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Production of Sugar Feedstocks for Fermentation Processes from Selected Fast Growing Grasses

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Abstract: This study showed that kraft cellulosic pulps from *Miscanthus giganetus* JM Greef and Deuter ex Hodk. and Renvoize, sweet sorghum and 5 other fast growing grasses may be easily enzymatically converted to glucose-rich sugar feedstocks. The scientific goal of the paper was to assess and compare the potential yield of hydrolysis and verify whether these grasses may be a source of sugars for fermentation processes. Kraft pulping was used as a pretreatment method and hydrolysis of the pulps was conducted using a commercial multienzyme preparation containing cellulases and xylanases at initial substrate concentrations of 0.476, 3.88 and 7.46% *w*/*v*, and 3 different enzyme loadings. Results showed that tall wheatgrass, striped tuber oat grass, tall fescue and smooth bromegrass may be efficiently converted to sugar feedstocks for biotechnology application, but that the simple reducing sugars yield is lower than for wood, due to lower cellulose content.

Keywords: cellulosic pulps; enzymatic hydrolysis; kraft pulping; alternative fibrous raw materials

1. Introduction

The growing demand for energy, fuels and chemicals, depletion of nonrenewable fossil resources and increasing concerns of environmental pollution have given rise to the hunt for renewable feedstocks for various branches of industry [1]. Recently in the scientific literature, much information concerning new energy sources and technology has been indicated. Published by Lazaroiu et al., results showed that use of animal waste fats in blends with diesel fuel for an experimental engine is possible [2] or that the combustion of solid biomass in the presence of hydrogen enriched gas (HRG) leads to positive synergistic effects, thus reducing not only the overall level of pollutants produced (NO_x, CO, CO_2, dust), but also the SO_2 concentration by injection of hydrogen or HRG [3]. Authors also widely described efficient and innovative technology for co-combustion with HRG of solid biomass [4]. It should be noted that one of the of most attractive renewable resources is the lignocellulosic biomass of fast-growing perennials [5] and annuals [6], including grasses [7]. Perennial grasses have been increasingly used as energy crops (mainly as solid biofuels and for biogas production) [8,9], because of the higher cellulose and lignin contents, as compared to the biomass of annual crops, that results in a higher heating value [10]. Fast growing grasses, such as switchgrass (*Panicum virgatum* L.) [11–13], *Miscanthus giganetus* JM Greef and Deuter ex Hodk. and Renvoize [14,15], and sweet sorghum (*Sorghum bicolor* L.) [16–18], have been most studied. Cultivars of the latter grass were successfully used for biogas production by anaerobic digestion, yielding 207–387 $m^3 \times t^{-1}$ methane [15]. Switchgrass (*Panicum virgatum* L.) is considered a potential feedstock for production of liquid fuels such as ethanol and value added co-products [11]. Studies on compositional changes of its biomass over the harvesting season showed that it is possible to optimize the harvesting process to ensure the complete biomass utilization for a variety of products [19,20]. Miscanthus is a popular fast growing nonfood feedstock, widely applied not only as an energy crop, but also as livestock feed and organic fertilizer [21,22].

The high percentage of lignin in woods and grasses ensures the high heating value, which is attractive when energy is produced. However, this phenolic, highly cross-linked polymer negatively affects biomass conversion to glucose and other fermentable sugars, because it not only forms a seal protecting fibers of cellulose and hemicelluloses from the attack of hydrolytic enzymes, but also binds the latter. Therefore, lignocellulosic biomass must be pretreated to partially remove lignin [23,24]. Another important objective of pretreatment is to reduce the crystallinity and degree of polymerization of cellulose to enhance its enzymatic digestion [25]. The promising plant biomass pretreatment technologies developed to date include acid or SO_2 pre-impregnated steam explosion [26–28], wet explosion [29], organosolv pretreatment [30,31], and alkaline hydrogen peroxide (AHP) pretreatment [29,32]. However, these processes ensure neither selective lignin removal nor economic gain [33,34]. To reduce the overall costs of lignocellulosic biomass to ethanol conversion, a dry biorefining approach with costs close to that of corn ethanol production, has been proposed [35].

One of the best established delignification technologies is kraft pulping [36]. This cost effective chemical pretreatment is commonly used in papermaking. Although kraft pulping also causes partial removal of hemicelluloses from the lignocellulosic biomass, the amount of waste is reduced to a minimum because the black liquor is incinerated to regenerate chemicals and produce heat, which is used to produce electric energy. Our former studies showed that pretreatment of wood and other lignocellulosic materials by the sulfate method before enzymatic hydrolysis resulted in high glucose percentages in enzymatic hydrolysates while the contents of other simple sugars and cellobiose were relatively low [23,37]. Therefore, in this study grass biomass was also pretreated by this method. Furthermore, we showed that cellulosic pulps obtained from *Miscanthus giganetus* JM Greef and Deuter ex Hodk. and Renvoize, switchgrass (*Panicum virgatum* L.), tall wheatgrass (*Agropyron elongatum* (Host). Beauv.), smooth bromegrass (*Bromus inermis* Leyss.) and tall fescue (*Festuca arundinacea* Schreb.) are potentially attractive for papermaking [38]. Thus, efficient conversion of these pulps to sugar feedstocks in the case of excess or too low a quality may help to establish a cost-effective utilization technology. Apart from the latter grasses, this study involved striped tuber oat grass (*Arrhenatherum elatius* (L.) P. Beauv. ex J. and C. Presl) and sweet sorghum (*Sorghum bicolor* (L.) Moench.). All these grasses are adaptable to a variety of soil and climatic conditions, and can easily be propagated feedstocks.

