Sugars Yield Obtained after Acid and Enzymatic Hydrolysis of Fast-growing Poplar Wood Species

Andrzej Antczak,[a,*] Monika Marchwicka,[a] Jan Szadkowski,[a] Michał Drożdżek,[a] Jakub Gawron,[b] Andrzej Radomski,[a] and Janusz Zawadzki[a]

The potential of fast-growing poplar species was evaluated for bioethanol production. The yields of glucose and xylose from acid and enzymatic hydrolysis were compared. The hydrolysis processes were performed on raw and extracted wood. The extracted wood was obtained by action of a chloroform and 96% ethanol (93:7 w/w) mixture. Additionally, to enhance the enzymatic hydrolysis efficiency, a liquid hot water (LHW) pretreatment was used. The acid hydrolysis turned out to be a good method to verify the biomass potential for bioethanol production. After acid hydrolysis of raw and extracted biomass, high total sugars yields were obtained (between 626.2 to 808.5 mg/g raw or extracted biomass), while in the case of enzymatic hydrolysis the total sugars yields were very low (between 45.5 to 68.9 mg/g raw or extracted biomass). The LHW pretreatment greatly enhanced the enzymatic digestibility of the studied wood. The average glucose yield from enzymatic hydrolysis was up to 602.0 mg/g pretreated biomass and was higher than that from acid hydrolysis (the maximum yield was 566.9 mg/g extracted biomass). As a result of the LHW pretreatment, up to 91.3% of the hemicelluloses were removed from the solid fraction. From the obtained glucose and xylose results, it was concluded that *Populus trichocarpa* wood had a higher potential for bioethanol production than *P. deltoides* x *maximowiczii* wood. The presence of extractives (low molecular substances) in raw poplar wood (up to 2.8%) had a low impact on the yield from acid and enzymatic hydrolysis.

INTRODUCTION

A constant energy supply is the basis for the economic and social development of a country. A future increase in energy demand in both Poland and around the world is an inevitable phenomenon. Currently, the energy demand is mainly met with the use of dwindling fossil fuel resources (oil, coal, and natural gas). An alternative to conventional fuels is the use of renewable energy sources, such as biomass, biogas, wind, solar, and water energy. Bioethanol is a biofuel that is currently produced on an industrial scale from renewable energy sources, such as sugar cane (Brazil) and corn (USA). Unfortunately, the growth in demand for this fuel and the controversy associated with the use of edible raw material for fuel production have made it necessary to seek new biomass sources (Stolarski *et al.* 2013; Krzyżaniak *et al.* 2014). One of the sources that can replace edible raw materials is lignocellulosic biomass in the form of wood from fast-growing poplar species.

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Fast-growing poplars are characterized by a high biomass yield (usually 6T/ha·year to 12 T/ha·year, and 13 T/ha·year to 18 T/ha·year for special species) (Labrecque and Teodorescu 2005; Sannigrahi *et al.* 2010). Additionally, these poplar species can tolerate unfavorable environmental conditions, are suitable for plantations, and can be used for the reclamation of industrial areas. An important aspect during bioethanol production is the chemical composition of the biomass. Poplar wood is characterized by a high content of valuable polysaccharides, such as cellulose (44% to 52%) (Pettersen 1984) and glucuronoxylan (15% to 23%) (Willför *et al.* 2005). These polysaccharides are a source of simple sugars that can be obtained by enzymatic or acid hydrolysis and can be converted into bioethanol through fermentation.

Acid hydrolysis, a method that is well known and has been used on an industrial scale, was used in this study, in addition to the enzymatic hydrolysis process (Harris and Beglinger 1946; Wyman 1999). Acid hydrolysis, in this paper, was based on a laboratory method optimized and adapted for poplar wood, which allowed for the control of the sugars content from the beginning stages of tree growth (Antczak *et al.* 2012, 2014). Moreover, this method allowed for the assessment of the suitability of different biomass types (wood, bark, or leaves) for the production of bioethanol (Antczak *et al.* 2016). Generally, the enzymatic hydrolysis process can be regarded as advantageous compared to the acid hydrolysis process. The most important advantages of enzymatic hydrolysis include a higher process efficiency and the use of non-corrosive and milder conditions, and biodegradable and non-toxic reagents (Taherzadeh and Karimi 2007b; Verardi *et al.* 2012). Unfortunately, a cost-effective technology has not yet been developed for obtaining bioethanol from wood on an industrial scale. The main reasons for this are a high enzyme prices and problems related to the optimization of the speed and efficiency of enzymatic hydrolysis and fermentation processes (El-Naggar *et al.* 2014; Achinas and Euverink 2016; Álvarez *et al.* 2016). Additionally, an appropriate pretreatment of the lignocellulosic feedstock is necessary for a high yield from enzymatic hydrolysis (Taherzadeh and Karimi 2007b; Verardi *et al.* 2012; El-Naggar *et al.* 2014). A pretreatment step should overcome the recalcitrant structure of lignocellulosic biomass. An effective pretreatment method allows enzymes to have easier access to polysaccharides by removing lignin, increasing the porosity of biomass and decreasing the degree of cellulose crystallinity. Among the available pretreatment methods, the most common are physical (especially milling) and physicochemical (steam explosion, ammonia fiber expansion, and liquid hot water pretreatment), and are supported by chemical methods (action by oxidizing substances, acids, or bases) (Kumar *et al.* 2009; Zheng *et al.* 2009; Alvira *et al.* 2010; Marchwicka *et al.* 2015).

