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ORIGINAL

Conversion of various types of lignocellulosic biomass to fermentable sugars using kraft pulping and enzymatic hydrolysis

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Abstract The aim of this work was to assess the utility of seven different kraft pulps produced from softwood (pine), hardwood (poplar, birch and beech), wheat straw and hemp (bast and harl) as potential sources of sugar feedstocks for fermentation processes. The pulps contained low amounts of hemicelluloses (1.9–8.2% d.w.) and lignin (1.7–15% d.w.). The crystallinity index values ranged from 55% (wheat straw pulp) to 79% (hemp bast pulp), while the average DP varied from around 230 (hemp bast pulp) to 1482 (poplar and birch pulps). The results of enzymatic hydrolysis showed that not only the residual lignin content but also the cellulose crystallinity index decided on the sugar yields while the average polymerization degree had a weak impact. More reducing sugars were obtained from the hardwood pulps and wheat straw pulp (100% d.w.) than from the pine pulp (around 89% d.w.) and two hemp pulps (40.5% d.w. and 44.7% d.w. from the bast and harl pulps, respectively). Glucose was the dominating (69–79% w/w) soluble sugar in enzymatic hydrolysates of the pulps. The sugar profiles of these hydrolysates make them suitable sugar feedstocks for fermentation processes.

Introduction

Various types of lignocellulosic biomass have been increasingly used as renewable raw materials for the production of fuels and chemicals because of the depletion of fossil fuels, accumulation of greenhouse gases and other problems related to the

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growing pollution of natural environment. The principal factor deciding on the use of plant biomass for conversion to sugar feedstocks for fermentation processes is the overall content of cellulose and hemicelluloses (holocellulose) and the percentage of lignin. The latter, highly cross-linked polymer protects fibers of cellulose and hemicelluloses from the attack of hydrolytic enzymes. Therefore, lignin along with extractives, which also negatively affect the extent of enzymatic degradation of lignocellulosic biomass, must be removed before enzymatic hydrolysis of holocellulose. Delignification of vegetal biomass is carried out using various chemical, physicochemical and biological pretreatments (Chaturvedi and Verma 2013; Singh et al. 2016). Another objective of pretreatment, apart from partial lignin removal, is reduction in cellulose crystallinity, to make it more susceptible to enzymatic degradation. Pretreatment also has to be scalable, cost-effective and environmentally friendly. Lignocellulosic biomass such as softwood, hardwood, wheat straw and hemp is rich in holocellulose (above 70% on a dry weight, Table 1), abundant and easily available. Because of the high lignin level, varying between 16 and 28% d.w., each of these materials requires delignification before enzymatic digestion.

Despite intensive studies on the development of new pretreatment technologies, selective delignification of lignocellulosic biomass has not been achieved so far since the majority of pretreatments also cause partial removal of hemicelluloses. Furthermore, results of economic analysis of many pretreatments, taking into consideration costs of biomass, energy, chemicals and installation as well as treatment of waste water and other wastes, are variable making the economic gain uncertain. The economic gain is ensured by kraft pulping, which is one of the well-established, chemical pretreatments, commonly used in papermaking to partially remove lignin and hemicelluloses. In this technology, amounts of wastes are reduced to minimum because the black liquor is burnt to regenerate chemicals and produce heat, which is used to produce electric energy. In addition, the majority of substances that are contained in wood biomass, such as terpenes, resinous acids and lipids that are potent inhibitors of enzymatic hydrolysis and fermentation, are extracted as tall oil, which is a valuable raw material for chemical industry. Although kraft pulping has a long history in papermaking as an economically feasible and environmentally friendly process, it is underestimated in biotechnology.

