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# Perspective pretreatment method of beech and poplar wood and wheat straw in 2G biofuel production processing

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**Abstract**

Monosaccharides such as glucose, xylose and arabinose are the main monomer units of which cellulose and hemicelluloses are composed. The cellulose and hemicelluloses content in many biomass species makes them suitable for 2G bioethanol production. Today, when 1G bioethanol production is closely monitored due to its enormous consumption of food raw materials such as wheat or corn grains, larger companies are gradually moving to pilot operations of 2G bioethanol production. However, cellulose and hemicelluloses contained in biomass are only very slightly accessible to enzymes used in 2G bioethanol production. Therefore pretreatment methods such as steam explosion are very suitable to use for fractionation of cell structure. In this paper, we tested the cellulose accessibility. We compared the cellulose accessibility of wheat straw particles with wooden particles obtained from beech and poplar. Particle size was less than 0.7 mm. We identified the optimal conditions of steam explosion pretreatment at reaction temperature of 200 °C for wheat straw, poplar and beech wood particles. The main indicator of accessibility was concentration of monomers obtained from enzymatic hydrolysis. The concentration of monomer was determined by high performance liquid chromatography. The experimental results showed different accessibility measure for each type of biomass species.



## Introduction

Hexose and pentose sugars are suitable for bioethanol production, however, they are predominantly chemical components of starch, cellulose and hemicelluloses. In general, lignocellulosic biomass rich in cellulose and hemicelluloses include raw materials with worse cellulose accessibility due to their recalcitrant nature and complex structure (Stankovská *et al.* 2018). The composition of different lignocellulosic materials may vary considerably between each other (Jørgensen *et al.* 2007; Joshi *et al.* 2011; Bertero *et al.* 2012; Hazuchová *et al.* 2017; Kucharska *et al.* 2018) and this fact is justified by the genetic variability and diversity among different sources (Prasad *et al.* 2007; Iqbal *et al.* 2013; Shahzadi *et al.* 2014). Some lignocellulosic materials are less suitable for 2G bioethanol production. The disadvantages of materials which are less suitable or unsuitable for 2G bioethanol production include low availability of plants or crops in nature, presence of waxes and resins, low cellulose content, high cellulose crystallinity,

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high lignin and ash content, structure of lignin, presence of acetylated hemicelluloses and lignin-carbohydrate complexes, high degree of polymerisation, low pore volume and low specific surface area of cellulose (Thian Hong 2013). Although specific pretreatment methods can cause favourable changes in lignocellulosic material structure, less suitable or unsuitable materials such as softwood (pine, spruce and fir) without using more energy-intensive thermo-hydro-mechanical fractionation are not useful for large-scale 2G bioethanol production.

The potentially suitable lignocellulosic materials for utilization in 2G bioethanol production include beech wood, poplar wood, and also wheat straw which is an agricultural waste and much of this waste remains unused. The reasons for selection of the raw materials are good geographic and time availability, high holocellulose content and low prices whereas these raw materials are mostly waste or redundant materials. However, cellulose and hemicelluloses are poorly accessible in these materials and pretreatment processes are needed to be applied to obtain significantly enhancement of cellulose accessibility for the following production processes. One of the industrially utilized processes, also used in 1G bioethanol production, is enzymatic hydrolysis (EH). This process is necessary to use as a control process for cellulose accessibility level determination since it gives information about hydrolysate compositions which can vary significantly depending on biomass species and enzymatic hydrolysis conditions (Gigac *et al.* 2017; Pažitný *et al.* 2019a). As published, enzymatic hydrolysis gives a relatively good response for cellulose accessibility when the compositions of original and pre-treated samples are compared and it is also a major financial item in bioethanol production process due to high enzyme cost (Pažitný *et al.* 2019b). Optimal pretreatment conditions for each raw material can result in structural recalcitrance reduction and enhanced cellulose accessibility (Pažitný 2019).

