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The Influence of Selected Physico-Chemical Pretreatment Methods on Chemical Composition and Enzymatic Hydrolysis Yield of Poplar Wood and Corn Stover

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In the paper, three different physico-chemical pretreatment methods in relation to bioethanol production were compared. The wood of fast-growing poplar species (*Populus deltoides x maximowiczii* and *Populus trichocarpa* Torr. & A. Gray ex Hook) and corn stover were used as a feedstock. The chemical composition and enzymatic hydrolysis efficiency of the biomass before and after pretreatments were compared. On the basis of the results, it was concluded that the applied pretreatments changed the chemical composition of the raw materials. In the case of the acid and LHW (liquid hot water pretreatment) methods, up to 93.7% of hemicelluloses (especially pentosans) were removed. From among the selected pretreatment methods, the LHW and alkaline methods occurred to be the most interesting. Nevertheless, from the bioethanol production point of view, the LHW process of Populus deltoides x maximowiczii wood was the best because after enzymatic hydrolysis, high amounts of glucose (up to 600.9 mg/g pretreated biomass) and minor amounts of xylose (up to 37.9 mg/g pretreated biomass) were produced. Moreover, based on the chemical composition and sugar profile analysis, it was proved that the Populus trichocarpa wood also has a high potential for bioethanol production.

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Introduction

The depletion of natural resources, mainly fossil fuels, has significantly increased the interest in biomass, mainly of plant origin. Many years of research have shown that plant tissues are a rich source of various chemicals. These types of materials undergo natural renewal over a period of several to several dozen years,

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while absorbing significant amounts of carbon dioxide from the environment. One of the possibilities of processing wood, as well as other lignocellulosic biomass, is the possibility of converting it during the production of bioethanol.

The literature review shows that a significant part of the research is carried out on wood raw material, which is mainly a lignin carbohydrate complex (LCC) [Lee et al. 1999; Lu et al. 2009; Antczak et al. 2019; Shi et al. 2020; Marks and Viell 2021; Antczak et al. 2022; El Hage et al. 2022]. Generally, the bioethanol production process consists of three main steps: pretreatment, hydrolysis and fermentation. The main tasks of the pretreatment include: effective breakdown of the LCC, which hinders the decomposition of carbohydrates (cellulose and hemicelluloses), development of the specific structure of biomass in order to facilitate the availability of the material for enzymes to decompose them, reduction of the degree of crystallinity and the polymerization of cellulose, and the greatest possible elimination of lignin and non-structural substances. The effectiveness of this stage has a significant impact on the efficiency of subsequent processes, especially those as important as enzymatic hydrolysis.

The pretreatment methods can be divided into biological, physical, chemical and physicochemical. These methods, differing in the effectiveness of their action on individual biomass components, result in the formation of various inhibitors of biological processes as well as by-products, which may affect the efficiency of bioethanol production [Alvira et al. 2010; Kratky and Jirout 2011; Jönsson et al. 2013; Sjulander and Kikas 2022].

Acids are considered to be the most effective in destroying the woody xylem; therefore, they are the most often used in the pretreatment. Due to problems with the corrosion of equipment and the disposal of waste from concentrated acids, as well as the formation of inhibitors formed from carbohydrates (mainly pentoses and hexoses) as a result of degradation and dehydration reactions, which are furfural and furan derivatives, as well as organic acids (formic and acetic) and aromatic compounds derived from the decomposition of lignin, treatment with dilute acids is more often used [Fengel and Wegener 2003; Tomás-Pejó et al. 2011]. The treatment is carried out on a comminuted material (from chips to dust) with dilute acids (from 0.2% to 10%), at temperatures from 130°C to 210°C, using percolation, spray or batch reactors. The process takes from a few minutes to several hours. In the pretreatment of lignocellulosic materials, inorganic acids are utilized: sulfuric [Uçar 1990; Cao et al. 2009; Kumar et al. 2009; Janga et al. 2012; Shi et al. 2020], hydrochloric [Shi et al. 2012; Sun et al. 2014], phosphoric [Siripong et al. 2016], nitric [Yang et al. 2015] or organic acids: oxalic [Zheng et al. 2018], citric [Qiao et al. 2019] or acetic [Trzcinski and Stuckey 2015]. The biomass obtained after the pretreatment is characterized by a large amount of completely or partially degraded polysaccharides, a reduced degree of crystallinity and cellulose polymerization, and a lower content of lignin and hemicelluloses compared to the native raw material.

Alkaline pretreatment is characterized by significant removal of lignin from the lignocellulosic biomass and relatively low degradation of carbohydrates (especially cellulose); thus, it improves the susceptibility of cellulose and hemicelluloses to enzymatic hydrolysis, leading to a higher sugar yield compared to untreated biomass [Zheng et al. 2018; Bay et al. 2020; Dąbkowska-Susfał 2020; Woiciechowski et al. 2020]. Additionally, alkaline pretreatment involves the use of non-polluting and non-corrosive reagents, and it is often efficient under relatively mild process conditions, which are considered as the main advantage of this method. Furthermore, compared to acid pretreatment, this method leads to the generation of many fewer by-products such as furanaldehydes and aromatic compounds, which inhibit enzymes and the growth of microorganisms [Jönsson et al. 2013; Woiciechowski et al. 2020]. From the chemical point of view, alkaline pretreatment is based on breaking ester bonds between lignin and hemicelluloses and removing the acetyl and uronic acid substitutions in hemicelluloses [El-Naggar et al. 2014; Baruah et al. 2018]. Among known efficient alkaline pretreatment reagents, sodium hydroxide has been extensively studied and used to date in different loading and process conditions [Zheng et al. 2018; Wang et al. 2020].

