Chemical Changes within Solids during Liquid Hot Water Pretreatment of Wood

Viera Kučerová,[a,]* Eva Výbohová,[a] Vladimír Hönig,[b] and Iveta Čabalová [a]

The chemical properties of willow were studied following liquid hot water pretreatment. Three different temperatures of liquid hot water pretreatment were used (160, 180, and 200 °C) for periods of 30, 60, 120, and 240 min. The contents of extractives, lignin, holocellulose, glucose, and xylose was determined. Compared with the original sample, the results indicated that lignin and extractives content increased after pretreatment. Further, the content of xylose decreased significantly with increasing pretreatment time for all three temperatures. The highest glucose content was obtained under the conditions of 180 °C for 240 min. In addition, a significant correlation was found between the content of xylose in the solid fraction and the severity factor of treatment. To determine the changes in cellulose crystallinity, the total crystallinity index (TCI) and the lateral order index (LOI) were calculated from the Fourier-transform infrared spectroscopy spectra of cellulose. An increase in both of these structural characteristics was observed at all experimental temperatures.

Keywords: Changes of extractives; Lignin; Holocellulose; Glucose; Xylose; TCI; LOI

Contact information: a: Department of Chemistry and Chemical Technologies, Technical University in Zvolen, T. G. Masaryka 24, 960 01 Zvolen, Slovak Republic; b: Department of Chemistry, Czech University of Life Sciences Prague, Kamýcká 129, Prague 6, 169 21, Czech Republic;
** Corresponding author: viera.kucerova@tuzvo.sk*

INTRODUCTION

The lignocellulosic biomass of fast-growing wood is a potential renewable raw material and the basis for sustainable production of biobased chemicals, which are important for meeting global energy and chemical demands (Himmel *et al.* 2007). Forestry products and residues, agricultural by-products, energy crops, and municipal solid waste could be used to produce biofuels. In Slovakia, there are available fast-growing wood species, such as willow, which can be used as raw material for biorefinery. Lignocellulosic biomass is the most widespread source of carbohydrates, but this raw material is not readily fermentable. The structure of lignin surrounding cellulose and hemicellulose prevents the breakdown of cellulose and hemicellulose to their sugar components. Without pretreatment, expensive processing enzymes do not have access to complex hydrocarbons to release the desired monomeric sugars. Following enzymatic hydrolysis, extracted sugars are then available for conversion to biofuels or other organic products. The percentages of cellulose, hemicellulose, and lignin in the raw material of biomass are an important criterion for determining its suitability as an economically viable starting material, and to decide on the optimal path for its conversion (Sannigrahi *et al.* 2010). Therefore, the purpose of pretreatment is to disrupt the crystalline structure of inappropriate raw materials, remove or reduce hemicelluloses and/or lignin, and thereby modify the physiochemical properties of the plant cell wall so that the resulting biomass is more suitable for enzymatic deconstruction (Sun *et al.* 2014; Li *et al.* 2016). There are many available pretreatment technologies, including alkaline, concentrated and dilute acid, liquid hot water, ozonolysis, stream explosion, and CO_2 explosion (Kumar *et al.* 2017). Liquid hot water pretreatment is widely used as an effective method for the removal of hemicelluloses from biomass (Antczak *et al.* 2018) because it is environmentally friendly and only uses water as a reagent. In order to release the desired carbohydrates from the wall of the plant cells, the structure must be subjected to some form of pretreatment. This exposes the polysaccharides, which can subsequently be hydrolysed. The formation of monomeric sugars and further degradation to toxic compounds is restricted by controlling the pH of the liquid phase (Mosier *et al.* 2005; Kim *et al.* 2008). Understanding both the chemical and structural transformations of lignocellulosic biomass components during pretreatment will provide important insights into the mechanism of overcoming biomass recalcitrance. Structural features (such as the crystallinity of cellulose) are widely regarded as key characteristics that affect cellulose deconstruction performance, and their changes upon pretreatment are related to the reduction of biomass recalcitrance (Yang and Wyman 2008; Cao *et al.* 2012).

The present study investigated the composition of willow before and after liquid hot water pretreatment. The changes in glucose and xylose content within solids during liquid hot water pretreatment of willow wood were examined. The total crystallinity index (TCI) was used to evaluate the infrared crystallinity ratio.

