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THE COMPARISON OF ACID AND ENZYMATIC HYDROLYSIS OF PULP OBTAINED FROM POPLAR WOOD (*POPULUS* SP.) BY THE KRAFT METHOD

This paper compares the acid and enzymatic hydrolysis in relation to bioethanol production. The pulp obtained from wood of the fast-growing poplar species (Populus deltoides x maximowiczii and Populus trichocarpa Torr. & A. Gray ex Hook) was used as a feedstock. The delignification process by the Kraft method was carried out with 19% and 26% of active alkali (NaOH and Na_2S). The obtained sugars (xylose and glucose) were analyzed by a high-performance liquid chromatography. The results concluded that the enzymatic hydrolysis process is better than acid hydrolysis because a higher content of sugars (especially xylose) was obtained. Additionally, after the acid and enzymatic hydrolysis process from Populus trichocarpa pulp, more sugars were obtained than from the pulp of Populus deltoides × maximowiczii. The Kraft pulp from the wood of fast-growing poplar species is a good raw material for the production of glucose. However, in order to obtain a higher xylose content and finally increase the profitability of bioethanol production, a new method should be developed, or the Kraft method should be optimized.

Keywords: acid and enzymatic hydrolysis, Kraft pulp, poplar, bioethanol

Introduction

The topic related to renewable energy is currently of great interest [Fay and Golomb 2002; Drapcho et al. 2008; Stolarski et al. 2013; Krzyżaniak et al. 2014]. Bioethanol is an example of biofuel that can be produced from renewable sources such as sugar cane, maize or wood. The bioethanol made from feedstock that can be also consumed by humans (so-called first-generation bioethanol) is characterized by a low reduction carbon dioxide emission during its production

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and processing. This kind of production results in a shortage of agricultural land, which in turn leads to a rise in food prices. An alternative solution which avoids those disadvantages is the bioethanol production from lignocellulosic biomass (so-called second-generation bioethanol) obtained from fast-growing plantations of willow or poplar and residual vegetable mass, mainly stover. These specified raw materials fulfil the requirements of the sustainable development and greenhouse gas emission limitations recommended by Directive 2009/28/EC [Kupczyk et al. 2013].

The processing of lignocellulosic biomass to bioethanol consists of several stages: material pre-treatment, enzymatic hydrolysis and fermentation. The material pre-treatment and hydrolysis of polysaccharides are the most important steps because the yield of the resulting ethanol and the profitability of the process depends on them [Zhang and Lynd 2004; Dyk and Pletschke 2012]. The methods of biomass pre-treatment may be divided into different types: physical, thermochemical and biological [Sun and Cheng 2002; Kumar et al. 2009; Zheng et al. 2009]. In this paper, pre-treatment of lignocellulosic material with various concentrations of alkali reagents (primarily NaOH and Na_2S), known as the Kraft method, was performed. As a result of this treatment, the disintegration of ester and glycosidic bonds in lignin-carbohydrate complexes (LCC) occurs. This leads to changes in the lignin structure and to a large extent its subsequent dissolution, and a decrease in the degree of cellulose crystallinity as well as partial hemicelluloses dissolution [Singh and Trivedi 2013; Zawadzki et al. 2016]. After pre-treatment, the enzymatic hydrolysis of the lignocellulosic material is carried out. The main advantages of enzymatic hydrolysis are high process efficiency, no substrate loss due to chemical modification, the application of mild and non-corrosive conditions (lower reaction temperature, less acid pH) along with the use of biodegradable, nontoxic reagents. Unfortunately, a very significant disadvantage of the enzymatic hydrolysis is the high cost of the enzymes. Nowadays, a lot of studies aiming to reduce those expenses have been carried out [Samdhu and Bawa 1992; Taherzadeh and Karimi 2007b]. Furthermore, the acid hydrolysis is generally performed with the use of diluted or concentrated inorganic acids, such as sulfuric, hydrochloric and orthophosphoric acid, but organic ones are also used, for example, trifluoroacetic acid (TFA) [Kačik and Solár 1999; Antczak et al. 2012].

