

RESEARCH PAPERS

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HYDROTHERMAL PRETREATMENT OF POPLAR (*POPULUS TRICHOCARPA*) WOOD AND ITS IMPACT ON CHEMICAL COMPOSITION AND ENZYMATIC HYDROLYSIS YIELD

This paper focuses on the effect of liquid hot water pretreatment of fast-growing poplar wood in the context of bioethanol production. The milled Populus trichocarpa wood with a particle size of 0.43-1.02 mm was pretreated with liquid hot water method at temperatures range from 160°C to 205°C and then was subjected to enzymatic hydrolysis. The glucose and xylose content in the hydrolyzates were analyzed with high performance liquid chromatography. On the basis of results it was concluded, that increase of temperature in the hydrothermal pretreatment resulted in an increase of glucose and decrease of xylose content. However, increased temperature of the process led to inhibitor formation.

Keywords: poplar wood, liquid hot water pretreatment, enzymatic hydrolysis, bioethanol

Introduction

Nowadays due to concerns about environmental, economic and energy factors, as well as spread of governmental policies restricting extensive exploitation of traditional fossil fuels there has been increasing interest in new renewable energy sources. Plant-based biomass is regarded as an innovative sustainable energy carrier ranked as one of the most promising alternatives for conventional fossil fuels which have a detrimental effect on the greenhouse gases emission. What is more, around 200 billion tons of plant-based feedstock every year could be available [Piotrowski and Wiltowski 2004]. Through bioconversion of such

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abundant material liquid fuels may be procured such as cellulosic ethanol estimated to potentially limit greenhouse gases emission by 86% [Wang et al. 2007].

Bioethanol can be produced from plant-based biomass through direct fermentation processes (sugar-cane, beetroot) or hydrolysis and subsequent fermentation processes (potatoes, corn grain). However, high production costs and fact that the edible feedstock is used for biofuels manufacturing (which affects global food prices) causes continuous development in a new generation of environmentally friendly fuels, which would be more effective economically and with lower carbon dioxide emission. One source for such so-called second generation biofuels which is gaining more attention is lignocellulosic biomass from fast-growing plantation wood species due to their remarkable growth rate, good adaptability, as well as chemical composition tailored chiefly for energy purposes [Serapiglia et al. 2013; Antczak et al. 2016; Mota et al. 2017].

Depending on intended energy application different wood properties are required and suitability of the particular biomass for bioconversion depends on its chemical composition and structure. Generally, polysaccharides that could be used as substrate in the bioethanol conversion processes are a major component of plant cell walls. However, presence of lignin is a limiting factor for bioethanol procurement as it hinders the accessibility of cellulose microfibrils to enzymatic depolymerization. Therefore, genetically modified wood strives to contain less lignin and more cellulose [Kačík et al. 2012]. Especially fast-growing poplar wood is praised for high sugar content and productivity [González-García et al. 2012; Yavorov et al. 2014]. Among others poplar *Populus trichocarpa* wood is rich with cellulose (52.0-53.0%) and holocellulose (85.4-86.4%) [Antczak et al. 2019]. However, accurate knowledge of chemical composition of the very particular poplar wood is necessary to maximize the efficiency of the biomass utilization considering that significant differences in content are noticed even in terms of species or age of trees [Krutul et al. 2019].

Nonetheless, the chemical components of wood are strongly intermeshed and bonded with lignin forming the lignocellulosic matrix, which is resistant to hydrolytic enzymes and results in low digestibility of raw plant-based materials. Thus an appropriate pretreatment method is required as a necessary upstream process to increase polysaccharides susceptibility to the enzyme digestion. It is an essential step to make lignocellulosic biomass fibres (Lignin Carbohydrate Complex – LCC) more accessible to hydrolysis enzymes and prepare carbohydrates appropriately for subsequent hydrolysis and fermentation processes and that can be achieved by lignin removal and increase of the pore volume [Mosier et al. 2005; Alvira et al. 2010]. In some reports, also decrease in cellulose crystallinity was considered to influence hydrolysis efficiency favourably [Sinitsyn et al. 1991], although others described the opposite effect [Sannigrahi et al. 2010] or no correspondence at all [Kim and Holtzaple 2006]. Nonetheless, that additional pretreatment phase of cellulosic ethanol production

makes it more expensive than starch ethanol procurement. Therefore, feasible manufacturing of bioethanol from lignocellulose materials is one of the leading challenges in biorefinery [Sun et al. 2016].

