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The Effect of Lignin Content in Birch and Beech Kraft Cellulosic Pulps on Simple Sugar Yields from the Enzymatic Hydrolysis of Cellulose

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Abstract: The results of enzymatic hydrolysis of birch and beech kraft cellulosic pulps indicate that they may be promising feedstocks for fermentation processes including biofuel manufacturing. The aim of this study was to investigate whether birch and beech wood require the same degree of delignification by kraft pulping as pine wood. The differences observed in the efficiency of hydrolysis for the raw materials tested suggest that the differences in the anatomical structure of the examined wood in relation to pine wood is essential for the efficiency of the enzymatic hydrolysis process. The yields of glucose and other reducing sugars obtained from the birch and beech cellulosic pulps were similar (up to around 75% and 98.3% dry weight, and 76% and 98.6% dry weight, respectively). The highest glucose yields from cellulose contained in the birch and beech pulp were around 81.2% (at a Kappa number of 28.3) and 83.1% (at a Kappa number of 30.4), respectively. The maximum glucose yields and total reducing sugars of birch wood on a dry weight basis (39.8% and 52.1%, respectively) were derived from the pulp at a Kappa number of 28.3, while the highest yields of glucose and total reducing sugars of beech wood on a dry weight basis (around 36.9% and 48.2%, respectively) were reached from the pulp at a Kappa number of 25.3. To obtain the highest glucose yields and total reducing sugars of a wood on a dry weight basis, total lignin elimination from the birch and beech pulps was not necessary. However more in-depth delignification of birch and beech wood is required than for pine wood.

Keywords: cellulosic pulps; birch; beech; wood; kraft pulping; enzymatic hydrolysis

1. Introduction

In the European paper industry, birch pulps are the most widely used hardwood pulps. Beech pulps are often used as a substitute for birch pulps. Birch and beech wood contain around 40% and 41% dry weight (DW) cellulose and 36% and 33% DW hemicelluloses, respectively, and less lignin (20% and 22% DW, respectively) than softwoods (above 25% DW) [1–6]. Because of their high holocellulose content (more than 70% on a dry weight basis) and their high availability, birch and beech woods are not only excellent raw materials for papermaking but also promising feedstocks for biotechnology [7–9]. Wood together with other types of renewable lignocellulosic biomass have become a principal alternative energy resource because of their inputs to emissions of greenhouse gases and consumption of fossil energy. The use of lignocellulosic materials instead of starch and plant oils for the production of biofuels along with other bioproducts is attractive as it does not compete with food production.

However, enzymatic hydrolysis of cellulose or hemicellulose polysaccharides in lignocellulosic biomass appears much more complex than enzymatic starch saccharification. It results from the three-dimensional shape of plant cells, its crosslinked compact structure, lignin protective effect, and contents of resins and waxes. In plant biomass, the fibers of cellulose and hemicelluloses are coated with lignin, which restricts the access of enzymes that degrade polysaccharides [10]. Because lignin adsorbs cellulases and hemicelluloses unproductively the rate and degree of saccharification are reduced [11]. Thus, prior to enzymatic digestion, lignin has to be removed from the lignocellulosic biomass, the accessible surface area of cellulose has to be increased, and its crystallinity has to be reduced as crystalline regions of cellulose are not as prone to enzymatic degradation as the amorphous regions.

The papermaking process starts with the fragmentation into woodchips. Next, chemical delignification of chips and generating cellulosic pulps of a diverse range of residual lignin contents take place. One of the most popular wood delignification treatments is the sulfate method, which is a fully mature technology enabling total recovery of chemical substances used within the process, fulfilling the majority of the Twelve Principles of Green Chemistry (these principles mainly indicate ways to reduce the environmental and health impacts of chemical production) [12]. Annually, over 150 million metric tons (MMT) of cellulosic pulps are manufactured using the above-mentioned method. The delignification of wood by the sulfate method causes not only partial loss of lignin but also hemicelluloses. This can positively affect the results of certain fermentation processes because not all microbial species efficiently assimilate and metabolize pentoses, for example, wild type *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strains cannot produce ethanol from xylose [13].

Enzymatic hydrolysis of cellulosic pulps produced from low quality wood cannot be used in papermaking, due to the low average fiber length and severe depolimerization of cellulose. However, such pulps can be successfully used as a raw material for enzymatic hydrolysis, since they contain just a small amount of lignin and extractives which prevent enzymatic hydrolysis and fermentation [14].