The liquid hot water (LHW) pretreatment is an "autohydrolysis" process, relying on the deacetylation of the hemicellulose components in the biomass in hot water through the hydrolysis of labile ester bonds, which act as in-situ acid catalysts for the pretreatment as they create an acidic environment. Pressure is used in this method to keep the water in a liquid state at elevated temperatures (150-240 °C) and to cause changes in the structure of the lignocellulosic material. Contrary to the steam explosion (SE) method, LHW treatment does not cause rapid decompression of the biomass. The process is technologically simple to carry out. The treatment leads to the formation of complexes of lignin with polysaccharides, lignin droplets and condensation reactions [Ko et al. 2015b]. Moreover, the formation of pseudo-lignin was observed, especially under acid and hydrothermal pretreatment conditions [Shinde et al. 2018]. Maintaining the pH between 4-7 during the process enables the autohydrolysis of hemicelluloses to oligosaccharides, reducing the amount of simple sugars and inhibitors

in the form of furfural, 5-hydroxymethylfurfural and organic acids (mainly acetic acid) [Mosier et al. 2005; Alvira et al. 2010; Tomás-Pejó et al. 2011; Wilk and Krzywonos 2015; Jimenez-Gutierrez et al. 2021].

The aim of the studies was to compare the influence of selected pretreatment methods on the chemical composition and the efficiency of enzymatic hydrolysis. In this paper, the wood of fast-growing poplar species (*Populus deltoides* x *maximowiczii* and *Populus trichocarpa* Torr. & A. Gray ex Hook) and corn stover were used. The interesting and innovative aspect of these studies is the use of the wood of fast-growing poplar species, which have been acclimatized and are grown in field conditions in Poland. The unique wood material was compared with corn stover, a material often utilized in this type of research, coming from Polish resources. From among the known pretreatment methods, acid, alkaline and liquid hot water were compared. The pretreatments were carried out on the same feedstock and their effect on the chemical composition and the efficiency of enzymatic hydrolysis was studied. The available literature lacks such studies in which the effectiveness of several different pretreatments on the same material was compared.

Material and methods

In the studies, the debarked wood from the whole trunk of fast-growing poplar species (*Populus deltoides* x *maximowiczii* and *Populus trichocarpa* Torr. & A. Gray ex Hook) and corn stover were used. The poplars were from the Polish experimental field in Wolica owned by the Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology at Warsaw University of Life Sciences. The age of trees was 7 years. On the other hand, the corn stover came from Polish commercial fields. Before the studies, the material was air-dried, milled and sieved into particles with dimensions of 0.43 mm to 1.02 mm. All of the chemical substances were of an analytical grade and were purchased from Chempur (Poland) and Sigma-Aldrich (Germany).

Sugar profile analysis

The sugar profile analysis was done by the acid hydrolysis process, which proceeded according to the method described by Antczak et al. [2018], with minor modifications. The hydrolysis process was carried out on dried (60°C and a pressure of 0.4 kPa) raw materials (poplar wood and corn stover; fraction below 0.43 mm). The samples after the hydrolysis process (30 °C during 1 h and next 120 °C during 3 h in an oil bath) and 1 cm^3 of 2% sodium azide supplementation were neutralized with sodium carbonate to a pH about 5-6. After the neutralization, the filtered (0.22 μm nylon syringe filter) samples were analyzed by high-performance liquid chromatography (HPLC). All of the acid hydrolysis tests were performed fourfold and standard deviations were calculated.

Acid pretreatment method

Acid pretreatment was carried out in optimized conditions using sulfuric acid (H_2SO_4) with a concentration of 12%. The poplar wood and corn stover were treated with 12% H_2SO_4 for 4 h at 102 °C (boiling point) in a round-bottom flask supplied with a condenser and a magnetic stirrer with a heater. The optimal process conditions, i.e. concentration of H_2SO_4, pretreatment time and temperature, were selected based on our previous unpublished studies. The ratio of sulfuric acid to biomass was as follows: 100 cm^3 of 12% H_2SO_4 per 10 g of air-dried poplar wood (1.3 g of H_2SO_4 per 1 g of air-dried biomass) or 7 g of air-dried corn stover (1.9 g of H_2SO_4 per 1 g of air-dried biomass). After completion of the acid pretreatment, the reaction mixture was filtered under reduced pressure. The solid fraction was washed with distilled water until the filtrate reached a neutral pH. Two experiments were carried out according to the described procedure. The neutralized solid fractions were stored at 6°C until chemical composition analysis and enzymatic hydrolysis were performed.

Alkaline pretreatment method

Alkaline pretreatment was performed using a 2% solution of sodium hydroxide (NaOH). The corn stover biomass was treated with the 2% solution of NaOH for 3 h at 50 °C in a thermostated water bath shaker (180 rpm), whilst the poplar wood was treated in an autoclave (121 °C, 0.1 MPa) for 30 min. In all the cases the biomass loading equaled 50 g DM/dm^3 (0.41 g of NaOH per 1 g of absolutely dry biomass). The most efficient process conditions, i.e. the pretreatment duration time, temperature and concentration of NaOH for the poplar wood and corn stover were selected based on our previous unpublished studies. In these conditions, the biomass was activated, from which, as a result of enzymatic hydrolysis, the highest content of simple sugars was obtained. The reaction mixture was filtered under reduced pressure and the solid fraction was rinsed with distilled water until the filtrate reached a neutral pH. Five experiments were carried out under these conditions. The neutralized solid fractions were stored at 6 °C until the chemical composition analysis and enzymatic hydrolysis were performed.