The application of concentrated acid during processing allows a high content of sugar to be obtained, reaching up to 90% of the theoretical glucose yield, which results in a significantly larger amount of final ethanol production compared to using diluted acid. Nevertheless, the use of concentrated acid while performing hydrolysis leads to intense metal reactors corrosion, which makes it necessary to work with expensive special alloys or ceramic materials. Furthermore, the neutralization and regeneration of acids used for the hydrolysis reaction requires a high energy consumption, which leads to a decrease in the cost-effectiveness of the process [Taherzadeh and Karimi 2007a].

Conversely, the application of diluted acid is one of the most popular hydrolysis methods. This method can be generally used not only as an actual hydrolysis procedure, but also as a part of the pre-treatment process before enzymatic hydrolysis. It is distinguished by a low acid consumption and a short reaction time. However, the high temperature of the reaction and the large number of by-products are significant disadvantages [Taherzadeh and Karimi 2007a].

In the available literature, there are publications on the use of the Kraft method as a pre-treatment process before acid or enzymatic hydrolysis [Wu et al. 2014; Buzała et al. 2015; Palme et al. 2016; Przybysz-Buzała et al. 2016]. However, it is difficult to find information on studies, in which acid and enzymatic hydrolysis were compared, where sulfate pulp obtained from the fast-growing species of poplars were used as a feedstock. Therefore, from scientific and practical point of view, such a comparison is interesting and worthy of attention.

The aim of the studies was to verify the potential of pulp obtained from the fast-growing poplar species by the Kraft method (as a potential pre-treatment method) in relation to bioethanol production. To achieve this verification, acid and enzymatic hydrolysis were compared.

Materials and methods

The studies were carried out on the pulp obtained from wood of the fast-growing poplar species (*Populus deltoides* × *maximowiczii* and *Populus trichocarpa* Torr. & A. Gray ex Hook). The poplar feedstock was harvested from experimental fields in Wolica owned by the Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture and Landscape Architecture, Warsaw University of Life Sciences. The age of the trees was 2.5; 3 and 5 years. The debarked wood was used for further studies. The chemical composition of the studied poplar wood species is presented in table 1.

The delignification process by the Kraft method was made in cooperation with the Institute of Papermaking and Printing in Lodz. Poplar wood chips (particle size of 1.2-1.6 mm) were prepared by drilling larger pieces of poplar wood using an electric 6388AA Skil drill (Lowe's, USA) equipped with a Metabo drill (Poland) no 10 (10.0 mm in diameter). Then, the wood chips were subjected to a pre-treatment process under the following conditions:

1. the share of active alkali: NaOH (Chempur, Poland) and Na_2S (Chempur, Poland) was 19% and 26% respectively
2. sulfidity: 30%
3. module of liquid: 4
4. the maximum temperature of pulping: 160°C
5. time to achieve the maximum temperature: 2 h
6. pulping time in the maximum temperature: 2 h

7. cooling time of pulp to 40°C: below 20 min
8. cooling time of pulp to 25°C: below 30 min

Table 1. The chemical composition of studied poplar wood

Chemical composition [%]	*Populus deltoides* × *maximowiczii*			*Populus trichocarpa*		
	2.5 years	3 years	5 years	2.5 years	3 years	5 years
Extractives (in mixture of chloroform and ethanol 93:7 w/w)*	2.6 ±0.2	2.1 ±0.3	2.3 ±0.2	1.7 ±0.3	1.2 ±0.2	1.5 ±0.3
Lignin (acetyl bromide method)**	19.7 ±0.7	19.1 ±0.3	20.7 ±0.6	19.9 ±0.5	20.0 ±0.6	20.1 ±0.5
Cellulose (Kürschner and Hoffer method)***	51.2 ±0.4	51.7 ±0.5	51.6 ±0.3	52.0 ±0.4	53.0 ±0.3	53.0 ±0.2
Holocellulose (sodium chlorite method)****	82.1 ±0.3	82.0 ±0.1	83.5 ±0.1	85.4 ±0.2	85.7 ±0.2	86.4 ±0.5
Hemicelluloses (calculated from the difference in content of holocellulose and cellulose)	30.9	30.3	31.9	33.4	32.7	33.4

*Antczak et al. [2006].
**Johnson et al. [1961]; Iiyama and Wallis [1988].
***Saeman et al. [1954].
****Wise et al. [1946].