EXPERIMENTAL

Materials

Willow wood (*Salix alba* L.) was obtained from 15-year-old willow trees grown in the Zvolen region in the Slovak Republic. The bark was removed from the sampled tree. Samples from the trunk of a willow were chipped to dimensions of 2 × 2 × 10 mm (length × width × thickness). The wood chips were stored at room temperature prior to use.

Methods

Liquid hot water pretreatment

Wood chips (ten grams) were added to the stainless 0.3 L PARR 4848 reactor (Parr Instrument Company, Moline, IL, USA), and deionised water was subsequently added to obtain a solid to liquid ratio of 1:4. Water pretreatment was then performed at thermostat maintained temperatures of 160, 180, and 200 °C with the accuracy of temperature ± 2 °C for periods of 30, 60, 120, and 240 min. The pretreatment was stopped by submerging the reactor in an ice bath until the reactor temperature decreased to 20 °C. The pretreated solid was then separated by vacuum filtration and analysed for composition.

Severity factor

In this study, hot water pretreatment conditions corresponded to a severity factor (log R_0) from 3.24 to 5.32. The severity factor is defined as follows,

$$\log R_0 = \log\left[\mathrm{t}\,.\,exp\left(\frac{\mathrm{T}-100}{14.75}\right)\right] \tag{1}$$

where t is the reaction time in minutes (including pretreatment time), T is the hydrolysis temperature in °C, and 100 °C is the reference temperature (Overend and Chornet 1987).

Chemical analysis

Untreated and after-pretreatment samples were mechanically disintegrated to sawdust. Fractions measuring 0.5 to 1.0 mm were used for analyses.

Extractives were obtained in the Soxhlet apparatus with a mixture of ethanol and toluene according to ASTM D1107–96 (2013). Measurements were performed on three replicates per each pretreatment condition.

Lignin content was determined according to ASTM D1106-96 (2013). Data were presented as percentages of oven-dry weight per unextracted wood.

The samples were hydrolysed in a two-stage process for quantitative analyses of saccharides (Sluiter *et al.* 2011). The analyses of saccharides were carried out by high-performance liquid chromatography (Agilent 1200, Santa Clara, CA, USA), equipped with a refractive index detector and an Aminex HPX–87P column (Bio-Rad Laboratories, Hercules, CA, USA), at a temperature of 80 °C. Ultrapure water was used as a mobile phase, eluting at a flow rate of 0.6 mL/min. Measurements were performed on four replicates per each pretreatment condition. Data were presented per unextracted wood.

Cellulose was isolated using the Seifert method (1956). Holocellulose was isolated using the Wise method (1946).

Fourier-transform infrared spectroscopy (FTIR)

The samples of Seifert´s cellulose were analysed using attenuated total reflectance-Fourier-transform infrared spectroscopy (ATR-FTIR). The measurements were performed using a Nicolet iS10 FTIR spectrometer equipped with a Smart iTR ATR sampling accessory with a diamond crystal (Thermo Fisher Scientific, Madison, WI). The resolution was set at 4 cm^{-1} for 32 scans for each sample. The wavenumber range varied from 4000 to 650 cm^{-1}, and six analyses were performed on each sample. OMNIC 8.0 software (Thermo Fisher Scientific, Madison, WI) was used to evaluate the spectra.

The band 1429 cm^{-1} is associated with the amount of the crystalline structure of the cellulose, while the band at 898 cm^{-1} is assigned to the amorphous region in cellulose (Åkerholm *et al.* 2004). The ratio between the two bands was defined as an empirical crystallinity index proposed by Nelson and O'Connor (1964a) as a lateral order index (LOI). The ratio between the bands at 1368 and 2900 cm^{-1}, also proposed by Nelson and O'Connor (1964b) to be the total crystalline index (TCI), was used to evaluate the infrared crystallinity ratio.

Statistical analysis

For all parameters, multiple comparisons were first subjected to an analysis of variance (ANOVA), and the significant differences between the average values of the pretreated samples were determined using Duncan's multiple range test with a *p*-value of 0.05.

RESULTS AND DISCUSSION

The results of main wood components within solids before and after liquid hot water pretreatment of willow are given in Table 1. The close relationship between the extractives, lignin, and holocellulose content in a solid fraction, and the severity factor of liquid hot water pretreatment, was confirmed (Fig. 2). The content of cellulose, hemicelluloses, lignin, and extractives in wood depends on various factors, such as age, growth conditions, and season of timber harvesting. The degradation reactions and chemical changes of the wood components were observable by a change of colour. The intensity of wood darkening increased with increasing temperature and pretreatment time (Fig. 1). The increase in the intensity of wood darkening at treatment was also observed in other studies (Hrčka 2008; Tolvaj *et al.* 2010; Reinprecht and Hulla 2015; Kubovský *et al.* 2016; Vidholdová *et al.* 2017; Dzurenda 2018).