The objective of this work was to assess and compare the potential of seven fast growing grasses such as switchgrass (*Panicum virgatum* L.), *Miscanthus giganetus* JM Greef and Deuter ex Hodk. and Renvoize, sweet sorghum (*Sorghum bicolor* (L.) Moench. cultivar S-N), smooth bromegrass (*Bromus inermis* Leyss. Budzynska variety), tall fescue (*Festuca arundinacea* Schreb.), striped tuber oat grass (*Arrhenatherum elatius* (L.) P. Beauv. ex J. and C. Presl), and tall wheatgrass (*Agropyron elongatum* (Host). Beauv. cultivar A2) as a source of sugars for fermentation processes. The kraft pulps obtained in this study were subjected to enzymatic hydrolysis at three different enzyme loadings and three different initial substrate concentrations, of 0.476, 3.88 and 7.46% *w/v*, in order to evaluate the susceptibility to enzymatic digestion and potential utility for biotechnology, e.g., fuel ethanol production.

2. Materials and Methods

2.1. Raw Materials

The biomass of the grasses tested (*Miscanthus giganetus* JM Greef and Deuter ex Hodk. and Renvoize, *Festuca arundinacea* Schreb. Bromus inermis Leyss. *Arrhenatherum elatius* (L.) P. Beauv. ex J. and C. Presl, *Panicum virgatum* L. *Agropyron elongatum* (Host). Beauv. *Sorghum bicolor* (L.) Moench.) was harvested using a reel lawn mower ALKO 5001 R-II (ALKO, Germany), in the generative (blooming) phase when the contents of cellulose and other fibrous polymers were the highest. The biomass was dried to the humidity of 10% and chopped to 1.5 to 2.0 cm chaff using a MTD 475 petrol powered shredder (Briggs and Stratton, Viernheim, Germany), dedicated for disintegration of tree branches.

2.2. Chemical Composition of Grasses

Raw materials were disintegrated in a laboratory mill SM 100 (Retsch, Haan, Germany), screened through 0.63 mm wire, retained on 0.5 mm wire using a LPzE-3e vibration screener (Multiserv Morek, Brzeźnica, Poland) and subjected to analysis. The dry weight (DW) of all grasses was determined before pulping. The content of lignin was determined by a gravimetric method (Tappi T222 standard [39]) after the removal of extractives (Tappi T204 standard [40]). The content of holocellulose was determined according to the Tappi T249 standard [41]. Cellulose was quantified as alpha cellulose, according to the Tappi T203 standard [42]. The content of hemicelluloses was calculated as the difference between the holocellulose and cellulose contents. Ash content was determined by gravimetric method in compliance with the Tappi T211 standard [43]. All these assays were performed in triplicate for each raw material.

2.3. Cellulosic Pulps

Cellulosic pulps were prepared as described by Modrzejewski et al. [44]. Grass biomass (500 g DW) was suspended in 5 dm^3 of alkaline sulfate solution. The pulping conditions were as follows: active alkali 26% (260 g per batch), sulfidity 30%, liquid module 10.0, maximal digestion temperature 165 °C, heating time to maximal temperature 120 min, digestion time at maximal temperature 120 min, and cooling time to ambient temperature 15 min. The pulping conditions were optimized within the scope of our previous (unpublished) research activities.

Pulping was carried out using a 15 L laboratory heated digester PD-114 (Danex, Katowice, Poland) with agitation (3 swings per minute, swinging angle of 60°). The temperature was controlled using a dedicated RE82 driver (LUMEL, Zielona Góra, Poland) governed by a Lumel Process computer program that enabled recording of the data.

At the end of cooking, the content of the digester was cooled with 240 dm^3 of cold water, and a sample of residual base (0.5 dm^3) was withdrawn and characterized. The consumption of bases was expressed as the percentage of bases that underwent reaction with the fibrous materials (Table 2). Then the pulp was washed with 50 dm^3 of water and soaked overnight in 10 dm^3 of water to remove base residues. The fibrous biomass was disintegrated using a laboratory JAC SHPD28D propeller pulp disintegrator (Danex, Poland) at 12,000 revolutions, and screened using a PS-114 membrane screener (Danex, Poland) (0.2 mm gap). After screening, the pulps and shives were dried at room temperature (20 to 22 °C) for 48 h, and weighted to determine the yield of pulps and shives contents. The dry pulps were stored in hermetically closed vials before further experiments.

