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Effect of urea addition on giant reed ensilage and subsequent methane production by anaerobic digestion

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HIGHLIGHTS

- 90-Day ensilage of giant reed with 0% or 2% urea addition showed ~1% dry matter loss.
- Urea addition increased lactic acid accumulation during ensilage by about 4-fold.
- Urea addition reduced propionic acid accumulation during ensilage by 2–8 fold.
- Ensilage with urea addition significantly improved methane yield of giant reed.
- The methane yield was correlated with accumulation of lactate, acetate and ethanol.

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ABSTRACT

The effect of urea addition on giant reed ensilage and sequential anaerobic digestion (AD) of the ensiled giant reed was evaluated. The dry matter loss during ensilage (up to 90 days) with or without urea addition was about 1%. Addition of 2% urea enhanced production of lactic acid by about 4 times, and reduced production of propionic acid by 2–8 times. Besides, urea addition reduced degradation of cellulose and hemicellulose, and increased degradation of lignin in giant reed during ensilage. Ensilage with or without urea addition had no significant effects on the enzymatic digestibility of giant reed, but ensilage with urea addition achieved a cumulative methane yield of 173 L/kg VS, which was 18% higher than that of fresh giant reed. The improved methane yield of giant reed could be attributed to the production of organic acids and ethanol during ensilage.

1. Introduction

Giant reed (*Arundo donax* L.) is a C3 perennial rhizomatous grass that is currently widespread in India, China, the USA, Australia, Southern Africa, and the Mediterranean regions (Scordia et al., 2013). Different from typical C3 plants, which are less productive than C4 plants, giant reed has an unusually high photosynthetic capacity (Rossa et al., 1998), and thus achieves a biomass yield even higher than those of typical C4 plants, such as *Miscanthus* × *giganteus* (Corno et al., 2014). As a result, giant reed has been recognized as one of the most promising energy crops for providing biomass feedstocks for the production of fuels and value-added products (Corno et al., 2014). One approach for bioenergy production from giant reed is anaerobic digestion (AD), which is a well-established and widely-used technology for treating biomass and producing methane as energy (Yu and Schanbacher, 2010). AD of giant reed has been reported in a few publications, and is considered to be an effective and reliable method for harnessing energy from giant reed with low greenhouse gas emissions (Di Girolamo et al., 2013; Dragoni et al., 2011; Ragaglini et al., 2014; Yang and Li, 2014).

In order to maintain a viable biofuel supply chain, storage of raw biomass feedstocks is generally required. Ensilage is a well-known technology for wet biomass storage (Darr and Shah, 2012). During the ensilage, lactic acid bacteria (LAB) consume non-structural carbohydrates under anaerobic conditions, and produce organic compounds, such as lactic acid, acetic acid and ethanol. The accumulation of organic acids, mainly lactic acid, reduces the pH to about 4.0, thus preventing further degradation of biomass by inhibiting the activity of microorganisms (Zheng et al., 2011). Under favorable conditions, the silage can be stable for a

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long period, e.g. one year, with organic dry matter (ODM) loss of less than 10% (Herrmann et al., 2011). Besides being used for preserving wet feedstocks in the livestock industry, ensilage is also considered a promising biomass storage method for bioenergy production (Darr and Shah, 2012; Herrmann et al., 2011; Neureiter et al., 2005). The ensiled biomass is particularly suitable for production of methane by AD, because the organic acids and ethanol are also intermediates for biogas production in the AD process. Studies on ensilage of various crops, such as maize and grasses for methane production, have been reported (Neureiter et al., 2005; Pakarinen et al., 2011, 2008; Vervaeren et al., 2010; Yahaya et al., 2001). However, published data on ensilage of giant reed for methane production by AD is scarce.

Urea treatment has been frequently used in ensilage for improved silage quality, e.g. nutritive value and digestibility (Dias-da-Silva and SundstØl, 1986; Guedes et al., 2006; Mascarenhas-Ferreira et al., 1989; Sarwar and Khan, 2004). Breakdown of urea to ammonia via hydrolysis during 2–3 weeks of ensiling without any urease addition was reported by Ibrahim et al. (1985). In addition, urea treatment also increased lignin degradation during ensilage of hemp and, subsequently, increased its enzymatic digestibility by 46% (Pakarinen et al., 2011). To date, there have been no reports on urea treatment for ensilage of giant reed.

The objective of this study was to examine the effect of urea addition on giant reed ensilage, and subsequent methane production from ensiled giant reed via AD. Giant reed ensilage experiments with and without urea addition were conducted for different periods of time. Dry matter loss, lignocellulose degradation, and organic acid accumulation during the ensilage process were examined. Enzymatic hydrolysis and AD of giant reed with or without urea addition were then carried out, and the effects of ensilage and urea addition on enzymatic digestibility and methane production were studied. Finally, the correlation between the content of organic acids and ethanol in giant reed (fresh and ensiled) and the corresponding methane yields was further evaluated in order to explain the improved methane yield by ensilage.

