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Preliminary evaluation of five elephant grass cultivars harvested at different time for sugar production☆

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ABSTRACT

Five elephant grass cultivars, *Pennisetum purpureum*. cv. Huanan (Huanan), *P. purpureum*. cv. N51 (N51), *P. purpureum*. cv. Sumu No. 2 (Sumu-2), (*Pennisetum americanum* × *P. purpureum*) × *P. purpureum* cv. Guimu No. 1 (Guimu-1) and *P. americanum* cv. Tift23A × *P. purpureum* cv. Tift N51 (Hybrid Pennisetum), at three harvest stages were studied. With dilute sulfuric acid pretreatment followed by enzymatic hydrolysis, it is found that cellulose conversion of the five elephant grass cultivars harvested in August and September is higher than that harvested in October. The cellulose conversion for elephant grass cultivars harvested in August and September follows an order of Hybrid Pennisetum > Sumu-2 > Huanan > Guimu-1 > N51. This may be explained by the fact that lignification is gradually strengthened with time, inhibiting degradation of cellulose and hemicellulose. Moreover, cellulose conversions of Hybrid Pennisetum, Sumu-2 and Huanan harvested in August and September are higher based on hierarchical clustering results.

1. Introduction

The research on biomass conversion is receiving much attention all over the world as energy shortage and environmental pollution problems become more and more serious [1–3]. Bioconversion refers to the release and fermentation of sugars from biomass, in which carbohydrates are converted into sugars and the mixed sugars are further fermented microbially into ethanol or other chemicals. At present, there are two main procedures in sugar conversion process [4], concentrated acid or multi-step dilute acid pretreatment followed by enzymatic hydrolysis. Selig *et al.* have concluded that the overall effect of biochemical transformation is the best after dilute acid pretreatment for the present [5].

Since the 1980s, perennial grasses have been studied as energy crops. Nowadays, extensive research on perennial grasses is focused on switchgrass, miscanthus, Yi grass, bamboo reed, *etc.* [6,7]. Elephant grass belongs to high-yield perennial grasses of poaceae and pennisetum. Its dry matter biomass production is more than twice that of sugarcane and switchgrass per unit area. Thus, elephant grass is well-recognized as an energy crop. The root of elephant grass is transplanted from greenhouse to outside in April every year when the ambient temperature is above 4 °C. After three months of growth, the plant can reach 2 m in height, and cellulose and hemicellulose contents accumulate gradually. In October, when the temperature is below 4 °C, the root should be moved into the greenhouse again for survival. Therefore, the optimal harvest time for elephant grass is between August and October, and the annual dry biomass can reach 40–48 $t \cdot hm^{-2}$ [8–12].

Various factors affect the yield and composition of perennial grass biomass, such as cultivar, harvest time, and environmental conditions. It is found that cultivar and harvest time affect biomass yield and composition of switchgrass significantly. With the delay of harvest time, the mass fraction of cellulose, hemicelluloses and lignin increase gradually while sugar yield decreases [13–15]. In this paper, five elephant grass cultivars, including *Pennisetum purpureum*. cv. Huanan (Huanan) [16], *P. purpureum*. cv. N51 (N51) [17], Sumu No. 2 (Sumu-2) [18,19], (*Pennisetum americanum* × *P. purpureum*) × *P. purpureum* cv. Guimu No. 1 (Guimu-1) [20], and *P. americanum* cv. Tift23A × *P. purpureum* cv. Tift N51 (Hybrid Pennisetum) [21], are studied and the contents of cellulose, hemicellulose and Klason lignin are determined by National Renewable Energy Laboratory (NREL) method. In addition, sugar yield is investigated under the same pretreatment and enzymatic hydrolysis conditions to screen out the cultivar(s) with higher cellulose conversion to provide theoretical foundation for further study.

2. Methods

2.1. Feedstock materials

Samples of five elephant grass cultivars, Huanan (HN), N51, Sumu-2 (SM), Guimu-1 (GM) and Hybrid Pennisetum (HP), were obtained from

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Jiangsu Academy of Agricultural Sciences, Nanjing, China. They were planted in May 2011 and harvested in August, September or October in the same year. These elephant grass cultivars were dried at 105 °C for 12 h and milled until the samples passed a standard 2 mm sieve [24]. The samples were kept in the dryer at room temperature before composition analysis and pretreatment.

Elephant grass cultivars evaluated in this study are given in Table 1 with a description of their origins.

Table 1
Elephant grass cultivars evaluated in this study

Cultivar	Species	Germplasm origin	Reference
HN	*P. purpureum Schum.* cv. Huanan	Indonesia, 1960	Registered in 1990 in China [16]
N51	*P. purpureum Schum.* cv. N51	USA, 1985	Unregistered in China [17]
SM	*P. purpureum Schum.* cv. Sumu No. 2	Improved cultivar from 'N51', 2010, Nanjing, China	Registered in 2010 in China [18,19]
GM	(*P. americanum* × *P. purpureum*) × *P. purpureum* cv. Guimu No. 1 or (*P. americanum* cv. Tift23A × N51) × Mott	Improved cultivar from hybrid between 'Hybrid' and 'Mott', 2000, Guangxi, China	Registered in 2000 in China [20]
HP	*P. americanum* cv. Tift23A × *P. purpureum* cv. Tift N51	USA, 1981	Registered in 1989 in China [21]

2.2. Pretreatment of elephant grass with dilute H_2SO_4

Milled elephant grass was soaked in 1% (by mass) dilute sulfuric acid solution at room temperature with a solid loading of 10%(by mass). The presoaked biomass slurry was transferred to four Hastelloy C-276 batch tube reactors (5.1 cm × 11 cm, total volume 50 ml each, sealed with Swagelok fittings and Teflon gaskets at both ends) and pretreated in an oil bath (HH-S, Jiangsu, China) at 140 °C for 20 min. After the reaction was completed, the reactors were quenched in ice bath until the temperature dropped below 100 °C. The slurry was vacuum filtered immediately through a glass fiber filter with the temperature kept above 60 °C. The filtrate was collected with a 40 ml centrifuge tube and stored at −80 °C for the analysis of monosaccharides. The solid residue was washed with deionized water until the filtrate pH was above 6, and stored at 4 °C for composition analysis and enzymatic hydrolysis [22].