Pretreatment methods can be distinguished and classified as: physical, chemical, physiochemical and biological [Świątek et al. 2011]. One of the most common physiochemical method is liquid hot water (LHW) pretreatment which could be characterised by high efficiency, low reactor cost and general simplicity, as well as lack of chemical compounds or catalysts applied during process. Moreover, low temperature and pH level between 4-6 during pretreatment assures very limited equipment corrosion and allows to obtain 80% oligosaccharides and 20% monosaccharides with relatively small amount of by-products which may be likely to inhibit subsequent enzymatic hydrolysis process [Li et al. 2014]. In this method hot water is applied under pressure for a certain period of time in a high temperature environment (from 160°C to 240°C). Autohydrolysis of acetyl groups contained in hemicelluloses is triggered which leads to their further separation from cellulose. To date, LHW pretreatment has been studied in different wooden materials, however it still requires research and improvement in terms of process parameters [Imman et al. 2018]. Appropriate method as well as conditions of the pretreatment chosen for a particular biomass are critical for efficiency of the whole ethanol production process [Alvira et al. 2010].

The hydrolysis process converting the biomass biopolymers to fermentable sugars could employ two major categories of methods. The first method uses acids as catalysts, which are corrosive and cause formation of the fermentation inhibitory compounds [Antczak et al. 2018]. The second method, economically challenging, uses enzymes called cellulases, which can be produced by both bacteria and fungi. Cellulases are generally a mixture of several enzymes and at least three major groups of them may be distinguished: endoglucanase tearing into regions of low crystallinity in the cellulose fiber, creating free chain-ends; exoglucanase or cellobiohydrolase deteriorating the molecule further by extracting cellobiose units from the free chain-ends; β -glucosidase hydrolyzing cellobiose to produce glucose; additionally there are other supplementary enzymes that attack hemicelluloses, such as glucuronidase, acetyltransferase or xylanase [Sun and Cheng 2002]. Main advantages of enzymatic hydrolysis are high efficiency, eco-friendliness, limited temperature of 50-60°C and pH level between 4.5-5.5. Those mild conditions require limited energy and do not lead to the formation of inhibitory compounds either to equipment corrosion [Branco et al. 2018].

The aim of this research was to investigate the effect of the applied LHW pretreatment method on the 7-year-old fast-growing poplar wood (*Populus trichocarpa*) chemical composition as it is crucial criterion when determining both economical suitability of the feedstock as well as designating the optimum pathway for its conversion. Especially, suitable method and parameters of the

pretreatment have to be chosen accurately to that very specific biomass which was not subject to research so far. Final assessment of the hydrothermal treatment conditions was based on efficiency of its subsequent enzymatic hydrolysis. Additionally inhibitory compounds formation in liquid fraction obtained after pretreatment was analysed in consideration that enzymatic hydrolysis of pretreated whole slurry, including both pretreated solids and liquor, will likely be necessary to lower capital and operating costs in the future [Yang et al. 2011].

Materials and methods

In this study, freshly-harvested, air-dried stem-wood of fast-growing poplar *P. trichocarpa* was obtained from experimental field in Wolica owned by the Institute of Biology, Department of Genetics, Plant Breeding and Biotechnology at Warsaw University of Life Sciences. The fast-growing poplar, aged 7-year-old, was chipped and milled into particles with dimensions of 0.43 to 1.02 mm. Furthermore, Cellic CTec2 (Novozymes, Denmark), which is a mixture of cellulases, β -glucosidases and hemicellulases with density of 1.203 g/cm³ was used as a catalyst during hydrolysis.

