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Changes in poplar (*Populus trichocarpa*) wood porous structure after liquid hot water (LHW) pretreatment

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Abstract: *Changes in poplar (Populus trichocarpa) wood porous structure after liquid hot water (LHW) pretreatment.* The aim of this research was to investigate the effect of applying different hydrothermal pretreatment conditions on the porous structure of poplar wood. Porosity is recognised as an important factor considering efficiency of an enzymatic hydrolysis as a step of bioethanol production. Native poplar wood as well as solid fractions after pretreatment performed at different temperatures (160 °C, 175 °C and 190 °C) were analysed. Porous structure was examined with an inverse size-exclusion chromatography (ISEC) method. Results indicated a significant development of the porous structure of the biomass with increasing porosity along with the growing temperature of the LHW process. The temperature of 190 °C was chosen as the most promising condition of poplar wood LHW pretreatment in terms of the efficiency of the subsequent steps of bioethanol production. The obtained results were consistent with the previous experimental data procured during analysis of the LHW pretreated poplar wood and its subsequent enzymatic hydrolysis yield.

Keywords: liquid hot water, porosity, poplar wood, bioethanol production

INTRODUCTION

Nowadays transition from a fossil fuel driven energy to a more renewable and green options is taking place due to the growing environmental awareness, as well as the governmental policies. An enzymatically based cellulosic ethanol is one of the most promising alternatives for the traditional transportation fuels from the non-renewable sources (Yang *et al*. 2011). For instance the biomass from a fast-growing plantation wood is a very abundant and sustainable source for such ethanol procurement. Hybrid poplars are among the fastest growing temperate trees in the world and a very promising feedstock for the cellulosic bioethanol obtainment because their properties can be tailored especially for the biofuels production. Modifications that reduce the lignin content are among the most common techniques employed to better a poplar feedstock quality as it improves the enzymatic hydrolysis of polysaccharides (Sannigrahi *et al*. 2009).

However the efficient utilization of the lignocellulosic feedstock for the bioethanol production requires a certain pretreatment due to the natural recalcitrance of the plant cell walls to the direct enzymatic hydrolysis. Thus the main objective of a pretreatment is to maximize the cellulose susceptibility to the enzyme digestion which can be achieved mainly through overcoming the lignin-hemicellulose barrier (Chundawat *et al*. 2011). Furthermore, increasing biomass porosity also enhances the enzyme penetration. Wood has a certain level of the permeability through its porous structure which is strongly related to its internal microstructure, particularly porosity, pore size and the connectivity between pores (Tan *et al*. 2020).

A pore distribution ranges from the macro to the nano levels. Based on their size, pores may be divided according to IUPAC (1994) classification into: macropores (pore dimension above 50 nm), mesopores (pore dimension between 2 nm and 50 nm)

and micropores (pores dimension below 2 nm) (Lawrence and Jiang 2017). The main channel for the initial permeation of liquids from the exterior to the interior of the wood happens through macropores with diameter within the range of 15-400 μm such as vessels, tracheids and rays. Furthermore those elements are interconnected by the pit apertures with the diameters varying between 0.4 μm to 30 μm which classifies them also as the macropores. Moreover getting to the lumen of a fibre, the liquid starts to diffuse into a cell wall via the micropores and the mesopores with the diameters of few nanometres. Those micro- and mesopores in the cell wall or between the cellulose microfibrils have considerable specific surface areas and strong adsorption capacities. The porosity of molecular-scale dimensions that can be observed in the wood cell wall is a result of the partial filling of the space between the cellulose microfibrils with lignin, hemicelluloses or extractives (Yin *et al*. 2015).

The liquid hot water (LHW) method is one of the most common physiochemical pretreatments due to its relative low cost, high efficiency and general simplicity (Li *et al*. 2014). This method is based on applying hot water under pressure for a certain period of time in a high temperature environment. The main result is an autohydrolysis of acetyl and carboxylic groups contained in hemicelluloses which leads to their further separation from the cellulose. Another impact of such pretreatment is enlarging pore sizes which can also enhance the cellulase enzyme penetration into the biomass.

