EFFECT OF BIOTIC AND ABIOTIC PRETREATMENTS OF HORNBEAM WOOD ON ITS PROPERTIES INTERESTING FROM THE VIEWPOINT OF PULPING IN ALKALINE MEDIA. PART 2: CHEMICAL ALTERATIONS

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A series of comparable specimens of hornbeam wood were submitted to fungal and chemical pretreatments. Two strains of erosive white-rot fungi (*P. chrysosporium* and *T. versicolor*) and a lignin-selective fungus *C. subvermispora* were used. Chemical pretreatments were carried out with diluted sodium hydroxide, or sodium hydroxide and then by hydrogen peroxide, or per-acetic acid. Both biotic and abiotic pre-treatments modified the chemical composition of wood and were accompanied by its weight loss. The applied fungi apparently delignified the specimens, however at the expense of cellulose, especially when the erosive strains of fungi were used. The chemical pretreatments caused deep deacetyl-ation, and milder delignification of wood and did not cause an apparent loss of cellulose. Biotic pretreatments of hornbeam wood, despite their marked delignification effect, led to unexpected increase in the contents of residual lignin in the resulting kraft pulps. On the other hand, pulping of the chemically pre-treated chips yielded pulps with low contents of residual lignin and much higher brightness.

Key words: Pre-treatment; White-rot fungi; Alkali; Peroxides; Deacetylation; Wood constituents; NB oxidation; Kraft pulps; Yield; Residual lignin; Brightness.

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INTRODUCTION

Another option, apart from pulping the sound wood chips, may be pulping the chips with the properties modified by biotic, chemical, or mechanical pretreatments. The pretreatments may lead to partial delignification, released ultrastructure, and increased porosity of the chips, thereby improving their physical properties for mechanical, semichemical, or chemical pulping.

A problem often influencing the course of pulping in its initial phase is the impregnation of chips. The air entrapped in the vessels represents a pneumatic obstacle and prevents uniform impregnation of chips with the pulping media (Stamm 1953). Under such conditions impregnation of wood takes place only via diffusion of chemicals across the cell walls of the vessels filled with the pulping media (Zanuttini et al. 2005). An efficient and uniform impregnation of chips is reflected in reduced Kappa number, higher quality of the pulp, narrower distribution of *Kappa* number, and higher contents of

fibers at the average level in the pulp (Malkov et al. 2003). The uniformity of impregnation may be improved by presteaming, use of pressure, or by mechanical treatment of chips to increase their porosity due to formation of cracks, fissures, and microfractures (Malkow 2002). The application of vacuum may also effectively enhance penetration of the pulping media into chips. This is, however, impractical in ordinary digesters, and used mostly in laboratories (Rydholm 1965).

In the case of biotic pre-treatments the changes in the ultrastructure and physical properties of wood are related to enzymatic alterations of wood. Some of them, e.g. partial delignification, degradation of lipophillic extractives**,** and softened ultrastructure of wood may positively influence the pulping processes (Messner and Srebotnik 1994).

Alkaline pretreatments of chips result in their deacetylation. Deacetylation also occurs in the hydrothermal treatment of wood and during acid, neutral**,** and alkaline cooks. The proceeding deacetylation improves the accessibility of wood for water (Sumi et al. 1964) and diffusion of chemicals (Solár et al. 2008).

Deacetylation of chips taking place during kraft pulping may, under proper conditions of the cook, promote sorption of deacetylated xylan onto the fibres and increase the yield and mechanical strength of the pulp (Danielsson 2007). On the other hand, deacetylation and neutralisation of carboxyl groups in the chips during neutral sulphite semichemical (NSSC) and alkaline semichemical cooks reduces the alkalinity of a liquor (Sjöström 1993b; Zanuttini and Marzocchi 1997; Zanuttini et al. 2005). A way to avoid the excessive consumption of alkali and increase the efficacy of alkaline cooks may entail the pretreatment of chips by alkali, improving also their digestibility.

The aim of this article is to compare the impact of the applied biotic and combined alkaline/oxidation chemical pre-treatments of hornbeam wood on its composition, digestibility and the selected properties of the resulting kraft pulps.

MATERIAL AND METHODS

Wood Species

Model specimens of hornbeam wood (*Carpinus betulus* L.) were prepared from two 35cm long sections taken from the middle part of the tree trunk in approximately 1m distance. The tree age was 90 years. The sections were used to prepare specimens with dimensions of 2.5x2.5x1.0 cm. Their shortest dimension was parallel to the grain.