The main goal of this analysis was to assess the potential of birch and beech kraft pulps with Kappa numbers ranging from 19.5 to 65.3, and from 17.4 to 63.2, respectively, as feedstocks for the fermentation process, including production of biofuels. These pulps underwent enzymatic hydrolysis, with a multienzyme commercial preparation comprising cellulases and hemicelluloses. In order to define the influence of sulfate process lignin removal from wood on the effectiveness of enzymatic hydrolysis, pulps and woodchips from birch and beech underwent hydrolysis under the same conditions.

2. Materials and Methods

2.1. Woodchips

With the use of a 10 mm Metabo wood drill bit installed in a Milwaukee drill (Milwaukee Tool, the USA) birch and beech woodchips (0.43–0.8 mm) were obtained by drilling bigger logs of birch and beech wood. Next, to obtain woodchips with a fraction size of 0.43–0.8 mm, a LPzE-3e sieve shaker (Morek, Poland) was used. The fraction underwent enzymatic saccharification.

2.2. Kraft Wood Pulps

Birch (Kappa numbers from 19.5–65.3) and beech (Kappa numbers from 17.4–63.2) cellulosic pulps with a humidity level of 7–8%, were made out of woodchips (25 mm × 15 mm × 8 mm) by the application of the sulfate pulping process as stated by Modrzejewski [15]. After the previous mechanical removal of bark and gnarls located inside and outside the wood, the woodchips were prepared. The obtained sample materials were put into hermetic vials to prevent probable changes of the humidity level of the samples. Next, the sample chips were treated with freshly prepared NaOH and Na_2S solutions at a chip: solvent ratio of 1:4 weight/volume (w/v). Active alkali dosage ranged from 20% to 34%. Sulfidity, in all cases was equal to 30%. The removal of lignin was carried out in a 15 L PD-114 stainless steel reactor (Danex, Poland) with temperature adjustment (heater and cooling water jacket). The obtained woodchips suspensions were heated up for 120 minutes to reach 165 °C.

After 120 minutes of incubation at the same temperature, the suspensions were cooled down using tap water and pumped through the water jacket, to the ambient temperature of 22 ± 1 °C. In order to eliminate any alkali-soluble residues after delignification, the material was rinsed for several times and subsequently incubated overnight in demineralized water. Using the laboratory propeller pulp disintegrator (JAC SHPD28D, Danex, Poland), solids were subjected to disintegration at 12,000 rpm. for 4 minutes, while the fibers were screened using a PS-114 membrane screener with 0.2 gap screen (Dantex, Poland). The collected fibers were allowed to dry at an ambient temperature of 22 ± 1 °C for 48 h and weighed afterwards. Next, the chemical composition, Kappa number, and humidity of the fibers were examined. All samples were analyzed in triplicate. Analysis of the chemical composition of pulps and woodchips considered extractives, lignin, cellulose, hemicelluloses, and ash quantification. The lignin contents were determined gravimetrically according to the TAPPI T222 standard [16] following the elimination of extractives in compliance with the TAPPI T204 [17]. The holocellulose contents were determined in compliance with the TAPPI T249 standard as well [18]. Furthermore, cellulose was quantified as alpha cellulose (TAPPI T203) [19]. The hemicelluloses contents were estimated on the basis of the difference between the content of holocellulose and cellulose. Moreover, the content of ash was also determined according to the TAPPI T211 standard [20]. All of the above-mentioned samples were performed in triplicate for individual raw materials.

The yields of production of seven birch and seven beech cellulosic pulps with Kappa numbers from 19.5 to 65.3, and from 17.4 to 63.2, respectively, varied from 44.15% to 53.09%, and from 44.22% to 49.50% DW wood, respectively (Table 1).

Table 1. Kappa numbers and yields of birch and beech cellulosic pulps (on a dry weight basis).

Substrate	Kappa Number	Yield
		[% DW]
Birch	65.3 ± 0.4	44.15 ± 0.55
	52.1 ± 0.5	48.20 ± 0.46
	39.8 ± 0.3	49.26 ± 0.34
	33.4 ± 0.4	51.75 ± 0.31
	28.3 ± 0.2	53.09 ± 0.18
	24.6 ± 0.1	52.13 ± 0.27
	19.5 ± 0.1	51.11 ± 0.12
Beech	63.2 ± 0.3	44.22 ± 0.46
	51.6 ± 0.2	45.01 ± 0.34
	38.9 ± 0.6	46.22 ± 0.32
	30.4 ± 0.2	48.55 ± 0.15
	25.3 ± 0.3	49.50 ± 0.08
	22.0 ± 0.2	48.61 ± 0.26
	17.4 ± 0.2	46.13 ± 0.06

The results are reported as means ± standard deviation (SD).

