New approaches in pretreatment methods of beech particles in enzymatic hydrolysis – effect of impregnation time and reactor selection

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Abstract

Waste dendromass is one of the least used types of biomass and together with waste from the harvesting and processing of agricultural crops, belongs to the wastes that have a high potential for the second-generation ethanol production. Mass concentration of biofuel added to fossil fuels is established by law and this concentration is determined within the reference values for each year. For example, for 2021 it was 8.0% and for years from 2022 to 2030 the value will increase to 8.2%. Biofuels in the so-called reference value in the total fuel content have been mandatory since 2017, while the specific reference value is calculated from the energy content of the total amount of fuels placed on the market. This paper provides initial considerations and pilot results based on experimental data obtained in our workplace concerning the application potential of selected type of dendromass – beech particles from the point of view of their pretreatment by steam explosion and steam extrusion for potential production of liquid biofuels based on lignocellulosic biomass. We compared temperature courses of the mentioned pretreatment methods and pretreated samples were tested by enzymatic hydrolysis. Enzymatic hydrolysis results showed that steam explosion and steam extrusion led to concentration enhancement of fermentable monosaccharides compared to original samples depending on used pretreatment method.

Introduction

Biomass and especially waste dendromass is one of the solid fuels within renewable energy sources. Today it often replaces fossil fuels used in heating plants producing heat and hot water for households (Plešingerová et al. 2020; Nosek et al. 2021). In recent years, however, attention has focused not only on the production of biopellets and other fuel products based on dendromass, but also on research into the disintegration of dendromass through its pretreatment and hydrolysis, especially enzymatic hydrolysis (Wang et al. 2013; Pažitný et al. 2020a and 2020b). Dendromass-based hydrolysates can then be used in the alcoholic fermentation process. The composition of hydrolysates depends on the structure of the used lignocellulosic material, and on the other hand, the resulting composition of the hydrolysates may affect the reformulation of the feedstock mixture, which is more suitable for the production of the second-generation biofuel. In essence, it is an optimization of the composition of
input lignocellulosic raw materials. In addition, information obtained during enzymatic hydrolysis process and also from the material composition optimization can be potentially used in enzymology and in the wood processing industry (Pažitný 2019). The composition of different lignocellulosic materials may vary considerably between each other type (Jørgensen et al. 2007; Joshi et al. 2011; Bertero et al. 2012; Hazuchová et al. 2017; Kucharska et al. 2018; Pažitný et al. 2020b) and the chemical composition of individual wood species is variable also. The wood composition can also depend on whether wood is softwood or hardwood (Prasad et al. 2007; Iqbal et al. 2013; Shahzadi et al. 2014; Pažitný et al. 2020b). However, wood is essentially composed of lignin and hemicelluloses (Côté 1968), which are tightly crosslinked and form a complex structure. In this way, the wood raw materials have a very strong and durable structure, while the rigid and highly resistant structure is caused in particular by the entanglement of lignin and hemicelluloses with cellulose (Yang et al. 2019), as shown in Fig. 1, in which a flowchart is depicted for organosolvolysis pretreatment of recalcitrant softwood including following processing steps up to the production of high value-added materials (chemicals, fuels and other value-added materials). The complex of the mentioned natural substances forms a solid and durable matrix, which prevents the release of sugar from cellulose (Demartini et al. 2013; Yang et al. 2020).

![Flowchart for sugar and ultrafine organosolv lignin production from recalcitrant softwood](image)

**Fig. 1.** Flowchart for sugar and ultrafine organosolv lignin production from recalcitrant softwood (Yang et al. 2020).