Fig. 1. Influence of pretreatment temperature and time on the colour of willow

During pretreatment (160, 180, and 200 °C), the amount of extractives increased significantly in comparison with the original sample (Table 1). The content of extractives increased as a consequence of the release of degradation products of lignin and saccharides in the extraction mixture, and/or due to their condensation reactions with the extractives originally present in the untreated wood. The increase in extractives at treatment was also observed in other studies (Todaro *et al.* 2013; Kučerová *et al.* 2016; Kačík *et al.* 2016; Sablik *et al.* 2018). Under more intensive conditions, the intermediates and extractives originally present in the wood can be degraded (Esteves *et al.* 2008).

Among all the given chemical components of wood, lignin is the most resistant to thermal degradation. The relative amount of lignin increased at the temperatures 160, 180, and 200 °C and at times 30, 60, 120, 240 min. This relative lignin content increase is due to both its higher thermal stability in comparison with saccharides, and changes in its structure resulting from the simultaneous de- and re-polymerisation reactions of lignin (Li *et al.* 2007; Wang *et al.* 2012; Ko *et al.* 2015a,b).

The holocellulose content decreased with pretreatment time for all three temperatures. The minimal content of holocellulose was 47.16 % for 240 min at 200 °C. This decrease was accompanied by the growth of degradation reactions of hemicellulose, because content of xylose decreased by pretreatment (Fig. 3). The decrease in holocellulose after the LHW pretreatment was also observed by Antczak *et al.* (2018).

Table 1. Main Wood Components and Severity Factor within Solids Before and After Liquid Hot Water Pretreatment of Willow

Temperature (°C)	Time (min)	Extractives (%)	Lignin (%)	Holocellulose (%)	Severity factor
20	0	5.27 ± 0.01	24.44 ± 0.11	70.31 ± 0.20	-
160	30	13.17 ± 0.01	18.78 ± 0.04	66.72 ± 0.13	3.24
	60	17.87 ± 0.07	19.72 ± 0.23	62.45 ± 0.16	3.54
	120	22.36 ± 0.08	19.45 ± 0.27	58.09 ± 0.21	3.85
	240	23.41 ± 0.34	20.46 ± 0.21	57.03 ± 0.11	4.15
180	30	22.20 ± 0.01	17.04 ± 0.08	61.65 ± 0.26	3.83
	60	24.00 ± 0.03	17.03 ± 0.03	59.57 ± 0.20	4.13
	120	24.04 ± 0.26	18.51 ± 0.20	58.39 ± 0.05	4.43
	240	19.86 ± 0.11	22.97 ± 0.01	57.98 ± 0.14	4.74
200	30	24.46 ± 0.10	19.13 ± 0.55	56.41 ± 0.18	4.42
	60	21.15 ± 0.14	24.99 ± 0.16	54.76 ± 0.06	4.72
	120	17.85 ± 0.24	29.00 ± 0.40	52.85 ± 0.24	5.02
	240	14.16 ± 0.34	37.98 ± 0.40	47.16 ± 0.13	5.32

Note: Data represent means ± SE (standard error)