Table 1 Average chemical composition of selected lignocellulosic materials

Biomass	Cellulose (% d.w.)	Hemicelluloses (% d.w.)	Lignin (% d.w.)	Extractives (% d.w.)	Minerals (% d.w.)
Softwood*	42	27	28	1–5	0.5–1
Hardwood*	44	33	20–22	2–4	0.5–1
Wheat straw*	38	36	17	3	6
Hemp bast**	80	14	6.0–6.6	–	5.8

* Przybysz (2007)

** Moriana et al. (2014)

In former reports, evidence was provided that pretreatment of wood and other sorts of lignocellulosic wastes by the sulfate method prior to enzymatic hydrolysis ensured the high yield of glucose and other simple sugars for fermentation processes (Buzała et al. 2015a, b). The crucial factors deciding on the extent of enzymatic pulp saccharification were the residual content of lignin and botanical origin of pulp. The published data showed that despite intensive studies on the development of new pretreatment technologies, kraft pulping may be still regarded a competitive pretreatment technology.

The aim of this work was to assess the utility of seven different kraft pulps produced from softwood (pine), hardwood (poplar, birch and beech), wheat straw and hemp (bast and harl) as potential sources of sugar feedstocks for fermentation processes. It was attempted to find the relationship not only between the residual lignin levels in kraft pulps and the extent of their enzymatic hydrolysis but also to estimate effects of the pulp's crystallinity index and average polymerization degree on enzymatic saccharification outcomes. The results of this study may help to establish a cost-effective industrial technology of utilization of superfluous kraft pulps and pulps that are characterized by insufficient quality to be used for paper production.

Materials and methods

Cellulosic pulps

Poplar, birch, beech and pine pulps were prepared by the sulfate method as described in Modrzejewski et al. (1969) from woodchips (25 mm × 16 mm × 8 mm), containing 7–8% moisture. Pulps from wheat straw and hemp harl and bast were prepared by the same method. The disintegrated materials were kept in hermetically closed vials to avoid any changes in humidity prior to the treatment with NaOH and Na_2S solutions [at the biomass:liquid ratio of 1:4 (w/v)], which were prepared freshly prior to the usage. Delignification processes were conducted in a 15-L stainless steel reactor with regulation of temperature. Suspensions of the disintegrated materials were heated for 120 min to achieve the temperature of 140–172 °C and incubated at this temperature for the next 120 min. Then, the temperature was decreased to around 25 °C using a jacket with cold tap water, and the insoluble residue was separated by filtration on Buchner funnel, washed several times with demineralized water and incubated overnight in demineralized water to remove residues of the alkali-soluble fractions. The solids were disintegrated for 3 min in a laboratory propeller pulp disintegrator (type R1 from Labor-Meks, Poland), and the fibers were screened using a membrane screener (0.2 mm gap). The fibers were collected and dried for 48 h at ambient temperature (22 ± 1 °C) and then weighed. Triplicate samples of these fibers were analyzed for the humidity (by the gravimetric method, after drying to constant weight) and residual lignin (Kappa number) contents. Kappa numbers of the pulps varied from 11 (for hemp bast) to 100 (for hemp harl), and their yields ranged from around 45 to 56% on a dry weight basis (Table 2), like in industrial conditions.

Table 2 Parameters of cellulosic pulp production from the selected materials

Biomass	Dose of bases A_{cz} (%)	T (°C)	Alkali consumption (%) relative to the dose applied	Pulp yield (%)	Uncooked fraction (%)	Kappa number
Pine (*Pinus sylvestris*)	22	172	96.9	40.4	11.8	31.4
Birch (*Betula pendula*)	20	165	94.5	53.1	4.8	28.3
Beech (*Fagus sylvatica*)	20	165	94.0	49.5	4.4	25.8
Poplar (*Populus tremula*)	20	160	84.4	52.3	1.5	15.4
Straw (*Triticum aestivum* L.)	18	160	96.6	52.3	4.8	30.8
Hemp–harl (*Cannabis sativa* L.)	15	140	99.6	68.7	3.5	100
Hemp–bast (*Cannabis sativa* L.)	15	140	98.4	86.2	0	11

Chemical composition of cellulosic pulps

Analysis of chemical composition of cellulosic pulps included quantification of extractives, lignin, cellulose and hemicelluloses. The content of lignin was determined by a gravimetric method in compliance with the Tappi T222 standard (Acid-Insoluble Lignin in Wood and Pulp) after the removal of extractives according to the Tappi T204 standard (Solvent Extractives of Wood and Pulp). The content of holocellulose was determined according to the Tappi Useful Method 249 (Cellulose in Pulp). Cellulose was quantified as alpha cellulose, according to the Tappi T203 standard (Alpha-, Beta- and Gamma-Cellulose in Pulp). The content of hemicelluloses was calculated as the difference between the holocellulose and cellulose contents. All these assays were performed in triplicate for each cellulosic pulp produced in this study.