The pulps were characterized in terms of the yield from digester, the yield after screening, the contents of shives, and the residual lignin content, expressed as the Kappa number. The average polymerization degree of cellulose contained in the pulps was determined by the viscometric method ISO 5351 [45]. Chemical composition of cellulosic pulps (cellulose, hemicelluloses, lignin, ash and extractives) were determined according to the same standards as for raw material.

2.4. Enzyme Preparation

A commercial, industrial grade enzyme preparation NS-81235, showing activities of cellulases and xylanases, was purchased from Novozymes A/S (Denmark). Activities of these enzymes were assayed by the 3,5-dinitrosalicylic (DNS) method [46] 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). Total reducing sugars concentration in NS-81235 was assayed using the DNS method. Cellulases activities in the NS-81235 enzyme preparation (temperature 50 °C, pH 5.0) were 69.66 ± 0.06 U/mL and for xylanases this activity reached 141.92 ± 0.56 U/mL. All the tests were carried out in at least triplicate.

2.5. Enzymatic Hydrolysis of Kraft Pulps

Enzymatic hydrolysis of the kraft pulps was conducted at 50 °C in a shaken water bath SWB-22 (Laboplay, Bytom, Poland) (30 shakes per minute) for 24 h, at three substrate concentrations of 0.476, 3.88 and 7.465% (*w/v*) and three loads of the enzyme preparation (0.1 mL/g, 0.05 mL/g and 0.033 mL/g). Cellulosic pulps were suspended in 0.1 M sodium-acetate buffer solution (pH 5.0, 20 mL) and incubated for 15 min in the water bath at 50 °C before addition of the diluted preparation NS-81235 (1 mL, with vigorous mixing) to initiate enzymatic digestion. All the hydrolysates were sampled just after the addition of the enzyme to determine the initial concentration of reducing sugars. After 24 h, all the hydrolysates were filtered through a medium-fast filter paper (Munktell Ahlstrom, Bärenstein, Germany) and the filtrates were analyzed for glucose concentration using a commercial diagnostic kit (Biomaxima, Lublin, Poland) employing glucose oxidase and peroxidase [47], and reducing sugars concentration according to Miller [46] using the alkaline DNS solution. Dry weight of the insoluble residues after enzymatic hydrolysis was determined gravimetrically after drying to constant weight at 105 °C.

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

2.6. Calculation of Hydrolysis Yield

Total reducing sugars concentration was expressed as mg hexoses in 1 mL of hydrolysate. The yield of reducing sugars was calculated using the correction factor of 0.9, to compensate for the addition of a water molecule during hydrolysis of each glycosidic bond [45]:

$$\text{Total reducing sugars yield} = \text{hexoses in hydrolysate (g)} \times 0.9/\text{initial dry weight of the sample (g)}, \quad (1)$$

The yield of glucose in the hydrolysates was calculated using the same correction factor of 0.9:

$$\text{Glucose yield} = \text{glucose in hydrolysate (g)} \times 0.9/\text{initial dry weight of the sample (g)}. \quad (2)$$

3. Results and Discussion

3.1. Characterization of Lignocellulosic Substrates and Cellulosic Pulps

The percentage contents of cellulose, hemicelluloses, lignin, extractives and ash in the seven raw materials are presented in Table 1. Among the grasses, the richest sources of cellulose were tall wheatgrass cultivar A2 (47.6% DW) and miscanthus (47.2% DW). Contents of hemicelluloses in grasses varied from 25.9% DW (miscanthus) to 47.4% DW (sweet sorghum, cultivar S-N). The richest source of lignin was tall wheatgrass cultivar A2 (14.5% DW), while the lowest lignin content was in striped tuber oat grass at 12.2%.

The consumption of bases during pulping was above 95% in all the cases (Table 2). The highest yields of pulp after screening were obtained from miscanthus and tall wheatgrass cultivar A2 (around 47.1 and 44.2% DW, respectively). The shives content and Kappa number for these pulps were similar (Table 2). Kraft pulping is known to remove not only a part of lignin but also most of the hemicelluloses from the plant biomass. Table 3 presents the chemical composition of the obtained pulps, while Table 4 presents a fraction of the given chemical compound that was retained in the cellulosic pulp. The rest was dissolved in cooking liquor (black liquor). In all investigated kraft pulps cellulose content is above 90% while lignin content is below 2.2%. Most of the hemicelluloses and extractives were dissolved during cooking. Approximately less than 7% of the hemicelluloses present in the raw material were retained in the pulp after cooking. High hemicelluloses content in raw material and the loss of the majority of this compound during delignification led to low yields of pulps from the digester. Only in the case of miscanthus was the yield of pulp similar to the yield for wood.

Table 1. Chemical composition of the tested lignocellulosic materials.