Except of organosolvolysis, a possible approach is also pretreatment by thermo-hydro-mechanical procedures (Sandberg et al. 2013). So far, the most suitable solution seems to be steam explosion and steam extrusion, the so-called continuous steam explosion with similar mechanism such as in case of organosolvolysis. The mentioned pretreatment methods are very suitable and promising physico-chemical methods that modify lignocellulosic materials with resistant nature (Stankovská et al. 2018; Pažitný et al. 2021). These methods were originally developed for other purposes e.g., for extrusion of plastics (Huang and Liao 2002) and food products (Menis-Henrique et al. 2020). In the group of thermo-hydro-mechanical pretreatment methods, explosive processes using water, but also ammonia (Wyman et al. 2005) and sulfur dioxide (Wang et al. 2018) are significantly promoted, especially in softwood pretreatment (Galbe and Zacchi 2007; Hu and Ragauskas 2012; Jönsson and Martín 2016). Additionally, new more efficient processes such as alkaline extrusion are being developed, but so far it has been realized mainly on mechanically less resistant lignocellulosic materials such as wheat straw or similar lignocellulosic materials (Thian Hong 2013). Although alkaline pretreatment methods are chemical pretreatment methods, their combination with thermo-hydro-mechanical pretreatment methods causes even more extensive disruption of the dendromass recalcitrant structure, with fractionation by chemical agents and shear forces. Steam explosion is a type of chemo-thermo-mechanical pretreatment method, which causes decomposition of lignocellulosic materials by chemical modification by *in situ* formation of acids. The generated acids mainly cause depolymerisation of hemicellulosates, and mechanical disintegration occurs due to high
shear forces acting on lignocellulosic material immediately after energy or pressure release. The disruption of the structure of the lignocellulosic fibers and their bulk density reduction during this process are caused by a sudden drop in pressure which occurs at the end of set residence time. Compared to steam explosion and its chemical modifications, steam extrusion provides a continuous pretreatment with high shear and thermal stress, which is caused by rotational movement of a screw inserted in the extruder cylinder within a short time. In the field of dendromass pretreatment by steam extrusion method, very few published papers are probably due to the need to procure better and more mechanically resistant extruders. We assume that the main reason for not using dendromass in industrial scale production of liquid biofuels is, that the dendromass is processed for other purposes e.g., for mentioned solid fuel (biopellets) production. The most significant effect on the extrusion course has temperature or the temperature regime in which the extrusion is performed. For structurally simpler substances of organic origin, in particular based on lignocellulosic materials with relatively low molecular weight, lower extrusion temperature up to 140 °C is recommended (Li et al. 2021). In contrast, for more complex or chemically resistant substances, and in case of inorganic compounds, the extrusion temperature should be raised in the range from 150 °C to 300 °C (Ji et al. 2021).

The aim of this work was to study steam explosion and pilot steam extrusion with the inclusion of their machine preparation with respect to their temperature regimes, as well as the impregnation regimes of the selected lignocellulosic substrate with emphasis on different impregnation periods and concentration of released monosaccharides.

**Experimental**

**Lignocellulosic materials**

Beech particles (*Fagus sylvatica* L.) with uneven size distribution were obtained from company Ing. Jozef Fraňo Drevoúprava (Pezinok, Slovakia). The beech particles were not ground on mills, but they were directly impregnated with deionized water. In case of the impregnation method of preparation of swollen particles for steam explosion, the impregnation time was up to 15 min at room temperature, and in case of impregnation method of preparation of swollen particles for steam extrusion this time was at least 12 – 24 h (overnight), also at room temperature.

**Enzyme reagent**

Enzyme mixture Cellic CTec3, prepared as a stabilized enzyme complex for immediate use and supplied by Novozymes A/S (Bagsværd, Denmark), was used for enzymatic hydrolysis. After pretreatment, this enzyme mixture was used to degrade the lignocellulosic feedstock to fermentable monosaccharides. Enzyme activity was measured in the laboratory at 1,700 BU (Biomass Hydrolysis Units)/g of product.

