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Influence of acid concentration and time of the hydrolysis of birch and spruce wood on the content of reducing sugars

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Abstract: *Influence of acid concentration and time of the hydrolysis of birch and spruce wood on the content of reducing sugars.* The paper presents results of the study the effect of concentration of sulfuric (VI) acid and time of hydrolysis process on the effectiveness of this process determined by the share of reducing sugars in hydrolytic solutions. One species of hardwood (*Betula verrucosa Ehrh*) and one species of softwood (*Piea abies* L. *Karst*) were selected. Chemical composition of both types of wood were analysed. The hydrolysis process was carried out under various conditions using variable parameters: sulfuric acid - from 0.8% to 72, time - from 4h to 7h. As an outcome describing the hydrolysis process, the following calculations and analyses were adopted: the weight loss and the content of reducing sugars (Bertrand method and HPLC analysis). Considering the weight loss, content of reducing sugars and glucose concentration in the obtained hydrolysates, the conducted research shows that deciduous wood gets destroyed more easily than softwood, using all variants of hydrolysis conditions.

Keywords: biomass, acid hydrolysis, birch, spruce, sugars

INTRODUCTION

The depletion of stocks that are primarily fossil raw materials caused significant increase of the interest in natural resources of plant origin. Research conducted for many years has shown that plant tissues, and in particular woody biomass, can be a rich source of various chemical substances. Amongst many directions of chemical processing of wood, the saccharification process of biomass to obtain simple sugars – semi-products for the production of bioethanol [Mousdale 2010] and other substances (methanol, furfural, hydroxymethylfurfural), constituting components of liquid biofuels [Eseyin and Steele 2015, Addepally and Thulluri 2015], deserve attention.

A number of chemical compounds undergo hydrolysis processes, and in the case of plants, primarily polysaccharides. The products of this process are monosaccharides, such as: glucose, mannose, galactose, xylose, arabinose and others. In addition, in hydrolysates we identify: glucuronic acids, furfural, humic compounds and others. Biomass hydrolysis may lead to partial or complete degradation of polysaccharides, depending on the process conditions [Prosiński et al. 1962]. The polysaccharides that make up the cell wall generally do not hydrolyse in cold water. The hydrolysis process can be slightly accelerated with increase of the temperature. Satisfactory results of these reactions can be obtained using some catalysts, for example concentrated or diluted acids (organic and inorganic). The hydrolysis of certain polysaccharides can also be carried out without catalysts at raised temperatures and pressures.

Due to global warming, the European Parliament and the Council of Europe approved documents aimed at limiting the adverse effects in environment. The milestone of European legislation related to bioenergy was published in 2009 as "climate&energy package", consisting of six legal acts, two of which [Regulation No 443/2009/EC, Directive 2009/30/EC] refer to problems concerning the use of transport biofuels and the other four concern issues related to energy production [Decision No 406/2009/EC, Directive 2009/28/EC, Directive 2009/29/EC, Directive 2009/31/EC]. According to the analysis of the documents, the issue of rational use of energy, reduction of greenhouse gases related to its production and extensive use of renewable energy carriers constitute the basic assumptions of the climate and energy policy of the EU countries. On this basis, there is a strong interest in obtaining various types of solid biofuels, liquid biofuels of the first and second generation, components of liquid biofuels and semi-products used to obtain them from lignocellulosic materials. There are many publications on this subject in the literature. Nevertheless, there is no detailed information in this field relating to domestic species of wood and the requirements of biochemical processes used to obtain components of liquid biofuels.

The aim of the work was to study the effect of the concentration of mineral acid and the time of the hydrolysis process of selected species of deciduous and coniferous wood in laboratory conditions on the effectiveness of this process determined by the share of reducing sugars in hydrolytic solutions.

MATERIALS AND METHODS

One species of hardwood (birch) and one species of softwood (spruce) were selected for the study. Samples were collected from Puszcza Zielonka forest located in the Greater Poland Voivodeship from 60 and 90-year old birch trees (*Betula verrucosa* Ehrh.), and 100-year-old spruce (*Picea abies* L. *Karst*).