Polymerization degree

The degree of polymerization (DP) of the cellulosic pulps was determined by the capillary viscometry, using an Ubbelohde 1C viscometer (Labit, Poland) placed in a water bath, which was used to maintain the temperature at 25 °C. Pulp samples of known dry weight were weighed into 50-mL PE flasks and mixed with 25 mL of distilled water, a dozen of glass ballotines and several 5 mm pieces of pure copper wire until the suspensions were homogenous. Then, an aliquot (25 mL) of 1.0 M bis(ethylenediamine)copper(II) hydroxide solution was added into each bottle, and the samples were further intensively mixed until the pulps were completely dissolved. The capillary of viscometer was rinsed with each of these solutions before measurements of the time of flow through this capillary. The measurements of the time of flow were conducted in triplicate for each pulp, and the solvent was used as a reference for DP calculations. The DP assays were performed according to ISO 5351:2010 standard: Pulps—Determination of limiting viscosity number in cupriethylenediamine (CED) solution.

Crystallinity index of pulps

The X-ray diffraction (XRD) method was used to determine the crystallinity index of cellulosic pulps obtained from birch, beech, poplar and pine wood as well as from wheat straw and hemp (bast and harl) biomass. Each tested material was ground in an agate mortar, and then approx. 300 mg sample was placed in a vial (16 mm in diameter and 2.4 mm thick), gently pressed to obtain a flat surface and placed in an automatic (15 sites) autosampler. The measurements were conducted using a multi-function polycrystalline X'Pert PRO MPD diffractometer (PANalytical).

The used CuKα radiation was obtained through monochromatization of X-ray radiation using a nickel filter. The primary beam ran through a permanent slit (0.5°), Soller slits (0.04 rad) and an anti-scatter slit (1.52 mm). The secondary beam ran through an anti-scatter slit (5 mm), the nickel filter, Soller slits (0.04 rad) and a X'Celerator silicon strip detector. The measurements were carried out over the range of 2θ angles, varying from 10° to 40°. During the continuous scan (step of 0.0167°), the time of measurements for one step was 50 s. WAXFIT program was used to reduce the recorded data and conduct calculations.

Data reduction included data standardisation, Compton radiation removal and background approximation and removal.

Enzyme preparation

A commercial, industrial-grade enzyme preparation NS-22086, showing activities of cellulases and xylanases, was kindly supplied by Novozymes A/S (Denmark). Activities of these enzymes were assayed by the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959) at pH 5.0 and 50 °C for 0.5% carboxymethylcellulose and 0.5% birch xylan, respectively (reaction time of 5 min). Activities of both the glycosidases were expressed as micromoles of reducing sugars released from the polysaccharide substrates in 1 min (U). The filter paper activity was determined at pH 5.0 and 50 °C according to Adney and Baker (2008) and expressed as FPU/mL. Total reducing sugars and glucose concentrations in NS-22086 were assayed as described below.

