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Comparative Study of the Convertibility of Pretreated Miscanthus Straw Using Enzyme Preparations Produced by Different Recombinant Strains of *Penicillium verruculosum*

Dmitrii O. Osipov [1,*], Anna S. Dotsenko [1], Margarita V. Semenova [1], Alexandra M. Rozhkova [1] and Arkady P. Sinitsyn [1,2]

[1] Federal Research Center "Fundamentals of Biotechnology" of the Russian Academy of Sciences, 119071 Moscow, Russia; dotsenko-as@yandex.ru (A.S.D.); margs@mail.ru (M.V.S.); amrojkova@yahoo.com (A.M.R.); apsinitsyn@gmail.com (A.P.S.)
[2] Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russia
* Correspondence: d.osipov@fbras.ru

Abstract: Non-edible cellulosic biomass from perennial herbaceous plants is a promising and abundant feedstock for replacing slow-growing woody plants used in biotechnological applications. Herbaceous plant biomass, as other types of plant biomass, requires pretreatment before biochemical conversion. In this study, miscanthus straw was pretreated using different methods and subjected to enzymatic hydrolysis with *Penicillium verruculosum* enzyme complexes under laboratory conditions. The convertibility after enzymatic hydrolysis varied from 15% to 66%, depending on the pretreatment method. Dilute alkaline pretreatment showed the highest convertibility compared to other methods, reaching up to 66%. The efficiency of dilute acid pretreatment was relatively low compared to other methods. The maximum convertibility was 37% for sulfuric acid pretreatment (the least efficient) and 51% for nitric acid. Convertibility was almost equal with 43% for white liquor and 46% for hot water. The glucose-to-xylose ratio was 4.7:1 for dilute alkaline pretreatment and 11–13:1 for white liquor. Both sulfuric and nitric acid resulted in a low xylose content in the enzymatic hydrolysates. Low-xylose hydrolysates with less than 2% of the glucose amount can be produced by hot water pretreatment. Preparation C, enriched with endoglucanase I from *T. reesei* and endoglucanase II from *P. verruculosum*, was found to be the most effective of the different enzyme preparations (EPs) tested.

Keywords: miscanthus straw; recombinant enzymes; pretreatment

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1. Introduction

Annual and perennial herbaceous plants are promising feedstocks for green biorefinery and have several advantages compared to woody biomass. These fast-growing plants have a high biomass yield. For instance, switchgrass has a dry biomass yield ranging from 14 to 27 t/ha [1], and the average yield of dry amaranth biomass is 19.1 t/ha [2]. *Miscanthus sinensis* hybrids can produce up 25–41 t/ha of dry matter, depending on their location and growing conditions [3–5], dry wheat straw and maize stalks production are up to 15 and 25 t/ha of dry matter, respectively, which are comparable with the annual biomass production of fast-growing trees [6]. High productivity is associated with C4-photosynthesis often found in the species of *Poaceae* and *Amaranthaceae* grasses like food and feed crops such as sugar cane, maize, sorghum, bermudagrass, and amaranthus. A group of these plants originated under the high atmospheric levels of carbon dioxide and dry warm conditions [7], making them effective carbon-dioxide-fixing autotrophs. Despite their origin, some C4-species are cold-tolerant plants capable of abundant biomass production in high latitude environments [8,9]. *Miscanthus sinensis* hybrids such as *Miscanthus* × *giganteus* and *M. sinensis* var. Soranovsky [10] have lost seed propagation; therefore, they are propagated vegetatively using rhizomes. *Miscanthus* × *giganteus* is limited to the

temperate zone, while var. Soranovsky can grow even in Siberian climate conditions. var. Soranovsky may show invasiveness in warmer regions, whereas *Miscanthus* × *giganteus* does not due to its triploid nature [11].

Miscanthus has a wide range of applications, including agriculture, soil remediation, carbon farming, and bioenergy feedstocks. It can be cultivated in Mongolia, Central and Far Eastern Russia, and northern parts of China [12]. Unlike many other crops, miscanthus is harvested in late winter or early spring, as the full maturation and drying of its stems and leaves are necessary for the easy processing of biomass. The timing of miscanthus harvest is earlier than the start of the main agricultural season. This provides an advantage when processing the crop.