Material	Cellulose	Hemicelluloses	Lignin	Extractives	Ash
	% DW				
Miscanthus giganteus	47.2 ± 0.1	25.9 ± 0.8	17.8 ± 0.8	2.6 ± 0.6	6.5 ± 0.2
Tall wheatgrass cultivar A2	47.6 ± 0.4	29.4 ± 0.7	14.1 ± 0.6	3.0 ± 0.2	5.9 ± 0.1
Smooth bromegrass (Budzynska cultivar)	35.4 ± 0.1	40.2 ± 0.4	13.7 ± 0.5	4.2 ± 0.5	6.5 ± 0.2
Tall fescue	34.4 ± 0.3	40.9 ± 0.3	14.0 ± 0.8	3.8 ± 0.6	6.9 ± 0.3
Striped tuber oat grass	39.7 ± 0.4	39.4 ± 0.7	12.2 ± 0.2	2.8 ± 0.4	5.9 ± 0.3
Switchgrass	40.7 ± 0.7	30.4 ± 0.6	17.4 ± 0.9	4.1 ± 0.4	7.4 ± 0.3
Sweet sorghum cultivar S-N	32.9 ± 0.3	47.4 ± 0.5	12.8 ± 0.6	3.1 ± 0.2	3.8 ± 0.4

Note: Values are the means ± standard deviation (SD).

Table 2. Kraft pulping conditions * and characteristics of cellulosic pulps obtained by the sulfate method.

Material	Consumption of Bases	The Yield of Pulp from Digester	The Yield of Pulp after Screening	Shives	Kappa Number	DP
	%	%	%	%	-	-
Miscanthus giganteus	98.80 ± 0.03	47.82 ± 0.46	47.12 ± 0.31	1.47 ± 0.18	14.31 ± 0.16	1649 ± 26
Tall wheatgrass, cultivar A2	98.64 ± 0.05	44.95 ± 0.28	44.22 ± 0.39	1.62 ± 0.12	13.82 ± 0.24	1822 ± 41
Smooth bromegrass (Budzynska cultivar)	98.84 ± 0.03	34.67 ± 0.28	34.58 ± 0.16	0.27 ± 0.02	14.42 ± 0.16	1623 ± 57
Tall fescue	99.35 ± 0.04	34.08 ± 0.29	34.02 ± 0.23	0.18 ± 0.02	12.71 ± 0.24	1663 ± 38
Striped tuber oat grass	99.51 ± 0.04	33.23 ± 0.39	33.19 ± 0.29	0.12 ± 0.02	8.86 ± 0.25	1563 ± 44
Switchgrass	99.32 ± 0.06	34.47 ± 0.31	34.09 ± 0.22	1.11 ± 0.04	13.71 ± 0.12	1640 ± 52
Sweet sorghum cultivar S-N	95.87 ± 0.06	15.49 ± 0.23	15.46 ± 0.31	0.22 ± 0.09	7.54 ± 0.14	1310 ± 29

* Dry weight of chaff 500 g, maximal digestion temperature 165 °C, heating time to maximal temperature 120 min, digestion time at maximal temperature 120 min, sulfidity 30%, active alkali 260 g, effective alkali 221.00 g, liquid module for grasses 10.0. Note: Values are the means ± standard deviation (SD).

The values of the Kappa number ranged from around 7.5 for the pulp from sweet sorghum to around 14.4 for the smooth bromegrass pulp. Corresponding results were obtained for lignin content which varied from 1.1 up to 2.2%. In general, the pulps derived from the grasses were characterized by the significantly lower values of the Kappa number, as compared to the reported hardwood and softwood pulps, which were delignified under the same conditions [24,37,38]. It means that this material is susceptible to delignification using the kraft method.

Table 3. Chemical composition of cellulosic pulps.

Material	Cellulose	Hemicelluloses	Lignin	Extractives	Ash
	%				
Miscanthus giganteus	91.5 ± 0.4	3.6 ± 0.2	2.2 ± 0.2	0.3 ± <0.1	2.4 ± 0.2
Tall wheatgrass, cultivar A2	91.7 ± 0.6	4.1 ± 0.3	2.0 ± 0.2	0.2 ± <0.1	2.0 ± 0.1
Smooth bromegrass (Budzynska cultivar)	90.0 ± 0.5	5.4 ± 0.4	2.1 ± 0.3	0.3 ± <0.1	2.2 ± 0.2
Tall fescue	90.1 ± 0.7	5.3 ± 0.4	1.7 ± 0.1	0.3 ± <0.1	2.6 ± 0.1
Striped tuber oat grass	92.2 ± 0.6	4.6 ± 0.2	1.1 ± 0.1	0.2 ± <0.1	1.9 ± 0.1
Switchgrass	91.4 ± 0.6	3.5 ± 0.1	2.0 ± 0.1	0.2 ± <0.1	2.9 ± 0.2
Sweet sorghum cultivar S-N	92.7 ± 1.2	5.1 ± 0.2	1.1 ± 0.3	0.1 ± <0.1	1.0 ± 0.1

Note: Values are the means ± standard deviation (SD).

The longest cellulose chains were contained in the tall wheatgrass pulps (DP of 1822) while the DP of fibers from the other cellulosic pulps ranged from 1310 (for sweet sorghum) to 1663 (for tall fescue pulp) (Table 2). Although the degree of polymerization of the pulps was determined by the most widely applied viscometric technique, which is a relatively simple method [48,49], producing slightly different

DP values as compared to more sophisticated techniques, such as gel permeation chromatography, membrane osmometry, and cryoscopy, means that the results measured by the viscometric method are generally regarded as reasonable and reflect the differences in the length of fibers contained in various cellulosic pulps.