Enzymatic hydrolysis

Enzymatic hydrolysis processes were conducted at 50 °C, at 1.3% (w/w) substrate concentration. Samples of the above listed substrates (around 0.3 g wet mass, 0.28 g dry weight) were suspended in 0.1 M sodium acetate buffer solution (pH 5.0, 20 mL) and incubated for 15 min in a water bath at 50 °C. Then, 1 mL of the preparation NS-22086 (diluted sixfold in the same buffer) was added (with vigorous mixing) to each of the substrate suspensions to initiate enzymatic digestion. All the hydrolysates were sampled just after addition of the enzyme (to determine initial concentrations of glucose and total reducing sugars) and after 1, 3, 6, 24, 48 h of the process (to follow the progress of enzymatic hydrolysis). All samples of enzymatic hydrolysates were filtered through a medium–fast filter paper, and the filtrates were subjected to analyses. Dry weight of the insoluble residues after enzymatic

hydrolysis was determined gravimetrically after drying to constant weight at 105 °C. Microscopic observations of fibers subjected to enzymatic treatment were conducted at 200× magnification using a MPI3/SK12 PZO (Poland) optical microscope.

Analysis of enzymatic hydrolysates

Reducing sugars concentration was determined according to Miller (1959) using the alkaline DNS solution. Mono- and disaccharide profiles of the hydrolysates were determined by HPLC using an Ultimata 3000 Dionex liquid chromatograph equipped with a Rezex RPM-Monosaccharide Pb^{2+} column (8 μm, 7.8 × 300 mm) and a Shodex-RI-10 refractive index detector. The temperature of the column and RI detector was 80 and 40 °C, respectively. Samples of hydrolysates were filtered through a nylon syringe filter (0.45 μm) before HPLC analysis. The volume of injected samples was 10 μL. HPLC-grade water (Sigma) was used as the mobile phase at a flow rate of 0.6 mL/min. Results of sugar resolution were recorded over 35 min. Glucose concentration was also determined according to Barham and Trinder (1972), using a commercial diagnostic kit employing glucose oxidase and peroxidase (BioMaxima, Poland). The assay was conducted according to the instruction from the manufacturer of the kit.

Both hydrolysis processes and analyses of the hydrolysates were carried out in at least triplicate. Their results are presented as mean ± standard deviation (SD).

Calculations of hydrolysis yield

Glucose and total reducing sugars yields from the pulps, woodchips and fines dry weight were calculated according to the equations:

$$\text{Glucose yield} = \text{Glucose in hydrolysate (g)} \times 0.9/\text{Initial dry weight of the sample (g)}$$

$$\begin{aligned}\text{Total reducing sugars yield} = {} & (\text{Hexoses in hydrolysate (g)} \times 0.9 \\ & + \text{Cellobiose in hydrolysate (g)} \times 0.95 \\ & + \text{Pentoses in hydrolysate (g)} \times 0.88)/\text{Initial dry weight of the sample (g)}\end{aligned}$$

The correction factors of 0.9, 0.88 and 0.95, corresponding to hexoses, pentoses and cellobiose (Van Dyk and Pletschke 2012), were used in the calculations to compensate for the addition of a water molecule during hydrolysis of each glycosidic bond.

To determine the impact of lignin content on the degree of cellulose saccharification, glucose yields from cellulose contained in the pulps and woodchips were calculated according to Kumar and Wyman (2009):

$$\begin{aligned}\text{Glucose yield} = {} & [\text{Glucose in hydrolysate (g)} + 1.053 \\ & \times \text{Cellobiose in hydrolysate (g)}]/[1.111 \\ & \times \text{Initial amount of glucan (g)}]\end{aligned}$$

Results and discussion

Characterization of cellulosic pulps

Kraft pulping is a severe and highly efficient technology of lignocellulosic biomass pretreatment that was proved by the high cellulose content in the seven kraft pulps, ranging from around 76% d.w. for the hemp harl pulp to around 96% for the hemp bast pulp, and the low concentrations of extractives (up to around 0.48% d.w. in the hemp harl pulp) (Table 3). The percentage of hemicelluloses varied between around 1.9% for the hemp bast pulp and around 8.2% for the hemp harl pulp. In addition, lignin concentration was the lowest (around 1.7%) for the hemp bast pulp and the highest (around 15%) for the hemp harl pulp. Percentages of these three polymers and extractives in the hardwood, softwood and wheat straw pulps were similar (89–95% d.w. cellulose, 2–5.5% d.w. hemicelluloses, 2.3–4.7% d.w. lignin and 0.06–0.09% d.w. extractives). The low concentrations of hemicelluloses and lignin in the seven cellulosic pulps are advantageous because it was observed that not only the content of lignin but also the percentage of residual hemicelluloses decide on the extent of enzymatic cellulose degradation (Kabel et al. 2007). According to the latter authors, cellulose degradability increased with the decrease in the amount of xylan remaining after lignocellulosic biomass pretreatment.