The chemical composition of raw materials was determined based on the classical research methods (Browning 1967, Prosiński 1984, PN 92/P-50092) and the pH was determined using the Gray method [Gray 1958]. The acid hydrolysis of wood was carried out in laboratory conditions. Based on the literature [Prosiński 1984, Fengel and Wegener 1984, Springer and Harris 1982, Harris et al. 1984, Mosier et al. 2005, Kumar et al. 2009] the following parameters were determined as a constant: type of mineral acid - sulfuric(VI) acid, hydromodule 1/10 (w/v), temperature (boiling point), atmospheric pressure. Next, the variable parameters were determined: sulfuric(VI) acid (0,8% - 72% (w/w) for birch wood and 1% - 72% for spruce), time of the process (from 4 h to 7 h).

As an outcome describing the hydrolysis process, the following calculations and analyzes were adopted:

- the weight loss of the analyzed materials in relation to the original mass of samples,
- the content of reducing sugars in hydrolysates determined according to the Bertrand method,
- the content of monosaccharides in hydrolysates determined quantitatively on the basis of chromatographic analysis (HPLC).

Each hydrolytic mixture (wood material and sulfuric(VI) acid solution) was placed in a flat bottom flask equipped with a reflux condenser and a magnetic stirrer with heating. After the end of hydrolysis the reaction mixture was filtered under reduced pressure.

To calculate the weight loss of the tested materials the solid residue was neutralized by water and dried to a constant mass at 105±2°C. The hydrolysate solution was neutralized by 5% sodium bicarbonate and analyzed for the content of reducing sugars according to the Bertrand method, and for monosaccharides by liquid chromatography (HPLC).

The concentration of reducing sugars in hydrolysates was determined by the Bertrand method, which consists of the oxidation of the hydrolysate with an alkaline copper solution (Fehling's reagents: copper (II) sulfate pentahydrate, potassium sodium tartrate in a sodium hydroxide solution).The resulting copper solution was reduced to obtain a cuprous oxide precipitate, which was further dissolved in a solution of iron(III) sulfate and sulfuric(VI) acid. The iron(II) sulfate(VI) obtained as a result of this reaction was oxidized with a potassium permanganate solution in an acidic medium. The volume of titrant allowed to calculate the amount of reduced copper. The quantitative content of reducing sugars in analyzed hydrolysates was calculated using data's from the table of the dependence of copper and glucose.

The analysis of monosaccharides content (glucose, cellobiose) was performed using HPLC technique with a high performance Waters 2695 liquid chromatograph which included the 2414 Refractive Index detector [RI - Waters, Milford, MA, USA]. The results were developed using the Empower ™ 1 software. For the analysis of the obtained hydrolysates a dedicated for the sugars separations Bio-Rad Aminex HPX-87H (USA) column was used.

RESULTS

In the first stage of the research, the chemical composition of selected wood raw materials was determined, which is summarized in Table 1. According to literature [Prosiński 1984], percentage chemical composition of wood depends on the tree species, it's age, habitat conditions or period of harvesting. These differences were confirmed in the analyzes of raw materials used in this work.

Table 1. Chemical composition of birch and spruce wood.

Determination	Unit	Birch wood	Spruce wood
Moisture	[$\%_d$]	6.56	8.55
Solubility in cold water	[$\%_d$]	1.08	0.73
Solubility in hot water	[$\%_d$]	2.22	2.42
Solubility in 1% NaOH	[$\%_d$]	15.79	12.89
Solubility in EtOH	[$\%_d$]	1.76	1.27
Pentosans	[$\%_d$]	25.45	9.58
Seifert cellulose	[$\%_d$]	41.04	47.96
Lignin insoluble in H_2SO_4	[$\%_d$]	18.47	25.65
Lignin soluble in H_2SO_4	[$\%_d$]	7.49	0.06
Lignin	[$\%_d$]	25.96	25.71
pH	-	4.45	4.58

d - dry

Acids are among the most destructive substances in wood tissue. According to literature [Prosiński 1984] in the first phase of the reaction of acid with wood, hemicelluloses transform into pentoses and hexoses, followed by furfural and organic acids, mainly formic and acetic acid. As the hydrolysis conditions progress, mainly by increasing the acid concentration or the process temperature, hemicelluloses and cellulose are further degraded.