Pretreatments

For bio-degradations, white-rot fungi *Trametes versicolor*, *Phanerochaete chrysosporium* (erosive strains), and *Ceriporiopsis subvermispora* (lignin-selective strain) were used. All biotic pre-treatments lasted 30 days. Biotic and abiotic pre-treatments were described in detail in Part 1 of this paper (Solár et al. 2008).

Chemical pre-treatments comprised: **1**/ a 48-h treatment of comparable series of the specimens with 2.5 % NaOH; the selected time of action of alkali was determined based on the preliminary evaluation of penetration sodium hydroxide solution into the specimens in axial direction, and the necessary mean of the time was increased for 8 h; **2**/ 48-h treatment of the specimens with 2.5 % NaOH with a subsequent 72-h oxidation treatment with 7.5 % H_2O_2; **3**/ the same pretreatment as in 2, however with the addition

of dicyandiamide delignification activator (0.028g/g o.d. wood.); **4**/ the same alkaline treatment followed by oxidation with 8 %, or 4 % per-acetic acid. The ratio of wood to solution of alkali and oxidation agent was constant at 1:5, and the pre-treatments were carried out at 20 ^{o}C.

Bio-mimetic delignification of the test specimens was performed as a standard to estimate the delignifiation efficacy of biotic and chemical pretreatments. The 4-aminopyridine/tertial butylhydroperoxide/Cu^{2+} complex in concentration of 1.5 multiple of that proposed by Messner et al. (2003) was used. The wood to bio-mimetic complex ratio equalled to 1:16, the temperature of treatment was 50 ^{o}C, and the time of delignification was 72 h.

Kraft Pulping

The model chips with dimensions of 0.3x0.3x1cm (longer dimension parallel to the grain) were used for cooks. Chips were cut from the test specimens after measurements of their physical properties. Pulping was performed with a liquor of 21.5% sulphidity and 16.0% of active alkali on the o. d. weight of the chips. The dry wood to liquor ratio was 1: 4.2. The air-dry chips with known moisture content were used for pulping. A 20-min impregnation of chips at an ambient temperature was followed by a 15-min preheating of the contents of 50 ml stainless autoclaves to 100 ^{o}C. The time to reach the pulping temperature of 170 ^{o}C was 60 min, and the cooking time also was 60 min.

Bleaching of Pulps for Viscosimetry

Hydrogen peroxide activated by dicyandiamide was used for bleaching the pulps (Chen 1996). The pulp consistency was 5%. Amounts of chemicals in the bleaching solution, based on the weight of oven dry pulp, were: H_2O_2 – 40%; NaOH – 5%; Chelaton III – 1%, and dicyandiamide – 1.5%. Time and the temperature of bleaching: 60 min at 30 ^{o}C and 120 min at 62 ^{o}C. Bleached pulps were washed with 5% acetic acid, then with deionised water to neutral pH, and finally with acetone.

Chemical Analyses

- Weight loss of the specimens due to the pretreatments was calculated from "oven-dry" weight of both the sound and the pretreated series of the specimens. The dry weight of wood was determined using 3 specimens from each series. Drying was at room temperature in a desiccator. The drying media were 98% H_2SO_4, and then P_2O_5.
- Extract in wood was determined using the fraction of sawdust with dimensions of 0.35 – 1.0mm. Extraction was performed with an ethanol/toluene mixture, applying the standard procedure D 1107-96. The extraction time was 8 hours.
- Lignin in wood and pulps was determined by the TAPPI T-13m method (ASTM Standard D 1106-96). For pulps a spectroscopic method of Iiyama and Wallis (1988) was also used.
- Cellulose was determined following the method of Kűrschner and Hoffer (KH), and 3 delignification steps were used (1929).
- Acetyl groups in the samples were determined by the method of Solár et al. (1987).
- Total hydrolysis of KH cellulose was carried out according to the method of Saeman et al. (1954). Monosaccharides in the hydrolysates of cellulose preparations were

determined by gas liquid chromatography (GLC) of aldnitrilacetates, using a CHROM 5 device equipped with a FID detector (Kačík et al. 1993).