The traditional cellulose model describes cellulose chains as containing ordered, crystalline and amorphous (less ordered) regions. The X-ray diffraction method considers the contributions from crystalline and amorphous cellulose to the entire XRD spectra and is generally regarded an accurate technique of the crystallinity index measurement, although the crystallinity values obtained by this method may be higher than those obtained by the other methods (Park et al. 2010; Cao and Tan 2005; Driemeier and Calligaris 2011). The X-ray diffractograms of the seven kraft pulps, which were produced as described above, suggested the presence of

Table 3 Chemical composition, crystallinity index and average polymerization degree of the seven kraft pulps

Pulp	Cellulose (% d.w.)	Hemicelluloses (% d.w.)	Lignin (% d.w.)	Extractives (%)	Crystallinity index (%)	DP
Poplar pulp	95.55 ± 0.12	2.06 ± 0.08	2.31 ± 0.06	0.08 ± 0.04	69	1482 ± 20
Pine pulp	91.08 ± 0.08	4.12 ± 0.04	4.71 ± 0.04	0.09 ± 0.03	70	1127 ± 16
Birch pulp	92.26 ± 0.09	3.42 ± 0.06	4.25 ± 0.08	0.07 ± 0.05	67	1482 ± 22
Beech pulp	91.22 ± 0.14	4.85 ± 0.08	3.87 ± 0.04	0.06 ± 0.03	65	1345 ± 35
Wheat straw pulp	89.78 ± 0.10	5.51 ± 0.06	4.62 ± 0.02	0.09 ± 0.04	55	1350 ± 18
Hemp harl pulp	76.29 ± 0.34	8.22 ± 0.29	15.01 ± 0.26	0.48 ± 0.05	64	464 ± 8
Hemp bast pulp	96.11 ± 0.09	1.88 ± 0.03	1.65 ± 0.02	0.36 ± 0.06	79	230 ± 7

amorphous and crystalline regions. The values of crystallinity index of these pulps ranged from 55 to 79% (Table 3). This index was the lowest for the pulp from wheat straw and the highest for the pulp from hemp bast, which was characterized by the lowest lignin content (around 1.7% d.w., Kappa number of 11) and the lowest average polymerization degree (around 230). Further, the DP of the pulp from hemp harl, which was characterized by the highest lignin content (around 15% d.w., Kappa number of 100), was low (around 464). The average polymerization degree was the highest in case of the poplar and birch pulps (around 1482). The DP of the wheat straw pulp (around 1350) was alike the DP of the pulp from beech (around 1345) and higher than DP of the pine pulp (around 1127). The degree of polymerization of the pulps was measured by the viscometric technique, which is the most widely used method (Karimi and Taherzadeh 2016). Although the DP values determined in this relatively simple way are not strictly the same as those measured using more advanced techniques, for example gel permeation chromatography, membrane osmometry and cryoscopy, the viscometric technique produces reasonable DP values and enables to compare the effects of lignocellulosic biomass pretreatment.

Enzymatic hydrolysis of cellulosic pulps

To determine the effect of the lignin and hemicellulose contents, crystallinity index and average polymerization degree of the pulps on their susceptibility to enzymatic degradation, these pulps were subjected to enzymatic hydrolysis under identical conditions, as described in "Materials and methods" section, using the multienzyme preparation NS-22086. The latter preparation was selected in a former study (Buzała et al. 2015a) as an efficient biocatalyst, enabling the nearly complete hydrolysis of various cellulosic pulps and fines from papermaking as well as partial hydrolysis of diverse woodchips and chopped wheat straw. This preparation displays the activities of cellulases (80.6 U/mL for CMC and 112.12 FPU/mL at 50 °C and pH 5.0) and xylanases (192.5 U/mL at 50 °C and pH 5.0) (Buzała et al. 2015b). Because of the relatively high content of reducing sugars (66.6 mg/mL), including glucose (42.0 mg/mL), this multienzyme preparation was diluted sixfold before mixing with the substrates, and the amounts of sugars contained in this solution were discounted when the yields of substrates saccharification were calculated.

