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

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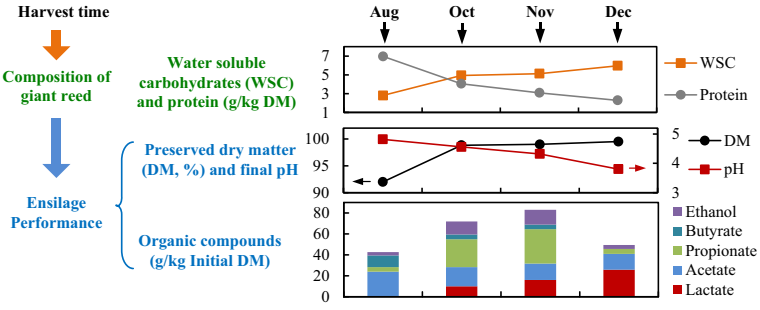
HIGHLIGHTS

- Harvest date affected composition of giant reed and ensilage performance.
- Water soluble carbohydrates in giant reed increased from August to December.
- Late-harvest resulted in higher ensilage quality than early-harvest.
- Late-harvested giant reed could be less digestible than early-harvested giant reed.
- Ensiled giant reed harvested at different times showed a comparable digestibility.

GRAPHICAL ABSTRACT



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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.

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.

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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 heterofermentative 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 heterofermentative bacteria during the ensilage process (Dreihuis et al., 1999; Oude Elferink et al., 2001). The organic acids, especially lactic acid, can decrease 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 the 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,b).

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 the 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) and organic dry matter (ODM) contents, carbon to nitrogen (C/N) ratio, and water soluble carbohydrate (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 acids and in 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. Methods

2.1. Feedstocks

Giant reed was planted in April 2013 at the Ohio State University (OSU) research farm (Columbus, OH, USA) 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 ± 3 °C). According to a 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 non-ensiled and 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). The cellulase 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 h, 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, 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:

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

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