2.3. Enzyme Activities

The commercial enzyme with cellulases and xylanases activities (NS-22086), was provided courtesy of Novozymes A/S (Denmark). The activity of both enzymes was estimated by the 3,5-dinitrosalicylic acid method (DNS) [21]. The reaction was conducted for 5 minutes at a temperature of 50 °C and pH 5.0 for both 0.5% carboxymethylcellulose (CMC) and 0.5% birch xylan. Activities of the above-mentioned glycosidases were assessed as micromoles of sugars yielded from the polysaccharide substrates in 1 minute. (U). As recommended by Adney and Baker [22], the activity of filter paper was measured at pH 5.0 and 50 °C and assessed as filter paper units per milliliter. A description of the NS-22086 tests for the contents of total reducing sugars and glucose concentrations are presented in the following sections.

2.4. Enzymatic Hydrolysis

The suspension of substrates (0.3 g DW each in the 20 ml solution of 0.1 M sodium-acetate buffer with pH 5.0) was prepared and subjected to incubation in a water bath at a temperature of 50 °C for 15 minutes. Next, 1 ml NS-22086 aliquot was diluted 1/6 in identical buffer and then added via vigorous mixing individually to those suspensions for enzymatic digestion initialization. Enzymatic digestion was performed at a temperature of 50 °C. For the woodchips, hydrolysis lasted 72 h, and for pulp the process was stopped after 48 h. All hydrolysates of enzymes were clarified through a medium-fast filter paper and their filtrates were analyzed. Enzymatic hydrolysis was performed at enzyme: proportions of substrate for cellulases approximately 44,73 U: 1 g DW and for xylanases 106,84 U: 1 g DW (approximately 1 ml NS-22086 formulation per 1.8 g DW pulp).

2.5. Analytics

As stated by Miller [21], the concentration of the reducing sugars was carried out by the application of alkaline DNS solution. Mono- and disaccharide profiles of the hydrolysates were developed by high-performance liquid chromatography (Ultimata 3000 Dionex with the column type Rezex RPM-Monosaccharide Pb^{2+} (8 µm, 7.8 × 300 mm) and the detector type Shodex-RI-10). The analysis was carried out at a temperature of 80 °C for the column and 40 °C for the RI detector. Prior to chromatographic analysis, the filtration of the hydrolysates samples was carried out using a nylon syringe filter (pore size 0.45 µm). The injected samples had a volume of 10 µl. For the mobile phase high-performance liquid chromatography (HPLC), grade water (Sigma) was used (flow rate: 0.6 ml/min). The results of sugar break down were registered for over 35 minutes. In order to confirm the HPLC results, the concentration of glucose was verified again as advised by Barham and Trinder [23], with the use of a commercial diagnostic test using glucose oxidase and peroxidase (Biomaxima, Poland). The tests were performed following the instructions enclosed in the diagnostic kit.

All hydrolysis reactions and hydrolysates analytics were conducted at least in triplicate. The results are reported as means ± standard deviation (SD).

2.6. Yield Calculations

Equations (1) and (2) are applied to calculate glucose and total reducing sugars yields from the pulps and woodchips dry weight (according to Kumar and Wyman [24] with modification presented by Buzała et al. [25]):

$$G_Y = G_h \times 0.9/S_{dw}, \quad (1)$$

$$T_{RS} = (H_h \times 0.9 + C_h \times 0.95 + P_h \times 0.88)/S_{dw} \quad (2)$$

where, G_y indicates glucose yield, G_h is the glucose in hydrolysate (g), S_{dw} means initial dry weight of the sample (g), T_{RS} denotes total reducing sugars yield, H_h represents hexoses in hydrolysate (g), C_h is the cellobiose in hydrolysate (g), and P_h is the pentoses in hydrolysate (g).

The correction factors of 0.88, 0.9, and 0.95, for pentoses, hexoses, and cellobiose, respectively [26], were applied in the calculations to recompense the additional water molecule in the hydrolysis process for individual glycosidic bonds.

To define the influence of lignin content on the saccharification degree of cellulose, glucose yields contained in cellulose obtained from the pulps and chips were estimated as stated by Kumar and Wyman [24], according to Equation (3):

$$G_Y = (G_h + 1.053 \times C_h /1.111) \times G_{ia} \quad (3)$$

where, G_{ia} is the initial amount of glucan (g).

