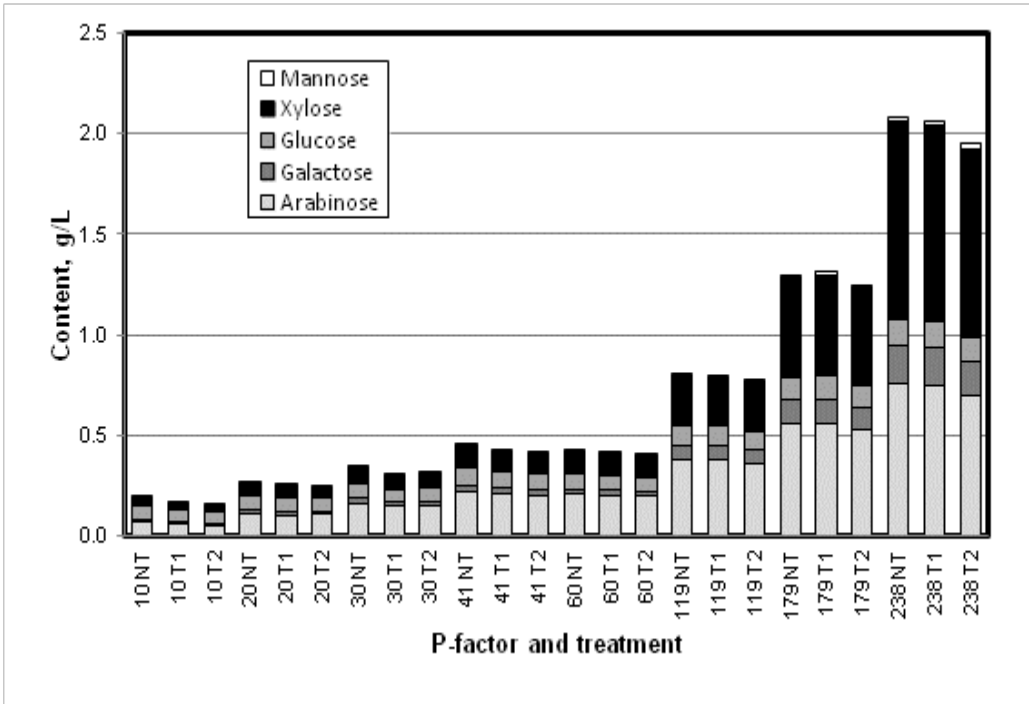

Fig. 4. Content of the free monosaccharides in different hydrolysates not treated with XAD-4 (NT), treated with neutral XAD-4 (T1), and treated with basic XAD-4 (T2). The numbers on the horizontal axis labels refer to the P-factor (10–238).

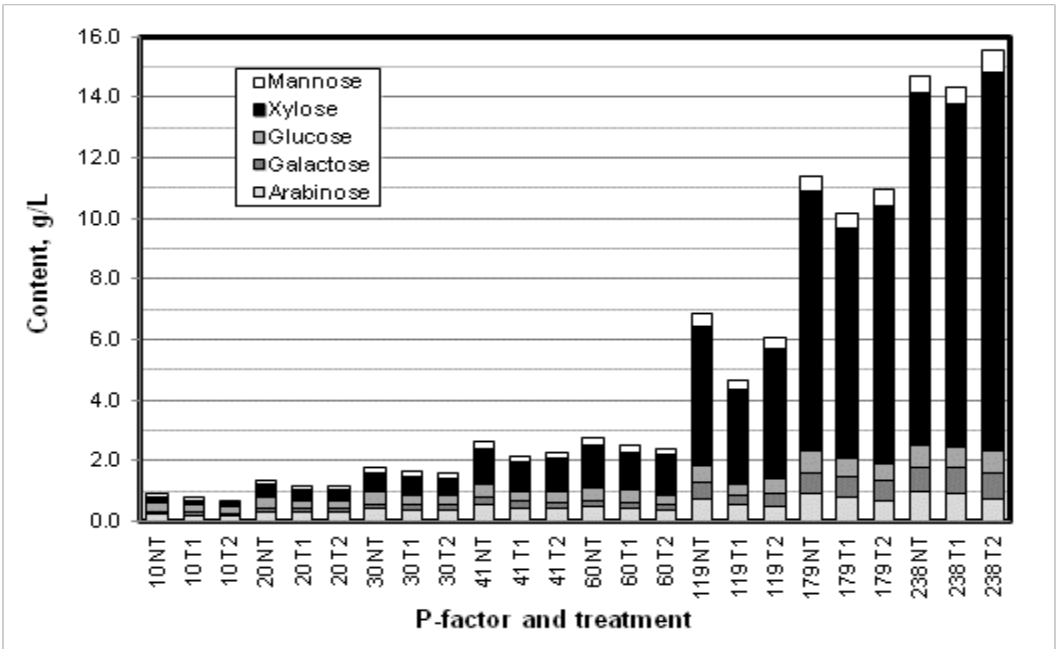

Fig. 5. Content of the total carbohydrates in different hydrolysates not treated with XAD-4 (NT), hydrolysates treated with neutral XAD-4 (T1), and hydrolysates treated with basic XAD-4 (T2). The numbers on the horizontal axis labels refer to the P-factor (10–238).