LHW pretreatment method

Before the liquid hot water pretreatment process, about 20 g of the biomass (with an absolute humidity of 7%) was treated with distilled water (200 cm^3) in order to swell the material. The swelling process of the biomass was realized in a beaker using a magnetic stirrer for 20 min at 75 °C. Next, the material was quantitatively transferred into a stainless steel reactor with a capacity of 250 cm^3 and a respective amount of water was added. The ratio of solid to liquid was 1:12.5. The LHW process was performed at 190 °C and the residence time of biomass in the reactor was 15 min. The above conditions (temperature and residence time) were selected based on our previous studies [Akus-Szylberg et al. 2018; Akus-Szylberg et al. 2020]. After the reactor reached the set temperature and time, it was then cooled rapidly in a bath filled with ice and sodium chloride. Next, the reaction mixture was filtered using a Büchner funnel and the solid fraction was washed with distilled water until the filtrate reached a neutral pH. Three experiments were carried out according to this procedure. The neutralized solid fractions were stored at 6 °C until chemical composition analysis and enzymatic hydrolysis were conducted.

Chemical composition analysis

The chemical composition of the raw materials and the materials obtained after the acid, alkaline and LHW pretreatments was determined on the basis of standard methods. The following determinations were made:

- extractives content - substances soluble in 96% ethanol (analysis is performed in a Soxhlet apparatus) [PN-92/P-50092; Sluiter et al. 2008]
- cellulose content by the Seifert method using a mixture of acetylacetone, dioxane and 36% hydrochloric acid [Seifert 1960; PN-92/P-50092]
- Klason lignin content by the Tappi method utilizing 72% sulfuric acid [TAPPI T222 om-02]
- pentosans content by the Tollens method - pentosans hydrolysis with 12% hydrochloric acid to furfural and its precipitation with barbituric acid [Prosiński 1984; Kačik and Solár 1999]
- holocellulose content using sodium chlorite in the presence of glacial acetic acid [Wise et al. 1946; PN-92/P-50092]
- hemicelluloses content calculated as the difference between the holocellulose and cellulose contents.

The absolute moisture content of the raw materials for analysis was in the range of 7.0-8.2%, while the materials after acid treatment 4.2-6.4%, after alkaline treatment 7.5-24.3%, and after LHW treatment it was from 5.4 to 5.9%. The percentage of extractives and the content of cellulose, lignin, pentosans, holocellulose and hemicelluloses were calculated based on absolutely dry biomass. All of the chemical composition analyses were done in duplicate or triplicate and standard deviations were calculated.

Enzymatic hydrolysis process

The enzymatic hydrolysis was performed on the raw materials and materials obtained after acid, alkaline and LHW pretreatments. The enzymatic hydrolysis process was conducted according to earlier studies [Antczak et al. 2018] with a cellulose concentration of 1% w/w (0.1 g of enzyme per 0.1 g of cellulose). A 2% water solution of sodium azide was utilized as a substance preventing the growth of microorganisms during the hydrolysis. The enzymatic hydrolysis process was carried out in sealed screw-capped test tubes and the total volume of the mixture was 10 cm^3. Cellic CTec2 enzyme (Novozymes, Denmark) was used, with an activity of 148 FPU/cm^3 determined according to Adney and Baker [1996]. The samples were hydrolyzed during 72 h by means of a mixer (RM-2M, Elmi, USA) with rotation (25 rpm) placed in a laboratory drier at 50°C. After the process, the content of simple sugars in the supernatant was determined by the HPLC method. All the enzymatic hydrolysis tests were done in triplicate and standard deviations were calculated.

HPLC analysis

The monosaccharide concentrations in the hydrolysates were determined by the HPLC method. Analyses were conducted employing the Varian 365 CL System (Varian, USA) equipped with a refractive index detector (Smartline 2300, Knauer, Germany) and Rezex RHM-Monosaccharide H+ (8%) (300 × 7.8 mm) column with a SecurityGuard Cartridge-Carbo-H (4 × 3.0 mm) (Phenomenex, USA), which during the analyses were thermostated at 60 °C. 0.001N H_2SO_4 at a flow rate of 0.4 cm^3/min was used as a mobile phase.

Results and discussion

The results of the chemical composition of the raw materials and materials obtained after the acid, alkaline and LHW pretreatments are presented in Tables 1-3.

Table 1. Chemical composition of *Populus trichocarpa* wood before and after acid, LHW and alkaline pretreatments

Parameter	Raw wood [%]	Acid pretreatment [%]	Alkaline pretreatment [%]	LHW pretreatment [%]
Extractives	1.5 ±0.1	3.8 ±0.1	0.7 ±0.1	17.4 ±0.5
Cellulose*	44.6 ±0.1	63.9 ±0.6	57.5 ±0.1	75.6 ±0.1
Holocellulose*	81.7 ±0.3	71.2 ±0.2	73.9 ±0.3	82.0 ±0.1
Lignin	19.7 ±0.1	27.9 ±0.3	20.4 ±0.2	19.5 ±0.1
Pentosans	18.2 ±0.1	0.1 ±0.0	13.3 ±0.1	2.3 ±0.1
Hemicelluloses* (calculated)	37.1	7.2	16.5	6.4
Solid recovery	-	66.2	74.6	68.5

* Results should be treated only in approximate and comparative way, as used methods did not allow completely pure substances to be obtained; errors (±) refer to standard deviations.