In recent years, the liquid hot water (LHW) process has often been tested as a pretreatment (Li *et al.* 2014; Michelin and Teixeira 2016; Li *et al.* 2017; Imman *et al.* 2018). The LHW process involves using liquid water at a high temperature and pressure to break up the lignocellulosic complex. The temperatures used usually range from 160 °C to 240 °C, and the residence times range from a few minutes to an hour. In the LHW process, similar to steam explosion, the autohydrolysis of raw materials proceeds quickly. However, in contrast with steam explosion, the LHW process does not have a rapid decompression stage. The most important effect of the LHW process is to dissolve hemicelluloses (in the liquid fraction) and separate them from the solid fraction. Because of this treatment, the solid fraction is less contaminated with inhibitors, which would have lowered the hydrolysis and fermentation efficiencies. The hemicelluloses and products of their decomposition (oligosaccharides) in the liquid fraction can also be taken into account in

bioethanol production technology (Sreenath *et al.* 1999; Gírio *et al.* 2010). Another interesting application for these substances are production of xylitol, furfural, or lactic acid (Gullón *et al.* 2012; Luo *et al.* 2018). These applications are even more important due to the fact that currently there are no suitable microorganisms that would effectively produce bioethanol from pentoses on an industrial scale.

Another problem, concerning production of bioethanol that is still poorly studied is the presence of extractives in lignocellulosic biomass. Compounds such as tannins, fatty acids, phenolic acids, glycosides, and sterols are substances that are found in poplar biomass and can affect the hydrolysis and fermentation processes even in small amounts (Luo *et al.* 2002; Fengel and Wegener 2003; Szadkowska *et al.* 2016). There are only a few publications in the literature that have studied the influence of extractives on the hydrolysis yield (Thammasouk *et al.* 1997; Tamaki and Mazza 2010; Li *et al*. 2016; Smit and Huijgen 2017).

Unfortunately, these publications did not study the use of wood from fast-growing poplar species. Therefore, in this paper, the acid and enzymatic hydrolysis processes were performed on raw and extracted materials from poplar species. Special varieties of fast-growing poplar species (*Populus deltoides* x *maximowczii* and *P. trichocarpa*), distinguished by their high content of polysaccharides, were selected for this study. The utilized poplar species are a valuable source of glucose and xylose, *i.e.*, simple sugars that are important in bioethanol production. From scientific and practical points of view, the use of fast-growing poplar species is interesting and worthy of attention.

EXPERIMENTAL

Materials

Debarked wood from fast-growing poplar species (*P. deltoides* x *maximowiczii* and *P. trichocarpa* Torr. and A. Gray ex Hook) was used in this study. The wood came from the experimental field at Wolica, Department of Genetics, Plant Breeding and Biotechnology, Faculty of Horticulture, Biotechnology, and Landscape Architecture at Warsaw University of Life Sciences. The tree ages were 2.5 years, 4 years, and 5 years. The chemical composition of the studied poplar wood is shown in Table 1.

The poplar wood was ground into sawdust (size fraction below 0.43 mm). Then, the obtained raw material was divided into two parts. One part was extracted in a Soxhlet apparatus according to the NREL (National Renewable Energy Laboratory) procedure (Sluiter *et al.* 2008b) using a mixture of chloroform and 96% ethanol (93:7 w/w) for 10 h. This solvent was used because it is better at dissolving polar and non-polar low molecular substances (such as tannins, fatty acids, phenolic acids, glycosides and sterols), which are present in poplar wood, compared to ethanol alone. Moreover, this solvent is non-flammable and less toxic than benzene, which was formerly used to wood extraction (Antczak *et al*. 2006). Prepared in this way, the raw and extracted wood were subjected to the acid and enzymatic hydrolysis processes. Additionally, the second part of the size reduced raw wood (5-year-old *P. deltoides* x *maximowiczii* and *P. trichocarpa*) was pretreated using the LHW process before enzymatic hydrolysis. All of the chemical substances were of analytical grade and purchased from Chempur (Piekary Śląskie, Poland).

Table 1. Chemical Composition of the Studied Poplar Wood

Chemical Composition (%)	P. deltoides x maximowiczii			P. trichocarpa		
	Tree Age (years)					
	2.5	4	5	2.5	4	5
Absolute moisture of the raw wood (drying and weighing method); Krutul (2002); Sluiter et al. (2008a)	5.0 ± 0.3	6.2 ± 0.3	5.1 ± 0.3	4.3 ± 0.3	5.3 ± 0.2	7.8 ± 0.3
Absolute moisture of the extracted wood (drying and weighing method); Krutul (2002); Sluiter et al. (2008a)	n.d.	8.2 ± 0.1	n.d.	n.d.	8.7 ± 0.1	n.d.
Extractives (mixture of chloroform and ethanol 93:7 w/w); Antczak et al. (2006); Sluiter et al. (2008b)	2.6 ± 0.2	2.8 ± 0.1	2.3 ± 0.2	1.7 ± 0.3	1.5 ± 0.3	1.5 ± 0.3
Lignin (acetyl bromide method); Johnson et al. (1961); Iiyama and Wallis (1988)	19.7 ± 0.7	19.9 ± 0.5	20.7 ± 0.6	19.9 ± 0.5	19.1 ± 0.3	20.1 ± 0.5
Cellulose* (Kürschner-Hoffer method); Saeman et al. (1954); Krutul (2002)	51.2 ± 0.4	51.4 ± 0.2	51.6 ± 0.3	52.0 ± 0.4	52.8 ± 0.2	53.0 ± 0.2
Holocellulose* (sodium chlorite method); Wise et al. (1946)	82.1 ± 0.3	82.7 ± 0.5	83.5 ± 0.1	85.4 ± 0.2	85.5 ± 0.5	86.4 ± 0.5
Hemicelluloses* (calculated as the difference between the holocellulose and cellulose contents)	30.9	31.3	31.9	33.4	32.7	33.4

*Results should be treated only in an approximate and comparative way, as the methods used did not allow for completely pure substances to be obtained; n.d. – Not determined; the errors (±) refer to standard deviations – at least three replications were done to obtain the average values.