Thus the objective of this research was to investigate the effect of applying different temperatures of the hydrothermal treatment conditions on the porous structure of the biomass from poplar wood intended for further bioethanol production via the enzymatic hydrolysis process.

MATERIALS AND METHODS

Raw material

A stem-wood of a 7-year-old fast-growing poplar *P. trichocarpa* harvested at the end of winter was subject to this research. The poplar wood was obtained from an experimental field in Wolica owned by the Institute of Biology, Department of Genetics, Plant Breeding and Biotechnology at the Warsaw University of Life Sciences. The wooden material was debarked, dried and then chipped and milled into the particles with dimensions from 0.43 mm to 1.02 mm. Then, the hydrothermal process was performed.

LHW pretreatment

Before the actual pretreatment the biomass (20 g) was soaked in the distilled water at 75°C using a magnetic stirrer for 20 min to remove out air and cause a swelling of the biomass. After that it was quantitatively placed in a stainless steel reactor with respective amount of water in order to apply solid to liquid ratio of 1:12.5. Next the reactor was placed in an oil bath that was pre-set and subsequently maintained at 160 °C, 175 °C and 190 °C for 20 min prior to a rapid cooling ending the reaction. The solid and liquid fractions were separated by filtration with a Büchner funnel. The solid fraction was than washed with the distilled water until the pH reached value of 7. Both neutralized solid and liquid fractions were stored at 6 °C until further investigations.

ISEC analysis

The porosity of the native wood and solid fractions obtained after the LHW pretreatment were examined using an inverse size-exclusion chromatography (ISEC)

(Radomski 2015; Zawadzki *et al*. 2016). A procedure was done with a high-performance liquid chromatography (HPLC) system which consisted of: LC-20AD pomp, DGU-20A degasser, CTO-20A oven, RID-10A differential refractive detector and CBM-20A controller (all above Shimadzu, Japan). The procured chromatographic data were processed with a LC Solution v.1.21 SP1 software (Shimadzu, Japan). The empty stainless steel columns (Macherey-Nagel, Germany) with 250 mm length and 4.0 mm internal dimension were used. The columns were filled with an analysed lignocellulosic material, both the native and the pretreated samples. To prevent a mechanical spoiling of the RID-10A detector filled columns were equipped with the metal filters 1/4 4.6 mm 5 μm on the both sides. For the pore distribution analysis in the researched material different analytic standards dissolved in the re-destilled water were used (Table 1): a set of 10 dextran standards (Dx) (Fluka, US), ethylene glycol (Sigma-Aldrich, Germany), a set of 3 poly(oxyethylene) standards (POE) (Polymer Standards Service, Germany), methanol (Chempur, Poland), maltose and glucose (both POCh, Poland).

Table 1. General characteristic of analytic standards used to determine the available pores distribution in the lignocellulosic material (Radomski 2015, Szadkowski 2019).

Labeling used at work	**Molar mass at peak maximum**	**The number average molar mass**	**The weight average molar mass**	**Hydrodynamic radius**
	M_p/(kg·mol^{-1})	M_n/(kg·mol^{-1})	M_w/(kg·mol^{-1})	r_η/nm
Dx1k	1.08	1.01	1.27	0.86
Dx5k	4.44	3.26	5.22	1.72
Dx50k	43.5	35.6	48.6	5.35
Dx80k	66.7	55.5	80.9	6.56
Dx150k	123.6	100.3	147.6	8.95
Dx270k	196.3	164.2	273.0	11.21
Dx410k	276.5	236.3	409.8	13.31
Dx670k	401.3	332.8	667.8	15.94
Ethylene glycol	0.06	0.06	0.06	0.30
POEn2	0.11	0.11	0.11	0.37
POEn4	0.19	0.19	0.19	0.47
POEn9	0.43	0.4	0.43	0.63
glucose	0.18	0.18	0.18	0.48
maltose	0.34	0.34	0.34	0.59
methanol	0.03	0.03	0.03	0.24