**Steam explosion and extrusion pretreatment of lignocellulosic materials**

Beech particles impregnated for thermo-hydro-mechanical pretreatment were subjected to steam explosion at 200 °C in a 2 L stainless steel batch reactor (Amar Equipments Pvt. Ltd., Mumbai, India) and at the same temperature on a continuously working extruder (Hungaromix Agrárfejesztő-Fővállalkozó Kft., Komárom, Hungary). In both cases of pretreatment, temperature regimes of the experiment was recorded. The temperature regime was recorded before the lignocellulosic substrate passed through the discharge valve in case of steam explosion. After the residence time of 10 min a sample of steam-treated dendromass was released from reactor by rapid depressurization of steam explosion reactor vessel. When a continuously working extruder for swollen particles was used, the temperature regime was measured at the nozzle of extruder. In contrast to steam explosion, the appropriate sample of dendromass began to load after reaching a temperature of 200 °C at the nozzle, without any residence time. Both laboratory experiments had a common parameter – the temperature was pre-set to 200 °C. After steam explosion and continuous steam extrusion
pretreatment were samples labelled as steam explosion pretreated (STEXP) samples or continuous steam extrusion pretreated (C-STEXP), respectively. The samples obtained by these pretreatment methods were subjected to enzymatic hydrolysis. Non-pretreated (NP) material as the original samples of dendromass was subjected to enzymatic hydrolysis, for comparison.

**Enzymatic hydrolysis of lignocellulosic materials**

The beech substrates obtained by pretreatment were subjected to hydrolytic cleavage to monosaccharides. Enzymatic hydrolysis of the non-pretreated and pretreated 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 h 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 h, respectively.

**Determination of monosaccharides in hydrolysates from pretreated dendromass**

Concentration of monosaccharides 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 and acetic acid) 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., Bratislava, 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 – glucose, xylose, and arabinose (GLU, XYL and ARA, respectively) and inhibitors. Hydrolysates were injected into the HPLC system through smartline 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., Prague, Czech Republic).

**Results and Discussion**

**Temperature regime of batch reactor for steam explosion**

As it shown in Fig. 2, the temperature regime of the steam explosion reactor heater changed during the experiment with different intensity.

![Fig. 2. Time course of recorded temperature of the steam explosion reactor heater provided with ceramic heating element.](image)

The steepest temperature increase was at the temperature of 158 °C after 24 min. Then the temperature rose more slowly and uniformly, and heating of the reactor heater filled with a sample of beech particles occurred in the reactor heating region in the temperature range from 24 °C to 200 °C i.e., for 49 min. The temperature was stabilized at the temperature of 200 °C. This temperature was set to end the experiment or for expected steam explosion, respectively. However, the sample of the impregnated beech particles remained in the reactor at this temperature and appropriate pressure for 10 min before the steam explosion. The period of 10 min is called residence time. After residence time of 10 min, and 59 min after beginning of heating, the valve on the reactor was electronically opened and a sample of beech particles impregnated with water was subjected to a steam explosion at 200 °C.
**Temperature regime of continuously working extruder for steam extrusion**

Fig. 3 shows that the temperature regime of the heating element of continuously working steam extrusion reactor differs significantly from the temperature regime of the batch reactor for steam explosion. The reactor heating region had termination as early as 45 min, which was 4 min earlier compared to the reactor heating region for the batch reactor for steam explosion (Fig. 3).

![Diagram](image)

**Fig. 3.** Time course of recorded temperature of the continuously working steam extrusion reactor heater provided with metal heating element.