This is confirmed by the results of calculations of weight loss in the analyzed samples (Fig. 1). The highest weight losses concerned wood materials treated with 72% sulfuric(VI) acid during 4-hour hydrolysis, where for birch wood the weight loss was 83,2% and for spruce – 76,6%. The lowest losses were calculated for the lowest concentrations - in the case of birch wood in the hydrolysis with 1% H_2SO_4 the weight loss was 26,5%, while in the reaction with spruce wood – 16,9%.

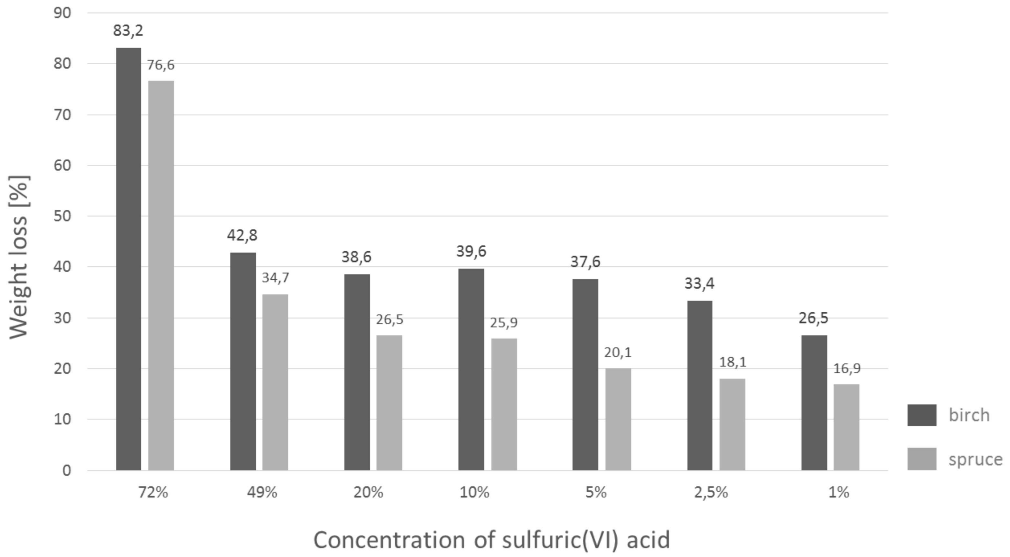

Figure 1. The effect of sulfuric(VI) acid concentration on the value of weight loss during 4-hour hydrolysis of birch wood and spruce wood.

Softwood is more acid resistant than hardwood. It is related to the chemical structure of coniferous wood, which has a smaller amount of hemicelluloses than deciduous, and with the resin content in coniferous wood [Prosiński 1984, Fengel 1984]. This dependence was confirmed by the hydrolysis tests carried out under the same conditions for both wood species (Fig. 1). Comparing the calculated weight losses of the two species, for each of the analyzed concentration of the acid used, birch wood have from a few to several percent more weight loss than spruce.

The effect of concentrated acids on polysaccharides varies depending on the polysaccharide structure, the type of acid used, as well as on its concentration and process temperature. When the concentration of acids increases, their hydrolysis effect increases gradually, causing swelling of the polysaccharides, and then their dissolution. A characteristic feature of the hydrolysis process carried out in the concentrated inorganic acids environment is, that it occurs at a deficiency of water. According to Prosiński et al. [1962], under these conditions cellulose first hydrolysate to glucose, which under the influence of acid, as a dehydrating agent, is again transformed into polysaccharide - a product of reverse (glucan), with a structure different from the starting cellulose. The reversible effect of acid increases with increasing its concentration, as well as with the concentration of monosaccharide [Prosiński et al. 1962].

