**Effect of harvest date on *Arundo donax* L. (giant reed) composition, ensilage performance,
and enzymatic digestibility**

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Abstract

Composition and ensilage performance of giant reed harvested in August, October, November, and December, were evaluated and compared. Generally, late-harvested giant reed had higher dry matter content, lower nitrogen content, and higher water soluble carbohydrates (WSC) content than early-harvested giant reed. During 90 days of ensilage, giant reed harvested in October, November, and December showed dry matter losses of about 1%, while giant reed harvested in August showed a higher dry matter loss of about 8%. During the ensilage process, more lactic acid was produced in late-harvested giant reed than in early-harvested giant reed. Late-harvested giant reed had a higher lignin content and lower enzymatic digestibility than early-harvested giant reed. However, enzymatic digestibility of all the giant reed biomass was improved by the 90-day ensilage process, reaching levels of 43-46%. In summary, ensilage could be used for storing giant reed biomass harvested at different times and for improving its digestibility.

Keywords: Giant reed; Ensilage; Harvest time; Energy crops; Enzymatic digestibility

1. Introduction

Arundo donax L., commonly known as “giant reed”, is a fast-growing perennial rhizomatous grass that has an average biomass yield of 30-40 tons of dry matter (DM) per hectare per year (Angelini et al., 2009). It can adapt to different types of soils and weather conditions, and requires very low cultivation inputs (Ge et al., 2016). As a result, giant reed has been considered a promising biomass feedstock for bio-refineries. Various biofuels, such as ethanol and methane, and bio-products, such as particle board, paper, and xylo-oligosaccharides, can be produced from giant reed biomass (Ge et al., 2016). Giant reed has recently been proposed as an energy crop for biogas production, and has been grown for bioenergy production on 25 farms in Italy since 2013 (Luca et al., 2015).

Storage of biomass feedstocks is a crucial step for sustainable bio-refining for the production of biofuels and bio-products. Ensilage is a traditional method for storing green crops, such as corn stover, in the livestock industry (Darr and Shah, 2012). During ensilage, lactic acid bacteria (LAB) break down free sugars in the biomass under anaerobic conditions, generating lactic acid, acetic acid, ethanol, and carbon dioxide through the glycolytic pathway, 6-phosphogluconate pathway or phosphoketolase pathway. Homofermentative LAB produce lactic acid as the main product, while hetero-fermentative LAB also produce acetic acid and ethanol (Egg et al., 1993; Herrmann et al., 2011). Besides, propionic acid can also be produced by propionic acid bacteria or hetero-fermentative bacteria during the ensilage process (Dreihuis et al., 1999; Oude Elferink et al., 2001). The organic acids, especially lactic acid, decreased the pH to around 4, which is low enough to inhibit growth of microorganisms (Herrmann et al., 2011; Pakarinen et al., 2011, 2008). Compared to acetic acid and propionic acid, lactic acid is more effective in decreasing pH due to its lower pKa value (3.86), and thus is more effective for

ensilage (Zheng et al., 2011). Besides preserving crops for livestock feed, ensilage has also been studied as a method of preserving lignocellulosic biomass for bio-based energy and products. Ensilage is particularly suitable for methane production by anaerobic digestion, since organic acids and ethanol that may inhibit fermentation processes are common intermediates for biogas generation in anaerobic digestion process (Darr and Shah, 2012; Herrmann et al., 2011; Liu et al., 2015a). To date, however, few studies on ensilage of giant reed have been reported (Liu et al., 2016, 2015a, 2015b).

Giant reed can be harvested at different times of the year (Ge et al., 2016; Lewandowski et al., 2003). According to the literature, the harvest date can significantly affect biomass composition, such as moisture, nitrogen, mineral, and carbohydrate contents, which could further affect the performance of the subsequent ensilage process (Nassi o Di Nasso et al., 2011; Vasco-Correa and Li, 2015). However, there are very few publications on composition of giant reed harvested at different times (Nassi o Di Nasso et al., 2011). To the best of authors' knowledge, no report on the effect of harvest date on the effectiveness of giant reed ensilage has been published.