The shortening of the heating period can be caused by a different electric power input, power, and type of heating element, but mainly the absence of the sample of beech particles impregnated with water during the heating phase of steam extrusion reactor i.e., in the reactor heating region of continuously working steam extrusion reactor (Fig. 3). The steepest temperature increase was at the temperature of 153 °C after 23 min. As in case of the batch reactor for the steam explosion, in this case the temperature rose more slowly and more uniformly. The heating of heating element of the steam extrusion reactor occurred in the reactor heating region in the temperature range from 24 °C to 200 °C i.e., for 45 min. The temperature stabilization occurred at a temperature of 200 °C, which was set temperature for starting of addition of water-impregnated beech particles i.e., for continuous steam explosion. At the indicated temperature the sample was passed through the inner body of the reactor for 10 min. This period is analogically called the residence time, as in case of a discontinuous steam explosion. After that period of 10 min, and 55 min after beginning of heating, the sample consisting of heated and milled water-impregnated beech particles was discharged through a nozzle with an internal slot size of 6 mm. The experiment was terminated in the beginning of the formation of blocking pieces, as the sample of beech particles was not passed through the nozzle, and it was necessary to terminate the experiment due to possible damage of the steam extrusion reactor and due to further sintering of the pretreated sample of the lignocellulosic substrate.

**HPLC chromatograms of STEXP and C-STEXP samples of dendromass**

The aim of this work was also to study the chemical compositions of hydrolysates obtained after enzymatic hydrolysis of NP, STEXP and C-STEXP samples of water-impregnated beech particles. The resulting monosaccharides and their concentration in hydrolysates are affected strongly by the original chemical composition of dendromass particles and the pretreatment temperature (Russ et al. 2016). However, removing hemicelluloses at high temperatures can effectively enhance cellulose digestibility (Zhao et al. 2012). Cellulose accessibility improvement for enzymes leads to high concentration of monomers due to dramatic changes in dendromass morphology after pretreatment aimed at enhancing the reactivity of dendromass. The monosaccharides obtained during enzymatic hydrolysis – glucose (GLU), xylose (XYL) and arabinose (ARA) were determined using HPLC (High Performance Liquid Chromatography) combined with external standard methods. These methods are used to perform an accurate qualitative and quantitative analysis of measured monosaccharides and inhibition products such as formic acid and acetic acid in hydrolysates from pretreated dendromass (Llano et al. 2017). According to scientific papers (Demčák et al. 2017; 2019; Ibrahim et al. 2020) it can be assumed that chemical structure of dendromass such as beech wood particles is mainly composed from cellulose, hemicelluloses and also lignin. The mentioned monosaccharides were formed just from holocellulose – cellulose and hemicelluloses during enzymatic hydrolysis of pretreated water-impregnated beech particles. The hydrolysates...
obtained from dendromass hydrolysis had different concentration of monosaccharides based on the specific chemical composition and porous structure of NP, STEXP and C-STEXP samples of pretreated beech particles. We also suppose some significant effect of the temperature regimes within two different reactors. HPLC analysis showed that the monosaccharides in obtained hydrolysates had a different elution time (Table 1). The elution time did not depend on concentration of hydrolyzed monosaccharides however, it depended on set-up and conditions of HPLC method (Pažitný et al. 2020b).

Table 1. Elution time of analysed monosaccharides in hydrolysates.

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

Fig. 4 demonstrates two HPLC chromatograms from hydrolysates of STEXP and C-STEXP samples pretreated at the temperatures of 200 °C. The hydrolysates based on pretreated dendromass were sampled after 72 h. All the chromatograms show the strong signal of GLU monomer (elution time of 11.80 min) and the weaker signals of C5 monosaccharides such as XYL (elution time of 12.60 min) and ARA (elution time of 13.85 min). Significant reduction of monosaccharides can be seen in case of beech wood hydrolysates obtained from C-STEXP compared to STEXP at the same pretreatment temperature. Since both reactors have a different temperature regime, this is also reflected in the influence of the temperature regime type on the formation of monosaccharides. Additionally, various impregnation regimes also play a role.