After the alkaline pre-treatment process, the material was subjected to a diffusion wash for a minimum of 12 h. Then, it was defibrated for 3 min in a laboratory propeller pulp defibrator (type R1 from Labor-meks, Poland), and the fibres were collected by centrifuging (300 rpm, 10 min). After the refining process, the pulp was subjected to washing and sorting to remove undigested elements. The characteristics of the studied pulp in relation to lignin content is presented in table 2.

The pulp was protected with 2% LiCl (Sigma-Aldrich, Germany) to prevent the growth of microorganisms. The material was stored in a refrigerator at about 6°C. The pulp, before the hydrolysis, was washed with distilled water (10 times with about 500 cm^3) on a Büchner funnel to remove lithium chloride. Then, after thorough pressing and filtration, the material was divided into two parts. The first part was pre-dried at 45°C to carry out acid hydrolysis. The pre-drying time was 3 days. After pre-drying, the pulp was ground to a form of sawdust (a fraction below 0.43 mm). Prior to the acid hydrolysis process, the material was dried to a constant weight in a vacuum drier at 60°C and under pressure of 0.4 kPa. Whereas in the case of the second part of the studied pulp, the samples were taken to determine the moisture content, and the remaining part was used to carry out enzymatic hydrolysis.

Table 2. The characteristic of pulp in relation to lignin content

Poplar species used to obtain pulp	Age [years]	Active alkali [%]	Lignin content [%]	
			Kappa number method*	Acetyl bromide method**
Populus trichocarpa	2.5	19	4.4	5.8 ±0.2
		26	2.4	5.2 ±0.4
Populus deltoides × *maximowiczii*	2.5	19	4.4	5.8 ±0.4
		26	2.7	4.2 ±0.3
Populus trichocarpa	3	19	8.5	10.4 ±0.3
		26	3.7	5.4 ±0.4
Populus deltoides × *maximowiczii*	3	19	9.0	11.2 ±0.7
		26	3.8	5.9 ±0.3
Populus trichocarpa	5	19	–	9.3 ±0.1
		26	–	5.6 ±0.2
Populus deltoides × *maximowiczii*	5	19	–	10.3 ±0.4
		26	–	5.6 ±0.4

*Dence [1992]; ISO 302 [2015].
**Marton [1967]; Iiyama and Wallis [1988].

Acid hydrolysis process

The acid hydrolysis of the pulp was made using trifluoroacetic acid (TFA) (Sigma-Aldrich, Germany) with an initial concentration of 99%. The method was described in detail by Kačik and Solár [1999] and later refined and adapted to the material of poplar wood by Antczak et al. [2014]. The TFA method was selected because of several important advantages, namely the high yield of simple sugars and the ability to shorten the hydrolysis process by evaporation of volatile TFA. The hydrolysis procedure using TFA was as follows: approximately 100 mg of poplar pulp was placed in a pear-shaped flask, with a volume of 50 cm^3, to which 4 cm^3 of 99%, trifluoroacetic acid was added. Then, the flask was stoppered and left for 24 h at room temperature. After this time the mixture was heated under reflux for 1 h in an oil bath at 120°C. Subsequently, the sample was cooled to room temperature and diluted to a concentration of 80% TFA by the addition of 1.5 cm^3 of distilled water. Then, the sample was further heated for 15 minutes. Finally, the sample was diluted to 30% TFA by adding 12 cm^3 of distilled water and the mixture was further heated for 1 h. After heating in the oil bath, the sample was filtered through a Schott G3 filter. During this time, the flask and filter were washed with 8 cm^3 of distilled

water (twice). The collected filtrate was quantitatively transferred to a pear--shaped flask with a volume of 100 cm^3. Then 1 cm^3 of 0.05 M sodium azide solution (Chempur, Poland) was added. The obtained hydrolyzate was subjected to a concentration process using a vacuum evaporator (Rotavapor R-215, Büchi, Switzerland) to remove acid and water. The precipitated sugars were dissolved in 10 cm^3 of distilled water and again evaporated to dryness. The operation was repeated twice. After the removal of the acid, the precipitated sugars were dissolved with distilled water and transferred to a 10 cm^3 volumetric flask. After the process, the sugar content (glucose and xylose) determination in the supernatant was done by HPLC (a high-performance liquid chromatography) method. All acid hydrolysis tests were done in fourfold and single standard deviations were calculated.