During the analysis the re-distilled water was used as a mobile phase. For the first 12 h a flow rate of 0.02 cm^3/min was used, and then it was increased by 0.02 cm^3/min every 0.5 h. After achieving the 0.5 cm^3/min flow rate and stabilizing the RID-10A detector signal, the standards from Dx 670 k to Dx 80 k were inserted into column. Then for standards from the Dx 50 k to Dx 12 k the flow rate of 0.7 cm^3/min was used and for the rest of the standards the flow rate of 1 cm^3/min was maintained. The applied changes in the flow rate were determined by desired size related improvement of particular standard separation during analysis. The column temperature during an analysis was maintained at 35 °C. Samples of the particular solid fractions were tested on a wet basis and then dried after analysis in order to calculate a weight of the particular load. Procured chromatographic data were processed with LC Solution v.1.21 SP1 software (Shimadzu, Japan) and the available specific volume of pores (V_p) was calculated according to methodology described by Radomski (2015).

RESULTS AND DISSCUSION

In the fig. 1. changes in the available specific volume of pores in the poplar wood after 20 minutes of the high temperature hydrolysis process performed at the different temperatures are shown. In all cases of the LHW pretreatment, despite the applied temperature, an increase in the available specific volume of pores could be observed compared to the control sample. However, the most significant development of the porous structure was noted in the biomass pretreated with the LHW method performed at 190 °C.

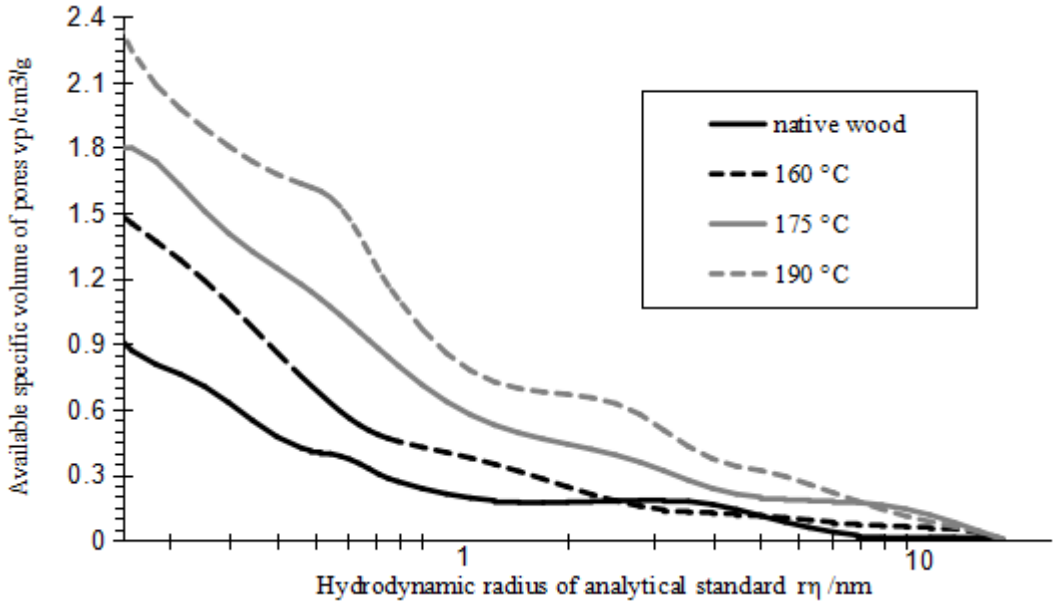

Figure 1. Changes in the available specific volume of pores in poplar wood after 20 minutes of the LHW pretreatment process performed at different temperatures.

In order to illustrate changes in the porous structure more effectively the division of the pore size ranges was implemented. The pores were grouped according to their radius size as it is shown in the fig. 2. In case of a native poplar wood the available specific volume of pores had an average of 0.91 cm^3/g, while the share of the pores from particular range was growing along with their decreasing size. Therefore, the most numerous were the smallest pores with a radius of less than 0.5 nm. Nevertheless, considering the changes in the porous structure of the biomass that can enhance the enzymatic hydrolysis yield, the macropores are of the biggest concern. As it was reported in literature, mostly the pores larger than 10 nm could greatly facilitate the accessibility of the enzymes, for example, cellulase from *Trichoderma reesei* has a radius of approximately 5 nm (Chundawat *et al*. 2011).