- Average degree of polymerisation of bleached pulps (DP) was calculated from the intrinsic viscosity of their solutions in $EWNN_{NaCl}$ complex (ISO 5351/2.1981).
- Nitrobenzene oxidation of extractive-free wood meals was carried out in stainless steel autoclaves (2 h at 180 ^{o}C with shaking the contents in 20 min. intervals). The oxidation products with added p-hydroxipropiophenone (internal standard) were isolated by extraction of the acidified reaction mixture. The products of NB oxidation were analysed by liquid chromatography (HPLC) using the Merck Hitashi L 6002 Intelligent pump equipped with a L 4250 UV-VIS detector and L 5025 Column thermostat. The conditions of the analysis were identical with those published by Kačík et al (1995).
- Brightness of unbleached kraft pulp was measured by the standard method STN ISO 3688 (50 0240):1994 with MgO used as a standard.

A student-t test was used to eliminate the data not fitting with the rest of them; the number of analyses within a determination varied from 3 to 5.

RESULTS AND DISCUSSION

Applied biotic and abiotic pretreatments of hornbeam wood specimens led to their weight loss and were accompanied by alterations in their composition**,** depending on the agent used (Table 1).

Table 1. Basic Analyses of Sound and Pre-treated Samples of Hornbeam Wood (%)

Pretreatment	Weight loss[a]	Extrac-tives	Acetyl groups	TAPPI lignin	Cellulose[b]
Sound wood	-	2.43	4.18 (4.08)	18.43 (**17.98**)	49.54 (**48.34**)
T. versicolor	16.51; v =12.0%	0.98	3.51 (2.90)	16.97 (14.02)	50.66 (41.85)
P. chrysosporium	10.86; v =18.5%	0.76	3,66 (3.24)	12.95 (11.46)	51.52 (45.57)
C. subvermispora	10.94; v =23.3%	1.23	3.69 (3.21)	10.14 (**8.92**)	53.47 (47.03)
2.5 % NaOH	8.47; v =10.1%	0.69	1.30 (1.19)	19.62 (17.83)	57.15 (51.84)
2.5 % NaOH/H_2O_2	11.69; v =19.4%	0.50	1.22 (1.07)	19.39 (17.04)	58.57 (51.47)
2.5 % NaOH/H_2O_2 + activator	10.53; v =7.4 %	0.57	1.24 (1.09)	18.73 (16.66)	58.35 (51.91)
2.5 % NaOH/8% peracetic acid	15.05; v =10.5%	1.86	1.00 (0.83)	14.08 (**11.74**)	62.23 (51.88)
2.5 % NaOH/4% peracetic acid	11.78;v =11.5 %	1.95	-	17.85 (15.44)	60.05 (51.95)
Bio-mimetic compl.	7.20; v =9.2 %	0.99	-	18.36 (16.87)	52.74 (48.42)

The data in parentheses are related to weight of wood prior to pretreatments and obtained by recalculation of the contents of wood components considering the weight loss of wood and extractives. [a] number of specimens pre-treated n = 14; [b] mean of 3 parallel determinations of KH cellulose; the data were not corrected for Klasson lignin content varying in the preparations from 0.06 to 0.10 %

Table 2 presents the amounts of wood components removed from hornbeam wood due to fungal and chemical pre-treatments.

Table 2: Amounts of Lignin, Cellulose and Acetyl Groups Removed from Hornbeam Wood in the Course of Biotic and Abiotic Pretreatments (%)

Pretreatment	Lignin	Cellulose	Acetyl groups
T. versicolor	22.03	13.43	28.92
P. chrysosporium	36.26	5.15	20.65
C. subvermispora	**50.39**	**2.71**	21.32
2.5 % NaOH	0.83	-7.23	70.83
2.5 % NaOH/H_2O_2	5.23	-6.47	73.78
2.5 % NaOH/H_2O_2 + activator	7.34	-7.39	73.28
2.5 % NaOH/8 % peracetic acid	**34.71**	-7.33	**79.66**
2.5 % NaOH/4 % peracetic acid	14.13	-7.46	-
Bio-mimetic complex	6.17	-0.17	-

The data in Tables 1 and 2 reveal a marked removal of lignin from the samples of the bio-degraded hornbeam wood. This observation becomes more striking when comparing the data related to weight of wood before the pre-treatments. Despite the effective delignification of a substrate, the applied white-rot fungi also removed relatively high portions of cellulose from wood (Table 2). In this respect, only the lignin-selective fungus *C. subvermispora* provided satisfactory results, removing 50% of lignin and only 2.7% of cellulose.