Table 2. Chemical composition of *Populus deltoides x maximowiczii* wood before and after acid, LHW and alkaline pretreatments

Parameter	Raw wood [%]	Acid pretreatment [%]	Alkaline pretreatment [%]	LHW pretreatment [%]
Extractives	1.7 ±0.1	4.0 ±0.1	0.5 ±0.1	17.2 ±0.1
Cellulose*	42.4 ±0.2	61.6 ±0.1	53.4 ±0.1	73.1 ±0.4
Holocellulose*	77.2 ±0.4	67.3 ±0.1	71.6 ±0.1	75.3 ±0.5
Lignin	23.5 ±0.1	32.9 ±0.1	23.0 ±0.1	25.0 ±0.1
Pentosans	16.9 ±0.1	1.4 ±0.1	14.1 ±0.1	1.0 ±0.1
Hemicelluloses* (calculated)	34.8	5.8	18.1	2.2
Solid recovery	-	68.4	76.9	71.3

* Results should be treated only in approximate and comparative way, as used methods did not allow completely pure substances to be obtained; errors (±) refer to standard deviations.

Table 3. Chemical composition of corn stover before and after acid, LHW and alkaline pretreatments

Parameter	Raw corn stover [%]	Acid pretreatment [%]	Alkaline pretreatment [%]	LHW pretreatment [%]
Extractives	6.0 ±0.1	8.4 ±0.1	0.9 ±0.1	17.3 ±0.5
Cellulose*	38.4 ±0.3	66.8 ±0.1	60.8 ±0.4	70.6 ±0.1
Holocellulose*	65.5 ±1.2	70.6 ±0.2	87.9 ±0.2	75.0 ±0.2
Lignin	21.4 ±0.2	35.6 ±0.2	8.9 ±0.1	26.7 ±0.2
Pentosans	24.4 ±0.1	1.5 ±0.1	24.0 ±0.1	2.7 ±0.2
Hemicelluloses* (calculated)	27.1	3.7	27.2	4.3
Solid recovery	-	53.8	54.7	54.4

* Results should be treated only in approximate and comparative way, as used methods did not allow completely pure substances to be obtained; errors (±) refer to standard deviations.

Based on the results presented in Tables 1-3, it was shown that all the pretreatments changed the chemical composition of the raw materials. In the case of the acid and LHW pretreatments, a very high loss of hemicelluloses, including pentosans was observed. The average result of the analysis of the pentosans content in the raw *Populus trichocarpa* wood (Table 1) was 18.2%. Treatment with sulfuric acid decreased the content of these substances to 0.1%. It was similar to the material after LHW treatment, where the share of pentosans was 2.3%. In the case of treatment by NaOH, such a significant decrease in the proportion

of pentosans was not noted. In the analyzed poplar sample, this share after hydrolysis was 13.3%. The average results of the analysis of pentosans content in the *Populus deltoides* x *maximowiczii* wood (Table 2) showed a similar relationship as in the *Populus trichocarpa* wood (Table 1) – the content of these substances in the raw material was 16.9%, after acid treatment 1.4%, after LHW 1.0%, and after NaOH treatment 14.1%. Also in the case of corn stover (Table 3), a significant loss of pentosans was observed. The average share of these substances in the raw material was 24.4%, after treatment with 12% H_2SO_4 it decreased to 1.5%, with LHW to 2.7%, while after treatment with NaOH it decreased slightly to 24.0%. The content of hemicelluloses obtained as a result of the calculation also indicates the strongest loss of these substances in the materials after H_2SO_4 and LHW treatment. In the wood of *Populus deltoides* × *maximowiczii* (Table 2), the share of hemicelluloses from the initial 34.8% dropped to 7.2% after acid treatment, to 18.1% after NaOH treatment, and to 2.2% after LHW. In *Populus trichocarpa* (Table 1), the share of these components in the raw material was 37.1%, while after pretreatments: 7.2% (H_2SO_4), 16.5% (NaOH), and 6.4% (LHW). In the corn stover (Table 3), the hemicelluloses before pretreatment were 27.1%, while after using sulfuric acid (VI) 3.7%, NaOH 27.2%, and LHW 4.3%.

The above losses in the hemicelluloses (up to 93.7% for LHW pretreatment of *Populus deltoides* × *maximowiczii* wood) are consistent with literature findings [Kim et al. 2009; Lu et al. 2012; Li et al. 2014, 2017; Antczak et al. 2022]. This is caused by the fact that due to the amorphous structure and the low degree of polymerization of hemicelluloses, they are easier to hydrolyze than cellulose and are the first to decompose [Fengel and Wegener 2003; Prosiński 1984; Gawron et al. 2014]. The hemicelluloses (especially pentosans) were more susceptible to hydrolysis in the acidic conditions of pretreatment than in alkaline conditions. The pretreatment with NaOH, under the applied conditions, regardless of the investigated raw material, contributed the least to the degradation of these structural substances. Probably the reason for this was the less severe conditions of alkaline treatment, such as the low concentration of NaOH (2%) and process temperature (50 °C for corn stover and 121 °C for poplar wood).

The intensive degradation of hemicelluloses after acid and LHW treatment was reflected in the increase in extractives. This is probably because the hemicelluloses degradation products were detected in the extracts, which was confirmed by other researchers [Li et al. 2017; Akus-Szylberg et al. 2020]. In the *Populus deltoides* × *maximowiczii* biomass, an increase in these components was recorded from 1.7% to 4.0% (acid treatment) and to 17.2% (LHW) (Table 2), in *Populus trichocarpa* from 1.5% to 3.8% (acid treatment) and to 17.4% (LHW) (Table 1) and in corn stover there was an increase in the share from 6.0% to 8.4% (acid treatment) and to 17.3% (LHW) (Table 3). The pretreatment with NaOH contributed to the greatest extent to the loss of extractives. In the *Populus deltoides* × *maximowiczii* biomass after alkaline treatment, their amount was 0.5%, in *Populus trichocarpa* 0.7%, and in the corn stover 0.9%.