Based on the results presented in Table 4, it can be concluded that kraft pulping is an effective pretreatment method, removing more than 94% of lignin and over 96% of extractives from the raw material. Both these compounds are the main inhibitors of enzymatic hydrolysis. However, the main drawback of this method is the dissolution of the majority of the hemicelluloses, which could be used as a source of simple sugars.

Table 4. Fraction of chemical compound retained in the pulp.

Material	Cellulose	Hemicelluloses	Lignin	Extractives	Ash
			%		
Miscanthus giganteus	92.7 ± 0.4	6.6 ± 0.6	5.9 ± 0.4	5.5 ± 0.2	17.7 ± 0.9
Tall wheatgrass, cultivar A2	86.6 ± 0.2	6.3 ± 0.5	6.4 ± 0.6	3.0 ± 0.4	15.2 ± 0.6
Smooth bromegrass (Budzynska cultivar)	88.1 ± 0.1	4.7 ± 0.5	5.3 ± 0.3	2.5 ± 0.4	11.7 ± 0.6
Tall fescue	89.3 ± 0.4	4.4 ± 0.4	4.1 ± 0.5	2.7 ± 0.5	12.8 ± 0.4
Striped tuber oat grass	77.2 ± 0.5	3.9 ± 0.2	3.0 ± 0.2	2.4 ± 0.6	10.7 ± 0.5
Switchgrass	77.4 ± 0.3	4.0 ± 0.4	4.0 ± 0.2	1.7 ± 0.2	13.5 ± 0.7
Sweet sorghum cultivar S-N	43.6 ± 0.2	1.7 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	4.1 ± 0.3

3.2. Enzymatic Hydrolysis of Cellulosic Pulps

To determine the effect of the pulp origin on the susceptibility to enzymatic degradation, the pulps were subjected to enzymatic hydrolysis under identical conditions, as described in Materials and Methods, using the multienzyme preparation NS-81235, which shows the activities of cellulases (69.66 U/mL) and xylanases (141.92 U/mL) (at 50 °C and pH 5.0). Because of the relatively high content of reducing sugars (198.71 mg/mL), this multienzyme preparation was diluted 10, 20 and 30-fold before mixing with the substrates and the amounts of sugars contained in the solution of NS-81235 were discounted when the yields of substrate saccharification were calculated. The enzyme loads to the pulp were 0.1 mL/g, 0.05 mL/g and 0.033 mL/g. The preparation NS-81235 was used as a replacement of the preparation NS-22086, which was used in our former studies [23]. The cellulases and xylanases activities of these two multienzyme preparations and the results of digestion of selected softwood and hardwood pulps were comparable.

3.3. The Effect of Enzyme Concentration and Pulp Origin

The effect of enzyme load (NS-81235 0.1; 0.05 and 0.033 mL/g) on the yields of glucose (Figure 1) and other reducing sugars (Figure 2) and the amounts of insoluble residues that remained after hydrolysis (Table 5) of the tested cellulosic pulps was determined at their concentration of 0.476% (*w/v*) corresponding to 0.1 g pulp dry weight per sample. At this concentration of the substrates, the concentrations of reducing sugars in the reaction mixtures were relatively low (up to 6 mg/mL), which prevented enzyme inhibition by the hydrolysis products. This resulted in the relatively high yields of glucose and other reducing sugars and small amounts of the insoluble residues. Inhibition of enzymes catalyzing hydrolysis of cellulose and other polysaccharides by high concentrations of glucose and other sugars released from the substrates is a commonly known phenomenon, reducing biomass saccharification yields [50].

The percentage of glucose among reducing sugars contained in the enzymatic hydrolysates varied from 72.9% (sweet sorghum) to 78.0% (switchgrass) w/w (Figures 1 and 2). It was not surprising that the highest glucose and total reducing sugars yields (up to 75.5% DW and 97% DW pulp, respectively, in the case of the tall wheatgrass cultivar A2) were obtained at the highest enzyme load (0.1 mL/g). However, certain grass pulps such as those from tall wheatgrass (cultivar A2), smooth bromegrass

(Budzynska cultivar), tall fescue, miscanthus, striped tuber oat grass, switchgrass and sweet sorghum (cultivar S-N) underwent advanced hydrolysis also at the lower enzyme loads (0.05 and 0.033 mL/g) that was reflected by the relatively high glucose and reducing sugars yields (Figures 1 and 2) and low amounts of insoluble residues after hydrolysis (Table 5).

