Effect of Acid–chlorite Pretreatment on *Miscanthus* Harvesting Date for Biosugar Production

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Miscanthus (Geodae-Uksae in Korean, GU) is a promising source of biomass for biosugar production due to its superior mass yield and minimal need for nitrogen fertilizer and herbicides. In this study, the biomass productivity, chemical composition, and physical properties of *Miscanthus* were investigated for two harvest dates. The total biomass of early harvest GU (EH-GU; 20.1 t DM/ha) was greater than that of the delayed-harvest GU (DH-GU; 18.0 t DM/ha). There was no noticeable difference in the carbohydrate content between the EH-GU and DH-GU, while the lignin content remarkable decreased after pretreatment. The enzymatic conversion rates of GU to biosugar increased after pretreatment, to approximately 86 to 90%. After enzymatic hydrolysis, 381 g and 147 g of glucose and xylose were produced, respectively. Consequently, 528 g of biosugar was obtained from 1 kg of EH-GU. Determining the optimal harvest date of *Miscanthus* has enabled a more efficient enzymatic hydrolysis and higher biosugar yields.

Keywords: Biosugar; Acid–chlorite pretreatment; Enzymatic hydrolysis; Miscanthus; Harvest date

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INTRODUCTION

Alternative biofuels and biochemical resources are required to sustain both economic growth and the environment. However, the production of non-petroleum liquid fuels and chemicals from food crops is not sustainable due to competition for materials and high production costs (Carroll and Someville 2009). Therefore, cheap and abundant non-food materials (*e.g.*, agricultural byproducts, woody biomass, or energy crops) are required as alternative biomass feedstocks, and processes must be developed that can efficiently and economically convert lignocellulosic and cellulosic biomass into biosugar (Choi *et al.* 2015b; Kim *et al.* 2016, 2017b). These processes produce sugar from biomass through saccharification, biofuels, and biochemicals from the sugar through fermentation (Choi *et al.* 2013). Cellulose and hemicellulose can be hydrolyzed to glucose and xylose, respectively, and subsequently converted to biofuels and biochemicals, such as ethanol or lactic acid, through fermentation (Demirbas 2011; Wi *et al.* 2015a).

Geodae-Uksae (GU; the Korean term for Giant *Miscanthus*), is a variety of *Miscanthus sacchariflorus* recently discovered in Korea that grows approximately 4-m tall, with an average stalk diameter of 1 cm, which is approximately twice as tall and thick as the common *M. sacchariflorus* (Moon *et al.* 2010). The mass yield of the dry stalk can be up to 20 to 30 t/ha, which is twice that of common *Miscanthus* (Moon *et al.* 2013). Due to its superior yield, GU is considered a good bioenergy crop, as the biomass cost affects the

economics of biofuel production. The carbohydrate composition of GU used in this study differs from those of previous studies (Hayes 2013; Wahid *et al.* 2015). This can be due to various factors, including the type of cultivar, stage of maturation, environmental conditions, agronomic conditions, storage time, and bulb section, and it is well known that plants have different chemical and physical properties at different developmental stages. Changes in biomass chemical composition can greatly affect the quality of feedstock for biofuel production (Kärcher *et al.* 2015; Frydendal-Nielsen *et al.* 2016). Therefore, the harvest date is an important factor that can affect not only the chemical composition but also biomass productivity (Ruf *et al.* 2017).

In general, *Miscanthus* plants used for paper and composites are harvested at the end of their growing season (Marín *et al.* 2009). The plants are allowed to dry, and then are processed to produce fibers. While the yield of an early harvest is low, there is an advantage for using biosugar in that there is reduced energy consumption during the pretreatment process for bioenergy production. For example, biomass from early-harvested plants contains low amounts of lignin (Frydendal-Nielsen *et al.* 2016), yielding biomass more suitable for biosugar production and requiring only mild pretreatment with low loadings of hydrolytic enzymes (Öhgren *et al.* 2007). Because GU biomass has many and varied applications, a better understanding of the effects of harvest date will enable more effective use of this resource. Efficient utilization of lignocellulose requires a pretreatment process to minimize lignin for effective enzymatic hydrolysis (Choi *et al*. 2012). A pretreatment is also necessary because GU biomass contains high concentrations of lignin. The acid-chlorite method, originally known as the Wise method, is oxidative treatment that can selectively remove lignin (Kumar *et al*. 2013). This method applied to sugar production from coffee residue waste and various hardwood (Kim *et al*. 2017a; Malgas *et al*. 2017) Furthermore acid–chlorite delignification implied to acid-cellulose nanofibrils from kenaf for the absorbent of cationic dye (Chan *et al*. 2015).