Various suitable thermo-mechanical or thermo-hydro-mechanical pretreatment methods were published by several authors while thermo-chemical pretreatments tend to generate soluble sugars, degradation products of sugars and lignin, various other soluble components, and organic acids (Larsson *et al.* 1999; Weil *et al.* 2002; Kumar and Wyman 2008, 2009; Stankovská *et al.* 2018; Xiong *et al.* 2020). These methods are physicochemical pretreatment methods and they include steam explosion (STEX), ammonia fiber explosion (AFEX) and liquid hot water (LHW) pretreatment. STEX seems to be the most suitable and universal physicochemical pretreatment method because LHW and AFEX have limited efficiency in wood biomass processing (Wyman *et al.* 2005). In case of AFEX, ammonia regeneration is required as well. Thus, the most perspective thermo-hydro-mechanical pretreatment seems to be the steam explosion procedure and its continuous alternative steam extrusion with the use of a low alkali concentration or without the use of any chemicals (Thian Hong 2013; Brezániová *et al.* 2016; Gigac *et al.* 2017; Lopez *et al.* 2019). Steam explosion is a promising pretreatment method for disintegration of the compact structure of hardwood such as beech or poplar wood and wheat straw and thrifty to hydrolysable components (Russ *et al.* 2016; Olguin *et al.* 2018; Pažitný *et al.* 2019a). Also many grass species such as *Miscanthus x giganteus*, *Panicum virgatum* (switchgrass), *Phalaris arundinacea* L. (reed canary grass) with high cellulose content belong to the plant species suitable for their conversion to 2G bioethanol (Pažitný *et al.* 2013; Pidlisnyuk *et al.* 2016). Some authors also applied the mentioned pretreatment method to *Miscanthus x giganteus* (Menardo *et al.* 2013). Miscanthus is a genus of about 20 species of perennial grasses native to Eastern Asia, Northern India and Africa (Heaton *et al.* 2010). Menardo's research team found that the intensity of pretreatment strongly affects the reduction of hemicelluloses at the level by about 15 % – 98 % between temperature ranging from 180 °C and 200 °C, however, the cellulose degradation is not affected by steam explosion pretreatment. Exotic grass species such as miscanthus are cultivated solely for experimental purposes in Slovakia but they may be a good starting point for experimental design of pretreatment processes.

The aim of our work was to optimize the pretreatment method for three waste materials based on biomass available in Slovakia. Beech wood, poplar wood and wheat straw particles were

subjected to the steam explosion pretreatment at various temperatures and the obtained hydrolysates were determined by high performance liquid chromatography (HPLC). The concentration of monosaccharides in hydrolysates showed the cellulose accessibility level for individual biomass materials.

## Experimental

*Lignocellulosic materials*

Lignocellulosic materials as sources of holocellulose for the steam explosion pretreatment were beech wood (*Fagus sylvatica* L.) obtained from Bukóza Píla JSC (Hencovce, Slovakia), poplar wood (*Populus alba* L.) obtained from municipal waste deposited on Fedinova Street (Bratislava, Slovakia) and wheat straw of winter wheat (*Triticum aestivum* L.) of variety Evina grown in the Bratislava Self-Governing Region (Senec, Slovakia).

*Mechanical pretreatment of lignocellulosic materials*

Lignocellulosic materials were pre-treated mechanically by dry milling in Brabender mill (Brabender®, GmbH & Co. KG, Germany). A finer fraction was obtained by a bottom sieve and a 0.7 mm mesh screen was finally used for the laboratory testing. The pre-treated biomass particles were impregnated with fresh water so that the final moisture content of the samples during steam explosion was at least 85 % (w/w). Water content in which the sample was soaked prior to steam explosion pretreatment was calculated to be 15 % (w/w) of the dry biomass. The samples were soaked in water at 20 °C for at least 30 min prior to steam explosion pretreatment.