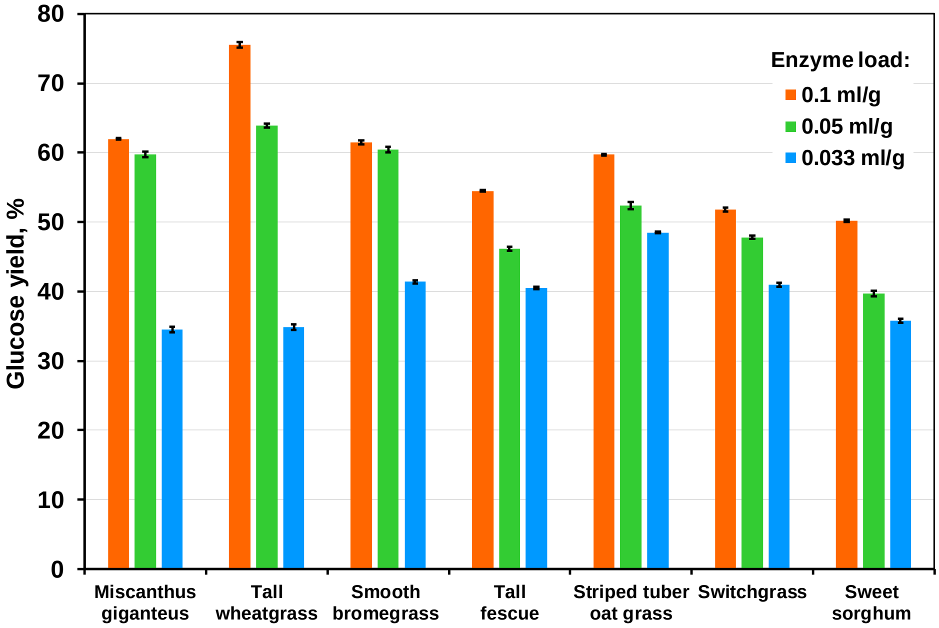

Figure 1. The effect of enzyme concentration on glucose yield from 7 cellulosic pulps of different origins (note: values are the means of triplicate assays).

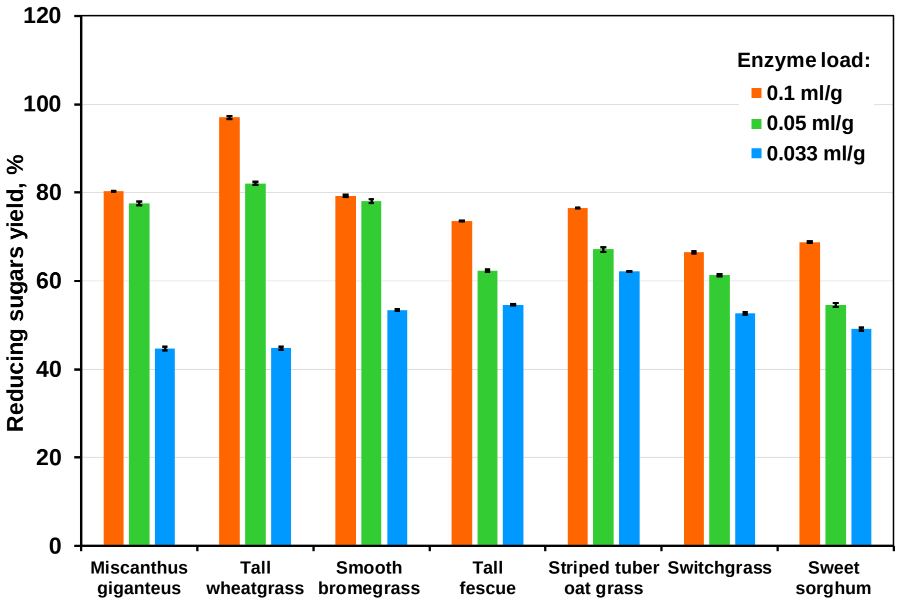

Figure 2. The effect of enzyme concentration on reducing sugars yield from 7 cellulosic pulps of different origins (note: values are the means of triplicate assays).

Table 5. The effect of enzyme load on the amounts of insoluble residues that remained after hydrolysis of the kraft pulps.

Pulp Origin	Insoluble Residues % DW Pulp NS-81235 Load		
	0.1 mL/g	0.05 mL/g	0.03 mL/g
Miscanthus giganteus	7.2 ± 0.1	8.4 ± 0.3	35.3 ± 0.1
Tall wheatgrass, cultivar A2	8.2 ± 0.3	8.7 ± 0.3	38.5 ± 0.4
Smooth bromegrass (Budzynska cultivar)	11.0 ± 0.1	11.7 ± 0.5	20.0 ± 0.3
Tall fescue	8.9 ± 0.2	11.2 ± 0.2	18.8 ± 0.4
Striped tuber oat grass	7.2 ± 0.1	8.4 ± 0.1	10.5 ± 0.3
Switchgrass	10.0 ± 0.5	11.4 ± 0.1	21.6 ± 0.3
Sweet sorghum cultivar S-N	8.4 ± 0.1	11.4 ± 0.1	32.1 ± 0.1

Note: Values are the means ± standard deviation (SD).