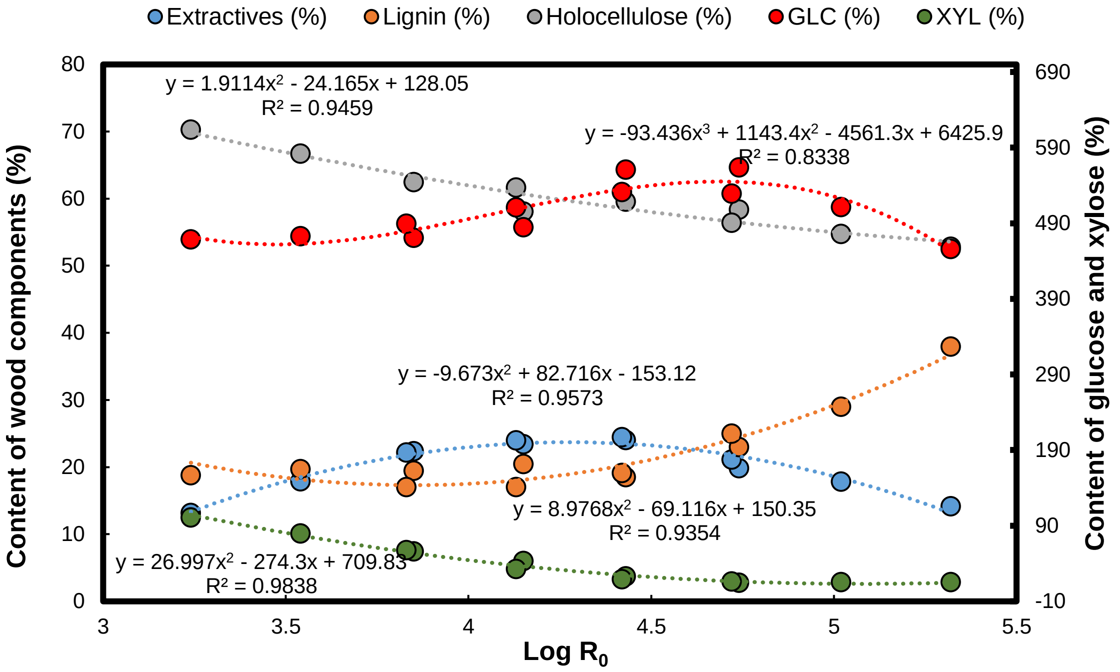

Fig. 2. Correlation between the content of wood components, glucose and xylose in solids after liquid hot water pretreatment of willow and the severity factor

The changes of neutral saccharide (xylose and glucose) compositions are given in Fig. 3. The native willow was predominantly composed of glucose (471 ± 0.08 mg.g^{-1}) and xylose (122 ± 0.08 mg.g^{-1}). The amounts of arabinose (1.9 ± 0.05 mg.g^{-1}), galactose (2.75 ± 0.06 mg.g^{-1}), and mannose (1.77 ± 0.09 mg.g^{-1}) were low. In this study, the content of xylose decreased significantly with pretreatment time for all three temperatures (160, 180, and 200 °C), as shown in Fig. 3. At 160 °C, the xylose content decreased by the pretreatment process from 122 to 43 mg.g^{-1}, which is a relative decrease of 65%. The content of xylose decreased by up to 88% from its original value at 180 °C, and decreased by up 87% at 200 °C. The experimental data confirmed thus the preferential solubilisation of hemicelluloses. In addition, a significant negative correlation was found between the content of xylose in the solid fraction and the severity factor of treatment (Fig. 2). This result agrees with the findings of Yanez *et al.* (2009) aimed at hydrothermal pretreatments on acacia dealbata at a temperature range of 170 to 240 °C.

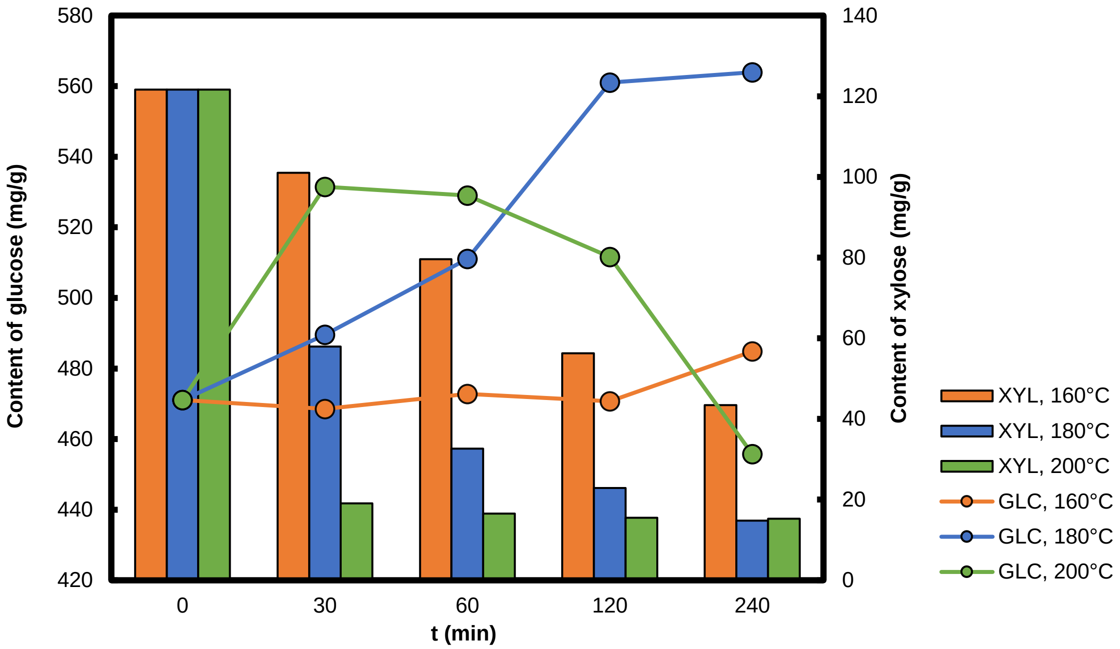

Fig. 3. The content of glucose and xylose in solids before and after liquid hot water pretreatment of willow