The final aim of the wood hydrolysis process is to obtain monosaccharides. Therefore the hydrolysis process with concentrated acids is divided into two stages. The first stage is the formation of secondary polysaccharides in concentrated acids (reversion). The second is their decomposition into monosaccharides by diluting the solution with a significant amount of water and heating to 100-150°C (inversion).

The described above mechanism of hydrolysis, carried out in the environment of concentrated inorganic acids has been confirmed in the study of this work. As concentrated inorganic acid the 72% and 49% H_2SO_4 was used. It was planned to carry out the hydrolysis process at room temperature for one hour in concentrated sulfuric(VI) acid. Then followed the process by 14-fold dilution of the hydrolysis solution, and held it at boiling point for 4 hours. Figures 2 and 3 present the results of determinations of weight loss and the content of reducing sugars under the assumed conditions.

In the case of using 72% H_2SO_4, the weight loss of birch wood was 83,2% (Fig. 2) and the content of reducing sugars 4,5% (Fig. 3). The use of 49% H_2SO_4 resulted in a weight loss of 42,8% (Fig. 2). The concentration of reducing sugars was 1,9% (Fig. 3).

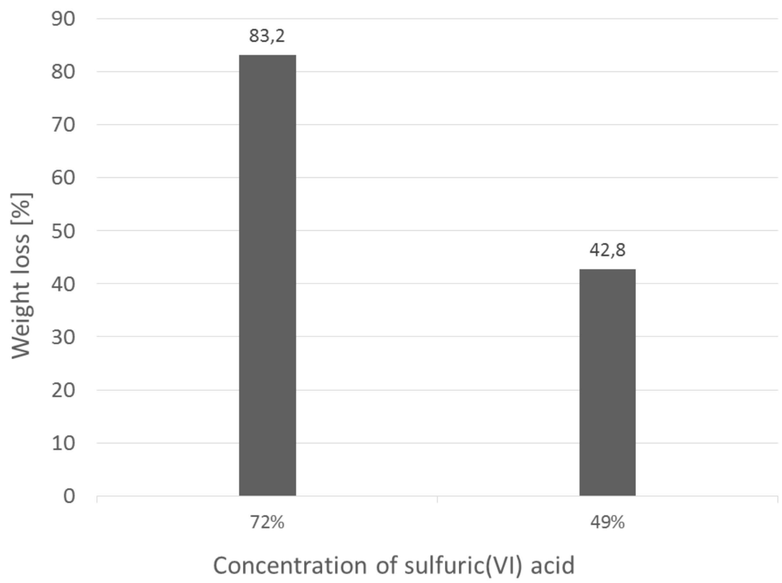

Figure 2. The weight loss of birch wood after the hydrolysis process using 72% and 49% sulfuric(VI) acid (hydrolysis conditions: 1 h room temperature + 4 h boiling point of reaction mixture after dilution).

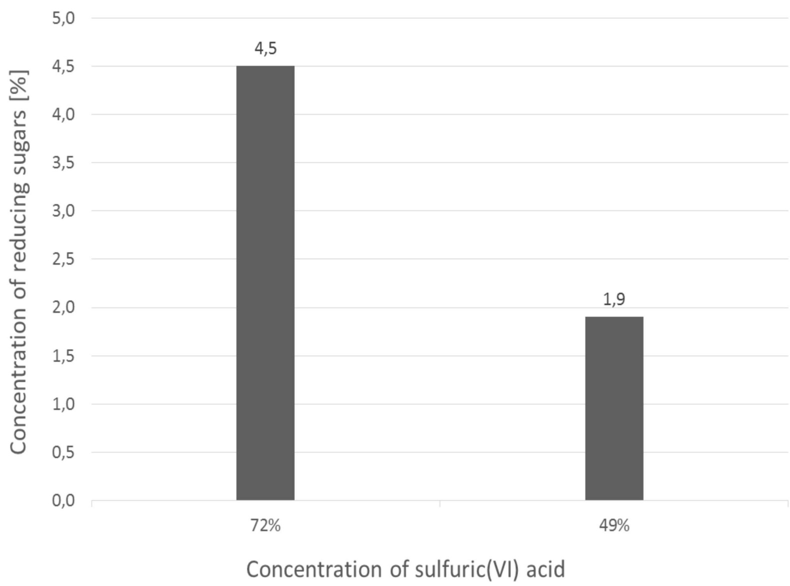

Figure 3. The content of reducing sugars in birch wood after the hydrolysis process using 72% and 49% sulfuric(VI) acid (hydrolysis conditions: 1 h room temperature + 4 h boiling point of reaction mixture after dilution).