3.4. The Effect of Pulp Concentration on Hydrolysis Yield

Despite the relatively high yields of glucose and other reducing sugars obtained from the seven kraft pulps at the initial concentration of 0.476% *w/v*, such a process is not attractive because of the low concentrations of hydrolysis products and the necessity of concentration of the hydrolysates before fermentation processes. In this study, reducing sugars contents in the hydrolysates obtained were not higher than 6 mg/mL. To increase the concentration of reducing sugars in the hydrolysates, the concentration of the kraft pulps was increased from 0.476% *w/v* to 3.88 and 7.46% *w/v*. The effect of pulp concentration on the yield of reducing sugars and amounts of insoluble residues remained after hydrolysis of the kraft pulps was determined at 0.1 mL/g load of NS-81235 preparation. The increase in pulp concentration from 0.476% *w/v* to 3.88% *w/v* meant that the concentrations of reducing sugars in the hydrolysates were increased around 10-fold (up to 57 mg/mL). Interestingly, the rise in pulp concentration from 3.88 to 7.46% *w/v* did not give rise to a significant increase in reducing sugars contents in the hydrolysates (Table 6), presumably because of the small volumes of liquid phase in the reaction mixtures, which caused diffusion problems. Pulp concentrations above 7.46% *w/v* were too high to enable agitation of the reaction mixtures (the buffer and enzyme solutions were completely absorbed by the pulps).

The data presented in Figure 3 and Table 6 demonstrate that in most cases the increase in the initial pulp concentration from 0.476% *w/v* to 3.88% *w/v* caused a decrease in the reducing sugars yield on a pulp dry weight basis. Further increase in the initial pulp concentration to 7.46% *w/v* resulted in a further decrease in reducing sugars yields and a rise in percentages of insoluble residues after hydrolysis. However, reducing sugars yields derived at the highest initial pulp concentration (7.46% *w/v*) from striped tuber oat grass (62.7% DW), sweet sorghum (62.0% DW), tall fescue (61.2% DW), tall wheatgrass (52.8% DW), and miscanthus (46.3% DW) were relatively high. In the case of switchgrass, the yields of reducing sugars and glucose (55.7 and 43.4% DW pulp, respectively) were relatively high only at the lowest pulp concentration (they dropped to 22.3 and 17.45% DW, respectively, at the higher pulp concentrations). The differences between the glucose and reducing sugars yields derived from the kraft pulps reflect the aforementioned differences in biomass composition.

Irrespective of the botanical origin of the seven kraft pulps, glucose was the principal reducing sugar contained in their enzymatic hydrolysates. The comparison of glucose yields from the grasses and kraft pulps, at the enzyme preparation load 0.1 mL/g and 3 pulp concentrations is presented in Table 6.

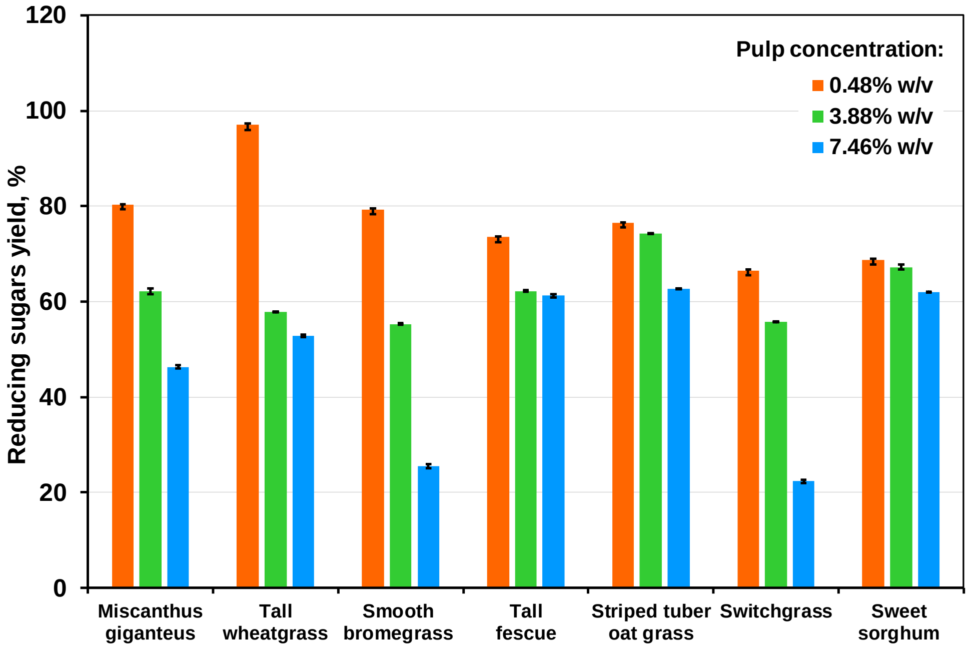

Figure 3. The effect of pulp concentration on the yield of reducing sugars after 24 h hydrolysis of the kraft pulps tested (note: values are the means of triplicate assays).

Table 6. Glucose yields from the grasses at 3 concentrations of kraft pulps (0.476, 3.88 and 7.46% *w/v*) derived from them (enzyme load 0.1 mL/g, 24 h hydrolysis at 50 °C).