Methods

Acid hydrolysis

The acid hydrolysis process was done on the raw and extracted wood (fraction below 0.43 mm) that was previously dried to a constant weight in a vacuum drier at 60 °C and a pressure of 0.4 kPa. Sulfuric acid (72%) was used for acid hydrolysis, and the process was done in accordance with the developed procedure for poplar wood described by Antczak et al. (2012, 2014). The weighed samples (about 100 mg) were placed in a pear-shaped flasks with a capacity of 50 mL, to which 1 mL of sulfuric acid at a concentration of 72% was added and were tightly closed with a stopper. Next, the samples were heated

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in a water bath at 30 °C during 1 h. After cooling to room temperature, 28 mL of distilled water was added into each of them. Then the samples were subjected to heating at a higher temperature (120 °C) under reflux for 3 h in an oil bath. Subsequently, the samples were cooled to room temperature and filtered through a Schott G3 filter. During this time, the flasks and filters were washed with 8 mL of distilled water (twice). The collected filtrates were quantitatively transferred to beakers with a volume of 150 mL. Afterwards, 1 mL of 0.05 M sodium azide solution was added, and the samples were neutralized with sodium carbonate to pH about 5. After the neutralization step, the samples were subjected to a concentration process using a vacuum evaporator (Rotavapor R-215, Büchi, Flawil, Switzerland). The concentrated hydrolysates were quantitatively transferred by using of distilled water into a volumetric flasks with a volume of 25 mL. The sugars results of glucose and xylose in the supernatant were analyzed by high-performance liquid chromatography (HPLC). All of the acid hydrolysis tests were done fourfold, and standard deviations were calculated.

Enzymatic hydrolysis

The enzymatic hydrolysis process was performed on the raw and extracted wood that had a known moisture content (Table 1). Cellic CTec2 enzyme (mixture of cellulases, β-glucosidases, and hemicellulases) (Novozymes, Bagsvaerd, Denmark) was used for enzymatic hydrolysis. The activity of enzyme was 129 FPU/mL, as measured with Whatman No.1 filter paper according to the NREL method (Adney and Baker 1996). The samples of poplar wood were weighed in sealed screw-capped test tubes with a volume of 10 mL. The cellulose concentration in the solution subjected to hydrolysis was 1% w/w. Then, 5 mL of a 0.1-M citrate buffer solution at a pH of 4.8 was added to each sample. Next, 0.1 mL of a 2% solution of sodium azide was added to each sample to prevent the growth of microorganisms during hydrolysis. The addition of distilled water was calculated so that that the total volume of the solution was 10 mL. Water was added to each sample before the enzyme addition. Subsequently, 0.333 mL of the 25% v/v Cellic CTec2 enzyme solution was added to each sample (0.1 g of enzyme per 0.1 g of cellulose). Finally, the test tubes were screwed tightly, and the samples were hydrolyzed for 72 h using a mixer (RM-2M, Elmi company, Calabasas, CA, USA) at a rotational speed of 25 rpm placed in a laboratory drier set to a temperature of 50 °C. After hydrolysis, the collected samples were stored in a freezer at -20 °C. The sugars results of glucose and xylose contents in the supernatant were analyzed by HPLC. All of the enzymatic hydrolysis tests were done in triplicate and single standard deviations were calculated.

LHW pretreatment

The LHW pretreatment process was performed on the size reduced (fraction below 0.43 mm) raw wood of 5-year-old *P. deltoides* x *maximowiczii* and *P. trichocarpa*). The pretreatment process was conducted in a stainless-steel reactor with a total volume of 250 mL. Before the LHW process, the raw wood (approximately 20g) was saturated with distilled water (200 mL) using a magnetic stirrer at 75 °C for 20 min. The saturation of wood with water at elevated temperature causes quick swelling of the material, which increases the effectiveness of the pretreatment process. Afterwards, the material and water were quantitatively transferred to the reactor, which was supplemented with distilled water to reach a total volume of 250 mL. The ratio of solid to liquid was 1:12.5. The pretreatment temperatures were 160 °C, 175 °C, 190 °C, and 205 °C. The residence time was 15 min. After the LHW process, the reactor was cooled by quenching it in a bath containing a

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mixture of ice and sodium chloride until the end of the reaction. The next day, the residual solid and pretreated liquor were separated through filtration with a Büchner funnel. The residual solid was washed with distilled water until a neutral pH was achieved, and then stored at 6 °C for enzymatic hydrolysis processing. Two pretreatment processes were performed at a given temperature. Then, the obtained washed residual solids from a given temperature were mixed, and the enzymatic hydrolysis process was performed according to the procedure described previously.

HPLC analysis

Before the chromatographic analysis, the samples were thawed and brought to room temperature. Subsequently, after enzymatic hydrolysis of the samples, they were denatured with heat for 15 min at 95 °C in a water bath. Then, the samples were centrifuged for 10 min on a laboratory centrifuge at 12000 rpm after the acid and enzymatic hydrolysis processes. Finally, each sample was filtered using a nylon syringe filter with a porosity of 0.2 μm.