3. Results and Discussion

3.1. Enzyme Preparation

The activities of cellulases and xylanases were demonstrated by the commercial preparation of NS-22086, used in the study for the hydrolytic process of birch and beech cellulosic pulps and birch and beech woodchips (0.43–0.8 mm). The possibility of producing glucose-rich hydrolysates from kraft pulps using NS-22086 has been described by Buzała et al. [25]. The NS-22086 enzyme preparation, as for many others, consists of reducing sugars, such as free glucose. The reducing sugars concentration of 68.6 mg/ml and the free glucose concentration of 42.0 mg/ml were taken into account for the calculation of the yields of enzymatic saccharification of the substrates. The six-fold dilution was performed prior to mixing with substrates in order to reduce the content of sugars added to reaction mixtures with the enzyme preparation. The enzymatic hydrolytic process was performed at the substrate ratio of 13.42 U: 0.3 g DW (18.69 filter per unit (FPU): 0.3 g DW) for cellulases, and the substrate ratio of 32.05 U: 0.3 g DW for xylanases. Cellulases activities in the NS-22086 enzyme preparation (temperature 50 °C, pH 5.0) were 80.6 ± 0.2 U/ml and 112.1 ± 0.9 FPU/ml; for xylanases this activity reached 192.5 ± 1.6 U/ml.

3.2. Cellulosic Pulps Chemical Composition

Cellulose was the key component of the kraft cellulosic pulps (Table 2). The content of cellulose improved with a lower Kappa number and oscillated from 76.7% DW maximum content of lignin in the beech pulp (Kappa number 63.2, 9.5% DW lignin) to 94.6% DW in the birch pulp with nearly no lignin content (Kappa number 19.5, 2.9% DW lignin). A positive correlation between hemicelluloses and lignin contents, as well as a negative one between hemicelluloses and cellulose contents, were revealed. The lignin content varied from 2.2% DW to 13.1% DW. Hemicelluloses content was significantly higher in the beech pulps as compared with the birch pulps at a similar Kappa number. Ash and extractives content ranged from 0 to 0.2% DW in the pulps at Kappa numbers below 40 (when the Kappa numbers were above 40 these values were slightly higher, up to 0.5% DW).

Table 2. Chemical composition of the birch and beech woodchips and kraft cellulosic pulps.

Substrate	Kappa Number	Cellulose	Hemicelluloses	Lignin	Extractives	Ash
				[% DW]		
Birch	65.3	80.8 ± 0.6	8.7	9.8 ± 0.4	0.4 ± < 0.1	0.3 ± < 0.1
	52.1	85.7 ± 0.6	5.9	7.8 ± 0.4	0.3 ± < 0.1	0.3 ± < 0.1
	39.8	88.8 ± 0.3	4.7	6.0 ± 0.3	0.3 ± < 0.1	0.2 ± < 0.1
	33.4	90.3 ± 0.8	4.3	5.0 ± 0.2	0.2 ± < 0.1	0.2 ± < 0.1
	28.3	92.3 ± 0.3	3.2	4.2 ± 0.3	0.1 ± < 0.1	0.2 ± < 0.1
	24.6	93.1 ± 0.5	2.8	3.7 ± 0.2	0.2 ± < 0.1	0.2 ± < 0.1
	19.5	94.6 ± 0.2	2.2	2.9 ± 0.3	0.1 ± < 0.1	0.2 ± < 0.1
woodchips (0.43–0.8 mm)	n/d *	45.3 ± 0.9	25.1	26.4 ± 0.3	2.6 ± 0.2	0.6 ± < 0.1
Beech	63.2	76.7 ± 0.8	13.1	9.5 ± 0.5	0.5 ± < 0.1	0.2 ± < 0.1
	51.6	82.7 ± 0.7	9.1	7.7 ± 0.3	0.3 ± < 0.1	0.2 ± < 0.1
	38.9	86.6 ± 0.4	7.3	5.8 ± 0.4	0.2 ± < 0.1	0.1 ± < 0.1
	30.4	89.3 ± 0.6	6.0	4.6 ± 0.3	0.0 ± < 0.1	0.1 ± < 0.1
	25.3	91.3 ± 0.3	4.8	3.9 ± 0.3	0.0 ± < 0.1	0.1 ± < 0.1
	22.0	92.4 ± 0.4	4.1	3.3 ± 0.2	0.1 ± < 0.1	0.1 ± < 0.1
	17.4	94.0 ± 0.2	3.3	2.6 ± 0.2	0.0 ± < 0.1	0.1 ± < 0.1
woodchips (0.43–0.8 mm)	n/d *	41.9 ± 0.3	31.2	22.7 ± 0.6	3.1 ± 0.2	1.1 ± < 0.1

The results are reported as means ± standard deviation (SD); * n/d, not determined.