In general, under varying conditions, neither free MSs nor OSs plus PSs were removed in significant amounts from the aqueous phase by the resin treatment. In addition, no clear differences were observed between the neutral and basic resin treatments. The resin treatment removed roughly one-third of the dissolved initial lignin (Fig. 6) and almost 90% of 2-furaldehyde. About 50% of HMF could also be recovered (Fig. 7) by this method.

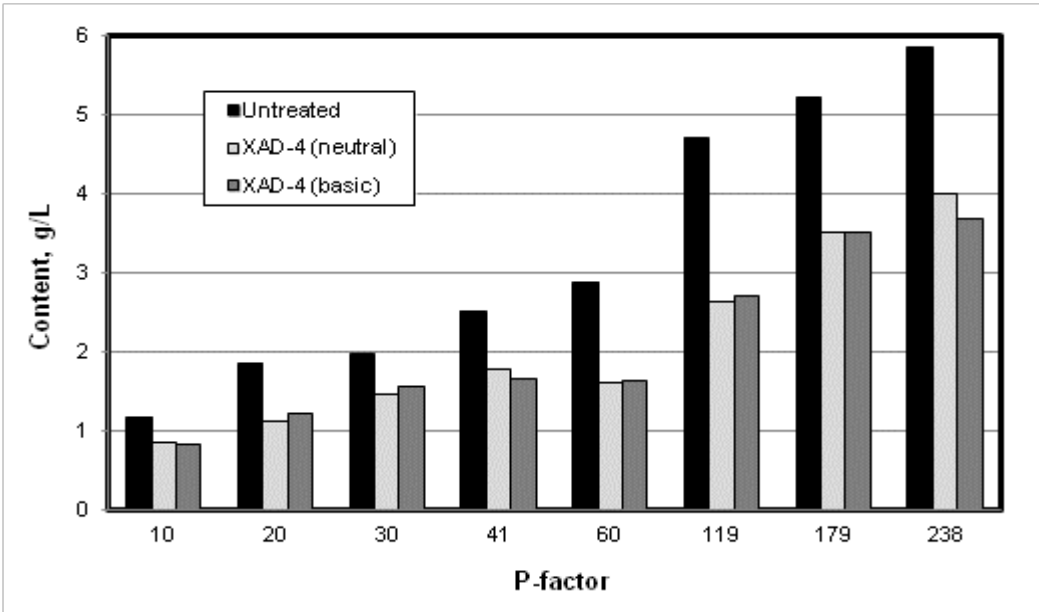

Fig. 6. Content of the dissolved lignin in different hydrolysates before and after the XAD-4 treatments