Enzymatic hydrolysis process

The enzymatic hydrolysis of the pulp was made on never dried, wet material. The wood was in an air-dry state (material without alkaline pre-treatment; milled totally to a fraction below 0.43 mm) and was also subjected to enzymatic hydrolysis. At first, the samples were weighed in sealed screw-capped test tubes with a volume of 10 cm^3 and the cellulose concentration was 1% w/w. Then, a 5 cm^3 of 0.1 M citrate buffer solution (Chempur, Poland) at pH = 4.8 was added to each sample. Next, 0.1 cm^3 of a 2% solution of sodium azide (Chempur, Poland) was added to each sample in order to prevent the growth of microorganisms during the hydrolysis. Subsequently, the addition of distilled water was calculated, so that the total volume of the solution was 10 cm^3. The distilled water was added to each sample prior to the addition of the enzyme. Finally, a 0.333 cm^3 of 25% v/v solution of Cellic CTec2 enzyme (the mixture of cellulases, β-glucosidases and hemicellulases) (Novozymes, Denmark) was added to each sample (0.1 g of enzyme per 0.1 g of cellulose). The test tubes were thoroughly sealed and samples were hydrolyzed over 72 h using a mixer (RM-2M, Elmi, USA) with a rotation of 25 rpm placed in the laboratory drier at 50°C. After the process, the collected samples were stored in a freezer at -20°C. The sugar content (glucose and xylose) determination in the supernatant was done by the HPLC method. All enzymatic hydrolysis tests were done in triplicate and single standard deviations were calculated.

Conditions of HPLC analysis

Before the chromatographic analysis, the samples were thawed and brought to room temperature. Subsequently, the enzyme was denatured by heating the samples for 15 min at 95°C in a water bath. In the next step, the samples were centrifuged for 10 min on a laboratory centrifuge at 12 000 rpm. Finally, each sample was filtered using a nylon syringe filter with a porosity of 0.2 μm.

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

– acetonitrile – water (80:20) $_v$ as the eluent,
– column – Genore Cosmosil Sugar-D (250 × 4.60 mm, Nacalai Tesque, Japan) connected with a guard column,
– oven temperature: 50 °C,
– flow rate: 2 cm^3/min,
– injection volume: 20 μL.

Based on the previously developed calibration curves [Antczak et al. 2014], under the conditions described, the content of sugar (glucose and xylose) in the hydrolyzates was determined. The developed equations of calibration curves for Genore Cosmosil Sugar-D column were as follows:

$$y = 689336\,x;\ R^2 = 0.9997 \text{ (xylose)} \quad (1)$$

$$y = 791397\,x;\ R^2 = 0.9995 \text{ (glucose)} \quad (2)$$

In the case of samples after enzymatic hydrolysis the conditions of HPLC analysis were as follows:

– redistilled water as the eluent,
– column – RHM-Monosaccharide (300 × 7.80 mm, Rezex, USA) connected with a guard column,
– oven temperature: 80°C,
– flow rate: 0.6 cm^3/min,
– injection volume: 20 μL.

Also, in these cases, based on the developed calibration curves, under the conditions described, the content of sugar (glucose and xylose) in the hydrolyzates was determined. The developed equations of calibration curves for RHM-Monosaccharide column were as follows:

$$y = 2911325\,x;\ R^2 = 0.9990 \text{ (xylose)} \quad (3)$$

$$y = 2838182\,x;\ R^2 = 0.9998 \text{ (glucose)} \quad (4)$$

Results and discussion

The results of the main sugar content (glucose and xylose) after acid and enzymatic hydrolysis obtained from fast-growing poplar species are presented in figures 1-4. In the case of enzymatic hydrolysis, the process was carried out on

wood (material without alkaline pre-treatment) and pulp (material after the Kraft method with 19% and 26% of active alkali share).