3.3. Enzymatic Hydrolysis of Birch and Beech Woodchips

To determine the correlation between birch and beech wood sulfate delignification and saccharification efficiency, the birch and beech woodchips which were obtained from the piece of wood that was used for the production of the birch and beech cellulosic pulps of diverse residual lignin

content, were subjected to enzymatic digestion under the same conditions as the pulps. The woodchips were digested for 72 h (this time was chosen taking into consideration the results of our previous study on woodchips hydrolysis [25]). The occurrence of lignin in the chips caused a relatively low degree of cellulose and hemicelluloses hydrolysis which was revealed by the small yields of glucose and total reducing sugars (Table 3), 10% and 26.19% on the birch DW wood, respectively, and 11.42% and 26.63%, on the beech DW wood, respectively. Increasing glucose content in the chips hydrolysates was noticeable for the initial 24 h of enzymatic digestion (Figures 1 and 2) and after this period of time the glucose concentration stabilized.

Table 3. Glucose and total reducing sugars concentrations and yields on enzymatic hydrolysates of samples *.

Substrate	Kappa Number	Glucose Concentration	Mean Glucose Yield		Total Reducing Sugars Concentration	Mean Reducing Sugars Yield	
		[mg/ml]	[% DW pulp]	[% DW wood]	[mg/ml]	[% DW pulp]	[% DW wood]
Birch cellulosic pulp	65.3	24.26 ± 0.21	55.07	23.76	29.14 ± 0.18	72.03	31.08
	52.1	27.47 ± 0.15	62.36	29.43	31.25 ± 0.18	77.25	36.46
	39.8	30.36 ± 0.06	68.92	34.43	36.12 ± 0.08	89.29	44.61
	33.4	31.99 ± 0.08	72.62	38.31	38.22 ± 0.09	94.48	49.84
	28.3	30.33 ± 0.02	74.98	39.81	39.68 ± 0.02	98.09	52.08
	24.6	32.63 ± 0.03	74.07	38.61	39.72 ± 0.02	98.19	51.19
	19.5	32.82 ± 0.01	74.50	38.08	39.75 ± 0.02	98.26	50.22
woodchips (0.43–0.8 mm)	n/d **	1.84 ± 0.01	n/d **	10.00	4.48 ± 0.09	n/d **	26.19
Beech cellulosic pulp	63.2	24.49 ± 0.33	60.53	25.56	30.81 ± 0.26	76.15	32.15
	51.6	26.26 ± 0.25	64.90	29.21	32.11 ± 0.13	79.37	35.72
	38.9	28.42 ± 0.07	70.24	32.47	37.52 ± 0.08	92.74	42.86
	30.4	30.01 ± 0.12	74.17	36.01	38.89 ± 0.19	96.12	46.67
	25.3	30.13 ± 0.16	74.47	36.86	39.39 + 0.05	97.36	48.19
	22.0	30.33 ± 0.12	74.96	36.44	39.45 ± 0.09	97.51	47.40
	17.4	30.74 ± 0.09	75.98	35.05	39.89 ± 0.05	98.60	45.48
woodchips (0.43–0.8 mm)	n/d **	2.09 ± 0.09	n/d **	11.42	4.63 ± 0.02	n/d **	26.63

* 50 °C, pH 5.0, 48 h, only the chips were digested for 72 h. ** n/d, not determined. ± standard deviation (SD).

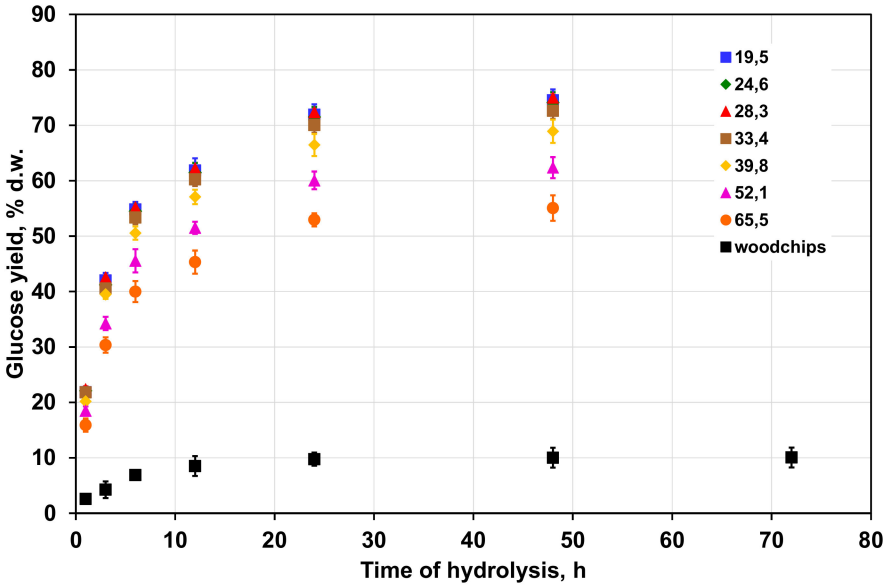

Figure 1. Yield of birch cellulosic pulps and woodchips hydrolysis by NS-22086 preparation (based on yields of glucose released from the substrates).