In this study, the composition of giant reed harvested at different times was analyzed. Dry matter (DM), organic dry matter (ODM), carbon to nitrogen (C/N) ratio, water soluble carbohydrates (WSC), protein, cellulose, hemicellulose and lignin contents in the giant reed biomass were determined. Ensilage of the giant reed biomass was conducted for different periods of time. Changes in DM, extractive, WSC, cellulose, hemicellulose, and lignin contents; pH; and organic acid and ethanol production during ensilage of the giant reed biomass were examined. Enzymatic hydrolysis of fresh or ensiled giant reed was carried out, and the effect of harvest date on enzymatic digestibility of fresh and ensiled giant reed was studied.

2. Materials and Methods

2.1. Feedstocks

Giant reed was planted in April 2013 at the Ohio State University (OSU) research farm (Columbus, OH, US.) and harvested in 2014 on August 26, October 3, November 6, and December 10. The harvested giant reed biomass was ground to pass through a 12 mm sieve using a shredder-chipper (Mighty Mac, Mackissic Inc., Parker Ford, PA, USA), and then ensiled. For each harvest of giant reed biomass, the harvesting, grinding, and initiation of ensilage were conducted in one day.

2.2. Ensilage of giant reed biomass

Ensilage was conducted by packing 1 kg of giant reed biomass into 1-gallon zipper bags (Ziploc Vacuum Freezer System, SC Johnson Inc., Racine, WI, USA) at room temperature ($25 \pm 3^\circ\text{C}$). According to previous study, moisture contents of 60-70% for ensilage had no significant effect on glucose yield (Liu et al., 2016). In this study, water was added to giant reed biomass to reach a moisture content of 60%, except for that harvested in August which had a moisture content of 68%. The presence of oxygen in the plastic bags was minimized by vacuuming the air out of the bags prior to storage. For each batch (i.e., harvest date) of giant reed, 24 identical bags were prepared. On days 0, 3, 7, 15, 30, 45, 60, and 90, three of the bags were randomly selected and the giant reed was thoroughly mixed. After sampling the mixture for composition analysis, the remaining samples were stored at -20°C for enzymatic hydrolysis. All tests were performed in triplicate.

2.3. Enzymatic hydrolysis of ensiled giant reed biomass

Enzymatic hydrolysis of ensiled giant reed using cellulase (Cellic CTec 2, Novozymes, Denmark) was conducted (in duplicate) according to a Laboratory Analytical Procedure (LAP) reported by the National Renewable Energy Laboratory (NREL) (Selig et al., 2008). Cellulase (Cellic CTec2, Novozymes, Denmark) was used in this study, and its activity was determined to be 137 FPU/ml based on the NREL LAP (Adney and Nrel, 2008). Samples supplemented with cellulase (60 FPU per gram of cellulose) were incubated at 50°C with shaking at 180 rpm for 72 hours, and each hydrolysate was filtered through a 0.2 µm nylon membrane filter prior to sugar analysis. The enzymatic digestibility was defined as the glucose yield from cellulose by enzymatic hydrolysis, and calculated as follows:

$$\text{Glucose yield (\%)} = 100 \times W_{\text{glucose}} / (f \times W_{\text{cellulose}}) \quad (1)$$

where W_{glucose} is the amount of glucose released from cellulose by enzymatic hydrolysis (i.e., glucose present in the sample and the enzyme solution before hydrolysis is subtracted); $W_{\text{cellulose}}$ is the amount of cellulose in the initial sample (determined by a method described in section 2.4); and $f = 180/162$, which is the conversion factor for cellulose to glucose (Cui et al., 2012).

2.4. Analytical methods

DM, ODM, total Kjeldahl nitrogen (TKN), and pH of samples were measured based on the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). A 5-g sample was suspended in 50 mL of de-ionized (DI) water prior to pH measurement. The C/N ratio was calculated based on total carbon (TC) and total nitrogen (TN) contents, which were determined using an elemental analyzer (Elementar Vario Max CNS, Elementar Americas, Mt. Laurel, NJ,

USA). Crude protein content was calculated by determining total organic nitrogen (TKN minus NH₃-N) and multiplying by a factor of 6.25 (Hattingh et al., 1967). DM loss during ensilage was attributed to the organic matter loss due to respiration of plants and activity of microorganisms converting sugar to carbon dioxide and other fermentation products during ensilage (Holzer et al., 2003). When the DM or volatile solids (VS) are determined by drying at 105°C, the volatile compounds are partially lost and cannot be calculated in measured TS. Thus, the VS used for calculating DM loss were corrected according to the following equation:

$$\text{Corrected TS (or VS)} = \text{TS (measured at 105 °C)} + \text{Ethanol} + 0.375 \text{ lactic acid} + 0.892 \times (\text{Acetic acid} + \text{Propionic acid} + \text{Butyric acid}) \quad (2)$$

where reported volatilization coefficients for ethanol, lactic acid, and total VFAs for silage dried at 100 °C were used (Kreuger et al., 2011).