The results of glucose assays during enzymatic hydrolysis of the pulps showed that the rate of this process was the highest within the first 6 h (Fig. 1), particularly in the case of pulps from which the highest amounts of glucose were obtained, such as the wheat straw (around 13 mg/mL), poplar (around 12.7 mg/mL), beech (around 12.6 mg/mL) and birch (around 12 mg/mL) ones (Table 4). The hydrolysis of pine pulp was slower, but the ultimate (after 48 h) glucose concentration (around 11.4 mg/mL) in its hydrolysate was also higher than that in the hydrolysates of hemp harl (around 8.2 mg/mL) and hemp bast (around 5 mg/mL) pulps. Despite the low contents of hemicelluloses (around 1.9% d.w.) and lignin (around 1.7% d.w., reflected by the low Kappa number of 11) and DP (around 230), hydrolysis of the latter pulp was the slowest (Fig. 1) that was correlated with the highest crystallinity index (79%). The high value of crystallinity index of bast fibers was also reported

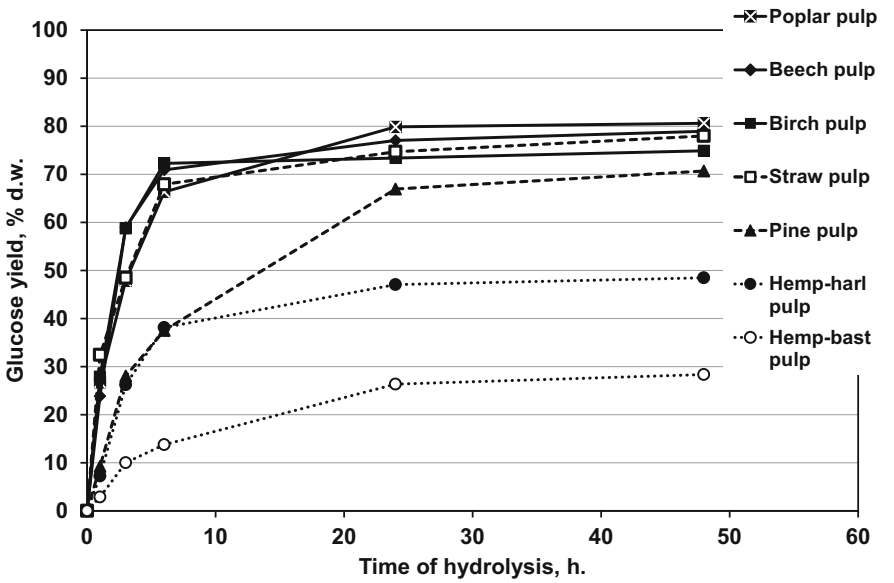

Fig. 1 Dependence of glucose yields obtained by enzymatic hydrolysis on the origin of cellulosic pulp

Table 4 Concentrations and yields of glucose and total reducing sugars in enzymatic hydrolysates of poplar, pine, beech, birch, wheat straw and hemp pulps (50 °C, pH 5.0, 48 h)