In this study, GU biomass was used as a biosugar resource, and the effect of harvest date on enzymatic hydrolysis was studied by analyzing the GU enzymatic hydrolysis, chemical composition, and physical properties. This research also considered the effect of pretreatment by acid–chlorite delignification, which has a significantly lower environmental impact and greater enzymatic hydrolysis efficiency (Davis *et al*. 2013; Kumar *et al.* 2013; Kim *et al.* 2017a; Malgas *et al*. 2017).

EXPERIMENTAL

Materials

The GU was planted in April of 2010 and was grown for six years in experimental fields in Muan (Korea) without nitrogen amendment. The dates of the growing days and harvests for different growth stages are shown in Table 1. The plants were harvested in September of 2016 (early harvest, EH-GU) and February of 2017 (delayed harvest, DH-GU). The average weight was estimated from three random blocks in the field at different areas. The daily biomass productivity was determined by the growing days. Plant samples were dried at 60 °C in an air-forced oven for three days and were stored under dry conditions at room temperature prior to use. All of the samples were ground in a Retsch mill equipped with a 0.5-mm sieve. The acid–chlorite pretreatment was applied to each sample for enzymatic hydrolysis analysis (Hubbell and Ragauskas 2010; Kim *et al.* 2017a). In this process, 10 g of GU were incubated in 0.8 mL of acetic acid and 4 g of sodium

chlorite at 80 °C for 1 h. This procedure was repeated three times each hour by adding chemicals and then neutralizing with distilled water and drying.

Table 1. Phenological Dates and Productivity of GU

	ST(JD)	GD (Harvest Day)	Average Weight (t DM/ha)	Productivity (kg DM/ha/Plant Day)
EH-GU	97	161	20.1 ± 1.1	124.8 ± 0.9
DH-GU	97	295	18.0 ± 1.6	61.0 ± 0.6

Abbreviations: ST = Sprouting time; JD = Julian day; GD = Growing days; values represent the average of three replicates

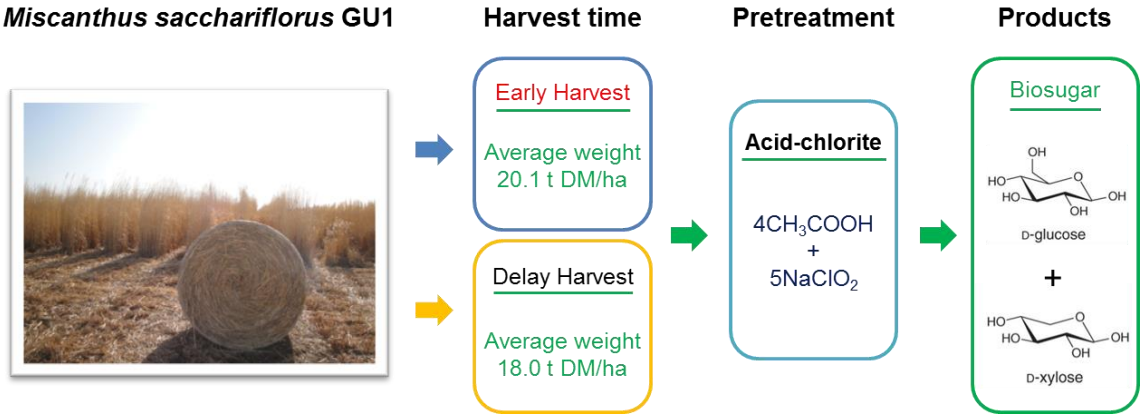

Fig. 1. Schematic representation of *Miscanthus* biosugar production processes

Methods

Determination of cell wall composition- Chemical composition

The authors determined the chemical composition for Klason lignin according to TAPPI T222 om-88 (1992), the organic solvent extractives (TAPPI T204 om-88 (1992)), and the ash content (TAPPI T211 om-85 (1992)) of the raw and pretreated GU using TAPPI standard methods.

Carbohydrate content

The determination of the structure of a polysaccharide of plant cell wall required knowledge of which sugars are present and in what amount. The alditol acetate derivatives or the formation of trimethylsilyl ethers are commonly used. When the present of uronic acids is suspected, the trimethylsilyl ethers method is preferred because uronic acid derivatives can be analyzed (Albersheim *et al*. 2011). In this study, alditol acetate was adapted to structural polysaccharide analysis. Structural carbohydrates of EH-GU and DH-GU were analyzed for their neutral sugar content using gas chromatography (GC) (Choi *et al*. 2012). The samples were hydrolyzed in 72% sulfuric acid for 45 min at room temperature, and diluted with distilled water to 4% sulfuric acid, followed by autoclaving for 1 h at 121 °C. The neutral sugar composition was measured with alditol acetates containing myo-inositol as an internal standard. The gas chromatograph (GC-2010; Shimadzu, Tokyo, Japan) used a DB-225 capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness) and operated with He at an injector temperature of 220 °C, a flame ionization detector (FID) at 250 °C, and an oven temperature programming of 100 °C for 1.5 min and 5 °C/min to 220 °C.