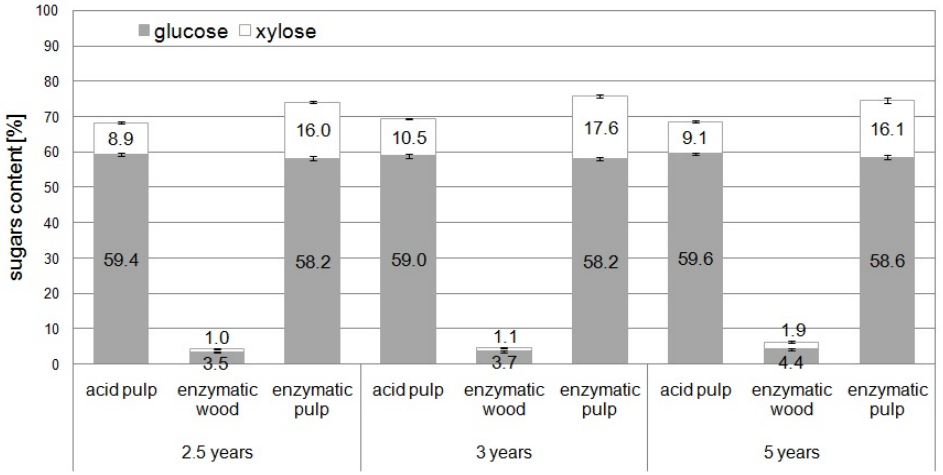

Fig. 1. The sugar content (glucose and xylose) after enzymatic hydrolysis of wood (before alkaline pre-treatment) and after acid and enzymatic hydrolysis of pulp obtained from *Populus deltoides* × *maximowiczii* with 19% of active alkali share

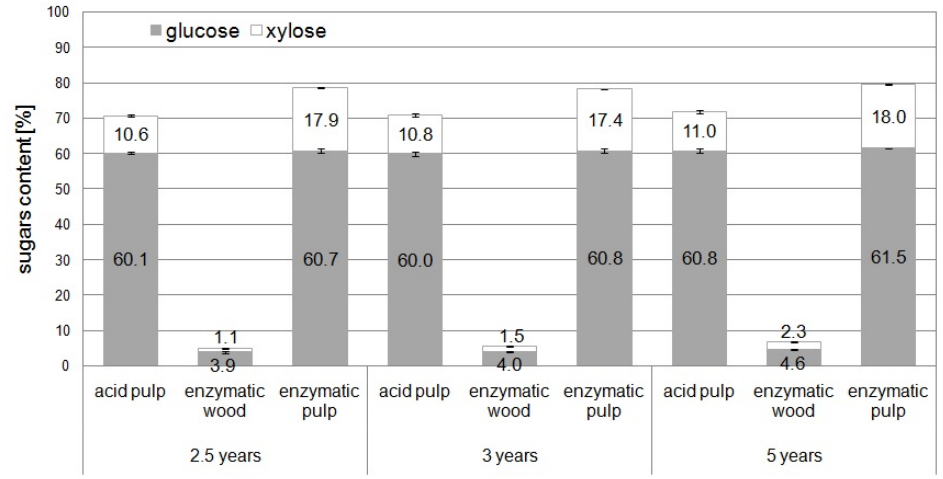

Fig. 2. The sugar content (glucose and xylose) after enzymatic hydrolysis of wood (before alkaline pre-treatment) and after acid and enzymatic hydrolysis of pulp obtained from *Populus trichocarpa* with 19% of active alkali share