Substrate	Mean glucose concentration (mg/mL)	Glucose yield		Mean reducing sugars concentration (mg/mL)	Total reducing sugars yield	
		% d.w. pulp	% d.w. wood, straw, hemp		% d.w. pulp	% d.w. wood, straw, hemp
Poplar pulp	12.67 ± 0.15	79.00	41.30	16.51 ± 0.18	100	52.30
Pine pulp	11.41 ± 0.23	70.72	28.57	14.47 ± 1.65	88.92	35.90
Beech pulp	12.62 ± 0.15	78.98	39.10	16.86 ± 1.04	100	49.50
Birch pulp	11.98 ± 0.30	74.87	39.76	15.90 ± 2.49	98.71	52.42
Wheat straw pulp	12.96 ± 0.07	77.99	44.06	17.67 ± 0.13	100	56.50
Hemp harl pulp	8.18 ± 0.05	48.47	33.30	11.08 ± 0.35	65.07	44.70
Hemp bast pulp	5.01 ± 0.08	28.34	24.43	7.24 ± 0.53	40.56	34.96

by Gümüşkaya and Usta (2006). However, the latter fibers were characterized by the much higher degree of polymerization (2411) than in this study. This discrepancy may be ascribed to the difference in the applied pretreatment methods because Gümüşkaya and Usta (2006) used the sulfite method. The biomass of hemp harl was more recalcitrant to kraft pulping than the biomass of hemp bast because the hemp harl pulp contained much more residual hemicelluloses (around 8.2 vs. 1.9% d.w., respectively), lignin (around 15 vs. 1.7% d.w., respectively) and

extractives (around 0.48 vs. 0.36% d.w., respectively). However, the hemp harl pulp had lower crystallinity index (64%) than the hemp bast pulp, and despite the high contents of lignin, extractives and hemicelluloses as well as higher DP (around 464 versus 230), it was hydrolyzed not only faster but also to a higher extent. The glucose and total reducing sugar yields obtained within 48 h from the hemp bast pulp, which was particularly recalcitrant to enzymatic digestion, reached only around 28 and 40.6% d.w. pulp, respectively, while the yields obtained from the hemp harl pulp were around 1.6-fold higher (around 48 and 65% d.w. pulp, respectively) (Table 4). The results presented in Fig. 1 and Table 4 suggest that the value of crystallinity index had a strong impact on the susceptibility of the compared cellulosic pulps to enzymatic degradation. This observation is consistent with the results reported by Cateto et al. (2011).

The highest glucose yields were obtained from poplar (79% d.w.), beech (nearly 79% d.w.) and wheat straw (nearly 78% d.w.) pulps (Table 4). These yields decreased with a rise in the Kappa number of these pulps (of 15.4, 25.8 and 30.8, respectively). In addition, the yields of total reducing sugars from these pulps were the highest (100% d.w.). The hydrolysis of birch pulp (Kappa number of 28.3) was only slightly less effective (around 75% d.w. glucose and 99% d.w. reducing sugars). In general, more reducing sugars, including glucose, were obtained from the hardwood pulps and wheat straw pulp than from pine (Kappa number of 31.4) pulp (around 89 and 71% d.w., respectively) and from both hemp pulps.

However, the short (around 1 mm) hemp bast fibers contain much less lignin (around 6.0–6.6% d.w.) (Moriana et al. 2014) than wood and straw biomass and the yield of hemp bast pulp was higher (86.2% d.w.) than the yields of hemp harl (68.7% d.w.), wheat straw (52.3% d.w.) and wood (40.4–53.1% d.w.) pulps (Table 2). Therefore, the yields of glucose and total reducing sugars from hemp bast (around 24 and 35% d.w., respectively) were comparable to those from pine wood (around 29 and 36% d.w., respectively) (Table 4). The yields of glucose and total reducing sugars from hemp harl (around 33 and 45% on a dry weight basis, respectively) were higher compared to those from hemp bast, but lower compared to wheat straw (around 44 and 57% d.w.), birch wood (around 40 and 52% d.w.), poplar wood (around 41 and 52% d.w.) and beech wood (around 39 and 50% d.w., respectively).