Grass	Glucose Yield					
	% DW Pulp			% DW Biomass		
	0.476% *w/v*	**3.88% *w/v***	**7.46% *w/v***	**0.476% *w/v***	**3.88% *w/v***	**7.46% *w/v***
Miscanthus giganteus	62.0 ± 0.1	47.9 ± 0.6	35.7 ± 0.4	29.2 ± 0.1	22.6 ± 0.6	16.8 ± 0.4
Tall wheatgrass, cultivar A2	75.5 ± 0.4	45.0 ± 0.1	41.1 ± 0.2	33.4 ± 0.4	19.9 ± 0.1	18.2 ± 0.2
Smooth bromegrass Budzynska cultivar	61.5 ± 0.3	42.9 ± 0.2	19.8 ± 0.3	21.3 ± 0.3	14.8 ± 0.2	6.8 ± 0.3
Tall fescue	54.5 ± 0.1	46.2 ± 0.2	45.4 ± 0.2	18.5 ± 0.1	15.7 ± 0.2	15.4 ± 0.2
Striped tuber oat grass	59.7 ± 0.1	58.0 ± 0.1	49.0 ± 0.4	19.8 ± 0.1	19.3 ± 0.1	16.3 ± 0.4
Switchgrass	51.8 ± 0.3	43.4 ± 0.1	17.4 ± 0.5	17.7 ± 0.3	14.8 ± 0.2	6.8 ± 0.5
Sweet sorghum cultivar S-N	50.2 ± 0.2	49.0 ± 0.5	45.2 ± 0.1	7.8 ± 0.2	7.6 ± 0.5	7.0 ± 0.1

Note: Values are the means ± standard deviation (SD).

The highest glucose yields, even at the high initial pulp concentrations in the reaction mixtures, were observed in case of tall wheatgrass (41.1–75.5% DW pulp), miscanthus (35.7–62.0% DW pulp), striped tuber oat grass (49.0–59.7% DW pulp), tall fescue (45.4–54.5% DW pulp), and sweet sorghum (45.2–50.2% DW pulp). However, because of the low yields of the latter pulp after screening, the yields of glucose on the sorghum dry weight basis were low (7.0–7.8% DW).

The highest glucose yields on a biomass dry weight basis were observed at the pulp concentrations of 0.476 and 3.88% *w/v* in the case of tall wheatgrass (33.4 and 19.9% DW biomass, respectively). At the highest initial pulp concentration (7.46% *w/v*) the greatest amounts of glucose were obtained from tall wheatgrass (18.2% DW biomass), miscanthus (16.8% DW biomass), striped tuber oat grass (16.3% DW biomass) and tall fescue (15.45 DW biomass).

Because cellulose is the main source of glucose contained in enzymatic hydrolysates, also the yield of glucose from this polysaccharide was calculated (Table 7). The data presented in Table 7 shows that at the lowest pulp concentration the highest cellulose-to-glucose yields were derived from the tall wheatgrass cultivar A2 (around 70% DW cellulose). However, glucose yields from miscanthus and smooth bromegrass (Budzynska cultivar) were only around 10% lower. In the case of miscanthus, tall wheatgrass, tall fescue and striped tuber oat grass, the yields of cellulose-to-glucose conversion were also relatively high (above 35% DW) at the highest pulp concentration. These glucose yields are comparable to that reported by Alvarez-Vasco and Zhang [34] who achieved from around 20 to 95% cellulose-to-glucose yield from softwood (two different Douglas fir samples) dependently on alkaline hydrogen peroxide (AHP) pretreatment conditions.

Table 7. Cellulose-to-glucose yields from the grasses at 3 concentrations of kraft pulps (0.476, 3.88 and 7.46% *w*/*v*) derived from them (enzyme load 0.1 mL/g, 24 h hydrolysis at 50 °C).

Grass	Glucose Yield		
	% DW Cellulose		
	0.476% *w*/*v*	**3.88% *w*/*v***	**7.46% *w*/*v***
Miscanthus giganteus	61.86	47.88	35.59
Tall wheatgrass cultivar A2	70.17	41.81	38.24
Smooth bromegrass (Budzynska cultivar)	60.17	41.81	19.21
Tall fescue	53.78	45.64	44.77
Striped tuber oat grass	49.87	48.61	41.06
Switchgrass	43.49	36.36	14.50
Sweet sorghum cultivar S-N	23.71	23.10	21.28

4. Conclusions

The results of this study demonstrate that it is not only miscanthus, switchgrass and sweet sorghum, (which have been increasingly applied as energy crops), but also less popular grasses such as tall wheatgrass, striped tuber oat grass, tall fescue and smooth bromegrass, that can be converted to sugar feedstocks for biotechnology using kraft pulping and enzymatic hydrolysis. The yields of pulps, as well as glucose and reducing sugars, depends on the contents of cellulose, hemicelluloses and lignin in the seven grasses. Kraft pulping caused the partial removal of not only lignin but also hemicelluloses and therefore the yield of pulp from sweet sorghum, which is rich in the latter heteropolysaccharides, was relatively low. This, in turn, resulted in the low glucose and total reducing sugars yields on a biomass dry weight basis (below to 8% DW).

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