Analysis of surface and relative crystallinity of biomass

The Brunauer-Emmett-Teller (BET) method was utilized to analyze the pore size and volume of the samples using nitrogen adsorption desorption isotherms at -196 °C in a surface-area analyzer (ASAP 2020; Micromeritics Co., Norcross, GA, USA) (Wi *et al.* 2015b). The sample was degassed for 1.5 h at 110 °C under vacuum to remove the moisture and any other contaminants. The total pore volume was analyzed by converting the amount of nitrogen gas adsorbed to the volume (cm^3/g at standard temperature and pressure (273.15 K, 101.325 kPa); STP) of liquid adsorbate. The relative crystallinity of the samples was measured by X-ray diffraction (XRD), using a diffractometer with Cu Kα radiation at 40 kV and 30 mA (X'Pert PRO MPD, PANalytical, Almeo, Netherlands) (Wi *et al.* 2015b). The crystallinity of each sample was revealed by the crystallinity index (CrI) (Segal *et al.* 1959).

Enzyme assays and enzymatic hydrolysis

Cellulase (C-TEC II, Novozymes, Franklinton, NC, USA) and xylanase (H-TEC, Novozymes, Franklinton, NC, USA) were used for enzymatic hydrolysis. Cellulase and xylanase activity were measured *via* methods used by Adney and Baker (2008) and Choi *et al.* (2015b), respectively. Cellulase and xylanase activities were 117 filter paper unit (FPU)/mL and 1090 fungal xylanase unit (FXU)/mL, respectively. Cellulase and xylanase were added to the biomass at concentrations of 4.8 to 19.2 FPU/g and 2.3 to 11.5 mg/g biomass, respectively. Enzymatic hydrolysis was conducted at 1% DM (dry matter, w/v) initial substrate loading in a 50-mL conical tube with a 0.05 M sodium citrate buffer (pH 5). To evaluate and compare the effects of different enzymes and their relative concentrations in the hydrolysis yields from GU, different volumes of cellulase and xylanase were added to 1% GU (w/v) (Table 4). Our study focused mainly on the glucose and xylose yield for biosugar production. Although arabinose, mannose and galactose were present, their concentrations in the GU were low. To identify the loading volumes that resulted from synergistic interaction, the enzyme mixtures (cellulase and xylanase) were added in different combinations to the GU. Enzymatic hydrolysis was performed at 180 rpm for 72 h at 45 °C. Hydrolysates were measured *via* high performance liquid chromatography (HPLC) with a refractive index detector (2414; Waters, Milford, USA), REZEX RPM (Phenomenex, Torrence, CA, USA) column (300 mm × 7.8 mm) at 85 °C and eluted with deionized water at a flow rate of 0.6 mL/min.

RESULTS AND DISCUSSION

Biomass Productivity

The biomass quality of *Miscanthus* was determined for two different harvesting dates to evaluate its potential as a biofuel and biochemical crop. The aboveground average dry weight showed different biomass yields for the different harvesting dates (Table 1). The total biomass yields were 20.1 t DM/ha and 18.0 t DM/ha for EH-GU and DH-GU, respectively. The GU produced 124.8 kg DM/ha of biomass with 161 growing days (EH-GU), and 61 kg/DM/ha of biomass with 295 growing days (DH-GU). Thus, the biomass yield of EH-GU was approximately twice as high as that of DH-GU; this may have been due to leaf shedding during plant senescence. Similar results have been reported by other studies (Amougou *et al.* 2011; Ruf *et al.* 2017), which reported that *Miscanthus* harvested in the spring yielded 20 to 30% less biomass than that harvested in autumn.

Chemical Composition of GU

In this study, the structural sugars analyzed using GC with alditol acetate method. Although NREL/TP-510-42628 method commonly used in recent study, some of sugars were presented overlap (Choi *et al*. 2012). The chemical compositions of EH-GU and DH-GU differed, as shown in Table 2. Although arabinose, mannose, and galactose were present, they appeared in low concentrations in the EH-GU and DH-GU. The authors mainly focused on major biosugar components, namely, glucose and xylose. Proportions of the major biosugar components, glucose and xylose, differed only slightly for the different harvest dates. The carbohydrates in EH-GU mainly consisted of 45.1% glucose and 20.1% xylose with 18.0% lignin, while DH-GU carbohydrates comprised of 47.4% glucose, 22.6% xylose, and 20.1% lignin. The difference in carbohydrates between the EH-GU and DH-GU was not substantial. Acid–chlorite pretreatment is used for delignification or holocellulose extraction (Kumar *et al*. 2013; Kim *et al*. 2017a). This pretreatment removed lignin and improved the efficiency of enzymatic hydrolysis.