As mentioned above, together with the concentration of acids, their hydrolyzing effect increases. Also the time of the hydrolysis process has the influence on its efficiency. Figures 4 and 5 presents the results of calculated weight loss and content of reducing sugars determination after hydrolysis of birch wood with different concentrations of sulfuric(VI) acid (0,8% - 20%) and different duration of the hydrolysis process (4 h and 7 h).

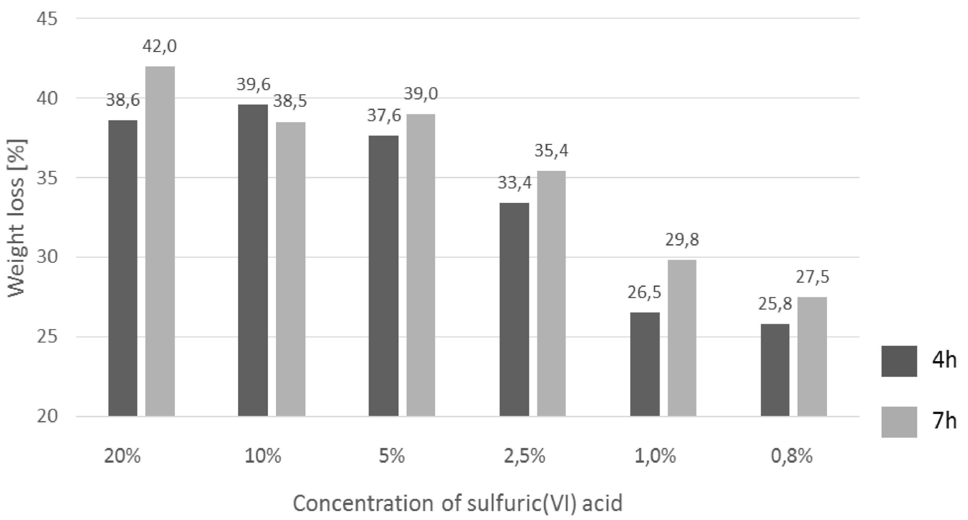

Figure 4. The weight loss of birch wood after the 4 h and 7 h hydrolysis process using 0,8% - 20% sulfuric(VI) acid.

The results of the weight loss calculations confirm the dependence of the hydrolysis efficiency on the acid concentration and the time of the reaction. The highest loss of weight (42%) was observed in the case of hydrolysis in 20% H_2SO_4 during the 7-hour heating of the mixture at a boiling point, the lowest – 25,8% in 0.8% H_2SO_4 during the 4-hour reaction. A noticeable high values of weight loses were observed in case of the hydrolysis with using 5% and 10% H_2SO_4. They both are close to the value of the loss of mass calculated for the hydrolysis carried out using 20% H_2SO_4 (Fig. 4).

Analyzing the content of reducing sugars in the above mentioned hydrolysates (Fig. 5), the highest level (2,0 – 2,1%) was found when 5% and 10% H_2SO_4 was used. A much lower content of the analyzed sugars was obtained in the case of hydrolysis with 20% H_2SO_4 – 1,2% (reaction time 4h) and 1,4% (reaction time 7 h). This can be explained by a partial reversion of monosaccharides to polysaccharides in these conditions. In other hydrolysates (2,5%, 1%, 0,8% H_2SO_4) the values of determined reducing sugars were in the range of 1,0 – 1,5%.