The cellulose content of each pretreated sample was significantly higher than in the raw material. In all the discussed types of biomass, the highest increase in cellulose content was recorded after LHW treatment. In *Populus deltoides* x *maximowiczii* (Table 2), it ranged from 42.4% in the raw wood to 73.1% in the material after LHW treatment, while in *Populus trichocarpa* (Table 1) from 44.6% to 75.6%, respectively and in corn stover (Table 3) from 38.4% to 70.6%. The apparent increase in the cellulose content was caused by the degradation of other components of the analyzed materials, such as hemicelluloses, as well as because of weight loss [Fengel and Wegener 2003; Akus-Szylberg et al. 2020].

By assessing the changes in the content of lignin after applying the pretreatment, it can be noticed that after acid and LHW treatment in the majority of the samples there was an increase in the proportion of this component, which like in the case of cellulose, was caused by a significant loss of other components (mainly hemicelluloses) as well as the loss of raw material mass caused by degradation. In the raw wood of *Populus deltoides* × *maximowiczii* (Table 2), 23.5% of lignin was determined after pretreatment with sulfuric acid 32.9%, and after LHW 25.0%. In the case of *Populus trichocarpa* (Table 1), 19.7% was determined in the raw wood before processing. Acid processing increased the share of this component to 27.9%, while after LHW treatment, lignin was at a similar level as in the raw material – the share was 19.5%. As for corn stover (Table 3), the lignin content in the material after pretreatment increased from 21.4% to 35.6% (acid treatment) and 26.7% (LHW). Another explanation for the increase in lignin content may be condensation reactions of lignin and polysaccharide molecules taking place in the acidic pretreatment environment, which was observed by other researchers [Ko et al. 2015a; Pu et al. 2015; Lu et al. 2016]. After using NaOH, no significant differences in the lignin content in the wood of the two poplar species were observed. The lignin content in the *Populus trichocarpa* wood before pretreatment was 19.7% and after 20.4%. On the other hand, in the raw wood of *Populus deltoides x maximowiczii* it was 23.5% and after NaOH pretreatment 23.0%. Hence, it can be assumed that the

lignin in poplar wood under the applied conditions was resistant to NaOH. Similar observations were reported in the literature. Bay et al. [2020] treated poplar wood in a more concentrated NaOH solution (8%) at 93 °C for 2 h and noticed only a slight increase in the lignin content, which they explained by a significant loss of hemicelluloses content in the solid fraction. On the other hand, a clear decrease in the amount of lignin occurred in the sample of corn stover (from 21.4% to 8.9%). This is consistent with the literature data on the effect of alkaline substances on herbaceous plant materials [Nlewem and Trash 2010; Cardona et al. 2014]. It is known that under the influence of alkali, lignin dissolves by breaking ethers and esters bonds, which explains the decrease in its content.

In order to assess the effectiveness of the pretreatments, the results of solid recovery presented in Tables 1-3 were compared. Based on the presented results, it can be concluded that, regardless of the examined lignocellulosic material, the lowest solid recovery occurred after acid pretreatment (53.8%-68.4%), higher after LHW pretreatment (54.4%-71.3%) and the highest after alkaline pretreatment (54.7%-76.9%). In turn, comparing the studied lignocellulosic materials, regardless of the employed pretreatment, the *Populus deltoides* × *maximowiczii* wood was the most resistant and the highest recovery of the solid fraction was obtained for it. Other researchers also obtained a high percentage of solid recovery after the pretreatment of poplar wood. Bhagia et al. [2016] reported that after LHW pretreatment at 180 °C for 12 min, the solid recovery from poplar wood was 76%, whereas after pretreatment in 0.05% H_2SO_4 at 140 °C for 192 min the solid recovery was 73%. Corn stover is a raw material much more susceptible to pretreatment than wood biomass. This is evidenced by the low solid recovery, which according to the results of this work was at the level of 53.8 - 54.7%. Analogical findings were observed by Mouthier et al. [2018] and Dąbkowska-Susfał [2020], who stated that after the pretreatment of corn stover in 2% H_2SO_4 at 160°C for 60 min, the solid recovery was 59.1% and in 2% KOH at 50 °C for 4 hours the solid recovery was 53.6%.

The results of the sugar profile analysis of raw poplar wood and corn stover are presented in Fig. 1.