2. Methods

2.1. Feedstock and inoculum

Giant reed biomass was harvested from the Ohio State University (OSU) research farm in Columbus, OH, USA on October 3, 2014, ground to pass through a 12 mm sieve using a shredder-chipper (Mighty Mac, Mackissic Inc., Parker Ford, PA, USA), and ensiled on the same day. Effluent from a mesophilic liquid anaerobic digester (KB BioEnergy, Akron, OH, USA) fed with sewage sludge was used as an inoculum for AD. Characteristics of giant reed biomass and the inoculum for AD are presented in Table 1.

2.2. Ensilage of giant reed

The processed giant reed biomass was ensilaged with and without urea addition. For ensilage with urea addition, the giant reed biomass was mixed with 16% (w/v) urea to reach a urea content of 2% (based on dry weight of biomass) and a moisture content of 40.6% (w/w). For ensilage without urea addition, the giant reed biomass was mixed with water to reach the same moisture content (40.6%). Ensilage was conducted by packing 1 kg of the mixture (giant reed biomass with additional water and/or urea) into 1-gallon-size zipper bags (Ziploc vacuum Freezer System, SC Johnson Inc., Racine, WI, USA). For each mixture, 12 bags were prepared. The bags were then vacuumed to minimize the presence of oxygen and placed at room temperature (25 ± 3 °C). Ensilage was run for different periods of time (30, 60, and 90 days). At each time interval including day 0, three bags were selected randomly from trials with and without urea treatment and the mixture in each of the three bags was taken out and mixed thoroughly. After sampling for composition analysis, the remaining silage samples were stored at −20 °C for AD and determination of enzymatic digestibility.

Table 1
Characteristics of giant reed biomass and inoculum for AD.

Parameters	Giant reed	Inoculum for AD
DM, %	43.53 ± 0.55	6.71 ± 0.01
ODM, % DM	92.19 ± 0.10	63.85 ± 0.05
TN, % DM	0.77 ± 0.03	3.83 ± 0.07
TC, % DM	49.98 ± 0.03	39.06 ± 0.52
C/N	64.55 ± 2.47	10.20 ± 0.06
pH	5.57 ± 0.03	7.99 ± 0.01
Extractives, % DM	21.80 ± 0.76	14.13 ± 0.91
WSC, % DM	4.94 ± 0.25	0.46 ± 0.08
Cellobiose, % DM	1.70 ± 0.08	ND
Glucose, % DM	1.47 ± 0.01	ND
Cellulose, % DM	27.73 ± 0.17	1.11 ± 0.05
Hemicellulose, % DM	15.65 ± 0.29	ND
Lignin, % DM	16.66 ± 0.38	NA
Crude protein, % DM	4.06 ± 0.66	ND
NH_3-N, % DM	0.06 ± 0.02	5.25 ± 0.52
Ash, % DM	7.81 ± 0.09	36.15 ± 0.05

DM – dry matter; ODM – organic dry matter; WSC – water soluble carbohydrates (a fraction of extractives); ND – not detectable; NA – not applicable.

2.3. Determination of enzymatic digestibility

Enzymatic digestibility of treated and untreated giant reed was determined according to a Laboratory Analytical Procedure (LAP) reported by the National Renewable Energy Laboratory (NREL) (Selig et al., 2008). The activity of the cellulase (Cellic CTec 2, Novozymes, Denmark) was measured according to an NREL LAP (Adney and Baker, 2008). Samples (in duplicate) 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:

$$\text{Enzymatic digestibility } (\%) = \frac{M_{\text{glucose}}}{f \times M_{\text{cellulose}}} \times 100$$

where M_{glucose} is the amount of glucose released from cellulose by enzymatic hydrolysis, $M_{\text{cellulose}}$ is the amount of cellulose in the sample (determined by a method described in Section 2.5), and $f = 180/162$ is the conversion factor for cellulose to glucose (Zeng et al., 2007).

2.4. Anaerobic digestion of ensiled giant reed

AD was set up by mixing fresh or ensiled giant reed, inoculum, and deionized (DI) water to obtain a feedstock to effluent (F/E) ratio of 0.5 (based on volatile solids, VS) and a total solids (TS) content of 5%. AD with only inoculum was also run as a control. All AD reactors were conducted in triplicate, and incubated at a mesophilic (37 ± 1 °C) condition for 30 days. A 5-L Tedlar gas bag (CEL Scientific, Santa Fe Springs, CA, USA) was connected to the outlet of each reactor for biogas collection. Biogas volume and composition were determined every 2–4 days.