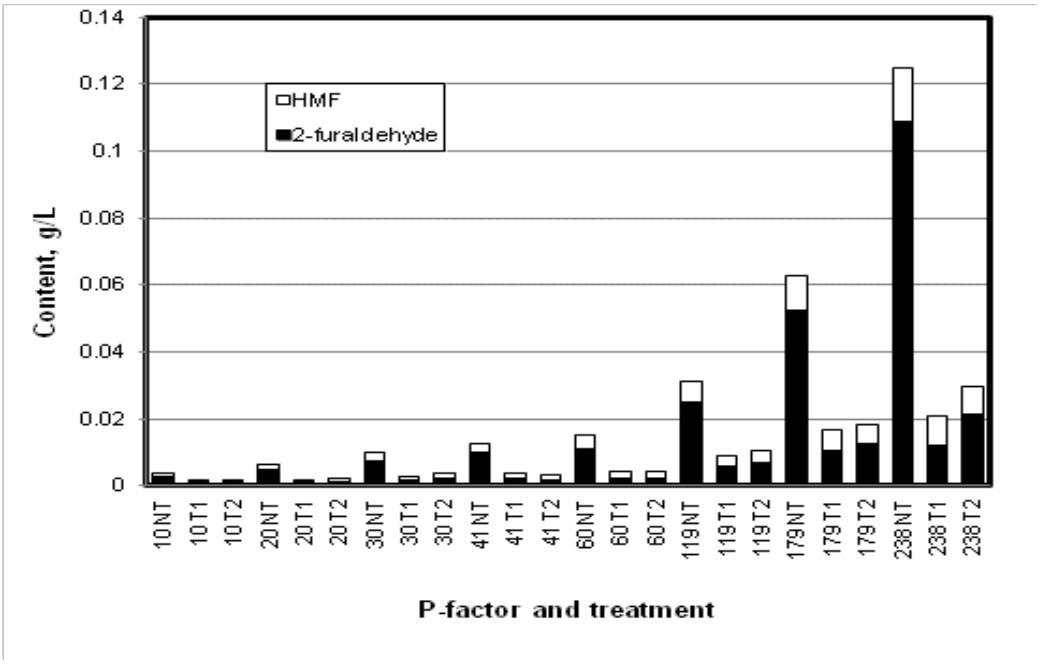

Fig. 7. Content of 2-furaldehyde and HMF in different hydrolysates not treated with XAD-4 (NT), hydrolysates treated with neutral XAD-4 (T1), and hydrolysates treated with basic XAD-4 (T2). The numbers on the horizontal axis labels refer to the P-factor (10–238).

Finally, it was noted (Fig. 8) that practically no volatile acids were removed by the resin treatment and that most of the acids remained in the hydrolysates.

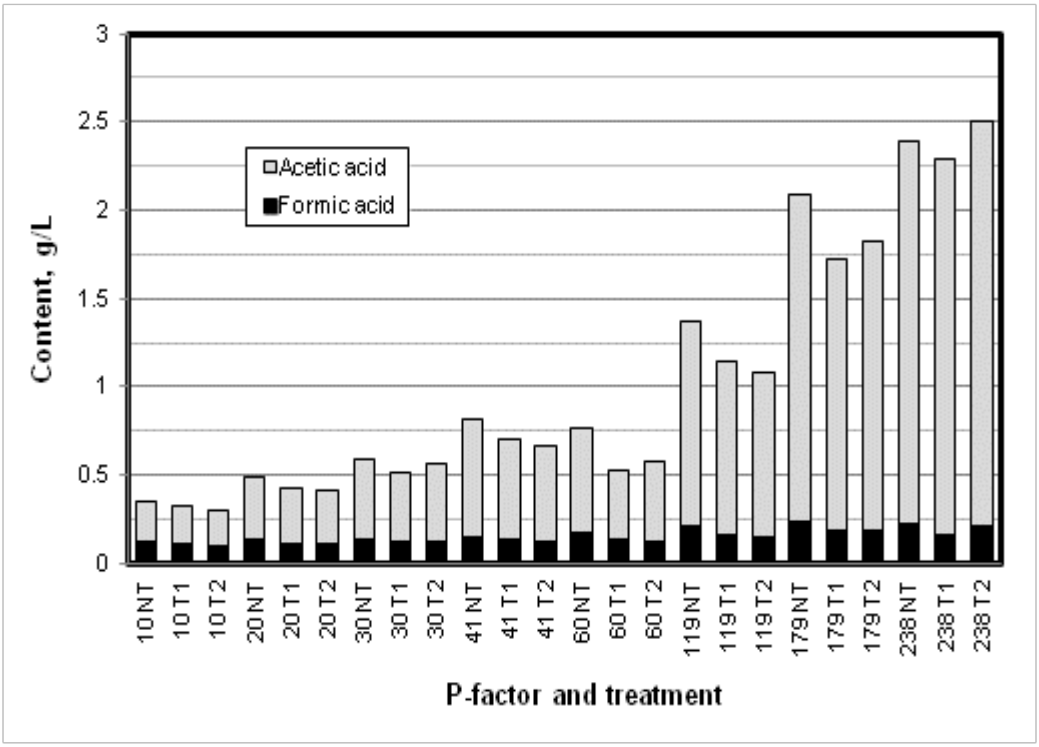

Fig. 8. Contents of volatile acids in hydrolysates not treated with XAD-4 (NT), hydrolysates treated with neutral XAD-4 (T1), and hydrolysates treated with basic XAD-4 (T2). The numbers on the horizontal axis labels refer to the P-factor (10–238).

CONCLUSIONS

1. Significant amounts of lignin and furanoic compounds were removed from hydrolysates containing carbohydrates (obtained from the autohydrolysis of hardwood chips prior to delignification), either by relatively simple methods such as solvent extraction (with ethyl acetate) or resin treatment (with XAD-4 resin).
2. Purification experiments caused no carbohydrate loss.
3. Volatile acids (formic and acetic acids) could not be removed from the hydrolysates in XAD experiments.

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