Previously to actual liquid hot water procedure the intended fraction of raw material (about 20 g) was treated with distilled water (200 cm³) aiming to filter out remaining air. During soaking at 75°C, water stirring was done for 20 min using a magnetic stirrer. Consecutive the biomass was quantitatively placed in a stainless steel reactor with the capacity of 250 cm³ and respective amount of water was added in order to apply solid to liquid ratio of 1:12.5. Then the reactor was placed in oil bath that was pre-set and subsequently maintained at 160°C, 175°C, 190°C and 205°C. A thermometer sensor was planted inside the tube routed through the reactor. Each treatment lasted for 20 min after the load inside the reactor reached the set temperature and, it was than cooled rapidly to end the reaction. The solid and liquid fractions were separated by filtration with a Büchner funnel. Solid was then washed with distilled water until pH reached 7.0, though so that the total amount of liquid in the beaker did not exceed 1.5 dm³, so that the volume of the filtrate is known. Two experiments were carried out at each given temperature and the obtained yield was blended. Both neutralized solid and liquid fractions were stored at 6°C until performing enzymatic hydrolysis and further composition investigations.

Thereafter, both untreated and treated solids were subject to the chemical composition analysis after drying for 6 h at 105°C. The extractives content was determined by extraction in mixture of chloroform and ethanol 93:7w/w following the procedure by Antczak et al. [2006]. Kürschner-Hoffer method [Kürschner and Hoffer 1929; Krutul 2002] was performed to cellulose isolation and content determination, while lignin, holocellulose and ash content were

examined according to PN-92/P50092 standard. Each sample was analyzed in triplicates and single standard deviations were calculated.

Enzymatic hydrolysis

To assess the enzyme digestibility of the treated poplar and determine the sugar content, the hydrolysis procedure was performed in triplicates for each material. Untreated biomass was taken through the same procedure as a reference. All the samples used were wet as collected from previous treatment phase with known moisture content. The specimens were weighed in 10 cm³ sealed screw-capped test tubes with cellulose concentration of 1% w/w. Subsequently 5 cm³ of a 0.1 M citrate buffer solution at a pH of 4.8 and 0.1 cm³ of a 2% sodium azide solution, preventing micro-organisms growth, were added to each sample. Then, distilled water was added in amount that was calculated so that the total volume of the solution was 10 cm³.

Furthermore, 0.333 cm³ of the 25% v/v enzyme solution was injected into each test tube (0.1 g of enzyme per 0.1 g of cellulose). Finally, screw-capped test tubes containing the enzyme hydrolysis preparations were placed in a mixer (RM-2M, Elmi, USA) and agitated at 25 rpm for 72 h at 50°C. After hydrolysis, the collected samples were stored in a freezer at -20°C until the sugar content in the supernatant was analyzed.

HPLC analysis

Samples were analyzed for glucose and xylose content using high-performance liquid chromatography (HPLC). Preceding the actual chromatography analysis samples were defrosted, brought to room temperature and denatured in a water bath at 95°C for 15 minutes. Afterwards, specimens were centrifuged at 12 000 rpm for 10 min and filtered using a nylon syringe filter with a porosity of 0.2 µm.

Sugars after hydrolysis were analyzed using HPLC system which consisted of LC-20AD pomp, DGU-20A degasser, CTO-20A oven, RID-10A differential refractive detector, CBM-20A controller (all above Shimadzu, Japan) and Rezex RHM-Monosaccharide column (300 × 7.80 mm) (Phenomenex, USA). Re-distilled water was used as the mobile phase at a flow rate of 0.6 cm³/min, injection volume of 20 µL and the column temperature was maintained at 80°C. Procured chromatographic data were processed with LC Solution v.1.21 SP1 software (Shimadzu, Japan).