The analysis of the sugars results after hydrolysis was performed using a HPLC system (LC-20AD, Shimadzu, Kyoto, Japan), which was equipped with a differential refractive detector (RID-10A, Shimadzu, Kyoto, Japan), pump (LC-20AD, Shimadzu, Kyoto, Japan), degasser (DGU-20A, Shimadzu, Kyoto, Japan), oven (CTO-20A, Shimadzu, Kyoto, Japan), and controller (CBM-20A, Shimadzu, Kyoto, Japan). The chromatographic data was processed using the LC Solution v.1.21 SP1 software (Shimadzu, Kyoto, Japan). The HPLC analysis conditions varied and depended on the hydrolysis process. For the samples that underwent acid hydrolysis, the conditions were as follows:

- Acetonitrile:water ratio of 80:20 (v/v) as the eluent,
- Column – Genore Cosmosil Sugar-D (250 mm ×4.60 mm, Nacalai Tesque, Kyoto, Japan) connected with a guard column,
- Oven temperature of 50 °C,
- Flow rate of 2 mL/min,
- Injection volume of 20 μL.

For the samples that underwent enzymatic hydrolysis, the conditions of the HPLC analysis were as follows:

- Re-distilled water as the eluent,
- Column – RHM-Monosaccharide (300 mm ×7.80 mm, Rezex, Torrance, USA) connected with a guard column,
- Oven temperature of 80 °C,
- Flow rate of 0.6 mL/min,
- Injection volume of 20 μL.

The sugars contents and yields after hydrolysis were determined based on the previously developed calibration curves (Antczak *et al*. 2014) or tests which were done in this work under the conditions described above.

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RESULTS AND DISCUSSION

Acid versus Enzymatic Hydrolysis of Raw or Extracted Biomass

To study the potential of fast-growing poplar species for the production of bioethanol, acid and enzymatic hydrolysis were performed. The resulting sugars contents and yields are presented in Figs. 1 and 2. Comparing the results, it was observed that the average glucose yield obtained from acid hydrolysis in all cases, regardless of the poplar species, was over 10 times higher than that from enzymatic hydrolysis. The average glucose yield after acid hydrolysis was between 473.9 to 566.9 mg/g raw or extracted biomass; whereas, after enzymatic hydrolysis was between 35.4 to 45.9 mg/g raw or extracted biomass. In turn, the average xylose yield obtained from acid hydrolysis was between 152.3 to 241.6 mg/g raw or extracted biomass, whereas after enzymatic hydrolysis was between 10.1 to 23.0 mg/g raw or extracted biomass. The results presented in this work are consistent with literature data obtained for different hardwoods. Uçar and Balaban (2003) used 77% sulfuric acid in hydrolysis process and obtained glucose content between 48.8% to 52.2%, whereas for xylose the content was between 14.5% to 20.6%.
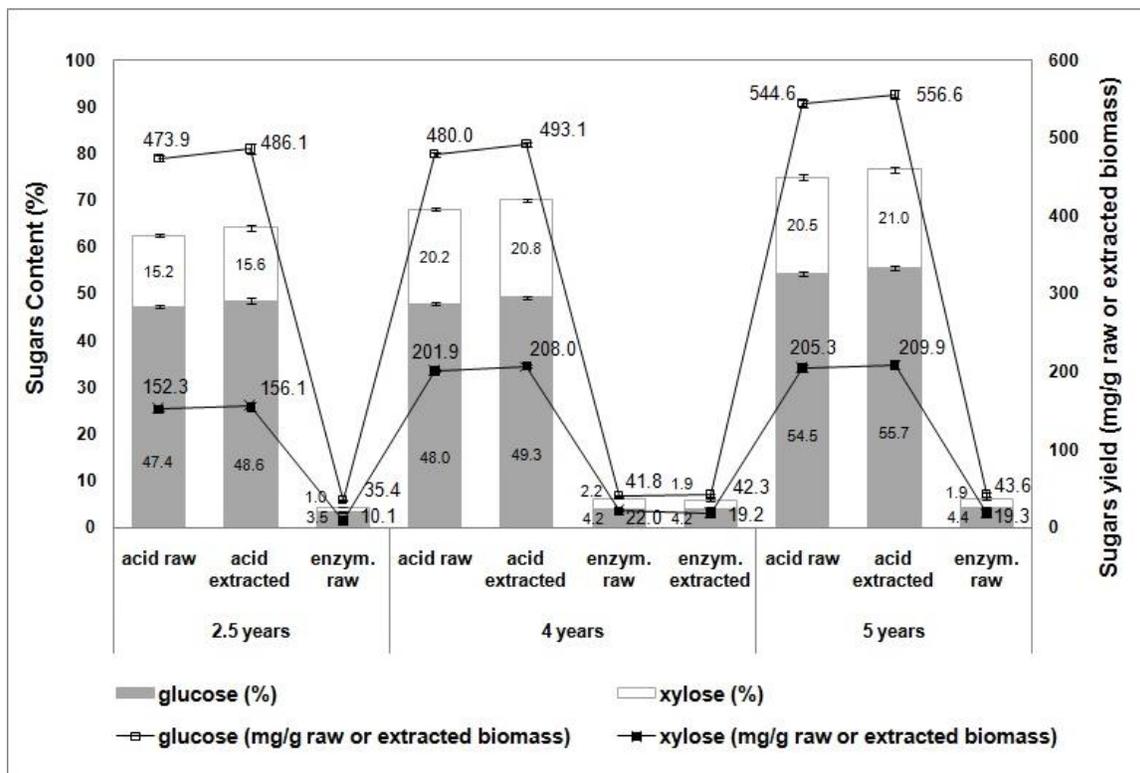