There was an increasing trend for both glucose and xylose, but the lignin concentration decreased after the pretreatment. The total carbohydrate content of GU increased from 67.7% to 93.9% in EH-GU, and from 73.3% to 91.7% in DH-GU. Lignin delignification was observed after pretreatment. The lignin content decreased from 18.0% and 20.1% to 2.1% and 3.8% in EH-GU and DH-GU, respectively. This is because pretreatment mostly remove lignin with some hemicellulose. This result was consistent with previous studies on switch grass and poplar biomass, in which a similar delignification was observed in the cell wall composition after acid-chlorite pretreatment among biomass (Kumar *et al*. 2013). The total carbohydrate content of EH-GU slightly increased more than that of DH-GU, which might have been due to the loss of lignin. After the pretreatment, the biosugar composition for EH-GU mainly consisted of glucose (45.1%) and xylose (20.1%), while DH-GU contained 47.7% glucose and 22.6% xylose (Table 2). The acid–chlorite pretreatment increased the biosugar and decreased the lignin content (Kim *et al*. 2017a). Therefore, this treatment is used in various biorefinery operations and is an important process in the production of biosugar.

Table 2. Chemical Composition of EH-GU and DH-GU With and Without Acid–chlorite Pretreatment

(% Dry Matter)	Sugar						Lignin
	Ara	Xyl	Man	Galc	Glu	Total	
Raw							
EH-GU	1.7 ± 0.2	20.1 ± 0.6	0.4 ± 0.1	0.4 ± 0.0	45.1 ± 2.5	67.7 ± 2.5	18.0 ± 0.1
DH-GU	1.9 ± 0.1	22.6 ± 0.5	0.7 ± 0.1	0.7 ± 0.2	47.4 ± 0.9	73.3 ± 0.6	20.1 ± 0.0
Pretreated							
EH-GU	1.8 ± 0.1	26.2 ± 0.3	0.5 ± 0.1	0.6 ± 0.1	64.8 ± 0.9	93.9 ± 0.8	2.1 ± 0.1
DH-GU	2.0 ± 0.3	27.4 ± 0.4	0.7 ± 0.1	0.8 ± 0.2	60.8 ± 2.5	91.7 ± 1.1	3.8 ± 0.0

Abbreviations: EH-GU = early-harvest *Miscanthus*; DH-GU = delay-harvest *Miscanthus*; Ara = arabinose; Xyl = xylose; Man = mannose; Galc = galactose; Glu = glucose; Values represent the average of three replicates

Biomass Surface Area and Relative Crystallinity

Surface analysis (BET)

Enzymatic hydrolysis is affected by cellulose crystallinity as well as the lignin and hemicellulose contents, porosity, and particle size. Surface area is also a major factor affecting enzymatic hydrolysis (Wi *et al.* 2015a). The specific surface areas of the dried GU samples were characterized by nitrogen adsorption. The data are summarized in Table 3 as the surface area, average pore diameter, and total pore volume. The EH-GU possessed a larger surface area, which may have been a consequence of the higher total volume. The surface area of EH-GU was 1.52 m^2/g, which was greater than the surface area (0.72 m^2/g) of DH-GU. The EH-GU also showed a higher surface area than DH-GU after acid–chlorite pretreatment. The average pore radius of EH-GU (23.44 nm) was less than that of DH-GU (28.50 nm), but after pretreatment, the average pore radius of DH-GU (24.57 nm) was less than that of EH-GU (37.64 nm), which might have been due to the loss of lignin (Meng and Ragauskas 2014).

Table 3. Comparison of BET, Mean Pore Diameter, and Total Pore Volume Between EH-GU and DH-GU With and Without Pretreatment

	BET	Mean Pore Diameter (nm)	Total Pore Volume (cm^3/g)
Raw			
EH – GU	1.52	23.44	0.009
LH – GU	0.72	28.50	0.005
Pretreated			
EH – GU	2.85	37.64	0.017
LH – GU	2.22	24.57	0.014