Miscanthus is considered a cluster feedstock in biotechnology due to its resistance to pests and diseases, ability to grow on poor soils, significant biomass productivity, and high cellulose content (up to 57%, comparable to wood [13]). In addition, it is potentially suitable for bioconversion after pretreatment under mild conditions due to its relatively low lignin content (13–17%) [14–16].

Pretreatment is required for plant materials containing cellulose to increase reactive ability during enzymatic hydrolysis. This is achieved through particle size reduction, the destruction of cellulose crystal structure, and the removal of lignin [17,18]. The most investigated methods for disrupting the hemicellulose matrix and delignification are pretreatment with dilute acids [19] and alkalis [20], organosolve [21,22], and eutectic [23] and ionic [24] liquids, as well as steam explosion [25,26]. Combined pretreatment methods are often the most effective method in increasing the convertibility of cellulose-containing materials to high-value products through enzymatic degradation.

The conversion of miscanthus into high-value products is currently under extensive study.

Table 1 presents the findings of prior research.

Table 1. The effectiveness of miscanthus biomass pretreatment at various conditions.

Pretreatment	Conditions	Effectiveness	Reference
Sulfuric acid	1.1% at 121.6 °C for 12.8 min	86.4% glucose conversion	[27]
	1.25% in 80% glycerol at 160 °C for 10 min	76.6% substrate convertibility	[28]
	1% at 130 °C for 30 min	79.07% pulp yield	[29]
	1% at 170 °C for 30 min	51.2% sugar yield	[30]
Nitric acid	3–6% at 90–95 °C for 10–12 h	61.4% convertibility	[31]
Alkali	4% sodium hydroxide at 50 °C for 168 h	75% of converted cellulose	[32]
	2% sodium hydroxide at 50 °C for 2 h	58.5% convertibility	[33]
	1–1.2% sodium hydroxide at 121 °C for 10 min	26.1% convertibility	[34]
	1–1.2% sodium hydroxide at 120 °C for 30 min	47.4% convertibility	[35]
Black liquor	0.6 M NaOH	64% convertibility	[36]
Hot water	200 °C for 15 min	35% convertibility	[37]

Miscanthus pretreatment is typically conducted using dilute (1–1.25%) sulfuric acid at temperatures of 120–130 °C for 10–30 min, resulting in 77% convertibility. Nitric acid is less commonly used for pretreatment and results in a lower convertibility of about 60% when carried out at temperatures below 100 °C for 12 h. Alkaline pretreatment has been demonstrated to be an effective method for removing lignin. Pretreatment with 1–4% aqueous alkaline solutions at temperatures ranging from 50–120 °C and various time intervals resulted in the conversion rates of 26–59%.

Only a few studies have been conducted on the use of industrial blends for delignification. For instance, black liquor pretreatment has resulted in high convertibility. Additionally, hot water can efficiently modify lignin and hydrolyze hemicelluloses at high temperatures for a short time.

Enzyme preparations based on the enzyme complexes of cellulases [38] of the *Penicillium verruculosum* (synonym *Talaromyces verruculosus*) mutant and recombinant strains have shown their efficiency in a wide range of tasks [39–41]. The high efficiency of hydrolysis under the action of the *P. verruculosum* enzyme complex is ensured by the balance of key hydrolytic enzymes–cellobiohydrolase I and II, 1,4-β-glucanase, and β-glucosidase [38]. Several years ago, a cellobiase producer was obtained based on the recipient strain *P. verruculosum* 537 [42]. The addition of cellobiase to the main enzyme complex of *P. verruculosum* allows one to increase the yield of reducing sugars by 20 to 30%, depending on the type of hydrolyzed plant material [41].

Reducing sugars obtained after the hydrolysis of miscanthus can be used as a raw material for bioethanol production [27,37], the synthesis of bacterial cellulose [31], and succinic [28] and lactic acid [43] production.

The aim of this study is to evaluate the convertibility of miscanthus straw pretreated by the different methods of enzymatic hydrolysis using *Penicillium verruculosum* recombinant enzyme preparations (Eps) of various compositions.