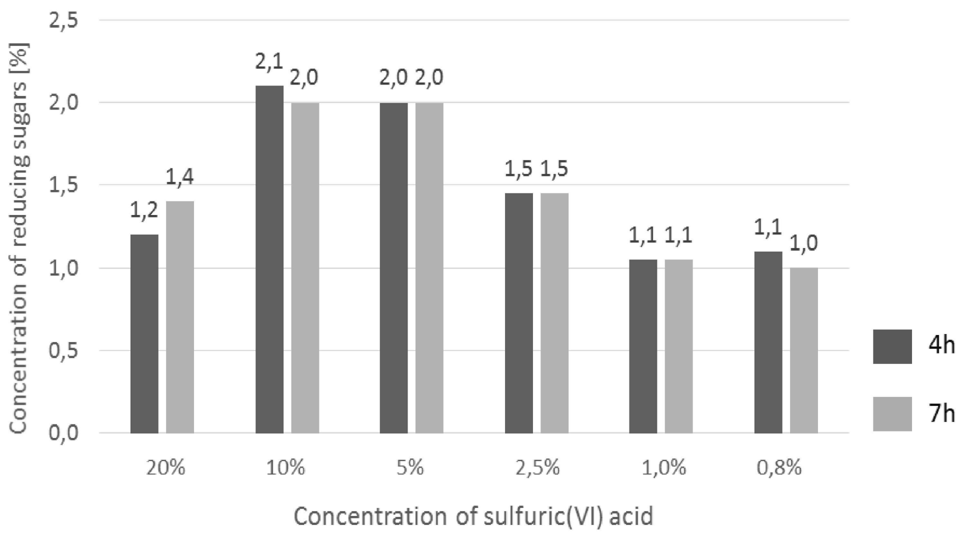

Figure 5. The content of reducing sugars in birch wood after the 4 h and 7 h hydrolysis process using 0,8% - 20% sulfuric(VI) acid.

Figure 6 presents the results of the reducing sugars analysis obtained for the spruce wood during hydrolysis process in 4 hours. The highest level (4%) was determined in the case of hydrolysis using 72% sulfuric(VI) acid. Similarly as in the process of hydrolysis of birch wood, the content of reducing sugars after using 20% H_2SO_4 was lower (1,3%) than their value after using 5% and 10% H_2SO_4 (1,6% and 1,7%).

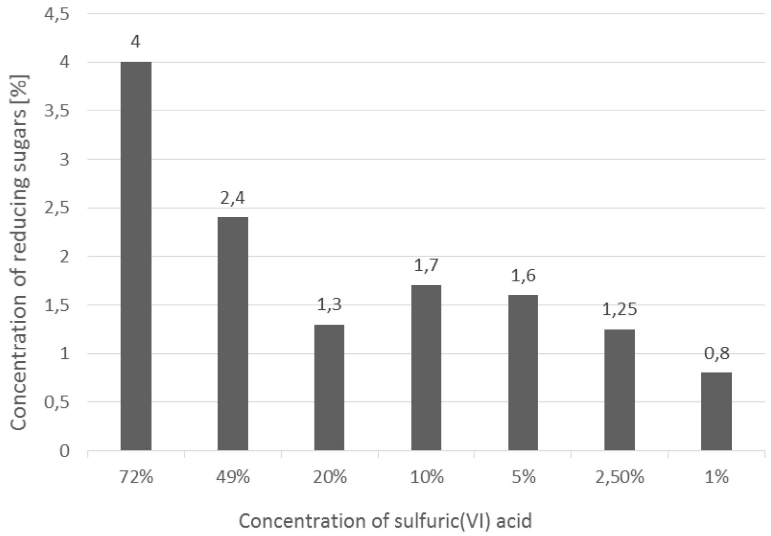

Figure 6. The content of reducing sugars in spruce wood after the hydrolysis process using 1% - 72% sulfuric(VI) acid during 4-hour hydrolysis.

Results of determination of reducing sugars concentrations obtained for the hydrolysates from spruce wood are lower than the concentrations obtained under the same conditions for birch (Fig. 3, 5, 6). This is related to the chemical composition of both species. In spruce wood, the amount of determinate pentosans was lower than in birch wood (spruce 9,58%, birch 25,45%) (Table 1). Because of amorphous structure and low degree of polymerization hemicelluloses, including pentosans, hydrolyze more easily than cellulose. Analysis of the content of soluble substances in 1% NaOH (Table 1), so hemicelluloses and small amounts of cellulose and lignin which goes to the extract, also showed their higher level in birch wood (15,79%) than in spruce (12,89%). The content of resin substances in coniferous wood affects the increased resistance of these species to destruction with inorganic acid.