Fig. 1. Sugars contents and yields after acid and enzymatic hydrolysis of the raw and extracted *P. deltoides* x *maximowiczii* wood (without LHW pretreatment)

Acid hydrolysis turned out to be a good method to verify the biomass potential for bioethanol production. After acid hydrolysis of raw and extracted biomass, high sugars yields were obtained, while in the case of enzymatic hydrolysis the sugars yields were very low. These results show, as was noted in the introduction, that an appropriate pretreatment step is necessary for a high yields of enzymatic hydrolysis. Confirmation of this essential statement are a results presented in other publication (Antczak *et al.* 2018), where analogous poplar biomass was used. In order to enhance enzymatic digestibility of poplar

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wood the Kraft pulping method was used. The alkaline pretreatment caused significant increase of sugars contents. After enzymatic hydrolysis of alkaline pretreated wood, the average glucose content was in the range 58.2% to 71.9% and for xylose between 15.8% and 18.8%. In publication Xu *et al.* (2011) a different chemical substances (sulfuric acid, sodium hydroxide and lime) were used in pretreatment step to enhance effectiveness of enzymatic hydrolysis of switchgrass and coastal Bermuda grass. The best method turned out to be lime pretreatment. At optimal enzyme loadings the maximum total sugars yield for switchgrass was 433.0 mg/g raw biomass, but for coastal Bermuda grass it was 429.7 mg/g raw biomass.
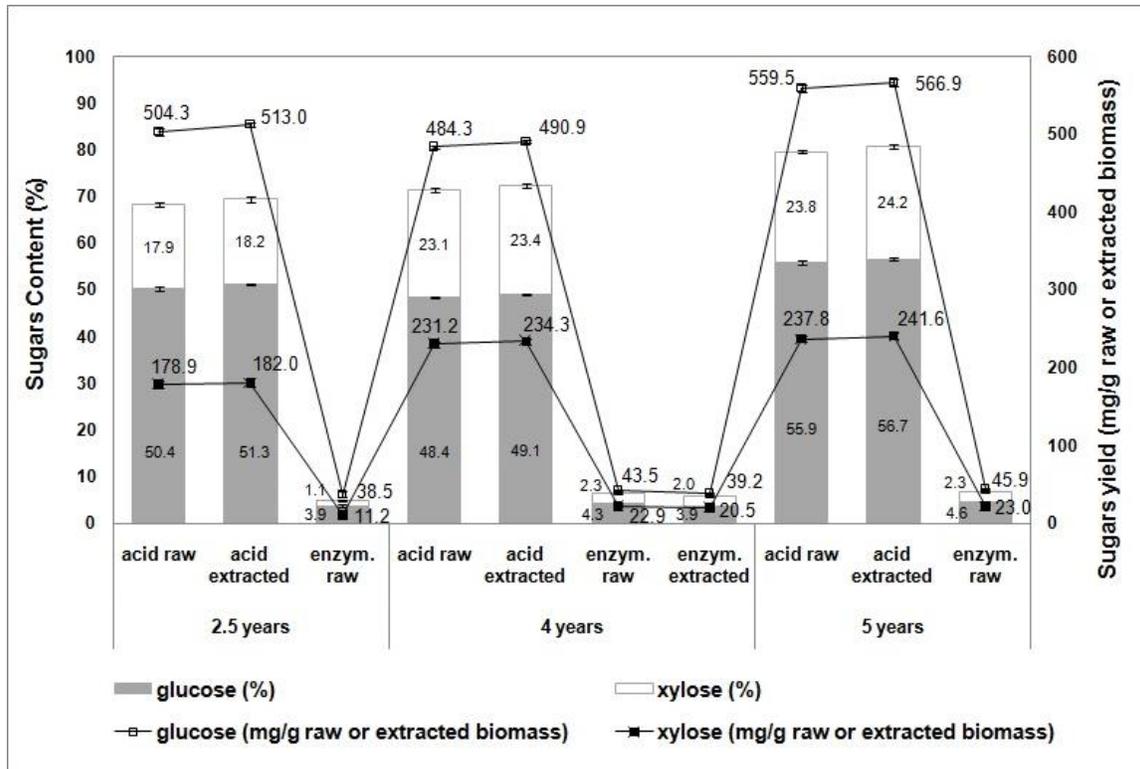

Fig. 2. Sugars contents and yields after acid and enzymatic hydrolysis of the raw and extracted *P. trichocarpa* wood (without LHW pretreatment)

The main disadvantage of acid hydrolysis method, especially important in bioethanol production technology, is partial degradation of sugars and formation of by-products, which inhibit bioethanol production in fermentation step. The most studied inhibitors are furfural, 5-hydroxymethylfurfural (HMF), acetic acid, formic acid, and levulinic acid (Jönsson *et al.* 2013). In order to minimize the formation of the inhibitors, dilute-acid hydrolysis is done in two or more stages. In the first stage, which is carried out under mild conditions, hemicelluloses are converted to monosaccharides. In the second stage, the residual solid (mainly cellulose) under more severe conditions, is hydrolyzed (Harris *et al.* 1984). Another very important factor, which will determine profitability of whole process of bioethanol production, especially on an industrial scale, is the cost aspect. The acid hydrolysis is an expensive process because it requires very expensive apparatus, which is made of special alloys or ceramics. Moreover, when sulfuric acid is used, large amounts of gypsum are produced in neutralization step. In addition the recovery of acid is

an energy consuming process (Taherzadeh and Karimi 2007a). Despite the above disadvantages, using acids in hydrolysis process is still of interest. A Dutch research group elaborated a process called "Biosulfurol", which has low overall cost of bioethanol production (van Groenestijn *et al.* 2006). In turn, Arkenol Inc. and Masada Resource Group (American companies) developed the concentrated acid hydrolysis processes, which in principle could be made economically viable and ready for commercial implementation (www.arkenol.com; www.masada.com).