2. Materials and Methods

2.1. Samples of Cellulose-Containing Substrate

The straw of *Miscanthus sinensis* var. Soranovski was obtained from PJSC Tatneft (Almetievsk, Russia). It was grown in Tatarstan, Russia, and harvested in 2022.

2.2. Substrates

Carboxymethylcellulose (CMC, sodium salt, medium viscosity), birch glucuronoxylan, glucose, xylose, fructose, and *p*-nitrophenyl-*β*-*D*-glucopyranoside were purchased from Sigma (St. Louis, MO, USA). Avicel PH105 microcrystalline cellulose was obtained from Serva (Heidelberg, Germany).

2.3. Cultivation of Recombinant Strains and Enzyme Preparations

Recombinant strains were cultured in 1 L KF-108/3 fermentor (Prointech LLC, Moscow, Russia) using a nutrient medium of the following composition (g/L), i.e., glucose (40), microcrystalline cellulose (40), wheat bran (10), corn extract (30), urea (2.5), KH_2PO_4 (14), $(NH_4)_2SO_4$ (10), $CaCl_2$ (0.6), $MgSO_4 \cdot 7H_2O$ (0.6), at pH 5.0 and 30 °C for 144 h. During fermentation, glucose and microcrystalline cellulose were fed three times. Biomass was removed from the culture fluid using centrifuge (Beckman Coulter, Brea, CA, USA) at 6000 rpm for 30 min, and the obtained supernatant was dried on a Mini Spray Dryer B-290 (Buchi AG, Flawil, Switzerland) to obtain dry EP.

2.4. Enzyme Activity Assays

CMCase and xylanase activities were determined by reducing sugars (RS) release at pH 5.0 and 50 °C after 10 min using the substrate concentration of 5 mg/mL in the reaction mixture [44]. Avicelase activity was determined by RS release at pH 5.0 and 50 °C after 60 min of enzyme reaction with Avicel PH105 (5 mg/mL) [45]. RS were assayed by Nelson–Somogyi method [46]. One unit of activity corresponded to the quantity of enzyme releasing 1 μmol of RS (in glucose equivalents) in one minute. The activity against p-NP-β-glucopyranoside was measured by determining the amount of p-nitrophenol released at pH 5.0 and 50 °C, following previously described methods [47]. One β-glucosidase unit of activity is the quantity of enzyme that liberates 1 micromole of *p*-nitrophenol in one minute. Protein concentration was determined according to the Lowry protein assay [48] using BSA as a standard.

2.5. Enzymatic Hydrolysis

Hydrolysis experiments were performed in triplicate in 2 mL Eppendorf tubes incubated at 50 °C and 250 rpm on a Biosan TS-100 shaker (Riga, Latvia) for 48 h. The reaction mixture, totaling 1.5 mL, contained 100 g/L miscanthus dry matter in 0.1 M Na-acetate

buffer at pH 5.0 with 10 mg/g dry matter protein loading of B151, C, CX or X enzyme preparation. To overcome cellobiose inhibition effects, 0.1 mg/g dry matter of EP F10 was added to the mixture. To prevent contamination, 1 mM NaN_3 and 100 µg/mL ampicillin were used. Nelson–Somogyi assay [46] was used to determine the concentration of RS in the reaction mixture.

Enzymatic convertibility was defined as a degree of conversion (48 h) to RS (in glucose equivalent) as a percentage per initial concentration of dry substrate, as described in the following equation:

$$\text{Convertibility } (\%) = \frac{\text{RS concentration} \times 0.9 \times 100}{\text{substrate concentration}}$$

where "RS concentration" is the RS concentration in reaction mixture after enzymatic hydrolysis, "substrate concentration" is the substrate concentration in reaction mixture before enzymatic hydrolysis, and 0.9 is the glucose equivalent conversion factor.