As expected, in the biomass after the 160 °C LHW pretreatment the total available volume of pores was higher than in untreated material and amounted to average 1.49 cm^3/g. An increase in the volume of pores from all ranges could be observed, with the most significant raise in case of pores with the 1÷5 nm radius, which more than doubled. Likewise, the volume of the smallest pores with a radius up to 1 nm increased, on the other hand the volume of the pores with the largest radius (>5 nm) stayed roughly the same after the pretreatment performed at 160 °C. Therefore pretreatment at such mild conditions appears to have limited advantage due

to the fact that majority of the available pores are not large enough to be accessible by enzyme molecules (Zeng *et al*. 2007).

In the biomass pretreated at 175 °C further increase of the pores share with radius size above the 0.5 nm was recognized compared to biomass pretreated at 160 °C. However, in comparison to the material treated at 160 °C the share of the smallest pores decreased. Similar as in the previous case, the most significant growth comparing to the untreated biomass, happened for the pores with radius size between 1÷5 nm - a nearly fourfold increase could be reported. Furthermore, more than a two-fold raise in the volume of pores with 0.5÷1 nm radius was observed in comparison to the control sample, as well as a near 80 % increase in the biggest pores share.

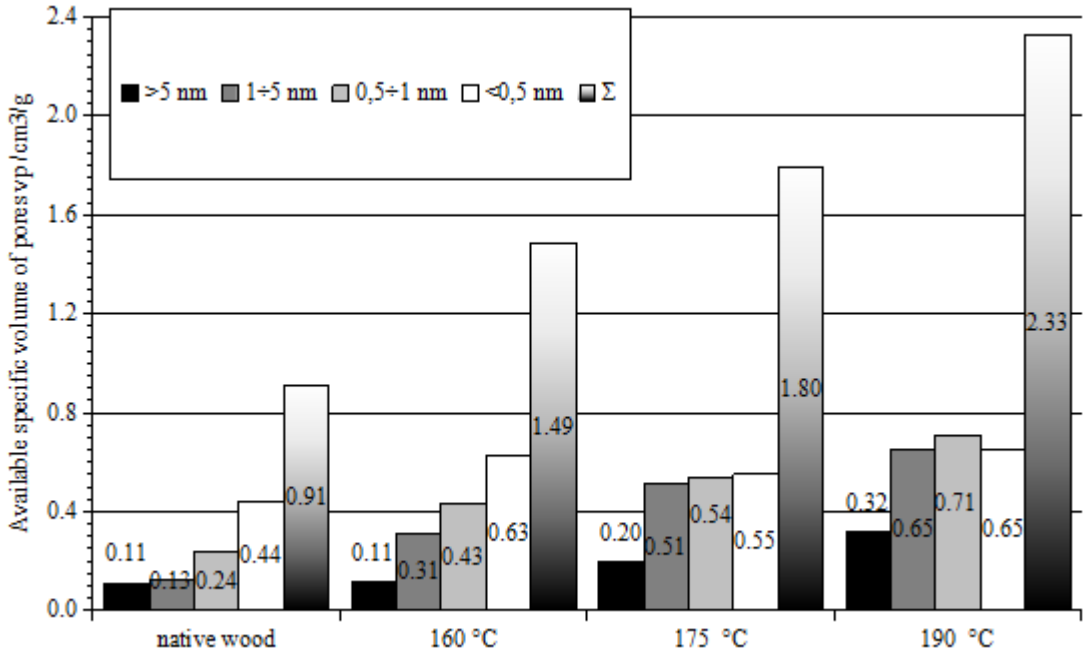

Figure 2. Changes in the available specific volume of pores in poplar wood after 20 minutes of the LHW pretreatment process performed at different temperatures within the selected ranges of the standards radius.