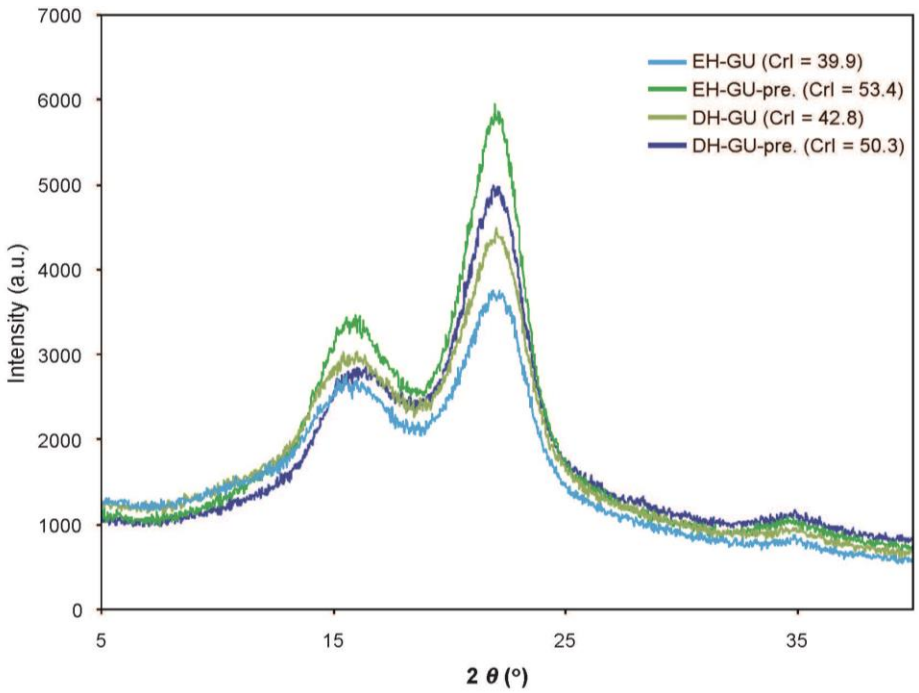

Fig. 2. XRD profiles of *Miscanthus* biomass; EH-GU = early-harvest *Miscanthus*; DH-GU = delayed-harvest *Miscanthus*; EH-GU-pre. = EH-GU with pretreatment; and DH-GU-pre. = DH-GU with pretreatment

Relative crystallinity of GU

The XRD experiments were conducted to analyze the crystallinity of GU prior to and after pretreatment (Fig. 2). The XRD spectra of GU showed a characteristic peak of cellulose Iβ that corresponded to the (110), (110), and (200) lattice planes. The EH-GU showed 39.9% CrI compared to 42.8% for DH-GU. Both the pretreated EH-GU and DH-GU showed increased CrI values of 53.4% and 50.3%, respectively. This may have been due to the removal of amorphous substances and lignin in the biomass. Other studies have also shown that pretreatment increases the biomass CrI value and enzymatic hydrolysis rate through removing lignin and amorphous hemicellulose (Wi *et al.* 2015b; Kim *et al.* 2017a).

Enzyme Optimization and Hydrolysis

The current cost of enzymes for biomass hydrolysis is a major obstacle for large-scale biosugar production. The minimum cellulase cost is estimated at $10/kg protein. Consequently, the amount of enzymes required for biomass hydrolysis must be reduced to commercialize the process (Klein-Marcuschamer *et al.* 2012; Choi *et al.* 2015a). In this study, the authors optimized the enzyme loading content of cellulase and xylanase to increase the biosugar yield.

Optimizing enzyme loading for hydrolysis

The authors varied the volumes of cellulase or xylanase to evaluate and compare the hydrolysis yields of different enzyme loadings obtained from different EH-GU and DH-GU (Fig. 3 and Table 4) samples. The three-dimensional profiles, which produced using Sigma plot 12 (San Jose, CA), show the influences of the harvest date and pretreatment with enzymatic hydrolysis on biosugar accumulation (Fig. 3). After 48 h of enzymatic hydrolysis, the biosugar content was analyzed *via* HPLC (Table 4). Because the released biosugar in hydrolysates measured without any pretreatment such as alditol acetate. The harvest date and pretreatment both affected enzymatic hydrolysis. The biosugar concentrations after cellulase (14.4 mg/g GU) and xylanase (9.2 mg/g GU) hydrolysis in EH-GU and pretreated EH-GU were 1.6 and 8.12 mg/mL, respectively. An increase was observed in the biosugar concentrations of the pretreated EH-GU when the enzyme loading increased from 4.8 to 14.4 mg of cellulase and from 0 to 9.2 mg of xylanase. However, the biosugar concentration did not further increase with the addition of more cellulase (19.2 mg/g GU) and xylanase (11.5 mg/g GU), which confirmed that 14.4 mg cellulase and 9.2 mg xylanase/g GU provided the most efficient dosage for enzymatic hydrolysis during biosugar production. The enzymatic hydrolysis process of DH-GU and pretreated DH-GU used the same enzyme loading but produced less biosugar than EH-GU.