2.6. Pretreatment

The dry miscanthus straw was ground using a PULVERISETTE 14 premium line rotor mill (FRITSCH, Oberstein, Germany). The rotor mill was equipped with a 200 µm sieve. The milled miscanthus straw underwent various pretreatment methods, including treatment with diluted sodium hydroxide (0.5–2%) at 100–180 °C for 15 to 60 min and diluted nitric acid (0.5–1.5%) at 100–180 °C for 15 to 60 min. The material was treated with 0.5–1.5% sulfuric acid at 100–170 °C for 15 to 60 min, with white liquor (90 g/L NaOH, 40 g/L Na_2S) at 160 °C for 15 to 30 min and a solid-to-liquid ratio of 1:4, and with hot water at 85–200 °C for 5 to 70 min. The concentration values used for the treatment of the material were based on the results of our previous study [41].

Pretreatment was conducted using 100 mL pressurized steel cylinders. The cylinder contained milled miscanthus straw and reaction solution with a solid-to-liquid ratio of 1:10 (*w*/*v*), except for white liquor pretreatment, which used a ratio of 1:4 (*w*/*v*). The cylinder was placed in a temperature-controlled oil bath. After the pretreatment process was complete, the reactors were cooled in cold water. The resulting slurry was filtered, and the remaining solids were pH adjusted and washed with water. The pretreated samples were stored in a refrigerator prior to enzymatic hydrolysis.

2.7. Chromatographic Analysis

The HPLC analysis was performed on an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) system equipped with a Diaspher-NH2 chromatographic column with an acetonitrile–water 75:25 mobile phase and a refractometric detector, according to the manufacturer's instructions. The retention times of standard solutions (1 g/L) at a flow rate of 1 mL/min and room temperature were as follows: xylose (6.7 min), arabinose (7.3 min), fructose (7.9 min), and glucose (8.9 min). Glucose, arabinose, fructose, and xylose were used as standards.

3. Results and Discussion

To investigate the convertibility of pretreated Miscanthus straw, different dry Eps were used as shown in Table 2. EP C and CX exhibited increased CMCase activity, while EP B151 and C showed increased Avicelase activity. EP CX and X demonstrated increased xylanase activity, and EP F10 showed increased β-glucosidase (cellobiase) activity.

Table 2. Activity of *P. verruculosum* enzyme preparations.

Enzyme Preparations	Protein, mg/g	Activities toward Various Substrates, U per 1 mg of Protein			
		Avicel, U/mg	CMC, U/mg	pNPG, U/mg	Xylan, U/mg
B151	950 ± 20	0.86 ± 0.04	13 ± 1.1	1.8 ± 0.1	19.8 ± 0.8
C	71 ± 3	0.76 ± 0.02	48 ± 2.9	0.73 ± 0.02	9.2 ± 0.3
CX	92 ± 5	0.53 ± 0.03	19 ± 1.2	0.58 ± 0.01	35 ± 1.5
X	90 ± 6	0.66 ± 0.02	5.2 ± 0.3	0.76 ± 0.03	66 ± 3.7
F10	660 ± 12	0.40 ± 0.03	3.4 ± 0.2	61 ± 3.7	3.3 ± 0.2

EP B151 containing cellulase and xylanase complex was obtained by *P. verruculosum* B151 strain [49]; EP C was obtained by recombinant *P. verruculosum* strain cultivation after the expression of *Trichoderma reesei* endoglucanase I (EG I) gene and *P. verruculosum* endoglucanase II gene; dry EP CX was obtained by recombinant *P. verruculosum* strain cultivation after the expression of *P. verruculosum* endoglucanase II (EG II) gene and *P. canescens* xylanase E (XylE) gene; dry EP X was obtained by recombinant *P. verruculosum* strain cultivation after the expression of xylanase E of *P. canescens* gene; dry EP F10 was obtained by recombinant *P. verruculosum* strain cultivation after the heterologous expression of *Aspergillus niger* β-glucosidase (cellobiase) gene [42]. Therefore, depending on the expressed gene, the Eps had corresponding increased activities compared to the wild type-based EP. EP C had the highest CMCase activity and relatively high Avicelase [50] activity since EG I is a processive enzyme like many other microbial Egs and this type of cellulases is very important for cellulose degradation [51–53]. EP CX contains EG II and XylE, which are able to degrade amorphous cellulose and xylan. Xylans are essential components of the plant cell wall that firmly anchor cellulose fibrils and protect them from cellulases [54,55]. EP X also contains XylE, but its activity against microcrystalline cellulose is higher, and its xylanase activity is the highest amongst all the presented Eps. The cellulolytic and xylanolytic activities of EP F10 are low, but the cellobiase activity is very high due to the high β-glucosidase content. This activity is important in overcoming the inhibition of cellulases by hydrolysis products.