*Steam explosion pre-treatment*

The prepared materials were subjected to steam explosion at different temperatures (180 °C; 200 °C; 220 °C, respectively) with corresponding pressures (12.4 bar; 15.4 bar; 29.2 bar, respectively) and residence time of 10 minutes. Before each pretreatment, a sample of particles (100 g o.d.) was impregnated in water (5 L) for 30 min. The pretreatment was carried out in a 2 L stainless steel batch reactor (Amar Equipments Pvt. Ltd., India), in which the impregnated particles were loaded at the top and heated to the required temperature. When the pre-set residence time concluded (10 min), the steam-treated biomass was released from reactor by rapid depressurization of steam explosion reactor vessel.

After the steam explosion pretreatment, the pre-treated materials were labelled as steam explosion pre-treated (STEXP) samples and they were subjected to cellulose accessibility control by enzymatic hydrolysis in contrast with non-pre-treated (NP) materials as the original samples of biomass.

*Cellulose accessibility control by enzymatic hydrolysis*

Enzymatic hydrolysis of the non-pre-treated and pre-treated biomass particles with Cellic Ctec3 at a dose of 15 % (w/w) (g Cellic Ctec3 / 100 g cellulose) was carried out at 50 °C, pH = 5.0 for 72 hours and 12.5 % (w/w) of the samples. The pH was adjusted continuously during the process using 0.1 N sulphuric acid or 0.1 N sodium hydroxide. The hydrolysate samples were sampled after 24, 48 and 72 hours, respectively.

*Determination of monosaccharides in hydrolysates*

Actual presence and concentration of mono-saccharides and inhibitors was determined using the procedure of National Renewable Energy Laboratory (Sluiter *et al.* 2008). Monosaccharides (glucose, xylose and arabinose) and generated inhibitors (formic acid, acetic acid and residual inhibitors) were determined in hydrolysates by HPLC method with Rezex ROA (organic acid) $H^+$ column. The HPLC system was supplied by Chromservis SK (Chromservis SK Ltd., Slovak Republic). Refractive index detector RI 101 Shodex was a part of the HPLC system. It was used for detecting and quantitative analysis of monosaccharides (GLU, XYL and ARA, respectively) and inhibitors. Hydrolysates were injected into the HPLC system through smartline

**Table 1.** Availability of STEXP samples for enzymatic hydrolysis testing of cellulose accessibility.

| Lignocellulosic material | Temperature [°C] | | |
|---|---|---|---|
| | 180 | 200 | 220 |
| **Wheat straw** | Available | Available | Available |
| **Poplar wood** | Unavailable | Available | Unavailable |
| **Beech wood** | Available | Available | Available |

universal mounting bracket for manual injection valves. Each sample was analysed three times. The mobile phase passing through column was 0.005 N sulphuric acid at a flow of 0.5 mL.min$^{-1}$ and temperature set to 30 °C. Chromatography data processing was performed by the software Clarity version 5.3.0.180 (DataApex Ltd., Czech Republic) and concentration of individual monomer and inhibition products present in hydrolysates were calculated by external standards of monitored monomers and inhibitors.

## Results and Discussion

### *HPLC chromatograms of STEXP samples of biomass*

The aim of this work was to study the chemical compositions of hydrolysates obtained after enzymatic hydrolysis of NP and STEXP samples of wheat straw, poplar and beech wood particles. The resulting monomers and their concentration in hydrolysates are affected strongly by the original chemical composition of biomass particles and the pretreatment temperature (Russ *et al.* 2016). Additionally, removing hemicelluloses at high temperatures can effectively increase cellulose digestibility (Zhao *et al.* 2012) and enhanced cellulose accessibility for enzymes provides high concentration of monomers due to dramatic changes in biomass morphology after pretreatment aimed at enhancing the reactivity of biomass (Podgorbunskikh *et al.* 2019). The resulting monomers – glucose (GLU), xylose (XYL) and arabinose (ARA) were determined using HPLC combined with external standard methods. These methods are used to perform an accurate qualitative and quantitative analysis of monitored monomers and also formic acid, acetic acid and residual inhibitors in lignocellulosic hydrolysates (Llano *et al.* 2017). According to several scientific papers (Kačík *et al.* 2012; Zhao *et al.* 2012; Kidalová *et al.* 2015; Demčák *et al.* 2017; Demčák *et al.* 2019; Ibrahim *et al.* 2020) it can be predicted that chemical structure of wheat straw, poplar and beech wood particles is mainly composed from cellulose, hemicelluloses and lignin. Those monomers were generated just from holocellulose made of cellulose and all of the hemicelluloses during the action of enzymes. Table 1 shows the available STEXP samples pre-treated at different temperatures. The samples were subsequently hydrolyzed.