Enzymatic conversion rate and concentration

The authors evaluated the enzymatic conversion of EH-GU and DH-GU with and without pretreatment, based on the enzyme loading results in Table 4. The conversion rates were calculated based on the total glucose and xylose of EH- and DH-GU. The cellulase and xylanase mixture was incubated with EH-GU for 48 h, and the glucose and xylose conversion rates reached 29.3% and 13.9%, respectively. For DH-GU, the conversion rates were much less at 27.9% for glucose and 12.8% for xylose. After pretreatment, the biosugar conversion rate increased in both the EH-GU (90.4% for glucose and 86.3% for xylose) and the DH-GU (87.3% for glucose and 85.8% for xylose) as shown in Fig. 4B.

Table 4. Enzyme Optimization under Different Enzyme Loadings for EH-GU and DH-GU with and without Acid–chlorite Pretreatment

Enzyme (mg/g GU)		EH-GU (mg/mL)		DH-GU (mg/mL)		EH-GU-Pre. (mg/mL)		DH-GU-Pre. (mg/mL)	
Cellulase	Xylanase	Glu	Xyl	Glu	Xyl	Glu	Xyl	Glu	Xyl
4.8	0	0.06	0.00	0.07	0.00	2.11	0.01	1.92	0.01
4.8	2.3	0.21	0.03	0.23	0.06	2.53	0.12	2.41	0.13
4.8	4.6	0.52	0.12	0.51	0.09	3.01	0.22	2.73	0.26
4.8	6.9	0.92	0.19	1.09	0.16	3.33	0.32	2.95	0.39
4.8	9.2	1.19	0.16	1.22	0.23	3.62	0.52	3.11	0.53
4.8	11.5	1.31	0.16	1.30	0.25	3.64	0.52	3.10	0.54
9.6	0	0.13	0.00	0.16	0.00	2.72	0.03	2.64	0.04
9.6	2.3	0.41	0.04	0.51	0.05	3.66	0.32	3.43	0.33
9.6	4.6	0.73	0.11	0.74	0.12	4.01	0.74	3.83	0.72
9.6	6.9	1.03	0.20	1.01	0.19	4.43	1.11	4.23	1.11
9.6	9.2	1.23	0.27	1.29	0.26	4.66	1.53	4.41	1.51
9.6	11.5	1.44	0.28	1.43	0.29	4.84	1.77	4.71	1.68
14.4	0	0.20	0.00	0.18	0.00	3.84	0.05	3.52	0.04
14.4	2.3	0.46	0.09	0.42	0.07	4.44	1.02	4.12	1.03
14.4	4.6	0.70	0.16	0.69	0.13	5.21	1.54	4.82	1.50
14.4	6.9	0.11	0.23	0.94	0.22	5.73	1.96	5.12	1.99
14.4	9.2	1.32	0.28	1.31	0.29	5.86	2.26	5.30	2.32
14.4	11.5	1.42	0.29	1.40	0.28	5.85	2.29	5.31	2.33
19.2	0	0.30	0.00	0.20	0.00	3.69	0.09	3.41	0.07
19.2	2.3	0.50	0.11	0.45	0.13	4.45	1.32	4.16	1.21
19.2	4.6	0.80	0.16	0.76	0.19	5.26	1.71	4.92	1.61
19.2	6.9	1.16	0.24	1.06	0.26	5.69	2.01	5.10	2.16
19.2	9.2	1.42	0.27	1.45	0.29	5.87	2.25	5.31	2.35
19.2	11.5	1.50	0.28	1.48	0.30	5.88	2.22	5.30	2.33

Abbreviations: EH-GU = early-harvest *Miscanthus*; DH-GU = delay-harvest *Miscanthus*; Glu = glucose; Xyl = xylose; Values represent the average of three replicates

The biosugar concentrations are shown in Fig. 4A. Different harvest dates led to differences in the biosugar concentration. The maximum glucose and xylose concentrations for the pretreated EH-GU were 5.9 and 2.3 mg/mL, respectively. However, the pretreated DH-GU produced 5.3 mg/mL of glucose and 2.3 mg/mL of xylose. In other words, the biosugar concentrations were 8.2 and 7.6 mg/mL for the pretreated EH-GU and DH-GU, respectively. This may be because the growing period and enzymatic hydrolysis are intimately related (Wi *et al.* 2015b). Compare with the analysis results in the switchgrass (Bals *et al*. 2010), the present study revealed similar effect of harvest date and enzymatic hydrolysis on bioenergy crop. The chemical composition and physical properties of the biomass strongly influence enzymatic hydrolysis (Chang and Holtzapple 2000; Himmel *et al.* 2007). No single factor is sufficient to prevent noticeable hydrolysis, because the relationships between the compositional and physical factors in cellulosic cell walls are complex (Choi *et al.* 2015). These results suggest that EH-GU can be a potential biomass material for biosugar production.