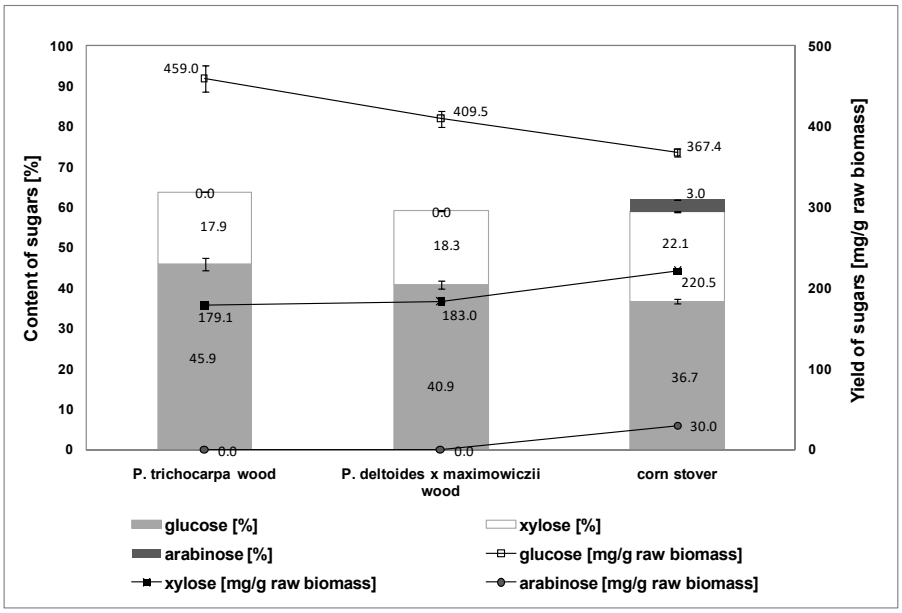

Fig. 1. Content and yield of sugars (glucose, xylose and arabinose) after acid hydrolysis of raw biomass – Populus deltoides x maximowiczii wood, Populus trichocarpa wood and corn stover

Based on the results presented in Fig. 1, it can be observed that from among the studied biomass materials, *Populus trichocarpa* wood has the highest potential for bioethanol production. After acid hydrolysis of the *Populus trichocarpa* wood, the highest average total content (63.8%) and yield (638.1 mg/g raw biomass) of sugars were obtained. Additionally, from this biomass the highest average glucose content (45.9%) was obtained. In turn, in the case of pentoses (xylose and arabinose), the highest contents (22.1% and 3.0% respectively) and yields (220.5 and 30.0 mg/g raw biomass respectively) from corn stover were achieved. Fehér et al. [2017] also found that corn stover is a good raw material for pentose production. As a result of the acid hydrolysis of corn stover with sulfuric acid under various conditions, they obtained 36-235 mg/g raw biomass of xylose and 17-57 mg/g raw biomass of arabinose. These results are well correlated with the data of the chemical composition presented in Tables 1 and 3. The *Populus trichocarpa*

wood possessed the highest average holocellulose content (81.7%) and the highest average cellulose content (44.6%), whereas the corn stover contained the highest average pentosans content (24.4%). Analogical relationships were obtained in earlier studies performed on poplar biomass described by Antczak et al. [2016; 2018]. In these studies, regardless of the researched biomass, the acid hydrolysis efficiency was always higher for the *Populus trichocarpa* species.

The results of the content and yield of sugars obtained after the enzymatic hydrolysis of raw or pretreated poplar wood and corn stover are presented in Figs. 2-4.

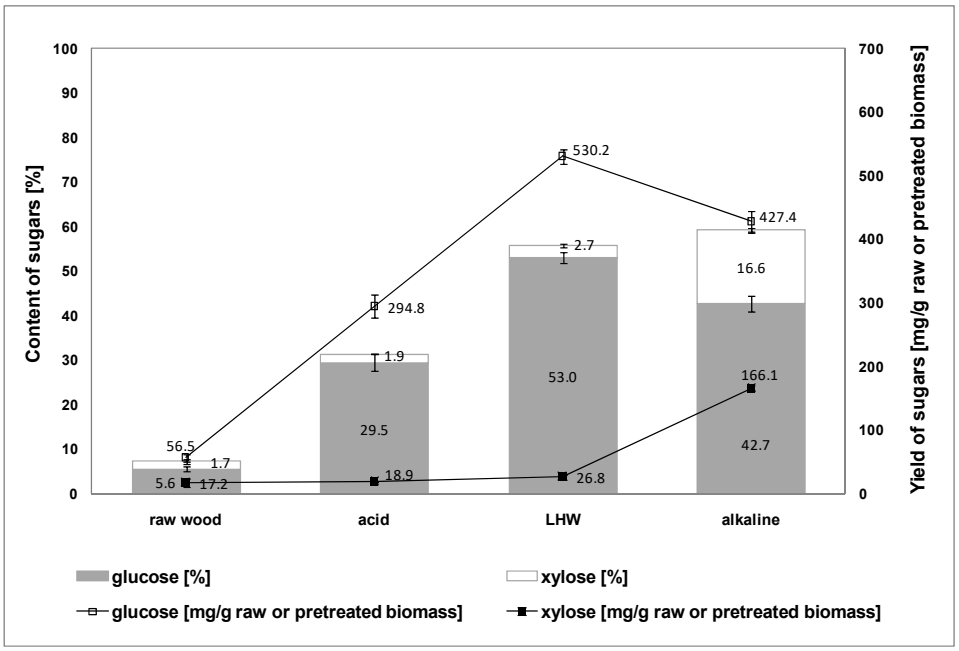

Fig. 2. Content and yield of sugars (glucose and xylose) after enzymatic hydrolysis of Populus *trichocarpa* wood before and after acid, LHW and alkaline pretreatments