Irrespective of the botanical origin of pulp, glucose was the dominating (69–79% w/w) soluble sugar in all the enzymatic hydrolysates (Table 5), which also contained cellobiose (9–25% w/w) and mannose (0.3–6.8% w/w). The highest amounts of these two reducing sugars (24–25% w/w cellobiose and 4.7–6.8% w/w mannose) were found in the hemp hydrolysates, which contained only around 69% w/w glucose. The hydrolysates of wheat straw pulp contained the least amounts of cellobiose (around 9% w/w) and the highest amounts of xylose (above 16% w/w). Xylose was not identified in the hemp hydrolysates, which also did not contain arabinose (like the hydrolysates of poplar and beech pulps). The relatively small amounts of xylose, mannose and arabinose in the hydrolysates were consistent with the low contents of hemicelluloses in the kraft pulps (Table 3). The high ultimate cellobiose concentration is usually ascribed to the inhibition of beta-glucosidase by glucose released by cellulolytic enzymes (Andric et al. 2010). However, the

Table 5 Mean percentage contents of glucose, cellobiose, xylose, arabinose and mannose among mono- and disaccharides contained in hydrolysates of tested pulps

Substrate	Glucose (% d.w.)	Cellobiose (% d.w.)	Xylose (% d.w.)	Arabinose (% d.w.)	Mannose (% d.w.)
Poplar pulp	77.96 ± 0.04	11.15 ± 0.03	10.27 ± 0.05	n.d.	0.62 ± 0.05
Pine pulp	78.88 ± 0.08	12.54 ± 0.06	3.45 ± 0.05	3.59 ± 0.05	1.54 ± 0.06
Beech pulp	74.86 ± 0.12	11.08 ± 0.06	13.31 ± 0.04	n.d.	0.75 ± 0.05
Birch pulp	75.36 ± 0.09	11.46 ± 0.05	12.29 ± 0.04	0.34 ± 0.02	0.54 ± 0.04
Wheat straw pulp	73.33 ± 0.11	8.98 ± 0.07	16.26 ± 0.05	1.06 ± 0.06	0.36 ± 0.06
Hemp harl pulp	69.15 ± 0.24	24.88 ± 0.09	n.d.	n.d.	4.65 ± 0.06
Hemp bast pulp	69.21 ± 0.04	23.98 ± 0.03	n.d.	n.d.	6.81 ± 0.04

n.d. not detected

concentrations of glucose in the hydrolysates obtained were too low to inhibit beta-glucosidase. Because the concentration of cellobiose was the highest in the hydrolysates of hemp pulps, which were the richest in extractives, presumably beta-glucosidase was inhibited by these compounds. The presence of cellobiose and xylose in hydrolysates of lignocellulosic biomass is not disadvantageous because these sugars may be converted by various microorganisms to value added products, including bioethanol. In contrast to wild-type *Saccharomyces cerevisiae* strains that cannot produce ethanol from xylose and cellobiose, the engineered strains co-ferment these sugars (Aeling et al. 2012; Hawkins et al. 2013). In general, the sugar profiles of the hydrolysates obtained in this study, particularly the high glucose level, enable their application as sugar feedstocks in fermentation processes.

Conclusion

The results of this work showed that kraft pulping is an efficient method of lignocellulosic biomass pretreatment, enabling the removal of the large part of lignin and hemicelluloses, and kraft pulps produced from pine, poplar, birch and beech wood, wheat straw and hemp (bast and harl) are potential sources of sugar feedstocks for fermentation processes. The hydrolysates obtained in this study were rich in glucose (69–79% w/w of soluble sugars) and contained also cellobiose (9–25% w/w), mannose (0.3–6.8% w/w) and xylose (3.5–16.3% w/w), with an exception of the hemp hydrolysates, which contained neither xylose nor arabinose (the latter was also absent in the hydrolysates of poplar and beech pulps). The extent of enzymatic hydrolysis was the highest in the case of the wheat straw and hardwood pulps (reducing sugar yields of 100% d.w., glucose yields of 75–79% d.w.), slightly lower for the pine pulp (around 89% d.w. reducing sugars and 71% d.w. glucose) and the lowest in the case of the hemp bast pulp (glucose and reducing sugar yields of only around 24 and 40.5% d.w., respectively). The results of this study provide evidence that various kraft pulps produced in paper mills that either are characterized by insufficient quality to be used for paper production or are

superfluous, may be efficiently converted to glucose and other fermentable sugars using suitable multienzyme preparations.

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