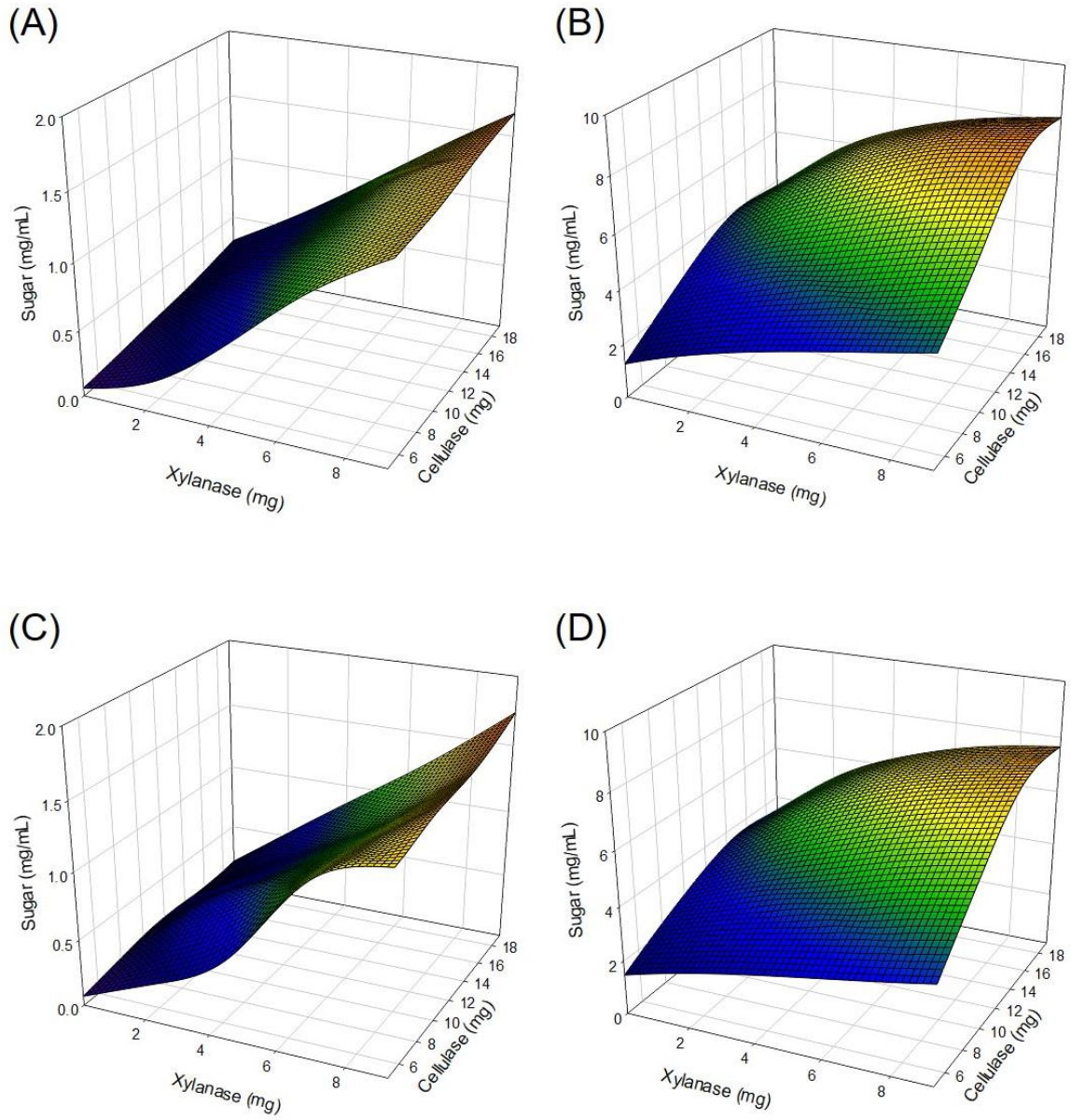

Fig. 3. Three-dimensional profiles of sugars *versus* pretreatment conditions and enzyme loading