Based on the obtained results, it can be observed that the applied pretreatment methods greatly enhanced the efficiency of the enzymatic hydrolysis of the lignocellulosic biomass under study. These correlations are in agreement with data presented by other researchers [Li et al. 2014; Michelin and Teixeira 2016; Imman et al. 2018; Woiciechowski et al. 2020; Antczak et al. 2022]. In the case of *Populus trichocarpa* wood, from among the employed methods the most effective was alkaline pretreatment (Fig. 2). As a result of this method using a 2% solution of NaOH in the autoclave (121 °C, 0.1 MPa) for 30 min, the *Populus trichocarpa* wood became the most susceptible to enzymatic hydrolysis. The obtained average total content of sugars was 59.3%, while the average total yield of sugars was 593.5 mg/g pretreated biomass. Also, high results of total content (55.7%) and yield (557.0 mg/g pretreated biomass) of sugars were obtained after enzymatic hydrolysis of the LHW treated *Populus trichocarpa* wood. Similar findings were obtained by Akus-Szylberg et al. [2020], who also studied the enzymatic hydrolysis efficiency of LHW pretreated 7-year-old *Populus trichocarpa* wood. The total content of sugars obtained after the enzymatic hydrolysis of LHW pretreated poplar wood at 190 °C during 20 min was a few percentage points lower and reached a value of 50.3% (47.0% of glucose and 3.3% of xylose). On the other hand, Antczak et al. [2022] obtained in similar conditions (190 °C, 15 min) of LHW pretreatment even higher results (66.3% of glucose and 7.5% of xylose), but they studied 5-year-old *Populus trichocarpa* wood. The acid pretreatment method occurred to be the least effective in terms of enzymatic hydrolysis efficiency. As the results of this method show, the average total content of sugars was 31.4%, while the average total yield of sugars was 313.7 mg/g pretreated biomass (Fig. 2).

In the case of the results presented in Fig. 3 for *Populus deltoides* x *maximowiczii* wood, it can be observed that from the bioethanol production point of view (high glucose content), the most efficient pretreatment method was LHW. After 15 min of LHW pretreatment at 190 °C, the average total content of sugars obtained during enzymatic hydrolysis was

63.9%, while the average total yield of sugars was 638.8 mg/g pretreated biomass. In turn, after the alkaline pretreatment, the average total content and yield of sugars achieved during enzymatic hydrolysis were significantly lower and reached values of 50.2% and 501.9 mg/g pretreated biomass, respectively. As earlier, the acid pretreatment with 12% H_2SO_4 for 4 h at 102 °C occurred to be the least effective in the context of the performed enzymatic hydrolysis. The obtained average total content (7.0%) and yield (69.8 mg/g pretreated biomass) of sugars were only slightly higher than the results obtained after the enzymatic hydrolysis of raw wood (5.7% and 56.8 mg/g pretreated biomass). Based on the results presented in Figs. 2 and 3, it can be concluded that *Populus deltoides* x *maximowiczii* wood was more recalcitrant than *Populus trichocarpa* wood. Analogical observations were presented by Antczak et al. [2018] for the 5-year-old poplar species. Probably, in order to achieve higher yields of enzymatic hydrolysis for *Populus deltoides* x *maximowiczii* wood, more severe alkaline or acid pretreatment conditions should be utilized.

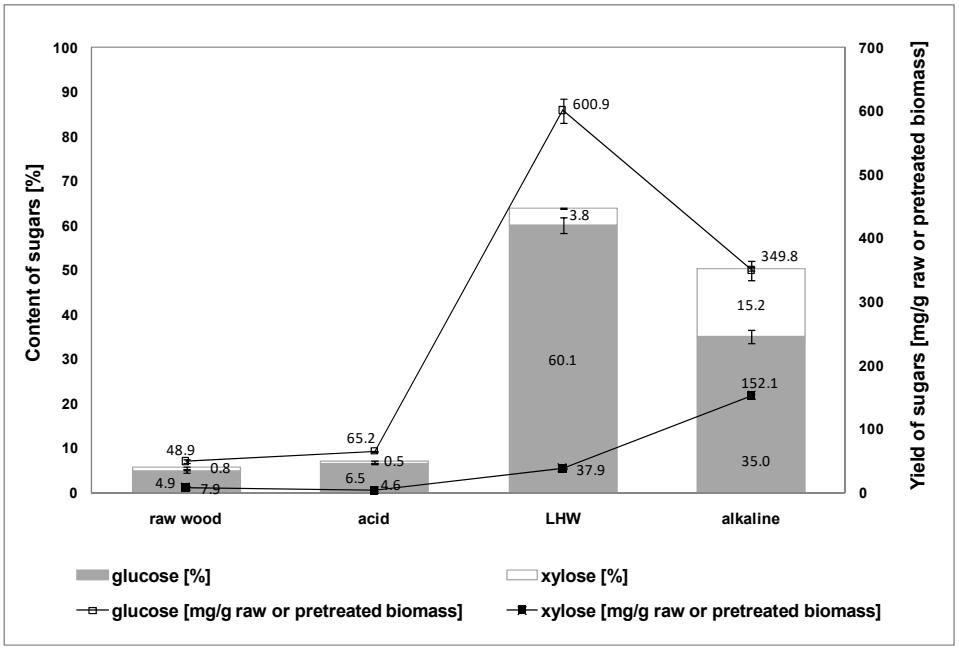

Fig. 3. Content and yield of sugars (glucose and xylose) after enzymatic hydrolysis of *Populus deltoides* x *maximowiczii* wood before and after acid, LHW and alkaline pretreatments

In Fig. 4 the results of the average total content and yield of sugars after the enzymatic hydrolysis of raw corn stover and corn stover pretreated by different methods are presented. In the case of corn stover, the alkaline pretreatment method occurred to be the most effective because the enzymatic hydrolysis proceeded with the highest efficiency. After this pretreatment, the average total content of sugars obtained during enzymatic hydrolysis was 65.9%, while the average total yield of sugars was 659.2 mg/g pretreated biomass. Also, high results of the total content (52.6%) and yield (526.3 mg/g pretreated biomass) of sugars were obtained after enzymatic hydrolysis of the LHW pretreated corn stover. Similar results were achieved by Akus-Szylberg et al. [2018]. After 20 min of corn stover LHW pretreatment at 190 °C the total content of sugars was 55.0%. Finally, similar to the other studied materials, the lowest average total content (29.7%) and yields (296.8 mg/g pretreated biomass) of sugars were obtained for the acid pretreatment. This method occurred to be the least effective, regardless of the investigated material.