Production and determination of monosaccharides

The average values for concentrations of monosaccharides measured by the HPLC method depend on enzymatic hydrolysis (EH) time and also on used pretreatment method. The qualitative HPLC method revealed the monosaccharides of both cellulose and hemicelluloses. The hydrolysates obtained from pretreated beech particles contained monomers GLU, XYL and ARA depending on the used pretreatment method. The highest total monosaccharide (GLU+XYL) concentration of 73.2 g.L⁻¹ was obtained after enzymatic hydrolysis of beech particles pretreated by steam explosion at the temperature of 200 °C (hydrolysis time of 72 h, STEX temperature of 200 °C). Significantly lower concentration of monosaccharides was found in the enzymatic hydrolysates of beech particles pretreated by steam extrusion (C-STEX) at the temperature of 200 °C after enzymatic hydrolysis for 72 h (55.2 g.L⁻¹). In case of NP sample the total monosaccharide (GLU+XYL) concentration was 6.0 g.L⁻¹ on average.

Production and determination of inhibition products

The main inhibitor for each hydrolysed sample was acetic acid which acted as a slight hydrolysis inhibitor (Yang et al. 2011). Concentration of total
inhibition products ranged from 4.4 g.L\(^{-1}\) to 4.9 g.L\(^{-1}\) among all the hydrolysates obtained from pretreated beech particles. Formic acid concentration was negligible and almost immeasurable. High concentration of acetic acid causes inhibition but a specific concentration may be useful in a specific pretreatment to coproduce xyloooligosaccharides and fermentable sugars (Lai et al. 2019). Among both the inhibition products, formic acid is the stronger cellulase inhibitor due to complete inactivation of enzymes (Panagiotou and Olsson 2007; Yang et al. 2011). However, formic acid had low concentration in all samples taken from hydrolysates of pretreated beech particles during enzymatic hydrolysis.

2G bioethanol production from dendromass

The results showed that beech particles were a dendromass resource suitable for 2G bioethanol production. Mentioned wooden material is good storable due to lower biodegradability when compared to agricultural wastes. Studied dendromass resource utilization and its thermo-hydro-mechanical pretreatment in 2G bioethanol production require further research. Temperature regimes in a batch and continuous reactor should be paid to the greatest attention. An important reason for the beech utilization in 2G bioethanol production is a large abundance within Slovakia, but also the entire Europe (Guckland et al. 2009).

Conclusions

Dendromass-based lignocellulosic materials are highly abundant and their renewable potential exceeds transformation efficiency or utilizability of biomass from agricultural crops, given by their relatively low voluminosity. Processing of waste dendromass at the mining site or waste obtained in the processing of wood raw materials in production plants provides raw material which is suitable for the production of fermentable monosaccharides. However, mechanical processing and impregnation of dendromass is followed by thermo-hydro-mechanical pretreatment. Pretreatment of beech particles showed a significant impact of selected reactor type. The very important parameter is also impregnation period of selected dendromass substrate. Beech particles that were pretreated at the temperature of 200 °C provided the higher concentration of monosaccharides compared to non-pretreated sample. Concentration of monosaccharides in hydrolysates was approximately twelve-fold for hydrolysates of water-impregnated beech particles within shorter period soaking (STEX samples) and approximately nine-fold for hydrolysates of water-impregnated beech particles within long period soaking (C-STEX) compared to average hydrolysates of NP sample of beech particles. Concentration of inhibition products was very low in all cases. Based on the obtained results, it may be considerably useful to process selected dendromass by thermo-hydro-mechanical pretreatment in 2G bioethanol production.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Bertero M, Puente GDL, Sedran U (2012) Fuels from bio-oils: Bio-oil production from different residual sources, characterization and thermal conditioning. Fuel 95: 263-271.

Côté WA (1968) Chemical Composition of Wood. In Kollmann FFP, Côté WA (Eds.), Principles of Wood Science and Technology, Springer-Verlag GmbH, Berlin, Heidelberg, pp. 55-78.

Demartini JD, Pattathil S, Miller JS, Li H, Hahn MG, Wyman CEJE (2013) Investigating plant cell wall components that affect biomass recalcitrance in poplar and switchgrass. Energy Environ. Sci. 6: 898-909.