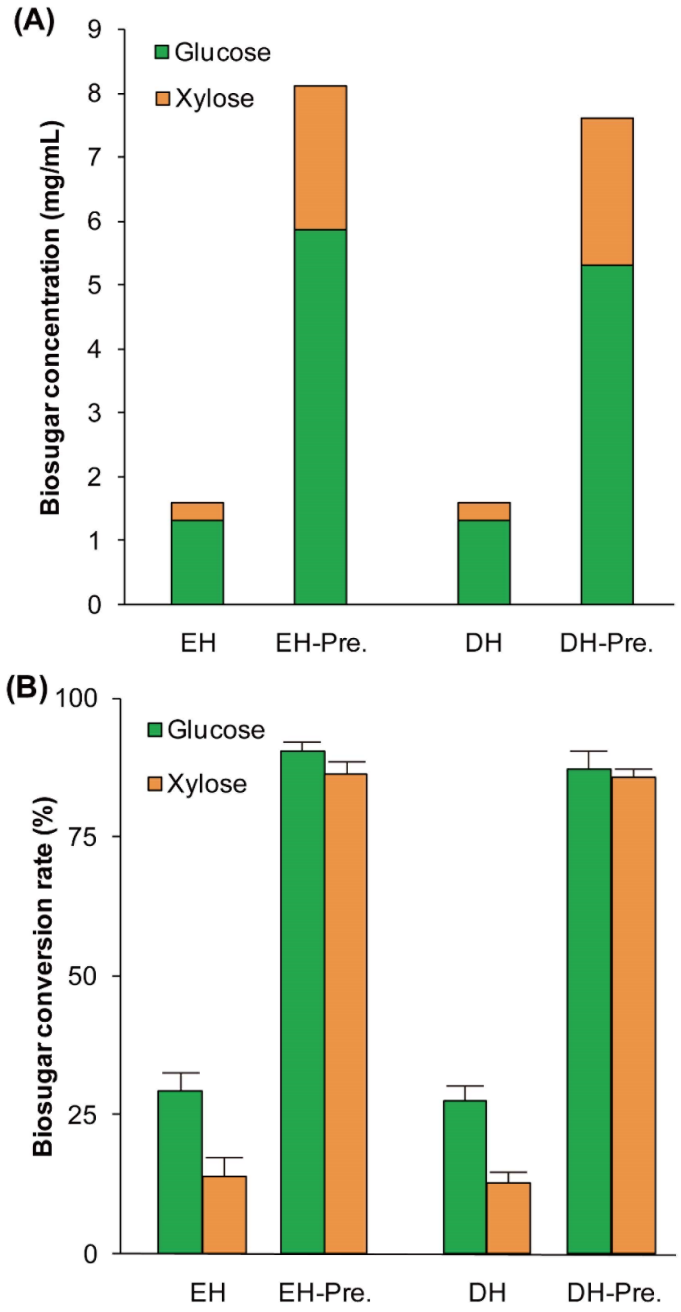

Fig. 4. *Miscanthus*-to-biosugar concentration (A) and conversion rates (B)

Overall Mass Balance

Figure 5 depicts an overall mass balance diagram that describes the pretreatment and conversion of GU to biosugar *via* enzymatic hydrolysis. It is shown that 1 kg of EH-GU yielded 451 g of glucose and 201 g of xylose. The pretreatment was processed with acetic acid and sodium chlorite at 80 °C. Enzymatic hydrolysis was performed on the pretreated EH-GU with 14.4 mg of cellulase and 9.2 mg of xylanase/g GU at 45 °C for 48 h. After enzymatic hydrolysis, 381 g of glucose and 147 g of xylose were produced for a total 528 g of biosugar from 1 kg of EH-GU.

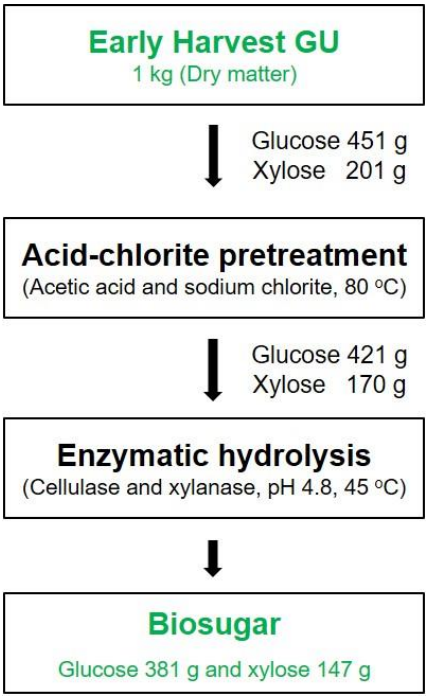

Fig. 5. Overall early-harvest *Miscanthus* mass balance

CONCLUSIONS

1. The harvest date of *Miscanthus* can affect the biomass yield and the quality and can be chosen to optimize biosugar production.
2. Biomass production was higher for early-harvest GU than delayed-harvest GU, and the former exhibited more efficient enzymatic hydrolysis, which was enhanced by acid– chlorite pretreatment.
3. These findings enabled the optimal harvesting of biomass from *Miscanthus* during the most appropriate growth periods, allowing more efficient enzymatic hydrolysis and higher biosugar yields.

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