In view of the above problems, it is currently believed that enzymatic hydrolysis will be the most cost-effective hydrolysis process in the future. The great hopes associated with enzymatic hydrolysis are due to several important advantages of this process, which concern both economic and ecological aspects. First, the enzymatic hydrolysis is carried out under mild, non-corrosive conditions; hence, the cost of the apparatus is much smaller. Second, the enzymatic hydrolysis process uses environmentally friendly and non-toxic chemicals. Moreover, it is possible to obtain a very high yields of enzymatic hydrolysis (close to 100%), which is difficult to achieve in acid hydrolysis process (Taherzadeh and Karimi 2007b). Despite the above advantages, the enzymatic hydrolysis process has its own problems. The main drawbacks of enzymatic hydrolysis are a long time of processing, a need for material pretreatment, and high enzyme prices. Fortunately, in recent years, the costs of enzymes have been gradually decreasing. Progress also has been achieved in the optimization of the enzymatic hydrolysis process. Thus, there is a good chance that a cost-effective bioethanol production technology based on enzymatic hydrolysis will eventually be developed and implemented on an industrial scale.

Influence of a Tree Age and Wood Chemical Composition

Based on the results presented in Figs. 1 and 2, it can be concluded that, the acid hydrolysis process efficiency increased remarkably with the tree age. Generally, the highest sugars yield was obtained from the 5-year-old poplar wood, and the lowest was from the 2.5-year-old wood. The relationships described above were related to the chemical composition of the wood (Table 1). The results that are presented in Table 1 indicated that the cellulose and hemicelluloses contents increased with the tree age. Similar observations were confirmed in earlier publication (Antczak *et al.* 2014). In these studies the acid hydrolysis process was used for *P. deltoides* x *maximowiczii* wood (0.5-year-old) and *P. alba* L. wood (30-year-old). It turned out that glucose content after acid hydrolysis was higher for *P. alba* L. wood than for *P. deltoides* x *maximowiczii* wood. In this case, the results were also connected with the tree age and chemical composition of the wood. The average cellulose content in 30-year-old *P. alba* L. wood was 44.0%, whereas in 0.5-year-old *P. deltoides* x *maximowiczii* wood it was 40.0% only. Also if we compare the results shown in Table 1, it is apparent that the *P. trichocarpa* wood contained more polysaccharides (by approximately 3%) than the *P. deltoides* x *maximowiczii* wood, which explained the higher hydrolysis efficiency. Analogous results were reported in another publication on the acid hydrolysis of wood, bark, and leaves from a previously described poplar species (Antczak *et al.* 2016). Regardless of the biomass tested, the hydrolysis efficiency was always higher for the *P. trichocarpa* raw material. On the basis of the obtained results, it was concluded that the *P. trichocarpa* wood had a higher potential for bioethanol production than the *P. deltoides* x *maximowiczii* wood.

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Influence of Extractives

Another aspect studied in this publication was the influence of extractives on the yields from the hydrolysis processes. As was mentioned in the introduction, compounds such as tannins, fatty acids, phenolic acids, glycosides, and sterols are extractives that are found in poplar biomass and can affect the hydrolysis or fermentation processes. Figures 1 and 2 show that the presence of extractives had a low impact on the acid hydrolysis yield. The average total sugars content obtained from the extracted wood (biomass treated a mixture of chloroform and 96% ethanol) was higher than that from the raw wood by approximately 1.5%. Higher differences (up to 12%) in the sugars content were observed in earlier studies using a weaker acid (trifluoroacetic acid) on *P. alba* wood (Antczak *et al.* 2014). When a stronger acid (sulfuric acid) was used, the extractives did not have such a large impact on the hydrolysis yield. During enzymatic hydrolysis, the removal of extractives was less important than during acid hydrolysis. The obtained average total sugars contents after enzymatic hydrolysis of the raw and extracted wood were at a similar level (approximately 6%). Additionally, the minor influence of the extractives on the hydrolysis yield was likely because of the low content of these substances in the studied wood (Table 1). Confirmation of this statement can be found in results presented by Li *et al.* (2016), who used as a feedstock the corn stover biomass which was consisted of a high content of extractives. Extractives-free corn stover presented higher cellulose enzymatic digestibility than raw corn stover after the same LHW pretreatment conditions. A total of 87.3% of cellulose was digested in extractives-free corn stover, compared to 71.0% in raw corn stover, after pretreatment at 200 °C for 20 min. Probably, some extractives could condense on corn stover after LHW pretreatment, which hinders cellulose hydrolysis.

Influence of LHW Pretreatment

To enhance the enzymatic hydrolysis efficiency, the LHW pretreatment process was used. The LHW pretreatment process was performed at different temperatures on the raw 5-year-old *P. deltoides* x *maximowiczii* and *P. trichocarpa* wood. The results of the glucose and xylose contents and yields are presented in Fig. 3.