Demčák Š, Bálintová M, Demčáková M, Zinicovscaia I, Yushin N, Frontasyeva MV (2019) Using of wooden sawdust for copper removal from waters. Nova Biotechnol. Chim. 18: 66-71.

Demčák Š, Bálintová M, Huráková M, Frontasyeva MV, Zinicovscaia I, Yushin N (2017) Utilization of poplar wood sawdust for heavy metals removal from model solutions. Nova Biotechnol. Chim. 16: 26-31.

Galbe M, Zacchi G (2007) Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv. Biochem. Eng. Biotechnol. 108: 41-65.

Guckland A, Flessa H, Prenzel J (2009) Controls of temporal
and spatial variability of methane uptake in soils of a temperate deciduous forest with different abundance of European beech (Fagus sylvatica L.). Soil Biol. Biochem. 41: 1659-1667.

Huzuchávová M, Chmelová D, Ondrejovič M (2017) The optimization of propagation medium for the increase of laccase production by the white-rot fungus Pleurotus ostreatus. Nova Biotechnol. Chim. 16: 113-123.

Hu F, Ragauskas A (2012) Pretreatment and lignocellulosic chemistry. Bioenergy Res. 5: 1043-1066.

Huang NX, Liao CM (2002) Prediction of parison swell in plastics extrusion blow molding using a neural network method. Polymer Test. 21: 745-749.

Ibrahim Q, Araujo PJ, Kruse A (2020) The effect of using different acids to catalyze the prehydrolysis stage on the organosolv delignification of beech wood in two-stage process. Renew. Energy 153: 1479-1487.

Iqbal HMN, Kyazz G, Keshavarz T (2013) Advances in the valorization of lignocellulosic materials by biotechnology: An overview. BioResources 8: 3157-3176.

Ji H, Wu G, Liu W, Sun W, Ding W (2021) Role of extrusion temperature on the microstructure evolution and tensile properties of an ultralight Mg-Li-Zn-Er alloy. J. Alloys Compd. 876: 160181.

Jönsson LJ, Martin C (2016) Pretreatment of lignocellulose: formation of inhibitory byproducts and strategies for minimizing their effects. Bioresour. Technol. 199: 103-112.

Jorgensen H, Kristensen JB, Fehly C (2007) Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels, Biopro. Bioref. 1: 119-134.

Joshi J, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L (2011) Lignocellulosic ethanol production: Current practices and recent developments. Biotechnol. Mol. Biol. Rev. 6: 172-182.

Kucharska K, Rybarczyk P, Holowacz I, Lukajtis R, Glinka M, Kamiński M (2018) Pretreatment of lignocellulosic materials as substrates for fermentation processes. Molecules 23: 2937.

Lai C, Jia Y, Wang J, Wang R, Zhang Q, Chen L, Shi H, Huang C, Li X, Yong Q (2019) Co-production of xylooligosaccharides and fermentable sugars from poplar through acetic acid pretreatment followed by poly (ethylene glycol) ether assisted alkali treatment. Bioresources Technol. 288: 121569.

Li J, Baker T, Sacripante GG, Lawton DJW, Marway HS, Zhang H, Thompson MR (2021) Solvent-free production of thermoplastic lignocellulose from wood pulp by reactive extrusion. Carbohydr. Polym. 270: 118361.

Llano T, Quijorna N, Andrés A, Coz A (2017) Sugar, acid and furfural quantification in a sulphite pulp mill: Feedstock, product and hydrolysate analysis by HPLC/RID. Biotechnol. Rep. 15: 75-83.

Menis-Henrique MEC, Scarlton M, Piran MVL, Clerici MTPS (2020) Cereal fiber: extrusion modifications for food industry. Curr. Opin. Food Sci. 33: 141-148.

Nosek R, Backa A, Durčanský P, Holubčík M, Jandačka J (2021) Effect of paper sludge and dendraam on properties of phytomass pellets. Appl. Sci. 11: 65.