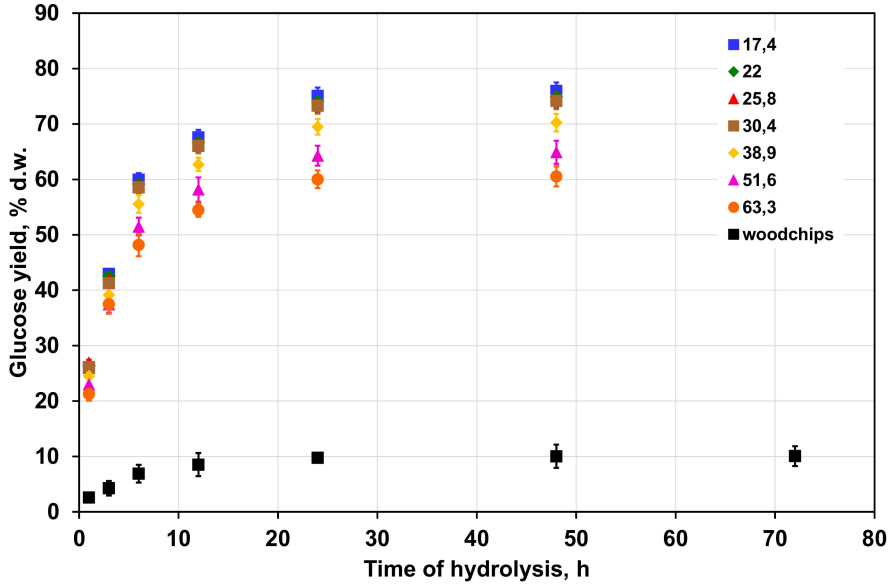

Figure 2. Yield of beech cellulosic pulps and woodchips hydrolysis by NS-22086 preparation (based on yields of glucose released from the substrates).

After 72 h of the birch and beech chips digestion, the enzymatic hydrolysates subjected to the high-performance liquid chromatography analysis revealed that glucose corresponded to about 41.1% and 45.1% of the sum of four soluble sugars contained in the hydrolysates, respectively. The level of its reducing dimer-cellobiose was slightly lower (32.1% for birch and 25.6% for beech DW) (Table 4). This could have resulted from the relatively high activity of cellobiohydrolase in NS-22086 as well as inhibition of β-glucosidase, in spite of very low concentration of glucose. With the exception of the above-mentioned sugars, hydrolysates consisted of mannose (23.2% DW for birch and 28.8% DW for beech woodchips) and xylose (3.7% DW for birch and 0.5% DW for beech woodchips) as well.

Table 4. Mean percentage contents of sugars among mono- and disaccharides contained in enzymatic hydrolysates of samples.

Substrate	Kappa Number	Glucose	Cellobiose	Xylose	Arabinose	Rhamnose	Mannose	Galactose
		[% DW]						
Birch cellulosic pulp	65.3	68.21	5.02	24.74	0.36	n/d *	0.46	1.21
	52.1	70.56	5.12	22.67	0.27	n/d *	0.31	1.07
	39.8	73.48	4.83	20.12	0.32	n/d *	0.41	0.84
	33.4	75.98	4.26	18.31	0.21	n/d *	0.33	0.91
	28.3	76.43	4.00	18.01	0.28	n/d *	0.36	0.92
	24.6	77.06	4.02	17.68	0.11	n/d *	0.25	0.88
	19.5	77.11	3.69	17.81	0.17	n/d *	0.18	1.04
woodchips (0.43–0.8 mm)	-	41.06	32.08	3.65	n/d *	n/d *	23.21	n/d *
Beech cellulosic pulp	63.2	66.76	5.09	26.33	0.42	0.38	n/d *	1.02
	51.6	71.39	4.84	21.95	0.39	0.35	n/d *	1.08
	38.9	74.58	4.69	18.93	0.34	0.39	n/d *	1.07
	30.4	75.44	4.02	18.98	0.31	0.36	n/d *	0.89
	25.3	76.49	3.94	17.96	0.35	0.31	n/d *	0.95
	22.0	76.89	3.66	17.87	0.28	0.32	n/d *	0.98
	17.4	77.05	3.51	17.92	0.26	0.30	n/d *	0.96
woodchips (0.43–0.8 mm)		45.09	25.6	0.51	n/d *	n/d *	28.80	n/d *

* n/d, not determined.