Cellulose, hemicellulose, and lignin contents were analyzed based on an NREL LAP (Sluiter et al., 2011). Briefly, biomass was extracted with DI water and ethanol in series using an automated extraction unit (Dionex ASE 300 extraction system, Thermo Scientific, Sunnyvale, CA, USA), and then the extractive content was determined. Extractive-free biomass was hydrolyzed into monomers via a two-step acid hydrolysis, and the concentrations of mono-sugars were measured by high performance liquid chromatography (HPLC) to determine the cellulose and hemicellulose content. The amount of cellulose was calculated from glucose using a conversion factor of 0.90. The amount of hemicellulose was calculated from the sum of xylose, arabinose, galactose, and mannose, using a conversion factor of 0.90 for C-6 sugars and 0.88 for C-5 sugars. Acid-soluble lignin was measured by ultraviolet-visible (UV-vis) spectroscopy (BioMate 3S spectrophotometer, Waltham, MA, USA), and acid-insoluble lignin was determined by gravimetric analysis. The HPLC (Shimadzu LC-20AB, Columbia, MD, USA) used for sugar

analysis of acid hydrolysis and enzymatic hydrolysis was equipped with a Biorad Aminex HPX-87P column and a refractive index detector (RID). Temperatures of the column and detector were maintained at 60 °C and 55 °C, respectively. HPLC grade water was used as the mobile phase with a flow rate of 0.3 mL/min.

In order to determine the content of lactic acid, acetic acid, propionic acid, butyric acid, and ethanol in the feedstock, a 10-g sample was suspended in 100 mL of DI water and incubated at 4 °C for 10 h (Liu et al., 2015a). The suspension was filtered through a 0.2 µm nylon membrane filter, and the filtrate was analyzed by a HPLC that was equipped with a Phenomenex Rezex RFQ-Fast Fruit H⁺ column (Phenomenex Inc., Torrance, CA, USA), a micro-guard cartridge (Catalog No.125-0129, 30×4.6 mm), a RID, and an ultra violet (UV) detector. Temperature of the column and the detector were set at 60 °C and 55 °C, respectively. H₂SO₄ (2.5 mM) was used as the mobile phase with a flow rate of 0.4 ml/min. RID was used to determine concentrations of lactic acid, acetic acid, and propionic acid. Since butyric acid and ethanol were difficult to separate from each other, their concentrations were determined based on the fact that butyric acid has a high UV absorbance at 190 nm, while ethanol does not have a significant absorbance at this wavelength. Briefly, butyric acid concentration was first determined via the UV detector. This concentration was used to calculate the RID peak area of butyric acid based on a pre-established calibration curve for butyric acid, and subtracted from the RID peak area for butyric acid/ethanol. The peak area after subtraction was then used to determine the ethanol concentration based on a pre-established calibration curve for ethanol.

2.5. Statistical analysis

Statistical significance was assessed by analysis of variance (ANOVA, $\alpha = 0.05$) using Minitab (Version 16, Minitab, Inc., State College, PA, USA).

3. Results and discussion

3.1. Effect of harvest date on composition of giant reed

DM content of giant reed harvested in August, October, November, and December were 32%, 44%, 43%, and 50%, respectively, while the ODM content varied slightly from 91% to 94% (Figure 1a). Generally, giant reed harvested at later dates (in October, November, and December) had lower moisture contents than that harvested at an earlier date (in August), thus a later harvest time could help reduce costs for biomass transportation and storage. However, it should be noted that giant reed leaves fall off in winter, which causes biomass loss (Lewandowski et al., 2003). This biomass loss was also found to be common for miscanthus, switchgrass, and reed canary, which stop growing in the autumn with significant leaf loss (around 20%) (Monti et al., 2008).