The obtained hydrolysates had different concentration of monomers based on the specific chemical composition and porous structure of NP and STEXP samples of wheat straw, poplar and beech wood particles with various holocellulose contents (Table 2). Moreover, the base bulk density of raw materials mildly affects the GLU and XYL yields of enzymatic hydrolysis and this physical property is different in case of the selected lignocellulosic materials (Vasco-Correa and Shah 2019). The bulk density extent can be an advantage when considering transport and logistics of lignocellulosic material of higher density exhibiting a more compact structure.

HPLC analysis showed that the monomers in hydrolysates had a different elution time (Table 3). The elution time did not depend

**Table 2.** The content of chemical components in selected lignocellulosic materials ([a]Russ *et al.* 2016; [b]Kačík *et al.* 2012; [c]Ibrahim *et al.* 2020).

| Lignocellulosic material | Lignin [%] | Cellulose [%] | Holocellulose [%] |
|---|---|---|---|
| [a]Wheat straw | 17.25 | 46.50 | 73.10 |
| [b]Poplar wood | 17.49 – 19.04 | 43.44 – 45.07 | 80.61 – 81.89 |
| [c]Beech wood | 24.30 | 43.00 | 65.70 |

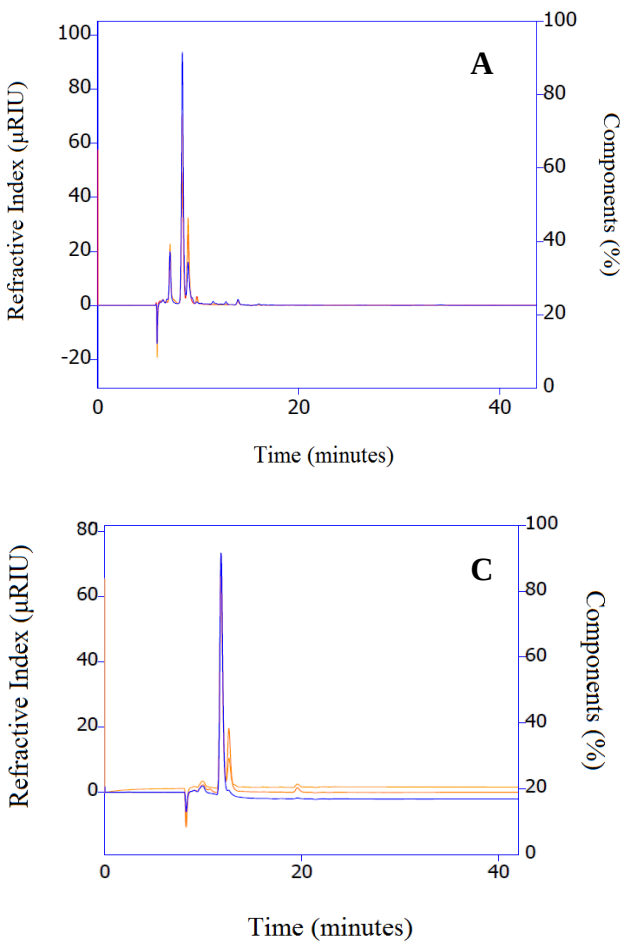


**Fig. 1.** HPLC chromatograms from hydrolyzates after 72 h of enzymatic hydrolysis of STEXP samples pre-treated at different temperatures (**A** – wheat straw particles, 180 °C, 200 °C, 220 °C; **B** – poplar particles, 200 °C; **C** – beech particles, 180 °C, 200 °C, 220 °C).