The average glucose content after enzymatic hydrolysis of wood without pre-treatment was between 3.5% to 4.6% and for xylose between 1.0% to 2.3% only. In turn, the average content of sugar after the enzymatic hydrolysis of pulp obtained during alkaline pre-treatment was much higher (for glucose between 58.2% to 71.9% and for xylose between 15.8% to 18.8%). Comparing the results, it may be observed that the pre-treatment process significantly increased the average contents of glucose and xylose obtained after enzymatic hydrolysis. Additionally, the results proved, that the enzymatic hydrolysis process after alkaline pre-treatment was even better than acid hydrolysis because especially for xylose, its content after enzymatic hydrolysis was over 1.5 times higher than after acid hydrolysis. Certainly, the main reason, in this case, is the greater susceptibility of xylan than cellulose to hydrolysis in concentrated acid, which may further result in a greater exposure of formed xylose to degradation in acid. The same relationships were observed in other studies [Wyman et al. 2005; Antczak et al. 2014; Gawron et al. 2014]. Hence, enzymatic hydrolysis was a more efficient process especially with regards to xylose. The average xylose content, regardless of the active alkali share, after acid hydrolysis was between 8.0% to 11.9% (figs. 1-4). On the other hand, after enzymatic hydrolysis, the average xylose content was higher (by approximately 7%) and was between 15.8% to 18.8% (figs. 1-4). However, based on the average hemicelluloses content in studied poplar wood (above 30%; presented in table 1) and literature information that poplar wood contains up to 23% of glucuronoxylan [Willför et al. 2005], it can be assumed that the xylose content was still underestimated. Probably, the main reason, in this case, is the strongly alkaline environment in the Kraft method. It is known that among all structural components, the hemicelluloses are the most susceptible to alkali [Fengel and Wegener 2003]. Hence, because of the alkaline pre-treatment, before the hydrolysis process, they most likely were dissolved. So, to receive an even higher xylose content and finally higher yields of bioethanol, a better method should be developed.

In the case of glucose, the average contents after acid and enzymatic hydrolysis were at a similar level and depended mainly on an active alkali share. For 19% of the active alkali, the average content of glucose was between 58.2% to 61.5% (figs. 1 and 2). However, for 26% of active alkali share the average content of glucose was much higher (by approximately 8%) and was between 64.3% to 71.9% (figs. 3 and 4). In this case, the difference in the content of glucose may be connected with the delignification degree of the studied pulp, for which the lignin content is presented in table 2. If we compare the results shown in table 2, we can see that the use of 26% of active alkali share, results in a greater removal of lignin from wood than for a 19% share. This is especially important for older wood, in which the lignin is more crosslinked and has a higher molar mass [Fengel and Wegener 2003]. Hence, with the use of more dilute alkaline solutions the delignification process may be more difficult and be less efficient. Similar results were obtained by other researchers [Buzała et al.

2015], who also used the Kraft method as a pre-treatment. After the enzymatic hydrolysis process, the hydrolysates were obtained, which mainly consisted of glucose.

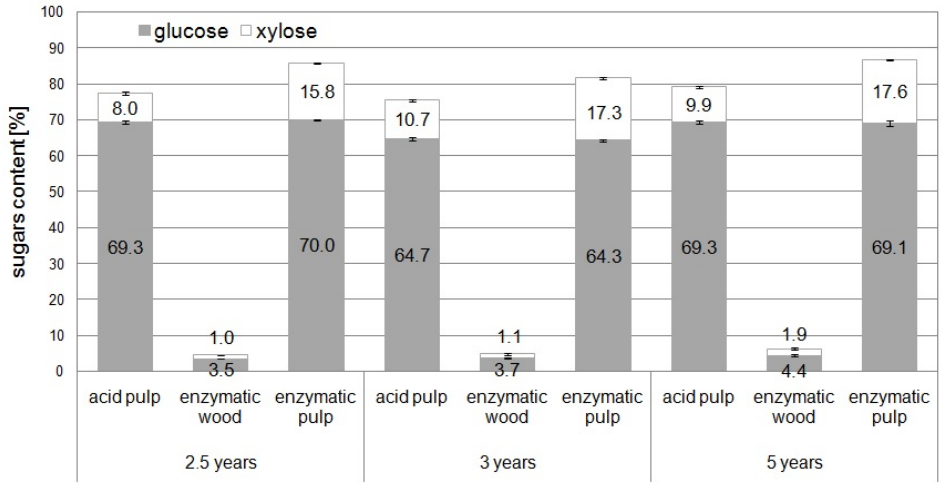

Fig. 3. The sugar content (glucose and xylose) after enzymatic hydrolysis of wood (before alkaline pre-treatment) and after acid and enzymatic hydrolysis of pulp obtained from *Populus deltoides* × *maximowiczii* with 26% of active alkali share