Late harvest also reduced the ash content from 9% to 6%, resulting in an increase in organic contents. Carbon contents (based on DM) in giant reed ranged from 50% to 46% from August to December (Figure 1b). Nitrogen content (based on DM) in giant reed decreased sharply and continuously from 0.86% in August to 0.44% in December (Figure 1b). As a result, the C/N ratio of giant reed increased by 43% (from 58 to 104) from August to December (Figure 1b). Similar to nitrogen content, protein content (based on DM) in giant reed also decreased from 6.97% in August to 2.29% in December (Figure 1c). There are two possible reasons for the reduced ash, nitrogen and protein contents in giant reed during their growth. One is translocation of nutrients, such as minerals and nitrogen, from the aboveground parts to the roots at the end of the growing season for (late fall and winter) (Smith and Slater, 2011). The translocated nutrients can be used for regrowth in the next year, which is common for rhizomatous grasses, such as

giant reed and miscanthus (Ge et al., 2016). The other reason is the loss of leaves in the fall, as leaves contain more minerals and nitrogen than the stems (Ge et al., 2016; Monti et al., 2008).

The WSC content (based on DM) in giant reed increased from 2.8% in August to about 5% in October, remained stable in November, and further increased to 5.96% in December (Figure 1c). Since WSC is the major substrate for lactic acid production by LAB, high WSC content generally results in high lactic acid levels and low pH during ensilage process, which are indicators of a successful ensilage (Weiland, 2010). Therefore, late-harvested giant reed (in December) may have better ensilage performance than early-harvested (in August) due to its higher WSC content.

3.2. Effect of harvest date on changes in composition of giant reed during ensilage

About 99% of DM was preserved during 90 days of ensilage, except for the August harvested giant reed which showed a DM loss of about 8% (Figure 2a). Extractives and WSC contents in giant reed biomass for all harvests were reduced sharply in the first 3 days of ensilage (Figure 2b and 2c). After 90 days of ensilage, giant reed harvested in December had more extractives and WSC remaining in the biomass and a lower pH than those harvested in August, October, and November (Figure 2b, 2c and 2d). The degradation of WSC in giant reed harvested in August, October, November and December were 92.1%, 91.4%, 92.7%, and 71.1%, respectively.

Generally, glucose and fructose in WSC are primary fermentation substrates for LAB, although pentoses from hydrolysis of hemicellulose are also fermented (Egg et al., 1993). During ensilage of giant reed harvested on different dates, more than 60% of cellobiose and glucose were consumed in 3 days (Table 1). After that, a small amount of cellobiose (about 2% of initial

DM) was maintained in giant reed harvested in December, which indicates that the pH in the ensiled biomass was low enough to prevent significant consumption of the cellobiose (Table 1).

During ensilage of giant reed harvested in August, October, November, and December, cellulose content in the biomass decreased by 8.6%, 5.1%, 3.6, and 3.3%, respectively (Figure 3a). More cellulose was preserved when harvest date changed from August through December. Hemicellulose was easier to degrade than cellulose during ensilage, and showed decreases of 12.3% (August), 12.4% (October), 5.0 (November), and 5.0% (December) (Figure 3b). Higher hemicellulose degradation than cellulose degradation has also been observed during ensilage of other feedstocks, such as corn stover and orchard grass (Ren et al., 2006; Yahaya et al., 2001). During the ensilage process, degradation of lignin in giant reed harvested in August, October, November, and December was in the range of 4.8%-6.8% (Figure 3c). Parkarinen et al. (2011) reported a lignin degradation of 3%-4% during 4-month ensilage of hemp, and no lignin degradation during ensilage of corn or fava beans. The relatively high lignin degradation in giant reed could result in improved digestibility, since lignin is the most recalcitrant component in lignocellulosic biomass (Meng and Ragauskas, 2014).

3.3. Effect of harvest date on organic acids and ethanol production during ensilage

Giant reed harvested at different times also showed differences in organic acid and ethanol production during the ensilage process (Figure 4). Lactic acid production was increased as the harvest date changed from August through December (Figure 4a), which was consistent with the pH profiles as illustrated in Figure 2d. It is known that sufficient WSC are preferred by homolactic fermentation during which lactic acid is produced as the main product (Piltz and

Kaiser, 2004). Therefore, high WSC content (Figure 1c) may have resulted in high levels of lactic acid (Figure 4a).