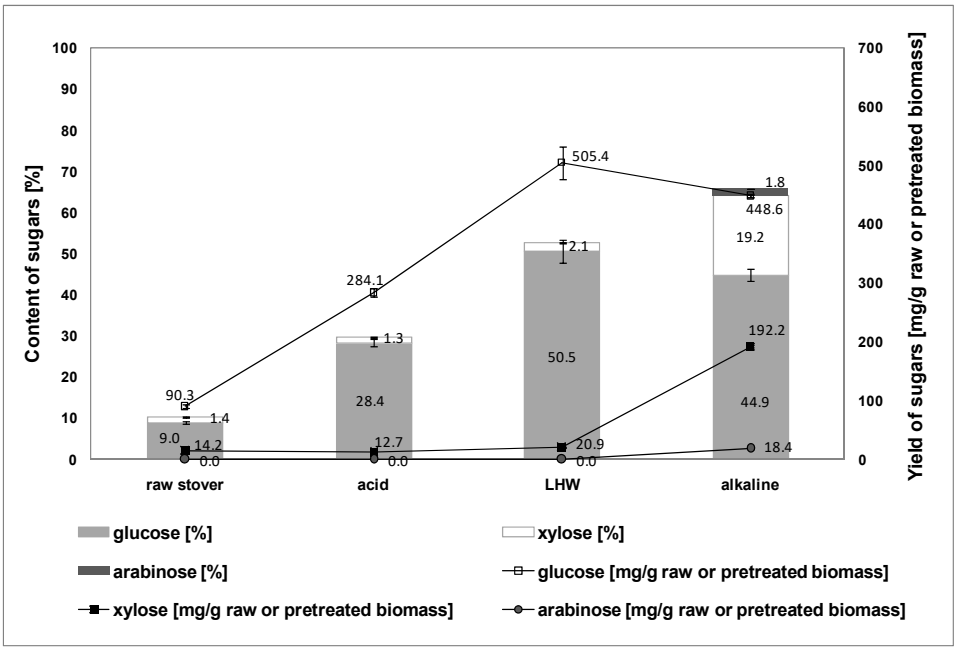

Fig. 4. Content and yield of sugars (glucose, xylose and arabinose) after enzymatic hydrolysis of corn stover before and after acid, LHW and alkaline pretreatments

If we compare two the best pretreatment methods (alkaline and LHW) studied in this work (Figs. 2-4), it can be observed that the results of the sugar content also differ in the profile of sugars obtained by enzymatic hydrolysis. Regardless of the studied material, as a result of the enzymatic hydrolysis of alkaline pretreated biomass, the obtained average glucose content was on the level of 35.0-44.9%. Besides the glucose, after enzymatic hydrolysis high amounts of xylose (15.2-19.2%) were achieved. Moreover, in the case of alkaline pretreated corn stover, small amounts of arabinose (1.8%) were also detected in the hydrolyzate. Analogical results were described in other publications in which alkaline pretreatment was also used [Antczak et al. 2019; Akus-Szylberg et al. 2021; Zborowska et al. 2022]. The LHW pretreated biomass had a more interesting sugar profile from the bioethanol production point of view. This biomass consisted mainly of cellulose (up to 75.6%) and the pentosans content did not exceed 2.7% (Table 1-3). Hence, after the enzymatic hydrolysis of LHW pretreated biomass, mainly glucose (50.5-60.1%) and minor amounts of xylose (2.1-3.8%) were produced. This sugar profile is even better owing to the fact that there are currently no suitable microorganisms that would effectively produce bioethanol from pentoses on an industrial scale.

Conclusions

In this paper, the influence of selected pretreatment methods on the chemical composition and enzymatic hydrolysis efficiency of poplar wood and corn stover were compared. On the basis of the performed experiments, the following conclusions were drawn:

1. The applied pretreatments changed the chemical composition of the raw materials. In the case of the acid and LHW (liquid hot water pretreatment) methods, a very high loss of hemicelluloses (up to 93.7% of *Populus deltoides* × *maximowiczii* wood), especially pentosans was observed, while for NaOH, such a significant decrease was not noted.
2. The applied pretreatments (acid, alkaline and LHW) greatly enhanced the enzymatic digestibility of the studied biomass. The LHW and alkaline methods occurred to be the most interesting. After the LHW pretreatment and subsequent enzymatic hydrolysis of *Populus deltoides* x *maximowiczii* wood, a total yield of sugar up to 638.8 mg/g pretreated biomass was obtained, while in the case of the alkaline method the total yield of sugars from corn stover was even higher and was up to 659.2 mg/g pretreated biomass.
3. The LHW pretreatment of *Populus deltoides* × *maximowiczii* wood, from the bioethanol production

point of view, occurred to be the best because after enzymatic hydrolysis, mainly glucose (up to 600.9 mg/g pretreated biomass) and minor amounts of xylose (up to 37.9 mg/g pretreated biomass) were produced.

4. Based on the chemical composition and sugar profile analysis, it was proved that from among the studied biomass materials, the *Populus trichocarpa* wood also has a high potential for bioethanol production.

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