Table 2 presents the results of the chromatographic analysis (HPLC), in which the glucose content was determined for selected samples of birch and spruce wood. The cellobiose content in all the samples was determined below the level of its detection (<0,01 mg/ml).

Table 2. The glucose content in hydrolysates from birch and spruce wood determined using liquid chromatography (HPLC).

Concentration of sulfuric(VI) acid		72%	49%	20%	10%	5%	2,5%	1%
Glucose content [mg/ml]	birch	28.53	11.97	5.32	-	0.38	0.16	0.05
	spruce	13.93	4.65	2.63	0.20	0.17	<0.05	<0.05

Obtained results indicate a proportional increase in the amount of glucose with increasing concentration of the acid used. The highest glucose content in birch and spruce wood was determined after hydrolysis with 72% H_2SO_4, respectively 28,53 mg/ml and 13,93 mg/ml. The lowest glucose concentration was determined in birch hydrolysate (0,05 mg/ml) obtained in the hydrolysis process with 1% H_2SO_4, whereas for spruce in hydrolysate obtained with 5% H_2SO_4 – 0,17 mg/ml. In the hydrolysates from spruce obtained after the reaction of 2,5% and 1% H_2SO_4 the glucose concentration was below the level of determination (<0,05 mg/ml). It is noticeable, that the results of glucose content determination in the hydrolysates after using 20%, 49% and 72% H_2SO_4 are at least 10 times higher than the glucose content in other post-hydrolysis solutions (hydrolysis with 10%, 5%, 2,5%, 1% H_2SO_4). This difference may result from the before mentioned amorphous wood structure - cellulose does not hydrolyze as easily as hemicelluloses.

CONCLUSIONS

As research conducted in laboratory conditions has shown, wood relatively easily undergoes a process of destruction caused by water action in presence of a mineral acid, such as sulfuric(VI) acid. During the research, a varied effect of water on wood tissue was observed in the presence of the catalyst used. The concentration of acid and the time of process directly influenced the effects of the hydrolysis. Considering the weight loss, content of reducing sugars, and glucose concentration in the obtained hydrolysates, the conducted research shows that in 4-hour hydrolysis process at the boiling point temperature, deciduous wood gets destroyed more easily than softwood, using all variants of sulfuric(VI) acid concentration.

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Streszczenie: *Wpływ stężenia kwasu i czasu przebiegu procesu hydrolizy drewna brzozy i świerku na zawartość cukrów redukujących.* Badania prowadzone od wielu lat wykazały, że tkanki roślinne, a w szczególności biomasa drzewna, mogą być bogatym źródłem różnorodnych substancji chemicznych. Spośród wielu kierunków chemicznego przerobu drewna na uwagę zasługują procesy scukrzania substancji roślinnej w celu otrzymywania cukrów prostych, które są między innymi półproduktami do wytwarzania bioetanolu i innych substancji stanowiących komponenty biopaliw płynnych. Celem badań było poznanie wpływu stężenia kwasu mineralnego oraz czasu przebiegu procesu hydrolizy wybranych gatunków drewna brzozy i świerka w warunkach laboratoryjnych na efektywność tego procesu określaną udziałem cukrów redukujących w roztworach pohydrolitycznych. Biorąc pod uwagę ubytek masy oraz zawartość cukrów redukujących i zawartość glukozy w otrzymanych hydrolizatach, z przeprowadzonych badań wynika, że drewno liściaste w procesie hydrolizy prowadzonej w temperaturze wrzenia mieszaniny reakcyjnej w ciągu czterech godzin w obecności kwasu siarkowego (VI) z zastosowaniem wszystkich wariantów stężeń, łatwiej ulegało destrukcji niż drewno iglaste.

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