Delignification of wood resulting from the chemical pretreatments was less significant, and the exception was only the pretreatment with 2.5% sodium hydroxide and then with 8% per-acetic acid. The delignification efficiency of this combination was equal to that of the erosive strain of the fungus *P. chrysosporium*. The use of the biomimetic system proposed by Messner et al. (2003), which was so extremely efficient in the case of delignification of microtonic slides of wood, did not show any advantage in comparison with the applied chemicals. Disadvantages of the application of this system in practice are the use of 4-aminopyridine - a known irritant, and of hydroperoxides as potentionally hazardous and explosive moieties.

Dedacetylation of hornbeam wood accompanied all pretreatments. While the application of white rot fungi led to only a moderate loss of acetyl groups - and part of them might be split off from the specimens during sterilization - the chemical pretreatments deacetylated wood to a much higher degree (Table 2). The residual contents of acetyl groups in the pretreated wood may result from an incomplete impregnation with the alkali. However, a minor amount of sodium acetate remaining in wood after pretreatments and extraction might also cause a positive error in the determination.

The pretreatments, except for the bio-mimetic one, caused an absolute increase in the apparent cellulose content of hornbeam wood (Tables 1 and 2). A similar phenomenon was observed in connection with plasticization of hornbeam wood with 25% aqueous ammonia at the ambient temperature. The increased amount of cellulose in the plasticized wood was proportional to the increased contents of pentosans in the

cellulose preparations (Solár and Melcer 1978). Sorption of deacetylated glucuronoxylans (hardwoods) and galacto-glucomanans (conifers) onto the cellulose during alkaline treatment of wood was reported by Marchessault et al. (1967) and Laffend and Swenson (1968). Data related to ratio of monosaccharides in KH cellulose are presented in Table 3.

Table 3. Relative Representation of Monosaccharides in the Hydrolysates of KH Cellulose Preparations Isolated from Sound and Chemically Pretreated Hornbeam Wood (%)

Sample/ Monosaccharide	Sound wood	$NaOH/H_2O_2$	$NaOH/H_2O_2$ with activator	NaOH/ 8 % per-acetic acid	NaOH/ 4 % per-acetic acid
D-glucose	**82.21**	78.19	78,22	78.67	78.35
D-xylose	15.08	**19.20**	**19.56**	**19.38**	**19.70**
D-mannose	1.58	1.43	1.41	0.97	1.05
L-arabinose	0.54	0.62	0.50	0.51	0.53
L-rhamnose	0.59	0.56	0.31	0.47	0.37

The relative increase in the representation of D-xylose in the hydrolysates of KH cellulose preparations isolated from the samples pretreated by alkali confirmed sorption of xylan onto the cellulose.

Some of the specimens of a series of chemically pretreated wood were cut to model chips, which were used for the kraft cooks. Table 4 presents the yields and some properties of the obtained unbleached pulps.

Table 4. Yield, Brightness, Content of Residual Lignin, Residual Lignin Content to Yield of Pulp Ratio, and Average Degree of Polymerisation of Pulp

Pretreatment	Yield (%)	Yield [a] (%)	Bright-ness (%MgO)	TAPPI lignin (%)	Lignin - UV (%)	TAPPI [b] Lignin-to-pulp ratio	DP
Sound wood	**55.53**	**55.53**	**23.12**	**4.07**	***7.83***	**0.073**	**1249**
T. versicolor	56.81	47.43	-	8.64	-	0.152	741
P. chrysosporium	58.31	51.98	-	7.01	-	0.120	856
C. subvermispora	55.02	49.00	-	6.72	-	0.122	867
NaOH	52.00	47.60	42.86	**1.10**	*3.65*	**0.021**	895
$NaOH/H_2O_2$	52.32	46.21	42.80	**0.53**	*2.74*	**0.010**	890
$NaOH/H_2O_2$ + activator	51.21	45.82	43.63	**0.51**	***2.60***	**0.010**	929
NaOH/per-ac. acid, 8%	54.41	46.22	**44.00**	**0.54**	*2.66*	**0.010**	934
NaOH/per-ac. acid, 4%	54.89	48.42	42.19	**0.60**	*2.81*	**0.011**	991