The chemical composition of *M. sinensis* var. Soranovski has been previously reported [56]. The above-ground part of the plant is characterized by the following composition (%): lipids 4.98, ash content 5.87, acid-soluble lignin 22.0, pentosans 21.0, and cellulose 53.1.

The intact and milled miscanthus straw convertibility was relatively low and did not depend on the type of EP, i.e., 8 and 11–13%, respectively. Thus, milling is not sufficient to obtain a high yield of RS in the course of enzymatic hydrolysis.

As we have shown previously [57], low-intensity milling followed by chemical or physicochemical pretreatment under mild conditions lead to an increase in the convertibility without the undesirable degradation of carbohydrates and the high accumulation of organic acids and phenol-containing inhibitors.

Thirteen samples of miscanthus straw were obtained using dilute alkali pretreatment (Table 3, here and further, in Tables, we present the best results from the point of view of convertibility achieved using various EP). EP C is the most efficient for the hydrolysis of alkali pretreated straw under virtually all conditions, but the favored conditions are 2% NaOH at 100 °C for 40 min (Figure A1) and 2% NaOH at 140 °C for 15 min with 66 and 65% convertibility accordingly. These results are possibly related to the amorphization and mercerization cellulose fibrils [57], which increases the number of cellulose molecules available for endoglucanases [58]. Pretreatment at higher temperature (180 °C) results in 22–28% lower glucose yield, i.e., 43 to 47 g/L. The lowest glucose yields are associated with EP CX and X, i.e., 31 and 35 g/L, respectively.

Table 3. Convertibility of miscanthus straw after alkali pretreatment.

NaOH, %	Temperature, °C	Time, min	Best EP	Glucose, g/L	Xylose, g/L	RS, g/L	Convertibility, %
0.5	100	40	CX	31 ± 1.8	10.4 ± 0.3	47 ± 3.4	42
1.25		15	C	51 ± 3.7	14.2 ± 0.6	60 ± 2.7	54
1.25		60	C	54 ± 2.8	14.0 ± 0.7	70 ± 3.1	63
2		40	C	56 ± 2.5	12.4 ± 0.4	73 ± 5.9	66
0.5	140	15	C	36 ± 1.2	11.1 ± 0.3	63 ± 3.4	57
0.5		60	C	47 ± 2.8	9.5 ± 0.2	57 ± 3.2	52
1.25		40	X	35 ± 2.9	9.8 ± 0.2	58 ± 4.1	53
2		15	C	60 ± 3.3	13.3 ± 0.4	73 ± 6.4	65
2		60	C	51 ± 3.5	12.7 ± 0.5	60 ± 4.5	54
0.5	180	40	C	47 ± 2.9	11.4 ± 0.5	56 ± 4.2	51
1.25		15	C	43 ± 2.3	11.9 ± 0.3	58 ± 3.9	52
1.25		60	C	47 ± 2.5	11.5 ± 0.6	56 ± 4.0	51
2		40	C	46 ± 2.9	12.2 ± 0.4	54 ± 2.3	48

At low alkali concentrations (0.5%), the pretreatment duration is a more important factor than at medium concentrations (1.25%). Longer pretreatment times can have the opposite effect at higher alkali concentrations (2%).

Dilute alkali pretreatment generally resulted in a high convertibility of miscanthus straw and generated the easily hydrolysable hemicellulose, resulting in a large proportion of xylose after enzymatic hydrolysis.

Thirteen samples of miscanthus straw were obtained using dilute sulfuric acid pretreatment (Table 4). This method of pretreatment conducted at mild temperatures (135 °C) could increase enzymatic convertibility to 27–37% compared to those at lower (22–30%) and higher temperatures (19–34%). Due to the solubilization of hemicelluloses and lignin at 170 °C, the glucose concentration was slightly higher than that at 135 °C, but the xylose concentration was minimal: 0.1–0.3 g/L with 1–1.5% acid for 40–60 min. Similarly, the prolonged pretreatment at elevated acid concentration at 170 °C led to the increase in cellulose crystallinity [59] and consequently to the resistance of the substrate to enzymatic hydrolysis. Temperatures around 100 °C are not sufficient to break down the hemicellulose/lignin matrix, thus resulting in both low glucose yield and total convertibility. There was not always a significant difference in the convertibility of straw treated with different concentrations of acid and different pretreatment times within the same temperature intervals. At high pretreatment temperature, the relationship between concentration/time and convertibility was inverse for the reason mentioned above.