In the samples, the highest content of glucose occurred at a temperature of 180 °C for 240 min (563.9 ± 1.49 mg.g^{-1}). The content of glucose increased up to 19.7% of its original value at this temperature. The glucose content increased approximately 12.8% during the first 30 min of the pretreatment at 180 °C (Fig. 3). Subsequently, the changes were negligible until a pretreatment time of 240 min, where the increase was significant in comparison to other samples. The relative increase in glucose content in the initial stages of pretreatment is due to easier hydrolysability of hemicelluloses compared to cellulose. However, at 200 °C, after 30 min of pretreatment, the content of glucose in the solid fraction gradually decreased. This can be caused by the release of hemicelluloses containing monosaccharide glucose (glucomannans) and the amorphous regions of cellulose into hydrolysate.

The ratio of glucose to xylose (Fig. 4) rapidly increased with pretreatment time at 180 °C (from 3.87 to 38.14, an increase about 10-fold). The calculated ratio increased by only 3-fold at 160 °C. These increases were involved in the degradation of hemicelluloses, mainly their rapid degradation at 180 °C, while hemicelluloses are the most thermally degradable constituents of wood. Changes at 200 °C were fast at the beginning of pretreatment, and then slowed down. This implies that almost all accessible hemicelluloses were removed from the willow at 200 °C during 30 min by hot water pretreatment.

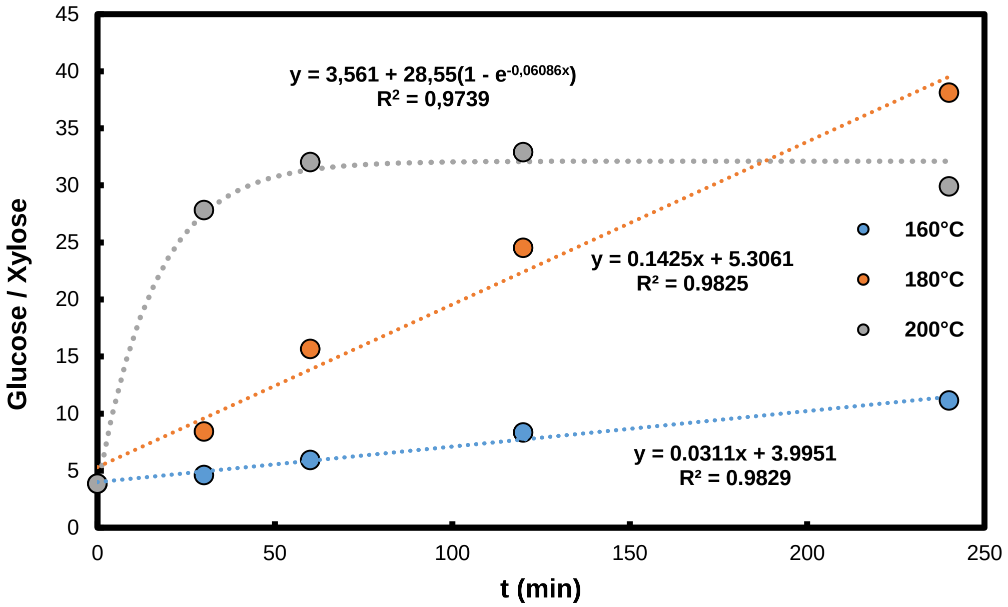

Fig. 4. Changes the ratio of glucose to xylose in solids after liquid hot water pretreatment of willow

Three hydroxyl groups in each cellulose unit are involved in a number of inter- and intramolecular hydrogen bonds. This results in the formation of various ordered crystalline arrangements. The ratio between the crystalline and the amorphous fractions has a significant impact on the physicochemical properties of the fibre (Smuga-Kogut *et al.* 2016), and on the accessibility of cellulose to reactions (Chen 2014).