[a]data expressed on the weight of wood prior to pre-treatment; [b]yield of pulp based on pretreated wood used in the calculation

As follows from Table 4, the pre-treatments of hornbeam wood influenced the yield of kraft pulps differently according to the agent used. The application of the erosive white-rot fungi moderately increased the yield of pulp. Pretreatment with the lignin-selective fungus *C. subvermispora* did not affect this value as compared to pulp from sound wood. The yield of pulps from fungally pre-treated samples expressed on the weight of wood prior to biodegradation was noticeably reduced, especially in the case of pre-treatment by *T. versicolor*. This observation is in good accordance with decomposition of cellulose by the applied fungi (Table 2).

Pulping the chips from chemically pre-treated wood resulted in a moderate reduction in the pulps yield as compared to pulp from sound wood. This reduction became more apparent when comparing results to corresponding data recalculated relative to wood prior to pretreatments. For recalculation of the pulp yield to weight of wood before the pre-treatments, the corresponding weight loss of wood was taken into consideration.

The yield of kraft pulps from chemically pre-treated samples of wood compared to the corresponding contents of KH cellulose was of the reverse trend (Tables 2 and 3). The sorption of xylan, so apparent in the KH cellulose preparations, was not reflected in the yield of kraft pulps. The correction of the pulps yield for the residual lignin may only diminish this difference. We are of the opinion that xylan that had sorbed onto cellulose in the course of alkaline pretreatments underwent a decomposition under the relatively severe conditions of the cook.

As presented in Table 4, all tested chemical pretreatments increased the brightness of the unbleached pulps by almost 100%, regardless whether oxidation steps after the alkaline treatment of wood were performed or not.

Data in Table 4 indicate a negative effect of fungal pre-treatments on the residual content of lignin in the kraft pulp. On the other hand, chemical pre-treatments of hornbeam wood led to deep reduction in the residual lignin in the pulps and increased noticeably the selectivity of the cook, as indicated by the residual TAPPI lignin-to-pulp ratios.

As seen in Table 4, the data concerning the lignin content in the pulp differed according to the method applied. The spectrophotometric method of Johnson et al. (1961) modified by Iiyama and Wallis (1988) provided several multiply higher values than the ASTM T 13-m method. This discrepancy between the obtained data cannot be simply explained by the acid-soluble fraction of lignin (representing 10 to 20% of hardwood lignin) passing into diluted sulphuric acid during the ASTM determination, or by 2-furalaldehyde formation during the solubilisation of the pulps before the spectroscopic determination. From the tested spectral modifications (Johnson et al. 1961; Iiyama and Wallis 1988; Hatfield et al. 1999) the lowest data and good reproducibility was provided the method modified by Iiyama and Wallis. Despite the discrepancy between lignin contents in the pulps determined by the applied methods, both of them confirmed better digestibility of the chemically pretreated chips.

The comparison of data related to lignin contents in the pre-treated wood samples (Table 2) and corresponding kraft pulps (Table 4) leads to a conclusion that a deep delignification of wood prior to pulping does not guarantee reduction in the residual content of lignin in the resulting pulp.

In connection with the low delignification efficacy of alkaline cooks of wood pretreated by white-rot fungi, it is interesting to mention the improved delignification of such a material under conditions of acid catalysed organosolv and acid sulphite cooks (Messner and Srebotnik 1994; Solár et al. 2001; Reinprecht et al. 2007). However, in some cases the properties of pulps were not always improved, and they depended on the fungus applied and the time of pretreatment. Different impacts of fungal pretreatment on the course of delignification in the alkaline and acidic media might stem from a different behaviour of the fungally pretreated wood in alkaline and acidic media, and the topochemistry of these processes.

The biotic and abiotic pre-treatments influenced the DP of kraft pulps negatively. The impact of the chemical pretreatments on the pulp DP might have been still acceptable and was less severe than that caused by the applied fungi (Table 4). The cause of the drop in the DP of pulps from biodegraded wood may dwell in degradation of cellulose in later stages of the pre-treatment (Table 2). Reduced DP of the pulps from chemically pretreated wood may hardly result only from chemical degradation of cellulose, especially if moderate alkaline pH, diluted H_2O_2, and ambient temperature of the pre-treatment were applied. Possibly, the extreme swelling of wood in the pre-treatment followed by its extreme shrinkage due to drying might mechanically reduce the DP of cellulose. Apparent mechanical degradation of cellulose when cutting the specimens to chips can be neglected because of all samples were cut in the same manner, and in their wet state.