Thirteen samples of miscanthus straw were obtained using dilute nitric acid pretreatment (Table 5). In general, pretreatment by dilute nitric acid was more effective than by dilute sulfuric acid but less effective than by sodium hydroxide. The results demonstrate inverse correlation between nitric acid concentration and substrate convertibility (35–37%). Longer pretreatment also caused relatively low glucose yield (10.8–36.0 g/L) and convertibility (11–36%).

The data display that straw with the maximum RS concentration (56 g/L) and convertibility (51%) could be obtained with 1.5% acid at 140 °C for 15 min in hydrolysis by EP C. Lower RS concentration (52 g/L) but higher glucose concentration (51 g/L) was obtained with 0.5% acid at 180 °C for 40 min. Low xylose yield is related with nearly complete hemicellulose dissolution at 180 °C. Under certain conditions (1–1.5% acid, 100 and 140 °C, 40–60 min), EP B151 showed the same results as EP C, possibly because of the high cellobiohydrolase activity [60] on the remaining crystalline cellulose.

Table 4. Convertibility of miscanthus straw after pretreatment with dilute sulfuric acid.

H_2SO_4, %	Temperature, °C	Time, min	Best EP	Glucose, g/L	Xylose, g/L	RS, g/L	Convertibility, %
0.5	100	40	C	19 ± 1.6	4.7 ± 0.3	25 ± 2.0	22
1		15	C	20 ± 1.1	3.9 ± 0.7	27 ± 1.1	24
1		60	C	25 ± 1.8	6.0 ± 0.4	34 ± 1.1	30
1.5		40	B151	23 ± 1.7	4.5 ± 0.3	32 ± 1.6	29
0.5	135	15	C	36 ± 1.5	6.2 ± 0.5	41 ± 1.8	37
0.5		60	C	24 ± 1.0	5.2 ± 0.3	30.1 ± 0.8	27
1		40	C	30.1 ± 0.9	5.3 ± 0.6	36 ± 2.0	32
1.5		15	C	32 ± 1.2	5.3 ± 0.5	35 ± 1.3	31
1.5		60	X	31 ± 1.3	6.6 ± 0.6	37 ± 1.2	34
0.5	170	40	C	38 ± 1.8	1.3 ± 0.1	38 ± 2.3	34
1		15	C	30 ± 1.6	1.7 ± 0.1	31 ± 1.6	28
1		60	C	22.7 ± 0.9	0.31 ± 0.04	23 ± 1.0	21
1.5		40	C	20.6 ± 0.8	0.14 ± 0.01	20.8 ± 0.5	19

Table 5. Convertibility of miscanthus straw after pretreatment with dilute nitric acid.

HNO_3, %	Temperature, °C	Time, min	Best EP	Glucose, g/L	Xylose, g/L	RS, g/L	Convertibility, %
0.5	100	40	C	21 ± 1.4	5.5 ± 0.2	33 ± 1.9	30
1		15	C	30 ± 1.6	7.3 ± 0.4	44 ± 2.5	40
1		60	B151	24 ± 1.1	6.4 ± 0.2	44 ± 2.4	40
1.5		40	B151/C	27 ± 1.1/ 26 ± 1.4	6.3 ± 0.2/ 8.3 ± 0.3	40 ± 1.6/ 40 ± 1.7	36/36
0.5	140	15	C	35 ± 1.5	8.0 ± 0.6	49 ± 1.2	44
0.5		60	C/X	35 ± 2.0	6.2 ± 0.5/ 8.3 ± 0.8	47 ± 1.3/ 47 ± 1.3	42
1		40	B151/C	42 ± 1.7/ 41 ± 2.0	4.7 ± 0.3/ 4.7 ± 0.2	48 ± 1.4/ 48 ± 1.7	43/43
1.5		15	C	47 ± 1.3	7.6 ± 0.3	56 ± 2.2	51
1.5		60	C	36 ± 1.1	2.4 ± 0.1	38 ± 1.7	35
0.5	180	40	C	51 ± 2.4	1.3 ± 0.1	52 ± 2.0	47
1		15	C	48 ± 1.8	2.0 ± 0.1	51 ± 2.1	46
1		60	C	10.8 ± 0.6	0.33 ± 0.01	11.7 ± 0.8	11
1.5		40	C	39 ± 1.5	0.72 ± 0.06	42 ± 1.5	37