The amount of sugar components (xylose and glucose) in hydrolysates was calculated based on calibration curves constructed according to equations (1) and (2) where „x” stands for concentration of an analyzed standard, „y” for peak area obtained from a chromatogram of the standard and „RT” for retention time in minutes:

$$y = 2761232 x; R^2 = 0.9996; RT = 11.10 \text{ (xylose)} \quad (1)$$

$$y = 2694228 x; R^2 = 1.0000; RT = 10.40 \text{ (glucose)} \quad (2)$$

Additionally chemical composition of liquid fraction obtained after LHW pretreatment was examined by high-performance liquid chromatography accordingly to method described above. Samples of particular liquid fractions were taken and checked for presence of organic compounds, that is carbohydrates (glucose, xylose and cellobiose) and inhibitors (acetic acid, levulinic acid, furfural and 5-hydroxymethylfurfural). The amount of each above substance was calculated using calibration curves corresponding to equations (3-8):

$$y = 1895963 x; R^2 = 0.9987; RT = 14.93 \text{ (acetic and levulinic acids)} \quad (3)$$

$$y = 2875979 x; R^2 = 0.9970; RT = 36.36 \text{ (furfural)} \quad (4)$$

$$y = 2723647 x; R^2 = 0.9937; RT = 26.58 \text{ (5-hydroxymethylfurfural)} \quad (5)$$

$$y = 2827001 x; R^2 = 0.9970; RT = 11.10 \text{ (xylose)} \quad (6)$$

$$y = 2916029 x; R^2 = 0.9977; RT = 10.40 \text{ (glucose)} \quad (7)$$

$$y = 2841706 x; R^2 = 0.9971; RT = 8.30 \text{ (cellobiose)} \quad (8)$$

Acetic and levulinic acids were combined due to their joint appearance on chromatograph spectrum.

Spectrophotometric analysis

The quantitative determination of lignin content in liquid fraction was performed by UV mini-1240 spectrophotometer (Shimadzu, Japan) according to Sluiter et al. [2012]. Samples for spectrophotometry examination were placed in quartz cuvettes with 1 cm thickness and an absorbance was measured at a 205 nm wavelength. Distilled water was used as a reference solution.

Results and discussion

In this work, the LHW pretreatment resulted in significant changes in extractives, cellulose and hemicelluloses content, which is presented in table 1. As can be seen, growing temperature of the liquid hot water pretreatment had an immense effect on hemicelluloses content decline. Extensive decrease of hemicelluloses content was probably caused by intense hemicelluloses degradation, of which products might had been detected as extractives. That is why with higher temperature of the process, the extractives content increased as drastically as the hemicelluloses content declined. In untreated biomass hemicelluloses constituted for 35.2%, while in material treated with the LHW procedure at 160°C, the hemicelluloses content was only 4%. Furthermore, for material after LHW pretreatment at 190°C hemicelluloses were effectively eliminated. The analogous relationships were observed in other studies [Ma et al.

Table 1. The chemical composition of 7-year-old poplar wood both untreated and pretreated with LHW process

Chemical composition [%]	Untreated wood	LHW pretreated wood			
		160°C	175°C	190°C	205°C
Extractives	1.7 ±0.1	7.3 ±0.4	14.7 ±0.2	17.5 ±0.3	20.7 ±0.2
Cellulose	50.2 ±0.4	72.3 ±1.0	78.7 ±0.4	82.9 ±0.4	82.8 ±0.1
Lignin	20.2 ±0.2	19.0 ±0.6	18.6 ±0.4	19.4 ±0.3	23.3 ±0.7
Holocellulose	85.4 ±0.6	78.3 ±0.4	81.1 ±0.1	81.8 ±0.1	74.3 ±0.4
Hemicelluloses*	35.2	4.0	2.4	0.0	0.0
Weight loss after pretreatment	-	11.0	25.0	32.3	40.7

*calculated from the difference in content of holocellulose and cellulose.