on monomer concentration, however, it depended on set-up and conditions of HPLC method. Fig. 1 demonstrates three HPLC chromatograms from hydrolyzates of STEXP samples pre-treated at different temperatures (180 °C; 200 °C; 220 °C). The hydrolysates were sampled after 72 hours. All the chromatograms show the strong signal of GLU monomer (elution time of 11.80 minutes) and the weaker signals of C5 sugars such as XYL (elution time of 12.60 minutes) and ARA (elution time of 13.85 minutes). Significant reduction of C5 sugars can be seen in case of beech and wheat straw hydrolysates obtained from STEXP at a lower temperature. The lignocellulosic biomass decomposition mechanism during thermo-hydro-mechanical refining processes is known. The rate of this decomposition is higher for hemicelluloses, much lower for cellulose and the lowest for lignin (Sandberg *et al.* 2013; Pažitný 2019). This explanation can be projected into the fact that the lower concentrations of C5 sugars correspond to lower peaks with equal elution time and they belong to the pretreatments at higher temperatures.

**Table 3.** Elution time of analysed sugar monomers in hydrolysates.

| Analysed component | Elution time [min] |
|---|---|
| **Glucose** | 11.80 ± 0.01 |
| **Xylose** | 12.60 ± 0.02 |
| **Arabinose** | 13.85 ± 0.02 |

*Production and determination of monosaccharides*

The average values for concentrations of monosaccharides determined by the HPLC method depend on enzymatic hydrolysis (EH) time and also on the steam explosion temperature (Fig. 2 – 4). The qualitative HPLC method revealed the monomers of both cellulose and hemicelluloses. The hydrolysates from wheat straw (Fig. 2), poplar particles (Fig. 3) and beech particles (Fig. 4) contained monomers GLU and XYL. In addition of these monomers, however, the enzymatic hydrolysates obtained from poplar particles also contained monomer ARA (Fig. 3). Various compositions of enzymatic hydrolysates and their

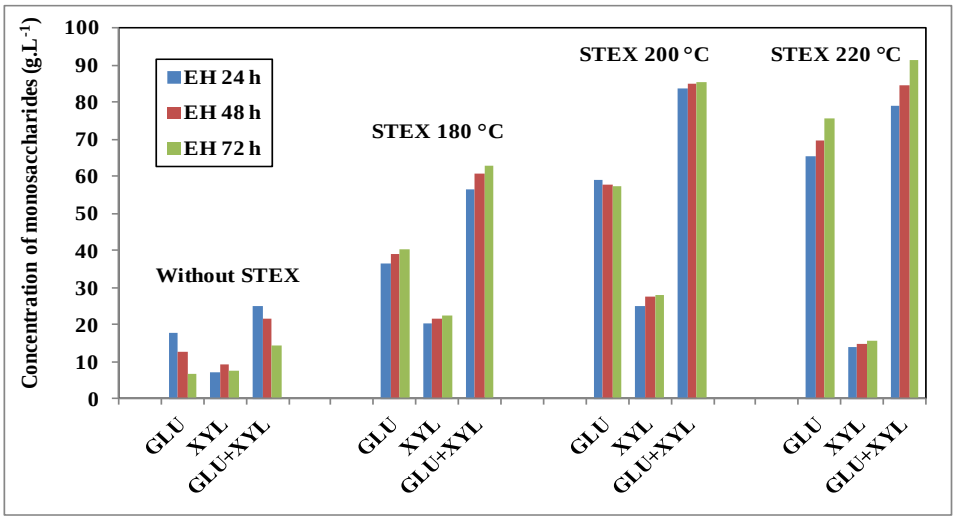


**Fig. 2.** Influence of steam explosion (STEX) temperature on concentrations of monosaccharides (GLU; XYL; GLU+XYL) in hydrolysates obtained during EH of wheat straw particles.