3.4. Enzymatic Hydrolysis of Birch and Beech Pulps

To conclude the influence of the residual lignin level on the birch and beech cellulosic pulps digestibility, fourteen pulps (seven from birch wood and seven from beech wood) at Kappa numbers ranging from 17.4 to 65.3 were digested by the preparation NS-22086. Glucose concentrations and total reducing sugars in the hydrolysates, including their yields, were monitored for over 48 h. These yields depended on the Kappa numbers of the tested fourteen pulps. The highest glucose level (above 70% DW) and reducing sugars level (above 92% DW) came from the birch and beech cellulosic pulps with Kappa numbers from 19.5 to 33.4, and from 17.4 to 38.9, respectively (Table 3). The pulp with the Kappa number 28.3 turned out to be the most appropriate to reach the maximum yields of glucose and total reducing sugars on the birch DW wood (39.8% and 52.1%, respectively). As presented in Figure 3 the birch pulp characterized by the Kappa number 28.3 reached the highest glucose yield from cellulose contained in the pulp (81.2% DW). Interestingly, while the Kappa number increased from 19.5 to 28.3, the glucose yields from cellulose from the birch pulps increased as well from 78.8% to 81.2% DW. Furthermore, with an increase of Kappa number to 65.3 the glucose yields decreased to 68.2% DW. Similar interplay was observed in the case of the beech pulps. In the latter case, the pulp at the Kappa number of 30.4 reached the highest glucose yield from cellulose (around 83.1% DW). However, comparatively, glucose yield from cellulose from the birch and beech chips reached 22.1% and 27.3% DW, respectively. The results prove that complete delignification is not required to enhance glucose yields from cellulose (the pulps at Kappa numbers of 28.3 and 30.4 contained around 4.2% and 4.6% DW lignin, respectively, which means approximately five- and six-fold less than the original wood).

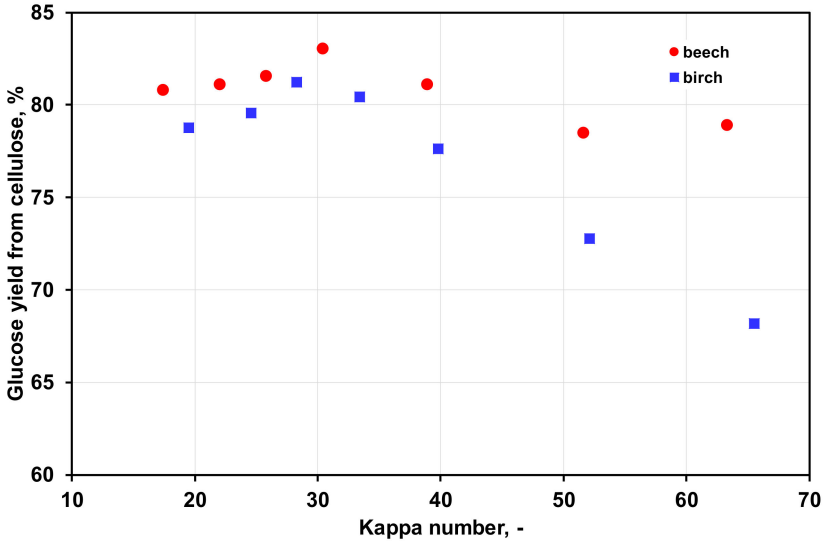

Figure 3. Mean glucose yields from cellulose contained in birch and beech kraft pulps.

The identification and quantification of sugars released by liquid chromatography analysis of cellulosic pulp hydrolysates are presented in Table 4. The HPLC results showed that not only glucose (66.8–77.1%) but also the hydrolysates of pulps consist of cellobiose (3.5–5.1%), arabinose (up to 0.4%), galactose (up to 1.2%) and relatively large amounts of xylose (17.7–26.3%). Both galactose and the two pentoses were released from the residual hemicelluloses contained in the pulps. Neither the hydrolysates of birch pulps nor the hydrolysates of birch woodchips contained rhamnose. Rhamnose was detected in beech pulp hydrolysates (up to 0.39%) despite the absence in the hydrolysates of beech chips. The hydrolysates of beech pulps did not contain mannose whose content in the birch pulp hydrolysates varied from 0.18% to 0.46%. Large amounts of the latter sugar were contained in the birch and beech woodchip hydrolysates (23.2% and 28.8% respectively). The time course of enzymatic hydrolysis of the birch pulps (Kappa numbers from 19.5 to 65.3) and birch woodchips are presented in Figure 1. The analogous data for beech pulps (Kappa numbers from 17.4 to 63.2) and beech woodchips

are presented in Figure 2. The hydrolysis of seven birch and seven beech pulps were much more effective in comparison to the hydrolysis of birch and beech woodchips. The yields of glucose released from these pulps within the first 6 h ranged between 40% and 55.2% for birch DW pulps and between 48.2% and 59.9% for beech DW pulps. Within 24 h, every single determined pulp almost entirely transformed into soluble sugars. The additional glucose and total reducing sugars slight increase after the next 24 hours did not exceed 2.5% for birch pulps and 0.8% for beech pulps and is assumed to be the result of soluble oligosaccharides and cellobiose hydrolysis.