2013]. Drastic hemicelluloses dissolution as a result of heat can notably increase cell wall extractability, indicating a loosening of the cell wall and drop in binding strength [Verardi et al. 2012].

Cellulose content in any treated biomass was significantly higher than in untreated feedstock (Table 1). The cellulose content increase was from 50.2%, in case of untreated material, to 72.3% in case of biomass treated at 160°C. With growing temperature of the LHW pretreatment, the cellulose content increased rather steadily, however to a smaller extent. Growth in cellulose content was probably a result of hemicelluloses loss and lignin deterioration [Fengel and Wegener 1984]. Therefore, a residual holocellulose is primarily cellulose with a small amount of hard to reach hemicelluloses. Holocellulose content tended to decrease selectively due to progressive dissolution of the material. However rapid decline at 205°C was observed which might be caused by serious material deterioration and loss of lignin that usually stays in holocellulose after delignification process [Kupczyk et al. 2013]. Generally, hydrothermal treatment had a limited impact on cellulose, primarily because severe degradation of cellulose occurs at temperature higher than 240°C [Sun et al. 2016].

Minor delignification could be observed in biomass after pretreatment (Table 1), due to general mass loss caused by dissolution. On the other hand, biomass after LHW procedure performed at 205°C had slightly increased lignin content. That could be result of intensive xylan degradation and lignin condensing with carbohydrate degradation products, which was already reported in different studies [Li et al. 2016; Lu et al. 2016].

Additionally, structural and chemical bounds between different macroparticles present in a cell wall might have caused difficulties during analysis of chemical composition of linocellulosic materials. That could hinder the selective separation of particular chemical components and resulted in some

inaccuracies in cumulative analysis of wood components [Fengel and Wegener 1984].

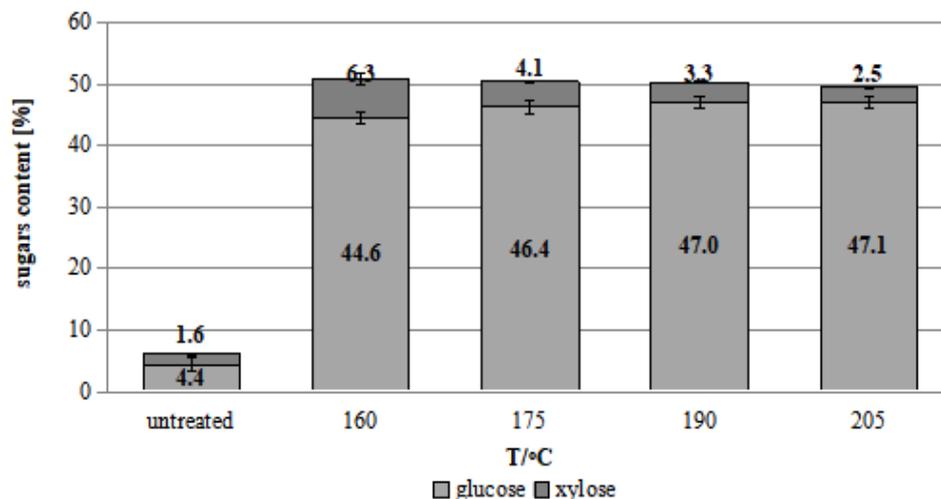


Fig. 1. The sugars content (glucose and xylose) after enzymatic hydrolysis of biomass obtained from *Populus trichocarpa* untreated and treated wood with LHW process at different temperatures

As shown in Figure 1. the liquid hot water process highly increased the amount of simple sugars (glucose and xylose) obtained from enzymatic hydrolysis compared to sugars content from untreated poplar wood. The same positive effect of hydrothermal pretreatment method was reported in other papers where improved enzymatic digestibility of pretreated material was connected to partial hemicelluloses removal and lignin transformation, reduced particle size and increased pore volume caused by thermal exposure [Brodeur et al. 2011; Lu et al. 2016].