Besides lactic acid, other organic compounds were also produced during the ensilage process (Figure 4), with the highest content of total organic acids and ethanol observed in giant reed harvested in November (Figure 4f). Other organic compounds were probably generated from the degradation of lactic acid by some heterofermentative bacteria (Dreihuis et al., 1999; Egg et al., 1993; Oude Elferink et al., 2001). Besides, acetic acid and ethanol can also be directly produced from free sugars by heterofermentative LAB (Egg et al., 1993). Giant reed harvested in August showed higher acetic acid and butyric acid contents than those harvested in October, November, and December (Figure 4b and 4d). Giant reed harvested in October and November had higher propionic acid and ethanol contents than others (Figure 4c and 4e). Giant reed harvested in August showed a low ensilage quality, as primarily evidenced by the high level of butyric acid which is a critical indicator of a bad ensilage (Dreihuis and Oude Elferink, 2000; Egg et al., 1993; Yang et al., 2006).

3.4. Effect of harvest date on enzymatic digestibility of non-ensiled and ensiled giant reed biomass

Enzymatic digestibility of non-ensiled giant reed decreased from 42% to 36% as the harvest date changed from August through December (Figure 5). It has been known that lignin is one of the major contributors to the recalcitrance of lignocellulosic biomass, and removal of lignin makes cellulose more accessible to enzymes and thus improves the enzymatic digestibility (Di Girolamo et al., 2013; Ge et al., 2016; Zheng et al., 2011). As shown in Figure 3C, the lignin

content of giant reed biomass increased with later harvest dates, which could partially explain the decrease of digestibility (Figure 5).

During the ensilage process, enzymatic digestibility of giant reed generally increased, which was also consistent with the decreased lignin contents as shown in Figure 3c. After 90 days of ensilage, giant reed was 8%, 14%, 15%, and 20% more digestible ($p<0.05$) than non-ensiled giant reed harvested in August, October, November, and December, respectively. All the ensiled giant reed biomass achieved enzymatic digestibility of about 43-46% (Figure 5). These results indicated that ensilage can effectively improve enzymatic digestibility of giant reed with lignin removal.

4. Conclusion

Harvest date significantly affected giant reed composition and ensilage performance. Compared to early-harvested giant reed, late-harvested giant reed showed lower moisture and nutrient contents, but higher WSC contents, resulting in better ensilage performance with lower DM loss, lower pH, and higher lactic acid production. Late-harvested giant reed had higher lignin contents and was more recalcitrant than early-harvested giant reed. However, the enzymatic digestibility of giant reed harvested at different times was improved to about 43-46% after 90 days of ensilage. In summary, ensilage was effective in preserving giant reed biomass harvested at different times and in improving its digestibility.

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References

1. Adney, B., Nrel, J.B., 2008. Measurement of Cellulase Activities, Renewable Energy. National Renewable Energy Laboratory Technical Report NREL/TP-510-42628.
2. Angelini, L.G., Ceccarini, L., Nassi o Di Nasso, N., Bonari, E., 2009. Comparison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance. *Biomass Bioenergy* 33, 635–643.
3. APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.
4. Cui, Z., Shi, J., Wan, C., Li, Y., 2012. Comparison of alkaline- and fungi-assisted wet-storage of corn stover. *Bioresour. Technol.* 109, 98–104.
5. Darr, M.J., Shah, A., 2012. Biomass storage: an update on industrial solutions for baled biomass feedstocks. *Biofuels* 3, 321–332.
6. Di Girolamo, G., Grigatti, M., Barbanti, L., Angelidaki, I., 2013. Effects of hydrothermal pre-treatments on Giant reed (*Arundo donax*) methane yield. *Bioresour. Technol.* 147, 152–159.
7. Dreihuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F., 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with. *J. Appl. Microbiol.* 87, 583–594.
8. Driehuis, F., Oude Elferink, S.J.W.H., 2000. The impact of the quality of silage on animal health and food safety : a review. *Vet. Q.* 22, 212–216.