Figures 1, 2, and 3 show that the LHW pretreatment process greatly enhanced the enzymatic hydrolysis efficiency. The improvement in the obtained glucose yield was particularly noticeable. The average glucose yield after enzymatic hydrolysis of the pretreated wood was between 382 to 602 mg/g pretreated biomass and was higher than that from acid hydrolysis. The xylose yield was low (between 18.9 to 74.8 mg/g pretreated biomass) and gradually decreased with the LHW process temperature. Similar observations were found by other researchers (Imman *et al.* 2013, 2018), who studied the influence of LHW pretreatment on enzymatic digestibility of agricultural residues. The raw biomass of corncobs was relatively resistant to enzymatic hydrolysis resulting in a low total sugars yield (41.3 mg/g pretreated biomass). Increasing pretreatment temperature and time led to higher sugars yield from enzymatic hydrolysis of the solid residues. The highest glucose yield of 794.1 mg/g was obtained using LHW pretreatment at 160 °C for 10 min. In a case of pentoses (xylose and arabinose), the yield was between 37.2 and 67.8 mg/g pretreated biomass. Using the data from Tables 1 and 2, it was calculated that up to 91.3% of the hemicelluloses were removed from the solid fraction. This observation was consistent with the literature findings and the most important effect of the LHW process, namely dissolving hemicelluloses (especially xylans) and obtaining a solid fraction that is less contaminated by inhibitors (Kim *et al.* 2009; Lu *et al.* 2012; Li *et al.* 2014, 2017).
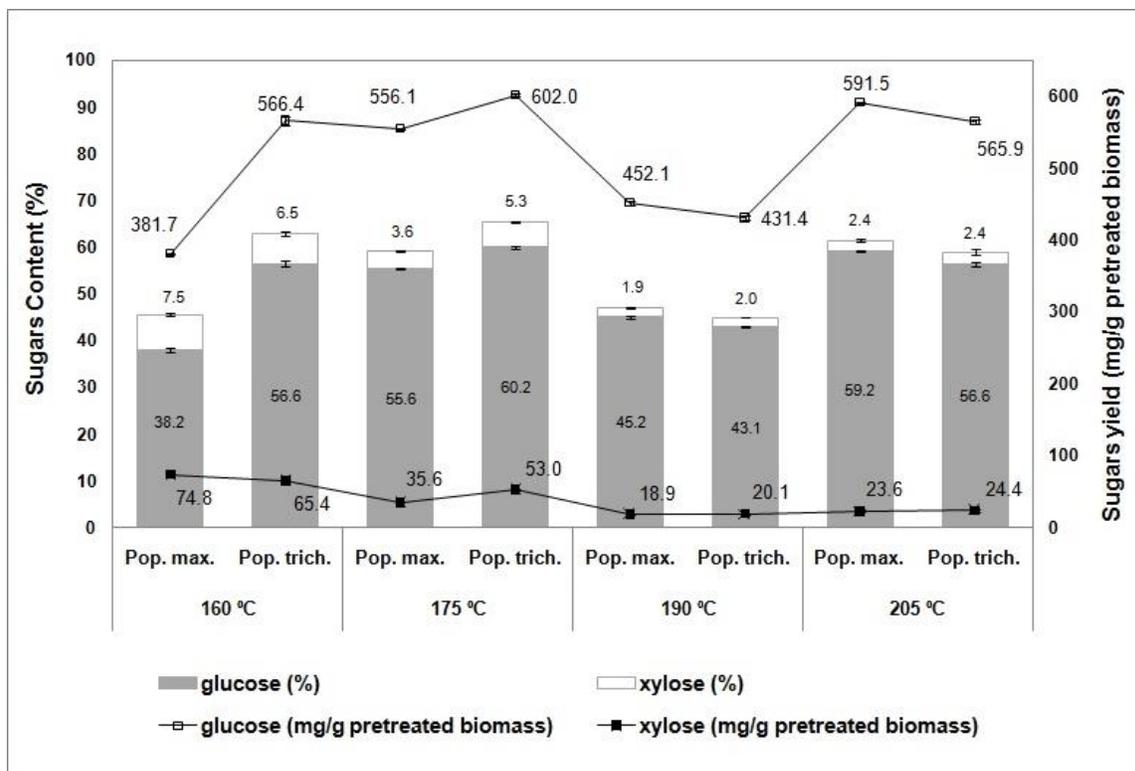
Fig. 3. Sugars contents and yields after enzymatic hydrolysis of the LHW-pretreated 5-year-old *P. deltoides* x *maximowiczii* and *P. trichocarpa* raw woods

Additionally, based on the results that are shown in Fig. 3, it was concluded that the sugars yield depended on the poplar species. The *P. deltoides* x *maximowiczii* wood was more recalcitrant than the *P. trichocarpa* wood. In the case of the *P. trichocarpa* wood, the highest average glucose yield (602.0 mg/g pretreated biomass) was achieved with the wood pretreated at 175 °C. In contrast, for the *P. deltoides* x *maximowiczii* wood, the highest average glucose yield (591.5 mg/g pretreated biomass) was obtained at 205 °C.