Panagiou G, Olsson L (2007) Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates. Biotechnol. Bioeng. 96: 250-258.

Pažitný A (2019) Steam explosion of wood particles from fibreboard and particle board with indirect control by enzymatic hydrolysis. Acta Chim. Slov. 12: 185-191.

Pažitný A, Boháčeck Š, Ihnát V, Šutý Š (2020a) Utilization of beech and poplar wastes in pretreatment processes for 2G biofuel production controlled by enzymatic hydrolysis. In Proceedings of Reviewed Joint Conferences “Wood, Pulp & Paper Polygraphia Academica”, Bratislava, SR, March 11th – 12th, 2020, pp. 253-257.

Pažitný A, Halaj M, Russ A, Boháčeck Š, Ihnát V, Skotnicová I, Šutý Š (2021) Steam explosion and steam extrusion pretreatment as auxiliary methods for concentration enhancement of monosaccharides from hydrolysates based on selected lignocellulosic materials. In ChemZi 17, Proceedings of Reviewed Joint Conferences “73. Zjazd Chemikov”, Vysoké Tatry, Horný Smokovec, SR, September, 6th – 10th, 2021, pp. 149-150.

Pešingerová B, Derin B, Vadász P, Medved D (2020) Analysis of deposits from combustion chamber of boiler for dendraam. Fuel 266: 117069.

Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour. Conserv. Recycl. 50: 1-39.

Russ A, Fišerová M, Letko M, Opálená E (2016) Effect of steam explosion temperature on wheat straw enzymatic hydrolysis. Wood Res. 61: 65-74.

Sandberg D, Haller P, Navi P (2013) Thermo-hydro and thermo-hydro-mechanical wood processing: An opportunity for future environmentally friendly wood products. Wood Mater. Sci. Eng. 8: 64-88.

Shahzadi T, Mehmood S, Irshad M, Anwar Z, Afroz A, Zeeshan N, Rashid U, Sughra K (2014) Advances in lignocellulosic biotechnology: A brief review on lignocellulosic biomass and cellulases. Adv. Biosci. Biotechnol. 5: 246-251.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Technical Report NREL/TP-510-42618. National Renewable Energy Laboratory (NREL), Golden, CO, USA.

Stankovská M, Fišerová M, Gigac J, Pažitný A (2018) Effect of alkaline extrusion pretreatment of wheat straw on filtrate composition and enzymatic hydrolysis. Cellulose Chem. Technol. 52: 815-822.

Thian Hong N (2013) Twin screw extrusion pre-treatment of wheat straw for biofuel and lignin biorefinery applications. Doctoral dissertation, Wolfson Centre for Materials Processing, Brunel University London.

Wang W, Yuan T, Cui B, Dai Y (2013) Investigating lignin
and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis. Bioresource Technol. 134: 381-385.

Wang Z, Wu G, Jönsson LJ (2018) Effects of impregnation of softwood with sulfuric acid and sulfur dioxide on chemical and physical characteristics, enzymatic digestibility, and fermentability. Bioresource Technol. 247: 200-208.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005) Coordinated development of leading biomass pretreatment technologies. Bioresource Technol. 96: 1959-1966.

Yang B, Dai Z, Ding SY, Wyman CE (2011) Enzymatic hydrolysis of cellulosic biomass. Biofuels 2: 421-450.

Yang X, Zheng A, Zhao Z, Xia S (2019) Overcoming biomass recalcitrance to enhance platform chemical production from soft wood by organosolvolysis coupled with fast pyrolysis. Cellulose 26: 9687-9708.

Yang X, Zheng A, Zhao Z, Xia S, Wang Q, Wei G, Huang Z, Jiang L, Wang S, Li H (2020) Maximizing production of sugar and ultrafine lignin particles from recalcitrant softwood by different acids-assisted organosolvolysis and fast pyrolysis. J. Clean. Prod. 276: 122827.

Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels, Bioprod. Bioref. 6: 465-482.