The highest content of glucose after hydrolysis (47.0% and 47.1%) was achieved at 190°C and 205°C respectively, as it is presented in Figure 1. However glucose content was not much lower after pretreatment at less severe conditions (160°C and 175°C) varying from 44.6% to 46.4%. In case of xylose obtained after hydrolysis, an opposite tendency was observed. Although, in all cases of pretreatment had increased xylose content comparing to untreated feedstock (1.6%), the highest average xylose content (6.3%) was obtained at 160°C and the decrease of xylose content (to the level of 2.5%) after enzymatic hydrolysis of biomass pretreated at 205°C was noticed. The results of xylose content (Fig. 1) very well correspond with the results of holocellulose and hemicelluloses contents (Table 1). The LHW pretreatment and enzymatic hydrolysis enabled depolymerization of hard to reach xylans. Similar findings were indicated in other studies [Martin-Sampedro et al. 2012; Trajano et al. 2015].

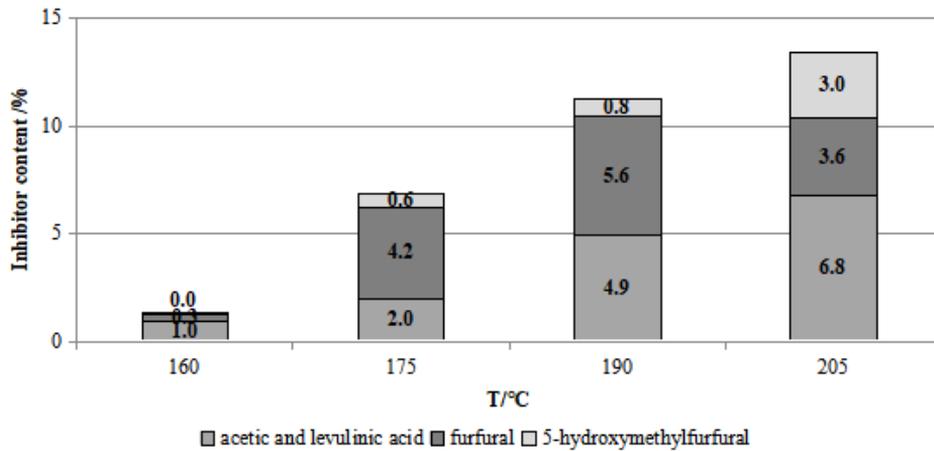


Fig. 2. The inhibitors content (acetic and levulinic acids, furfural and 5-hydroxymethylfurfural) in liquid fraction obtained after LHW pretreatment of *Populus trichocarpa* wood at different temperatures

In this work, the content of inhibitors (Fig. 2), produced during liquid hot water pretreatment, was also determined. Substances such as furfural, 5-hydroxymethylfurfural, acetic and levulinic acids, which are products of pentoses and hexoses degradation respectively are known inhibitory compounds to enzymatic hydrolysis and fermentation processes [Jönsson et al. 2013; Rahikainen et al. 2013; Kim 2018]. The total inhibitors content increased along with the temperature of the LHW process. The rapid raise could be observed from 1.3% in case of poplar wood treated at 160°C to 13.4% for biomass treated at 205°C.

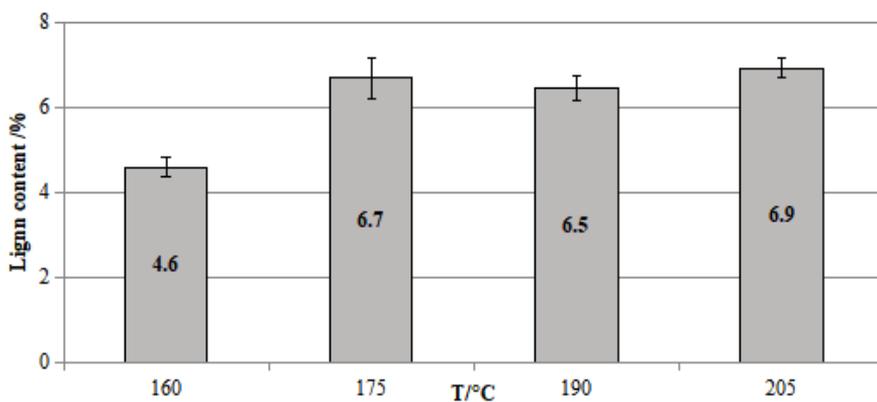


Fig. 3. The lignin content in liquid fraction obtained after LHW pretreatment process of *Populus trichocarpa* wood