The results of the glucose yield for the material that was pretreated at 190 °C were the most surprising (Fig. 3). One of the explanations of the obtained results may be the hypothesis that the drastic decrease in this sugar yield could be probably caused by a condensation reactions of lignin and cellulose. A confirmation of this phenomenon was seen in the results presented in Tables 2 and 3. Tables 2 and 3 show that the Klason lignin content gradually increased with the LHW process temperature. The highest average content of Klason lignin was achieved at 190 °C; likewise, under the same conditions, the average holocellulose and cellulose contents were the lowest. The temperature increase (up to 205 °C) probably caused the breakdown of the lignocellulosic complex, which resulted in a decrease in the Klason lignin content and an increase in the holocellulose and cellulose contents. This facilitated the enzymatic hydrolysis of the cellulose, which resulted in a higher glucose yield. Similar findings were observed in other studies. Lu *et al.* (2016) found that oxidization and condensation reactions occurred with lignin during the LHW pretreatment process. The condensation reactions may have increased the formation of high-molecular-weight lignin, which contributed to the decreased enzymatic hydrolysis efficiency for cellulose.

Table 2. Chemical Composition of the Washed Residual Solid Obtained from the 5-year-old *P. deltoides* x *maximowiczii* Wood after the LHW Pretreatment

Chemical Composition (%)	*P. deltoides* x *maximowiczii*			
	LHW Temperature (°C)			
	160	175	190	205
Extractives (mixture of chloroform and ethanol 93:7 w/w)	10.7 ± 0.1	16.2 ± 0.1	18.2 ± 0.3	20.6 ± 0.1
Acid insoluble lignin* (Klason lignin); TAPPI T222 om-02 (2006)	25.1 ± 0.1	28.6 ± 0.3	37.6 ± 0.4	25.8 ± 0.1
Acid soluble lignin (spectrophotometric method); TAPPI UM 250 (1985)	1.0 ± 0.1	1.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Cellulose* (Kürschner-Hoffer method)	62.5 ± 0.2	68.2 ± 0.4	61.2 ± 0.3	73.4 ± 0.3
Holocellulose* (sodium chlorite method)	72.6 ± 0.5	74.2 ± 0.6	66.5 ± 0.4	77.6 ± 0.1
Hemicelluloses* (calculated as the difference between the holocellulose and cellulose contents)	10.1	6.0	5.3	4.2

* Results should be treated only in an approximate and comparative way, as the methods used did not allow for completely pure substances to be obtained; the errors (±) refer to standard deviations – at least three replications were done to obtain the average values.

Table 3. Chemical Composition of the Washed Residual Solid Obtained from the 5-year-old *P. trichocarpa* Wood after the LHW Pretreatment

Chemical Composition (%)	*P. trichocarpa*			
	LHW Temperature (°C)			
	160	175	190	205
Extractives (mixture of chloroform and ethanol 93:7 w/w)	15.2 ± 0.0	17.4 ± 0.3	20.7 ± 0.2	23.2 ± 0.4
Acid insoluble lignin* (Klason lignin)	20.6 ± 0.1	21.8 ± 0.2	52.7 ± 0.6	29.6 ± 0.1
Acid soluble lignin (spectrophotometric method)	0.7 ± 0.1	0.8 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Cellulose* (Kürschner-Hoffer method)	67.7 ± 0.1	71.1 ± 0.2	48.1 ± 0.1	69.8 ± 0.1
Holocellulose* (sodium chlorite method)	75.4 ± 0.2	77.6 ± 0.2	51.5 ± 0.2	72.7 ± 0.3
Hemicelluloses* (calculated as the difference between the holocellulose and cellulose contents)	7.7	6.5	3.4	2.9

* Results should be treated only in an approximate and comparative way, as the methods used did not allow for completely pure substances to be obtained; the errors (±) refer to standard deviations – at least three replications were done to obtain the average values.

Ko *et al.* (2015) observed that the LHW process caused an increase in the lignin glass transition temperature. This change in the glass transition temperature also suggested that condensation reactions of the lignin molecules occurred during pretreatment, which inhibited the enzymatic hydrolysis process. Another very important factor influencing the efficiency of enzymatic hydrolysis is crystallinity. An increase of crystallinity was reported in various biomasses pretreated by LHW (Imman *et al.* 2013, 2015, 2018). A removal of the amorphous xylan and some lignin fractions causes the increase of biomass crystallinity, which may hinder enzymatic hydrolysis. Similar problems related to hydrolysis was reported by Gawron *et al.* (2014). In these studies ash wood (*Fraxinus excelsior* L.) was subjected to thermal modification at 190 °C under nitrogen. The increase of crystallinity was observed and caused incomplete acid hydrolysis of thermally modified ash wood.

CONCLUSIONS

1. The acid hydrolysis turned out to be a good method to verify the biomass potential for bioethanol production. After acid hydrolysis of raw and extracted biomass the high total sugars yields were obtained (up to 808.5 mg/g raw or extracted biomass), while in the case of enzymatic hydrolysis the total sugars yields were very low (up to 68.9 mg/g raw or extracted biomass).

2. The LHW pretreatment greatly enhanced the enzymatic hydrolysis of fast-growing poplar wood species. The average glucose yield from enzymatic hydrolysis was up to 602.0 mg/g pretreated biomass and was higher than that obtained from acid hydrolysis.

3. Based on the obtained glucose and xylose results, it was concluded that *P. trichocarpa* wood has a higher potential for bioethanol production than *P. deltoides* x *maximowiczii* wood.

4. The results show that the presence of extractives in raw poplar wood (up to 2.8%) had a low impact on the acid and enzymatic hydrolysis yields. This was likely caused by the low content of these substances in the studied wood.

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