To determine the changes in cellulose crystallinity, two parameters from FTIR spectra of cellulose were determined. These are the total crystallinity index (TCI) and the lateral order index (LOI). Whereas the TCI is proportional to the overall degree of cellular crystallinity in wood, LOI represents the ordered regions perpendicular to the chain direction (Široký *et al.* 2010). A comparison of the values of these parameters is listed in Table 2.

Regarding the total degree of crystallinity and the lateral ordering of cellulose chains, an increase in index values presenting both of these structural characteristics was observed at all experimental temperatures. These changes could result from the faster degradation of the amorphous cellulose regions, and from the reorganisation of the quasicrystalline cellulose region at higher temperatures (Bhuyian *et al*. 2000). The rate of changes in cellulose crystallinity is affected by treatment conditions and wood species (Bhuyian *et al*. 2000, Geffert *et al.* 2017, Kong *et al.* 2017).

The values of TCI increased due to liquid hot water pretreatment. The increase was in the range from 9.8% to 32.1%, depending on the conditions of the hot-water prehydrolysis (Table 2).

Changes in LOI values are listed in Table 2. Even under the mildest conditions of liquid hot water pretreatment (160 °C for 30 min), a 5.9 % increase in LOI was recorded. In the case of the most severe conditions, (200 °C for 240 min), the LOI more than doubled.

Driemeier *et al.* (2015) found that increasing crystallite mean lateral dimensions is a key structural modification promoted by hydrothermal treatments. With basis on their experimental observations, they argue that increasing crystallite lateral dimensions results from co-crystallization, which they define in terms of interaction of multiple aggregated crystallites. By removing non-cellulosic components, especially hemicelluloses acting as intercrystallite spacers, they propose that treatments promote direct contacts between aggregated cellulose crystallites. Co-crystallization and partial cellulose de-crystallization would result from such direct interfacing of cellulose crystallites.

Table 2. Total Crystallinity Index and Lateral Order Index of Cellulose and their Changes in Solids Before and After Liquid Hot Water Pretreatment of Willow

Temperature (°C)	Time (min)	TCI	Δ TCI	Δ TCI (%)	LOI	Δ LOI	Δ LOI (%)
160	0	0.255	-	-	1.598	-	-
	30	0.280	0.025	9.8	1.692	0.094	5.9
	60	0.290	0.035	13.6	1.750	0.152	9.5
	120	0.294	0.038	15.1	1.836	0.238	14.9
	240	0.301	0.046	18.2	1.923	0.325	20.3
180	0	0.255	-	-	1.598	-	-
	30	0.285	0.030	11.7	1.724	0.126	7.9
	60	0.297	0.042	16.4	1.940	0.342	21.4
	120	0.300	0.045	17.6	2.173	0.575	36.0
	240	0.317	0.062	24.2	2.325	0.727	45.5
200	0	0.255	-	-	1.598	-	-
	30	0.319	0.064	25.1	3.429	1.830	114.5
	60	0.333	0.078	30.7	3.429	1.830	114.5
	120	0.336	0.081	31.9	4.148	2.550	159.6
	240	0.337	0.082	32.1	5.000	3.402	212.9

CONCLUSIONS

The results present the changes in the chemical composition of wood and the structural changes of the wood components, as a consequence of the chemical reactions occurring during hot water pretreatment of willow.

1. The intensity of willow wood darkening increased with increasing temperature and pretreatment time during the liquid hot water pretreatment.
2. At lower temperatures and pretreatment times, a significant increase in the content of extracts compared to the original sample was recorded. However, their content decreased under more intensive conditions.
3. The relative amount of lignin increased at the pretreatment temperatures of 160, 180, 200 ° C and at times 30, 60, 120 and 240 min. The holocellulose content decreased with pretreatment time for all three temperatures.
4. The content of xylose significantly decreased with pretreatment time for all three temperatures. In addition, a significant correlation between the content of xylose in the solid fraction and the severity factor of treatment was found.
5. The highest glucose content occurred at 180 ° C for a pretreatment time of 240 min.
6. The changes of TCI and LOI may result from faster degradation of the amorphous cellulose regions, and from the reorganization of the quasicrystalline cellulose region at higher temperatures.

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under contract No. APVV-17-0583.

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Article submitted: Aug. 15, 2019; Peer review completed: Sept. 24, 2019; Revised version received: Oct. 21, 2019; Accepted: Oct. 30, 2019; Published: Nov. 6, 2019.
DOI: 10.15376/biores.15.1.38-48