Mild pretreatment conditions generate large amounts of easily hydrolysable hemicellulose that results in a large proportion of xylose after hydrolysis by EP B151 and X with elevated xylanase activity.

The effects of nitric acid concentration, pretreatment time, and temperature are quite complexly interrelated, but in many cases, shorter times are preferable.

The Kraft process is an industrial method used to convert wood into cellulose. This process involves the use of white liquor, a water solution of sodium hydroxide and sodium sulfide. Due to its high recovery rate, it is a well-established and widely used process for cellulose production in paper mills.

Two samples of miscanthus straw were obtained using this process (Table 6). The RS concentration varied from 34.5 g/L for 15 min to 48 g/L for 30 min. The convertibility of Kraft-style pretreated straw was higher than obtained by dilute sulfuric acid and comparable with the results obtained for high-temperature nitric acid pretreatment.

Table 6. Convertibility of miscanthus straw pretreated by white liquor solutions (at 160 °C).

Time, min	Best EP	Glucose, g/L	Xylose, g/L	RS, g/L	Convertibility, %
15	C	31 ± 1.4	2.8 ± 0.1	34.5 ± 0.8	31
30	C	42 ± 1.3	3.1 ± 0.2	48 ± 1.5	43

In comparison to alkaline pretreatment, the Kraft process appears to be inappropriate due to relatively low lignin content in miscanthus [15]. Similar convertibility was achieved with 0.5% NaOH at 100°C for 40 min and 9% NaOH/4% Na_2S at 160°C for 30 min. The white liquor turned black after pretreatment and could be recovered and reused, which is advantageous considering the high price of sodium hydroxide. This aspect of the study is promising for further study.

In both cases, EP C was the best option due to its low xylose content, which was less than 10% of the glucose concentration.

Improving the enzymatic convertibility of cellulose-containing materials through chemical means can be effective, but it requires reagent recuperation and waste processing technology. Although sulfuric acid pretreatment is low cost, it generates a large amount of non-biodegradable lignosulphonates. To reduce costs and avoid the formation of undesirable by-products, it is reasonable to avoid the pretreatment process using chemicals.

Nine samples of miscanthus straw were obtained by hot water pretreatment at different temperatures (Table 7). The experimental data demonstrated positive linear correlation between process temperature and convertibility and glucose concentration in enzymatic hydrolysis. Glucose and RS concentrations after 48 h hydrolysis with EP C were 41–45 and 49–51 g/L, respectively. This effect is related with autocatalysis caused by polysaccharide destruction and simple carbohydrates transformation to organic acids at high temperatures [61]. It explains the xylose yield obtained after enzymatic hydrolysis. The concentration increased until a short pretreatment at 180 °C (7.0 g/L) and then decreased to a minimum at 200 °C (0.9 g/L). This pretreatment is similar to dilute acid pretreatment, where hemicelluloses are released at low and mild temperatures and decomposed at high temperatures.

Table 7. Convertibility of miscanthus straw pretreated by water at elevated temperatures.