As a result of the LHW pretreatment performed at 190 °C the most significant development of the porous structure of the biomass took place. An increase of the available volume of pores from all the size ranges could be observed, compared both to the untreated biomass and all the samples of the biomass treated in the more mild conditions. After LHW pretreatment at 190 °C the most considerable growth happened in case of the volume of pores with the radius above 5 nm, it was almost three times larger than in case of the native feedstock. As to the biomass pretreated at 175 °C the available volume of pores from all ranges increased considerably, while compared to biomass pretreated at 160 °C the growth in case of the smallest pores volume was rather negligible - just a little over 4 %. Generally, the average available specific volume of pores in the biomass after the 190 °C pretreatment amounted to 2.33 cm^3/g, thus more than double that in untreated biomass. Similar findings were reported in literature for other lignocellulosic biomass with the LHW pretreatment at 190 °C said to generate and enlarge pores significantly, thus enhancing the enzyme accessibility resulting in the 3 to 4 times higher glucose yield upon hydrolysis (Zeng *et al*. 2007).

Furthermore, yield of the enzymatic hydrolysis of the studied LHW-treated biomass had been researched in previous study (Akus-Szylberg *et al*. 2020). The highest content of glucose had been achieved for biomass after pretreatment at 190 ºC, however it had not been much higher than in case of biomass treated with less severe conditions (160 ºC and 175 ºC), which does not confirm strong impact of the porous structure development on the hydrolysis efficiency. Some researches proved that pretreatment effectiveness and the extent of subsequent cellulose hydrolysis is associated with the accessible pore volume and the surface area (Lin *et al* 1985; Zhang and Lynd 2004). Grethlein (1985) found a linear correlations between the hydrolysis rate of the pretreated biomass and the pore size accessible to a molecule with a diameter similar to the size of individual fungal cellulase components. However, in other studies such approach was contested, reporting no correlation between the volume accessible to an enzyme-sized molecule and the digestibility of the cellulose (Ishizawa *et al*. 2007). For example, Lynd (1996) determined that the bacterium cellulase systems, which are much larger than that of the individual fungal enzymes have significantly higher specific activity. Both, bacterium and fungal enzyme systems appear to be similarly effective at the hydrolysis yields when acting on pretreated biomass. Furthermore, some researchers mentioned also possible cellulase entrapment in the pores, resulting in lower hydrolysis rates (Zhang and Lynd 2004). Therefore, this research area requires further investigation as the mechanism of the hydrolytic enzyme action is more complex than would be suggested by particle size effects alone (Zeng *et al*. 2007).

CONCLUSIONS

The porous structure analysis of the material obtained from native poplar wood and the LHW pretreated biomass indicated that the hydrothermal treatment significantly increased porosity of the lignocellulosic feedstock. Especially pretreatment performed at 190 ºC resulted in the formation of the large pores, which seem decisive considering the diameter of cellulase enzymes applied during process of the hydrolysis in the bioethanol production technology. The obtained results were consistent with the previous experimental data procured during analysis of the LHW pretreated poplar wood and its subsequent enzymatic hydrolysis yield (Akus-Szylberg *et al*. 2020).

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Streszczenie: *Zmiany w strukturze porowatej drewna topoli po obróbce gorącą wodą (LHW).* Celem badań było zbadanie wpływu zastosowania różnych warunków hydrotermalnej obróbki wstępnej na porowatą strukturę drewna topolowego.

Porowatość jest uznawana za ważny czynnik, biorąc pod uwagę efektywność hydrolizy enzymatycznej, jako etapu produkcji bioetanolu. W pracy przedstawiono analizę porowatości biomasy po procesie obróbki wstępnej gorącą wodą przeprowadzonym w różnych temperaturach (160 °C, 175 °C i 190 °C). W celu zbadania struktury porowatej materiału zastosowano odwrotną chromatografię wykluczenia przestrzennego (ISEC). W efekcie przeprowadzonych badań stwierdzono znaczące zwiększanie się porowatości materiału wraz z rosnącą temperaturą obróbki LHW oraz określono 190 °C, jako optymalną temperaturę obróbki wstępnej LHW drewna topoli. Uzyskane wyniki znajdują potwierdzenie we wcześniej prowadzonych badaniach dotyczących hydrolizy enzymatycznej drewna topoli poddanej obróbce wstępnej LHW.

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