- 302 9. Egg, R.P., Coble, C.G., Engler, C.R., Lewis, D.H., 1993. Feedstock storage, handling and
303 processing. *Biomass Bioenergy* 5, 71–94.
- 304 10. Ge, X., Xu, F., Vasco-Correa, J., Li, Y., 2016. Giant reed: A competitive energy crop in
305 comparison with miscanthus. *Renew. Sustain. Energy Rev.* 54, 350–362.
- 306 11. Hattingh, W.H.J., Thiel, P.G., Siebert, M.L., 1967. Determination of protein content of
307 anaerobic digesting sludge. *Water Res.* 1, 185–189.
- 308 12. Herrmann, C., Heiermann, M., Idler, C., 2011. Effects of ensiling, silage additives and
309 storage period on methane formation of biogas crops. *Bioresour. Technol.* 102, 5153–5161.
- 310 13. Holzer, M., Mayrhuber, E., Danner, H., Braun, R., 2003. The role of *Lactobacillus buchneri*
311 in forage preservation. *Trends Biotechnol* 21, 282–287.
- 312 14. Kreuger, E., Nges, I., Björnsson, L., 2011. Ensiling of crops for biogas production: effects on
313 methane yield and total solids determination. *Biotechnol. Biofuels* 4, 44.
- 314 15. Lewandowski, I., Scurlock, J.M.O., Lindvall, E., Christou, M., 2003. The development and
315 current status of perennial rhizomatous grasses as energy crops in the US and Europe.
316 *Biomass Bioenergy* 25, 335–361.
- 317 16. Liu, S., Ge, X., Liew, L.N., Liu, Z., Li, Y., 2015a. Effect of urea addition on giant reed
318 ensilage and subsequent methane production by anaerobic digestion. *Bioresour. Technol.* 192,
319 682–8.
- 320 17. Liu, S., Ge, X., Xu, F., Li, Y., 2016. Effect of total solids content on giant reed ensilage and
321 subsequent anaerobic digestion 51, 73–79.
- 322 18. Liu, S., Xu, F., Liu, Z., Li, Y., 2015b. Food waste addition for enhanced giant reed ensiling
323 and methane production. *Trans. ASABE* In press.
- 324 19. Luca, C., Pilu, R., Tambone, F., Scaglia, B., Adani, F., 2015. New energy crop giant cane

325 (Arundo donax L.) can substitute traditional energy crops increasing biogas yield and
 326 reducing costs. Bioresour. Technol. 191, 197–204.

327 20. Meng, X., Ragauskas, A.J., 2014. Recent advances in understanding the role of cellulose
 328 accessibility in enzymatic hydrolysis of lignocellulosic substrates. Curr. Opin. Biotechnol. 27,
 329 150–158.

330 21. Monti, A., Di Virgilio, N., Venturi, G., 2008. Mineral composition and ash content of six
 331 major energy crops. Biomass Bioenergy 32, 216–223.

332 22. Nassi o Di Nasso, N., Roncucci, N., Triana, F., Tozzini, C., Bonari, E., 2011. Seasonal
 333 nutrient dynamics and biomass quality of giant reed (Arundo donax L.) and miscanthus
 334 (Miscanthus x giganteus Greef et Deuter) as energy crops. Ital. J. Agron. 6, 152–158.

335 23. Oude Elferink, S.J., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., Driehuis, F.,
 336 2001. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by
 337 Lactobacillus buchneri. Appl. Env. Microbiol 67, 125–132.

338 24. Pakarinen, A., Maijala, P., Jaakkola, S., Stoddard, F.L., Kymäläinen, M., Viikari, L., 2011.
 339 Evaluation of preservation methods for improving biogas production and enzymatic
 340 conversion yields of annual crops. Biotechnol. Biofuels 4, 20.

341 25. Pakarinen, O., Lehtomäki, A., Rissanen, S., Rintala, J., 2008. Storing energy crops for
 342 methane production: Effects of solids content and biological additive. Bioresour. Technol. 99,
 343 7074–7082.

344 26. Piltz, J.W., Kaiser, A.G., 2004. Principles of silage preservation, in: Kaiser, A.G., Piltz, J.W.,
 345 Burns, H.M., Griffiths, N.W. (Eds.), Successful Silage. Dairy Australia and New South
 346 Wales Department of Primary Industries, Orange, NSW.

347 27. Ren, H., Richard, T.L., Chen, Z., Kuo, M., Bian, Y., Moore, K.J., Patrick, P., 2006. Ensiling

corn stover: effect of feedstock preservation on particleboard performance. *Biotechnol. Prog.* 22, 78–85.

28. Selig, M.J., Weiss, N., Ji, Y., 2008. Enzymatic Saccharification of Lignocellulosic Biomass, National Renewable Energy Laboratory Technical Report NREL/TP-510-42629. National Renewable Energy Laboratory Technical Report NREL/TP-510-42629.

29. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, D.C., 2011. Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory Technical Report NREL/TP-510-42618.

30. Smith, R., Slater, F.M., 2011. Mobilization of minerals and moisture loss during senescence of the energy crops *Miscanthus x giganteus*, *Arundo donax* and *Phalaris arundinacea* in Wales, UK. *GCB Bioenergy* 3, 148–157.