The presented observations are consistent with the results of our previous publication concerning evaluation of susceptibility of pine kraft pulp to enzymatic hydrolysis [11,27], which indicated that there is a maximal lignin content above which the hydrolysis yield is noticeable reduced. In the case of pine pulp, such maximal lignin content is equal to the Kappa number 50, which corresponds to approximately 7.5% lignin content [27]. The investigated hardwood pulps required removal of lignin at Kappa numbers below 35–40 in order to achieve the highest yield of glucose production. It is widely known that hardwood is easier to digest than softwood [28,29], therefore, due to the lower content of lignin in the pulp, it should be easier to undergo enzymatic hydrolysis [30,31]. However, it should be mentioned that deciduous wood is more anatomically complex, therefore, also a greater variation in the lignin distribution in the wood can occur [6,32]. The less complex structure of softwood (pine) allows for a more effective enzymatic hydrolysis with a similar higher Kappa number in the pulp [11]. The delignification rate is limited by diffusion in secondary walls and variations of physicochemical parameters of lignin in different morphological regions of plant tissues in external walls [29,33]. Hardwood is more susceptible to the delignification process than softwood also due to the increased consolidation of α-aryl-ether bonds (which are easier to hydrolyze), reduced susceptibility to lignin condensation reactions, as well as increased reactivity of β-aryl-ether bonds [28,29]. The results confirm the results of other researchers who proved that enzymatic hydrolysis of wood, after only mechanical grinding, enables production of several fold lower yield of glucose [34,35]. The yield of reducing sugars after hydrolysis of mechanically disintegrated wood chips was at least two times lower than for any of investigated cellulosic pulps.

4. Conclusions

Through the application of a suitable multienzyme preparation, which consists of cellulases and xylanases (i.e., NS-22086 by Novozymes), birch and beech cellulosic pulps (Kappa numbers of 19.5–65.3 and 17.4–63.2, respectively), are believed to be promising substrates for the production of glucose-rich enzymatic hydrolysates (content of glucose higher than 70% of soluble sugars). Kraft pulping caused not only the partial removal of lignin (2.6–9.8% DW) but also hemicelluloses, to 2.2–8.7% DW and 3.3–13.1% DW in birch and beech pulps, respectively. Enzymatic hydrolysis of residual hemicelluloses caused the hydrolysates to contain also xylose, arabinose, galactose, rhamnose (only the hydrolysates of beech pulps), and mannose (only the hydrolysates of birch pulps).

The yields of glucose and total reducing sugars were determined by the residual lignin contents in the pulps. This may result from cellulases and hemicelluloses limited by lignin, or access to polysaccharides. The yields of glucose and reducing sugars from the birch and beech cellulosic pulps remained similar (up to around 75% and 98.3% DW, and 76% and 98.6% DW, respectively). The maximum yields of glucose from cellulose contained in the birch and beech pulps were around 81.2% (at the Kappa number of 28.3) and 83.1% (at the Kappa number of 30.4), respectively. The maximum yields of glucose and total reducing sugars of birch wood on a dry weight basis (around 39.8% and 52.1%, respectively) were obtained from the pulp at a Kappa number of 28.3, while the highest yields of glucose and total reducing sugars of beech wood on a dry weight basis (around 36.9% and 48.2%, respectively) were obtained from the pulp at a Kappa number of 25.3. As a result, the complete delignification of the birch and beech pulps was not necessary to obtain the highest yields of glucose and total reducing sugars of wood on a dry weight basis.

Kraft pulping is underestimated in biotechnology and, in most cases, lignocelluloses are subjected to other pretreatments before fermentation processes. However, our results demonstrate that the sulfate method is an attractive technology for wood delignification because of the susceptibility to saccharification of the obtained cellulosic pulps. It is possible to obtain nearly four-fold more glucose from kraft pulps than from the woodchips under identical conditions of hydrolysis. The application of enzymatic saccharification of superfluous and low-quality cellulosic pulps, especially those that could not be used in papermaking and bioethanol or other bioproducts that are producted from the obtained hydrolysates, is believed to be a promising method of their valorization.

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