In order to estimate the alterations of lignin occurring during the applied pretreatments, the NB oxidation of selected samples of pretreated hornbeam wood was performed, and the products were analysed by HPLC. The results are given in Table 5.

Table 5. Yield of Products of NB Oxidation Expressed on Klason Lignin in the Pretreated Hornbeam Wood and their Molar Ratio

Sample /Yield of oxidation products	Syring-aldehyde (%)	Syringic acid (%)	Vanillin (%)	Vanillic acid (%)	Total yield on lignin in wood (%)	S/G molar ratio*
Sound wood	35.01	3.81	7.50	0.53	46.85	4.09
Pretreatment: 2.5 % NaOH	32.77	3.89	6.85	0.50	44.01	4.15
Pretreatment: *T. versicolor*	23.30	2.78	5.78	0.63	32.49	3.40
Pretreatment: *P. chrysosporium*	33.18	2.65	8.28	0.59	44.68	3.38
Pretreatement: *C. subvermispora*	32.07	3.86	7.69	0.78	44.40	3.55
Pretreatment: NaOH/ 7.5% H_2O_2+ activ.	20.11	7.41	4.86	1.02	33.40	3.91
Pretreatment: NaOH/ 8% peracet. acid	20.27	2.42	5.57	0.43	28.69	3.15

*related to couples syringyl aldehyde + syringic acid (S) and vanillin + vanillic acid (G) with their amounts expressed in moles

Pretreatment of hornbeam wood by white-rot fungi markedly reduced the syringyl-to-guaiacyl (S/G) ratio in the NB oxidation products of "in situ" lignin. Most probably, this drop resulted from the preferential decomposition of sinapyl structures in lignin due to their higher representation in hardwood lignin and less cross-linked structure.

Biodegradation of lignin reduced also the yield of products of NB oxidation, markedly in the case of application of *T. versicolor*. Pretreatment of wood by *P. chrysosporium* and *C. subvermispora* influenced the yield of NB oxidation products only slightly.

From the chemical pretreatments, the application of aqueous sodium hydroxide slightly altered the S/G ratio in lignin and moderately diminished the yield of NB oxidation products. Combined action of alkali and oxidation agent on wood reduced the S/G ratio and yield of NB oxidation products of lignin. An extreme drop in the S/G ratio in lignin was observed after application of per-acetic acid. On the contrary, the S/G ratio in lignin of wood pretreated with alkali and then by hydrogen peroxide was diminished only moderately. Characteristic for these two-step chemical pretreatments was a deep reduction in the yield of NB oxidation products of "in situ" lignin. The generally reduced yield of NB oxidation products of lignin in the pretreated wood may be attributed to:

- coupling of phenoxi- and derived radicals arising during fungal and combined alkali/peroxide pretreatments,
- enrichment of lignin with more condensed fractions due to preferential degradation of the less condensed ones, and
- extraction of more soluble, low molecular weight fractions of lignin from wood by aqueous solutions of sodium hydroxide, hydrogen peroxide, or per-acetic acid

The results of NB oxidation of "in situ" lignins, including diminished S/G ratio and the yield of products due to fungal and chemical pretreatments, did not provide information giving a satisfactory explanation of the difference between digestibility of chemically and fungally pretreated hornbeam wood under identical conditions of kraft pulping. It is possible that other analytical methods, e.g., FTIR, ^{1}H and ^{13}C NMR, UV spectroscopy of isolated lignins (MWL, Freudenberg´s or Pepper´s), GPC, and analysis for functional groups in lignin might provide more valuable information concerning alterations of lignin. The weak point of this approach, however, is the isolation of lignin preparation, its secondary alterations during isolation, and the presence of saccharidic impurities.