2.3. Enzymatic hydrolysis

An enzyme system, Cellic CTec2, purchased from Novozymes (Beijing, China), was used for enzymatic hydrolysis experiments. By the filter paper assay [23], the activity of this enzyme was measured and the value was 90 $FPU \cdot ml^{-1}$. The enzyme loading was 7 $FPU \cdot (g\ dry\ matter)^{-1}$ in the hydrolytic reaction and the pH value of the substrate was adjusted to 4.8 before enzyme addition with 50 $mmol \cdot L^{-1}$ acetate buffer. Then the reaction mixture (70 ml) of enzymatic hydrolysis was taken into flasks and incubated at 50 °C on a rotary shaker at 100 $r \cdot min^{-1}$. Aliquots of 0.5 ml were sampled at 12, 24, 36, 48, 60 and 72 h and centrifuged at 12,000 $r \cdot min^{-1}$ for 5 min. Finally, the supernatant was filtered through a 0.22 μm membrane and stored at −4 °C for further analysis.

2.4. Analytical procedure

The composition of elephant grass was determined by NREL standard laboratory analytical procedures [24], and components including cellulose, hemicellulose, Klason lignin, other extractives and ash were analyzed. Concentrations of monosaccharides were measured by HPLC (Shimazu, LC-20A) equipped with a refractive index detector. A Bio-Rad Aminex HPX-87H column was used for quantification of concentrations of sugars. The mobile phase of the column was 0.05 $mol \cdot L^{-1}$ H_2SO_4 at a flow rate of 0.6 $ml \cdot min^{-1}$ and the column temperature was 65 °C [25].

2.5. Statistical analysis

All treatments in this study were conducted in triplicate under the same conditions, with their average values reported. The squared Euclidean distance was used for distance measure and transform measure was rescaled to 0–1 range in cluster analysis. Based on the treatments, the similarity matrix was obtained and hierarchical clustering was performed with SPSS 18.0 software.

3. Results and Discussion

3.1. Composition of elephant grass cultivars and harvests

Five elephant grass cultivars harvested at three stages of growth were studied. Their total contents of cellulose, hemicellulose and Klason lignin were above 60% (dry mass) (Tables 2–4). No significant difference is found for chemical compositions among the five cultivars harvested at the same stage, while the contents of cellulose and hemicellulose increase gradually and the Klason lignin content increases dramatically with the delay of harvest time. It may be explained by the fact that the lignification is enhanced with time. Similar results have been observed by Xie *et al.* [26–28].

Table 2
Composition of elephant grass cultivars harvested in August

	Component/%				
	Cellulose	Hemicellulose	Klason lignin	Other extractives	Ash
HP	33.80 ± 0.13	17.89 ± 0.15	12.01 ± 0.07	4.34 ± 0.22	9.22 ± 0.03
GM	35.77 ± 0.26	16.86 ± 0.13	16.14 ± 0.08	6.78 ± 0.52	5.50 ± 0.04
N51	33.26 ± 0.21	17.33 ± 0.12	12.50 ± 0.11	5.08 ± 0.26	9.28 ± 0.05
SM	32.82 ± 0.19	16.43 ± 0.14	13.44 ± 0.08	5.81 ± 0.33	9.07 ± 0.04
HN	33.35 ± 0.17	16.08 ± 0.16	16.42 ± 0.08	7.28 ± 0.17	6.85 ± 0.05

Table 3
Composition of elephant grass cultivars harvested in September

	Component/%				
	Cellulose	Hemicellulose	Klason lignin	Other extractives	Ash
HP	36.02 ± 0.11	18.24 ± 0.14	16.62 ± 0.06	2.63 ± 0.02	8.79 ± 0.04
GM	36.93 ± 0.16	18.50 ± 0.12	19.33 ± 0.09	0.97 ± 0.07	8.61 ± 0.06
N51	35.31 ± 0.13	18.98 ± 0.16	18.57 ± 0.08	3.26 ± 0.03	7.47 ± 0.07
SM	38.10 ± 0.11	19.34 ± 0.12	19.34 ± 0.05	2.25 ± 0.10	6.69 ± 0.04
HN	35.65 ± 0.09	17.84 ± 0.11	19.31 ± 0.06	3.70 ± 0.06	6.97 ± 0.03

Table 4
Composition of elephant grass cultivars harvested in October

	Component/%				
	Cellulose	Hemicellulose	Klason lignin	Other extractives	Ash
HP	36.32 ± 0.14	19.41 ± 0.11	18.58 ± 0.06	2.91 ± 0.02	8.34 ± 0.06
GM	38.63 ± 0.16	19.20 ± 0.06	20.08 ± 0.10	3.49 ± 0.04	7.74 ± 0.07
N51	36.68 ± 0.12	18.19 ± 0.10	18.60 ± 0.08	2.98 ± 0.04	7.36 ± 0.03
SM	37.27 ± 0.10	17.90 ± 0.09	21.22 ± 0.04	4.39 ± 0.03	6.77 ± 0.07
HN	38.75 ± 0.13	18.27 ± 0.11	20.57 ± 0.05	4.58 ± 0.03	8.09 ± 0.08