31. Vasco-Correa, J., Li, Y., 2015. Solid-state anaerobic digestion of fungal pretreated *Miscanthus sinensis* harvested in two different seasons. *Bioresour. Technol.* 185, 211–217.

32. Weiland, P., 2010. Biogasproduction: current state and perspectives. *Appl. Microbiol. Biotechnol.* 85, 849.860.

33. Yahaya, M., Kimura, a, Harai, J., Nguyen, H., Kawai, M., Takahashi, J., Matsuoka, S., 2001. Effect of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass. *Anim. Feed Sci. Technol.* 92, 141–148.

34. Yang, H.Y., Wang, X.F., Liu, J. Bin, Gao, L.J., Ishii, M., Igarashi, Y., Cui, Z.J., 2006. Effects of water-soluble carbohydrate content on silage fermentation of wheat straw. *J. Biosci. Bioeng.* 101, 232–237.

35. Zheng, Y., Yu, C., Cheng, Y.S., Zhang, R., Jenkins, B., VanderGheynst, J.S., 2011. Effects of ensilage on storage and enzymatic degradability of sugar beet pulp. *Bioresour. Technol.*

371 102, 1489–1495.

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Captions for tables and figures

Table

Table 1 Effect of harvest dates on cellobiose and glucose in giant reed during ensilage

Figures

Figure 1. Effect of harvest date on (a) dry matter (DM) and organic dry matter (ODM) contents, (b) carbon and nitrogen contents and carbon to nitrogen (C/N) ratio, and (c) water soluble carbohydrate (WSC) and protein contents in giant reed biomass.

Figure 2. Changes in (a) dry matter (DM), (b) extractives, (c) water soluble carbohydrates (WSC), and (d) pH during ensilage of giant reed biomass harvested at different times.

Figure 3. Changes in (a) cellulose, (b) hemicellulose, and (c) lignin content during ensilage of giant reed harvested at different times.

Figure 4. Organic acids and ethanol production during ensilage of giant reed harvested at different times.

Figure 5. Enzymatic digestibility of fresh and ensiled giant reed harvested at different times.

Table 1 Effect of harvest dates on cellobiose and glucose in giant reed during ensilage

Ensilage time (d)	0	3	7	15	30	45	60	90
August								
Cellobiose (% of initial DM)	0.76 ± 0.12	ND	ND	ND	ND	ND	ND	ND
Glucose (% of initial DM)	0.34 ± 0.02	ND	ND	ND	ND	ND	ND	ND
October								
Cellobiose (% of initial DM)	1.73 ± 0.06	0.67 ± 0.19	0.31 ± 0.03	ND	ND	ND	ND	ND
Glucose (% of initial DM)	1.41 ± 0.05	0.20 ± 0.04	0.14 ± 0.01	ND	ND	ND	ND	ND
November								
Cellobiose (% of initial DM)	2.36 ± 0.04	0.68 ± 0.07	0.23 ± 0.03	ND	ND	ND	ND	ND
Glucose (% of initial DM)	1.24 ± 0.01	0.23 ± 0.03	0.13 ± 0.02	ND	ND	ND	ND	ND
December								
Cellobiose (% of initial DM)	2.24 ± 0.13	0.23 ± 0.03	0.25 ± 0.06	0.28 ± 0.07	0.29 ± 0.03	0.24 ± 0.05	0.26 ± 0.09	0.19 ± 0.01
Glucose (% of initial DM)	1.61 ± 0.09	0.60 ± 0.02	0.19 ± 0.05	0.38 ± 0.13	ND	ND	ND	ND

ND- not detectable; xylose, arabinose, galactose, and mannose were not detectable either.

FIGURES

Figure 1. Effect of harvest date on (a) dry matter (DM) and organic dry matter (ODM) contents, (b) carbon and nitrogen contents and carbon to nitrogen (C/N) ratio, and (c) water soluble carbohydrate (WSC) and protein contents in giant reed biomass.

Figure 2. Changes in (a) dry matter (DM), (b) extractives, (c) water soluble carbohydrates (WSC), and (d) pH during ensilage of giant reed biomass harvested at different times.

Figure 3. Changes in (a) cellulose, (b) hemicellulose, and (c) lignin content during ensilage of giant reed harvested at different times.

Figure 4. Organic acids and ethanol production during ensilage of giant reed harvested at different times.

Figure 5. Enzymatic digestibility of fresh and ensiled giant reed harvested at different times.