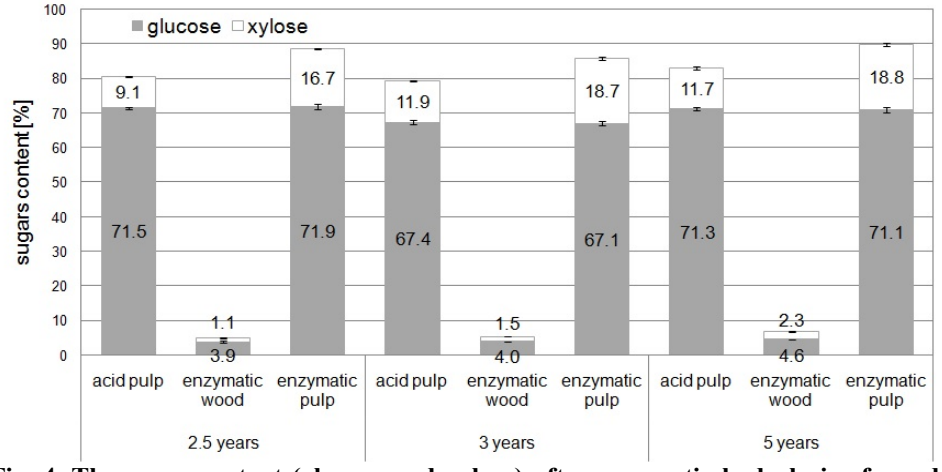

Fig. 4. The sugar content (glucose and xylose) after enzymatic hydrolysis of wood (before alkaline pre-treatment) and after acid and enzymatic hydrolysis of pulp obtained from *Populus trichocarpa* with 26% of active alkali share

Moreover, if we compare the sum of glucose and xylose content in figures 1-4, we can observe that after the acid and enzymatic hydrolysis process from *Populus trichocarpa* pulp more sugar (by approximately a few percent) were obtained than from pulp of *Populus deltoides* × *maximowiczii*. Similar findings were presented in earlier studies that focused on the acid hydrolysis of wood, bark and leaves of the previously discussed species of poplar [Antczak et al. 2016]. These differences are because the *Populus trichocarpa* wood was made up of more polysaccharides (cellulose: 52.0-53.0% and hemicelluloses: 32.7--33.4%) content than *Populus deltoides* × *maximowiczii* (cellulose: 51.2-51.7% and hemicelluloses: 30.3-31.9%) (table 1).

Finally, if we compare the sum of glucose and xylose content in figures 1-4, we can observe the slight impact of tree age on sugar content produced during the hydrolysis process. Generally, the highest sugar content was obtained during the hydrolysis process of pulp produced from the oldest poplar samples (5 years old). Surely, this relationship is also connected with the polysaccharides content in wood of the studied poplar species (table 1). Based on the results presented in table 1, we can see that the content of holocellulose (sum of cellulose and hemicelluloses) in wood increased with the age of poplar trees. The average holocellulose content in 2.5 year-old *Populus deltoides* × *maximowiczii* was 82.1%, whereas in the 5 year-old samples it was 83.5%. In turn, the average holocellulose content in 2.5 year-old *Populus trichocarpa* was even higher and was determined at a level of 85.4%. In the case of 5 year-old *Populus trichocarpa,* the average holocellulose content was the highest and amounted to 86.4%.

Conclusions

In this paper, acid and enzymatic hydrolysis of biomass from the fast-growing poplar species were compared. Based on the experiments performed, the following conclusions were drawn:

1. The Kraft method turned out to be a good pre-treatment method in relation to glucose production. However, in order to receive a higher xylose content and finally increase the profitability of the bioethanol production, a new method should be developed, or the Kraft method should be optimized.
2. The results proved that the enzymatic hydrolysis process after the Kraft method was better than acid hydrolysis, because of the higher content of sugar (especially xylose) that was obtained.
3. After the acid and enzymatic hydrolysis process from *Populus trichocarpa* pulp, more sugars were obtained than from the pulp of *Populus deltoides* × *maximowiczii*.

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List of standards

ISO 302:2015 Pulps - Determination of Kappa number

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