Temperature, °C	Time, min	Best EP	Glucose, g/L	Xylose, g/L	RS, g/L	Convertibility, %
85	40	C	12 ± 1.1	1.7 ± 0.1	17 ± 1.6	15
100	15	C	11 ± 1.0	1.7 ± 0.1	15 ± 1.3	14
100	60	C	15 ± 2.8	2.9 ± 0.2	21 ± 1.1	19
140	5	C/X	13.0 ± 0.5/ 13.7 ± 0.2	2.9 ± 0.1/ 1.5 ± 0.1	18 ± 1.6/ 18 ± 1.7	16/16
140	40	C	18 ± 1.4	4.9 ± 0.2	26 ± 1.3	23
140	70	C	20 ± 1.8	6.6 ± 0.3	32 ± 1.8	29
180	15	C	41 ± 2.9	7.0 ± 0.3	50 ± 2.9	45
180	60	C	41 ± 2.1	2.0 ± 0.1	49 ± 2.2	44
200	40	C	45 ± 2.6	0.93 ± 0.05	51 ± 2.7	46

4. Conclusions

The study investigated the effect of different pretreatment methods on the convertibility of miscanthus straw in enzymatic hydrolysis using *P. verruculosum* enzyme preparations. Dilute sodium hydroxide solution was found to be the most effective pretreatment method over a wide range of conditions. While dilute nitric acid pretreatment was also effective, it only worked in a narrower range of conditions. Based on the results, it was found that pretreatment with dilute sulfuric acid and hot water, as well as white liquor, were less effective. The effectiveness of the first two methods is dependent on temperature.

Among the different Eps, the most effective preparation was preparation C containing endoglucanase I from *T. reesei* and endoglucanase II from *P. verruculosum*. It was the best choice for the efficient saccharification of miscanthus straw samples obtained by any pretreatment method. EP B151 was the second most effective for enzymatic saccharification of straw pretreated with dilute sulfuric and nitric acid. EP X rarely showed efficacy after pretreatment with sulfuric and nitric acids and hot water. EP CX showed almost no efficiency.

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Appendix A

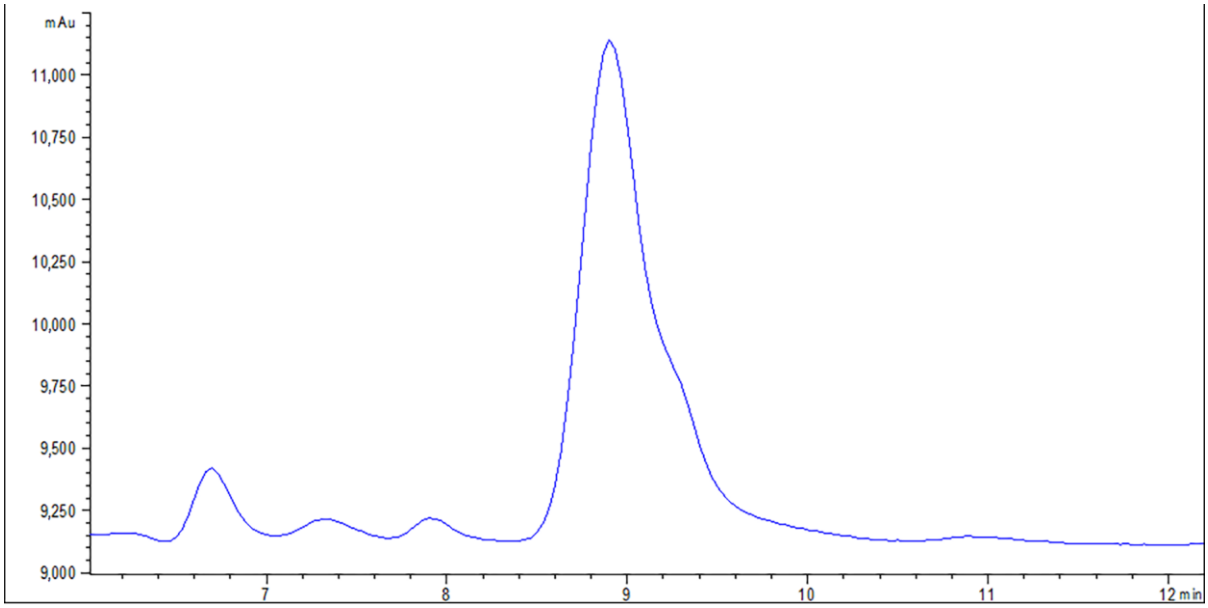

Figure A1. HPLC chromatogram obtained for the sample pretreated with 2% NaOH at 100 °C for 40 min.

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