CONCLUSIONS

The presented experimental data concerning the biotic and chemical pre-treatments of hornbeam wood, which was submitted further to small-scale kraft pulping, allow formulation of the following conclusions:

- The most effective fungus from the viewpoint of hornbeam wood delignification was the lignin-selective strain of *C. subvermispora*, with 50% of lignin and 2.7% of cellulose removed from a substrate.
- High delignification efficacy was provided by a 48-h treatment of wood by 2.5% NaOH connected with a subsequent 72 h lasting oxidation with 8% per-acetic acid; this combination removed 35% of lignin from wood.
- Contrary to fungal pretreatments, the chemical treatments increased the content of KH cellulose in hornbeam wood due to sorption of xylan onto cellulose under alkaline conditions of these pretreatments.
- All tested pretreatments resulted in deacetylation of hornbeam wood; in the case of fungal action the deacetylation was mild, while the chemical pretreatments removed up to 80 % of acetyl groups from wood.
- Chemical modifications of hornbeam wood noticeably reduced both the yield and residual lignin contents of the pulp compared to pulps from sound and fungally pretreated wood.
- The extent of hornbeam wood delignification due to the pre-treatments considered did not inevitably guarantee a low residual content of lignin in the resulting kraft pulp.
- Chemical pretreatments markedly increased the brightness of the unbleached pulps.
- All applied biotic and abiotic pretreatments of hornbeam wood reduced the pulps' DP; the deepest reduction resulted from pre-treatment by *T. versicolor*.

In closing, it is necessary to add that the improved digestibility of hornbeam wood resulting from the applied chemical pretreatments is formally a reflection of changed physical properties of such a material that were discussed in Part 1 of this article.

From the viewpoint of kraft pulping the most important change in the properties of pretreated wood is its increased accessibility for water or aqueous solutions of chemicals. This phenomenon might be considered as the principal condition for the enhanced diffusion of the liquor components into the chips and the delignification products from the chips into the liquor. Though this phenomenon at first is important, actually it is a secondary effect, arising from the chemical alterations of wood, especially deacetylation, cleavage of cross-links in the lignin-saccharidic matrix, and a partial decomposition and dissolution of lignin and hemicelluloses.

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LITERATURE CITED

ASTM Standard D 1106-96, Standard test method for acid insoluble lignin in wood (1998).

ASTM Standard D 1107-96 Standard Test Method for Ethanol-Toluene Solubility of Wood (Re-approved 2001).

Chen, J. (1996). "Process for delignification and bleaching of chemical pulps," *Canadian Patent* No. 2202150 http://patents.ic.gc.ca/claims?patent_number=2202150&language=X

Danielsson, S. (2007). "Xylan reactions in kraft cooking. Process and product considerations," Doctoral Thesis. Royal Institute of Technology. School of Chemical Sciences and Engineering. Department of Fibre and Polymer Technology. Stockholm 2007, 73 p.

Hatfield, R. D., Graber, J., Ralph, J., and Brei, K. (1999). "Using the acetyl bromide assay to determine lignin in herbaceous plants: Some cautionary notes," *J. Agric. Food Chem*. (47), 628-632.

Iiyama, K., and Wallis, A. F. A. (1988). "An improved acetyl bromide procedure for determining lignin in woods and wood pulps," *Wood Sci. Technol*. 22, 271-280.

ISO 5351/2. (1981). Cellulose in dilute solutions – Determination of limiting viscosity number. Part. 2. Method in iron (III) sodium tartrate komplex ($EWNN_{mod\ NaCl}$) solution.

ISO STN 3688 (50 0240). (1994). "Buničiny. Meranie difúzneho činiteľa odrazu v modrej oblasti spectra" (Pulps: Monitoring the diffusion portion of reflex in blue region of the spectrum).

Johnson, D. B., Moore, W. E., and Zank L. C. (1961), "The spectroscopic determination of lignin in small wood samples," *Tappi* 44(11), 793-798.

Kačík, F., Solár, R., and Baloghová, D. (1995). "Analysis of lignin nitrobenzene oxidation products by high performance liquid chromatography (HPLC)," *Drevársky výskum* (Wood Research) 40(3), 13-20.

Kačík, F., Solár, R., and Melcer I. (1993). "Stanovenie monosacharidov v dreve a drevných materiáloch metódou plynovej chromatografie" (Determination of monosaccharides in wood and lignocellulosics by the method of gas liquid chromatography), Zborník vedeckých prác Drevárskej fakulty TU vo Zvolene, p. 99-103.