individual monomers are caused by different representation of natural compounds. The highest total monosaccharide (GLU+XYL) concentration of 91.3 g.L$^{-1}$ was obtained after enzymatic hydrolysis of wheat straw particles pre-treated by steam explosion at the temperature of 220 °C (hydrolysis time of 72 hours, STEX temperature of 220 °C, green column in Fig. 2). Slightly lower concentration of analysed monosaccharides was found in the enzymatic hydrolysate of poplar particles pre-treated by steam explosion at the temperature of 200 °C after enzymatic hydrolysis for 72 hours (90.0 g.L$^{-1}$, Fig. 3). Generally, concentrations of monosaccharides were mildly higher in case of wheat straw due to higher cellulose content made accessible at higher temperature while hemicelluloses were decomposed. It is also relevant that original cellulose content in wheat straw is also mildly higher than in wood materials (Table 2).

*Production and determination of inhibitors*

The main inhibitor for each hydrolysed type of particles was acetic acid which acted as a slight hydrolysis inhibitor (Yang *et al.* 2011). Concentration of acetic acid ranged from 1.7 g.L$^{-1}$ however, the correlation of concentrations related to pretreatment temperatures was not significant. High concentration of acetic acid causes the process inhibition but its specific concentration may be useful in specific pretreatment to coproduce xylooligosaccharides and fermentable sugars (Lai *et al.* 2019). Formic acid concentration was the highest in wheat straw hydrolysates (2.0 g.L$^{-1}$), much lower concentration was in hydrolysates

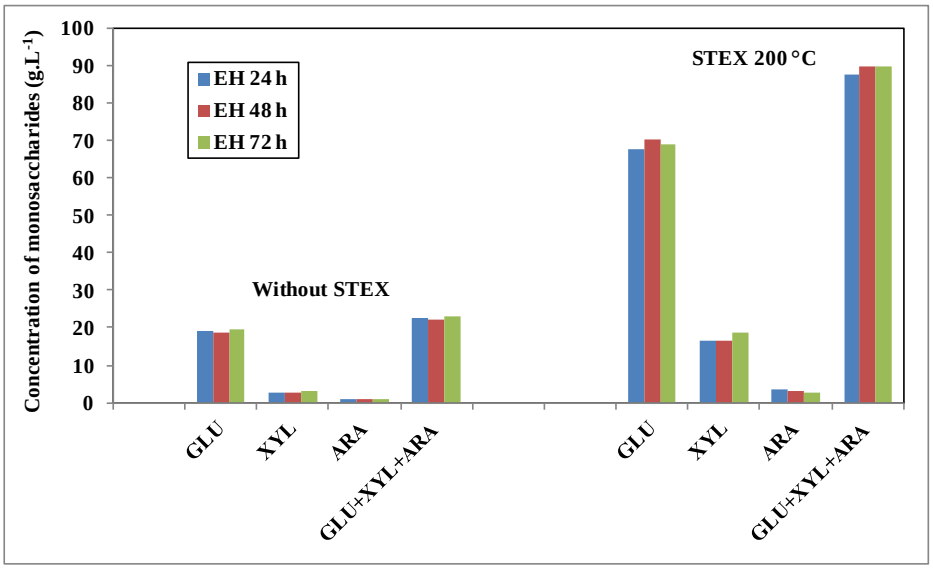


**Fig. 3.** Influence of steam explosion (STEX) at 200 °C on concentrations of monosaccharides (GLU; XYL; ARA; GLU+XYL+ARA) in hydrolysates obtained during EH of poplar particles.