Moreover, the content of soluble lignin, detected in all liquid fractions obtained after LHW pretreatment, indicated possible formation of other inhibitory compounds lowering hydrolysis efficiency. Lignin and its products degradation, likewise furan derivatives, can also delay enzymatic hydrolysis and fermentation processes [Kim et al. 2011; Jönsson et al. 2013].

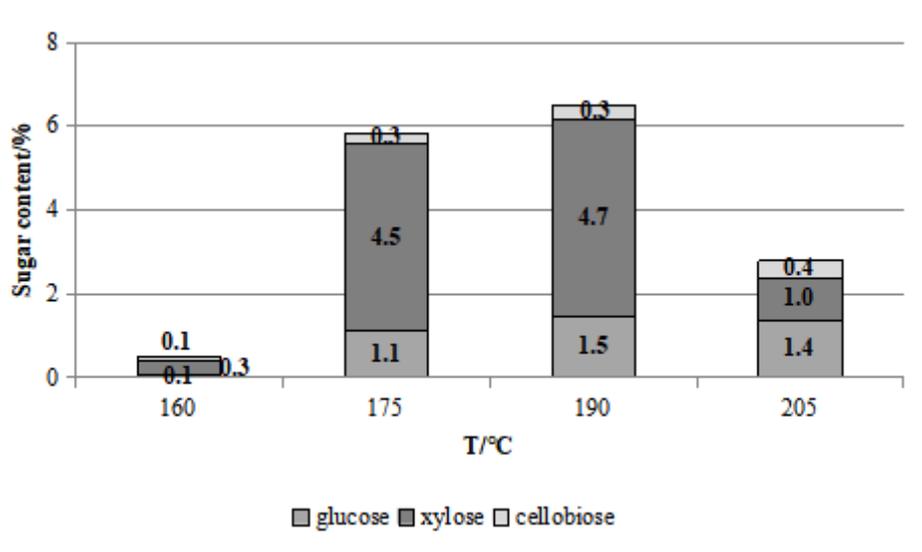


Fig. 4. The sugars content (glucose, xylose and cellobiose) in liquid fraction obtained after LHW pretreatment process of *Populus trichocarpa* wood

Sugar content in liquid fraction obtained after LHW pretreatment are shown in Figure 4. Together with growing temperature of the process glucose content tended to increase, as well as cellobiose content. On the other hand xylose content changed rather selectively which was also observed in other studies [Szadkowski 2019]. It developed rapidly after treatment at 175°C, stayed roughly the same for 175°C and 190°C to drastically decrease in case of biomass treated with hot water at 205°C.

Conclusions

In this research, the liquid hot water pretreatment resulted in significant growth in sugars content (xylose and glucose) in the enzymatic hydrolysates comparing to untreated material. Especially that significant increase occurred already at pretreatment performed at lowest temperature. With growing temperature glucose content increased steadily. However hydrothermal procedure proved to have negative effect on xylan content, which corresponds with chemical composition changes observed after pretreatment. Additionally, in all liquid fractions obtained after LHW pretreatment, the inhibitory compounds (lignin,

furfural, 5-hydroxymethylfurfural, acetic and levulinic acids) were detected. The inhibitors content increased along with the temperature of the LHW process.

The obtained results indicate that LHW pretreatment process of poplar wood positively affects enzymatic hydrolysis yield and shows great potential for large-scale application in biorefinery.

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