Kürschner, K., and Hoffer, A. (1929). "Ein neues Verfahren zur Bestimmung der Cellulose in Hölzern und Zellstoffen," *Techn. Chem. Papier und Zellstoff. Fabr*. 26, 125-129 in Kačík Fr., and Solár R. (1999). *Analytická Chémia Dreva* (Analytical Chemistry of Wood – Textbook). Technická univerzita vo Zvolene. ISBN 80-228-0882-0, 369 p.

Laffend, K. B., and Swenson, H. A. (1968). "Effect of acetyl content of glucomannan on its sorption onto cellulose and on its beater additive properties. I. Effect on sorption," *Tappi J*. 51(3), 118-123.

Malkov, S. (2002). "Studies on liquid penetration into softwood chips – Experiments, models, and applications," Dissertation Thesis for the DrSc. degree, Helsinki University of Technology, Laboratory of Pulping Technology Reports, Series A29, Espoo, 67 p.

Malkov, S., Tikka, P., Gustafson, R., Nuopponen, M., and Vuorinen T. (2003). "Towards complete impregnation of wood with aqueous solutions. Part 5: Improving uniformity of kraft displacement batch pulping," *Paperi Puu* 85(4), 215-220.

Marchessault, R. H., Settineri, W., and Winter W. (1967). "Chrystalization of xylan in the presence of cellulose," *Tappi J.* 50(2), 55-59.

Messner, K., Facker, K., Lampaipis, P., Gindl, W., Srebotnik, E., and Watanabe, T. (2003). "Overview of white-rot research–where we are today," In: *Wood Deterioration and Preservation*. ACS Symp. Ser. 845, Am. Chem. Soc., Washington DC, p. 73-96.

Messner, K., and Srebotnik, E. (1994). "Biopulping – An overview of developments in an environmentally safe paper making technology," *FEMS Microbiology Reviews* 13, 351-364.

Reinprecht, L., Solár, R., Geffert, A., and Kačík F. (2007). "Pre-treatment of spruce chips by fungi with aim to improve the pulp properties," *Wood Res*. 52(1), 1-13.

Rydholm, S. A. (1965). *Pulping Processes*. Interscience Publishers, New York, 1269 p.

Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millet, M. A. (1954). "Technique for the determination of pulp constituents by quantitative paper chromatography," Tappi 37(8), 336-343.

Sjöström, E. (1993). *Wood Chemistry. Fundamentals and Applications*, Academic, New York, p. 137.

Solár, R., Kačík, F., and Melcer, I. (1987). "Simple semimicro method for determination of O-acetyl groups in wood and related materials," *Nordic Pulp and Paper Research Journal* 2(4), 139-141.

Solár, R., Kurjatko, St., and Reinprecht, L. (2001). "Influence of hornbeam wood pretreatment by white-rot fungus *Phanerochaete chrysosporium* on the course of organosolv, kraft and neutral sulphite delignifications (Part. 1): Pulping and the selected properties of pulps)," *Wood Research* 46(3), 11-20.

Solár, R., Mamoň, M., Dibdiaková, J., Reinprecht, L., Pánek, M., and Hudec, J. (2008). "Effect of biotic and abiotic pretreatments of hornbeam wood on its properties interesting from viewpoint of pulping in alkaline media (Part 1: Physical properties)," *BioResourses* 3(4), 1278-1290.

Solár, R., and Melcer I. (1980). "Physico-chemical and chemical changes in the carbohydrate part of hornbeam wood in the process of wood plasticization with aqueous ammonia solution," *Cellulose Chemistry and Technology* 14(2), 197-202.

Stamm, A. J. (1953). "Diffusion and penetration mechanism of liquids in wood," *Pulp and Paper Magazine of Canada* 54(2), 54-63.

Sumi Y., Hale, R. R., Meyer J. A., Leopold A. B., and Ranby, B. G. (1964). "Accessibility of wood and wood carbohydrates measured with tritiated water," *Tappi J.* 47(10), 621-624.

Zanuttini, M., Citroni, M., Marzocchi, V., and Inalbon M. (2005). "Alkali impregnation of hardwood chips," *Tappi J*. 4(2), 28-30.

Zanuttini, M., and Marzocchi, V. (1997). "Kinetics of alkaline deacetylation of poplar wood," *Holzforsch*. 51, 251-256.

Zanuttini, M., Marzocchi, V., Mocchiutti, P., and Inalbon, M. (2005). "Deacetylation consequences in pulping processes," *Holz als Roh- und Werkstoff* 63, 149-153.

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