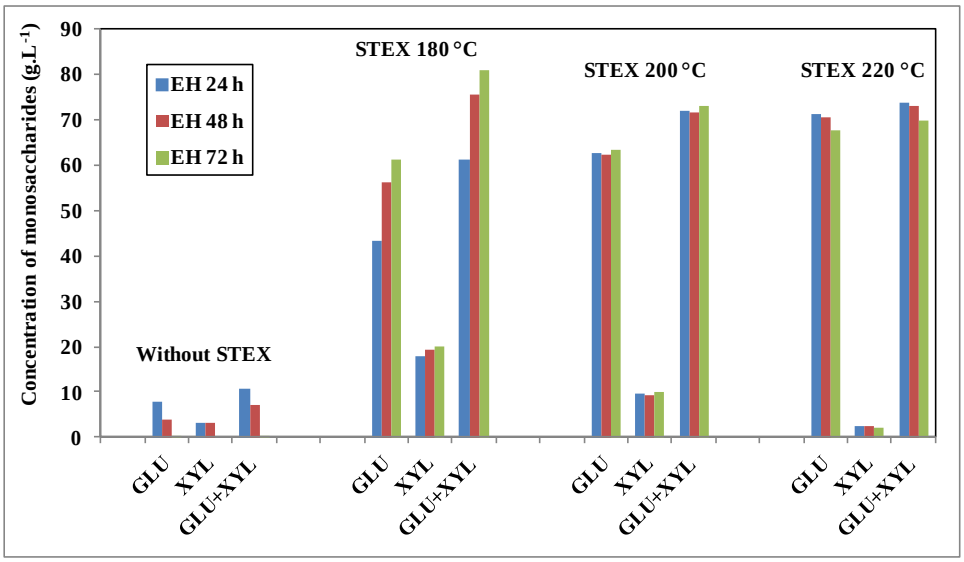


**Fig. 4.** I Influence of steam explosion (STEX) temperature on concentrations of monosaccharides (GLU; XYL; GLU+XYL) in hydrolysates obtained during EH of beech particles.

of poplar particles (0.0 $g.L^{-1}$ – 1.2 $g.L^{-1}$) and the lowest concentration in hydrolysates of beech particles (0.0 $g.L^{-1}$ – 0.3 $g.L^{-1}$). HPLC method also identified other inhibitors such as 5-hydroxymethylfurfural and furfural, however, their concentration was not significant. Of all the inhibitors, formic acid is the strongest inhibitor to cellulases due to complete inactivation of enzymes (Cantarella *et al.* 2004; Panagiotou and Olsson 2007; Yang *et al.* 2011). Formic acid concentration generating in enzymatic hydrolysis affects selection of suitable raw material for 2G bioethanol production. Formic acid had low concentration in all samples taken from mixtures during enzymatic hydrolysis. According to our results wheat straw is a less suitable raw material than wooden materials, especially when pre-treated at high temperatures as formic acid concentration mildly increases with steam explosion temperature.

*2G bioethanol production from selected biomass species*

The obtained results showed that wheat straw, poplar and beech particles are biomass resources suitable for 2G bioethanol production. However wheat straw is a more seasonal biomass and vice versa mentioned wooden materials are better storable due to lower biodegradability when compared to wheat straw. Combination of all studied biomass resources and their mixing before thermo-hydro-mechanical pretreatment in 2G bioethanol production require further research. However, 2G bioethanol based on the studied biomass resources is not only suitable for use in biofuels but also as the main component in disinfection materials inactivating viruses including coronaviruses and other pathogens (Zhao and Gao 2020).

## Conclusions

Pretreatments of selected raw materials based on different temperature showed a significant impact of steam explosion temperature. In two biomass pretreatment cases – wheat straw and beech particles that were pre-treated at three different temperatures increase of concentration of monosaccharides was found with enhancing temperature. The highest total concentration of detected monosaccharides (91.3 $g.L^{-1}$) was obtained by enzymatic hydrolysis of wheat straw particles pre-treated by steam explosion at the temperature of 220 °C and slightly lower in case of hydrolysed poplar particles pre-treated by steam explosion at the temperature of 200 °C (90.0 $g.L^{-1}$). Both hydrolysates were sampled after hydrolysis time of 72 hours. It is important to be careful when using wheat straw as a raw material for 2G bioethanol production due to higher concentration of formic acid generated at higher temperature of steam explosion pretreatment (2.0 $g.L^{-1}$) because this compound is the strongest inhibitor of cellulases by complete inactivation of enzymes. Based on the obtained results it may be considerably useful to combine the selected raw

materials before thermo-hydro-mechanical pretreatment in 2G bioethanol production. The starting point for pretreatment of selected biomass resources appears to be the optimum steam explosion temperature at 200 °C.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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