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Comparison between ensilage and fungal pretreatment for storage of giant reed and subsequent methane production

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HIGHLIGHTS

- Ensilage enhanced biomass preservation and methane yield of giant reed.
- Fungal pretreatment reduced glucose yields of giant reed harvested in August and October.
- Fungal pretreatment decreased methane yield of giant reed despite of harvest time.
- Ensilage was more suitable than fungal pretreatment for giant reed storage and AD.

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ABSTRACT

Ensilage and fungal pretreatment of giant reed harvested from August through December were compared based on their effects on feedstock preservation, glucose yield, and subsequent methane production via anaerobic digestion (AD). Compared to fungal pretreatment, ensilage obtained lower total solids (<1.2%) and cellulose (<3.5%) losses, and comparable hemicellulose degradation, except for giant reed harvested in August. Ensilage increased glucose and methane yields by 7–15% and 4–14%, respectively, for giant reed harvested from August through December. Fungal pretreatment failed for giant reed harvested in August and October with reduced glucose yields, and was effective for that harvested in November and December, with about 20% increases in glucose yield. However, hydrocarbon losses during fungal pretreatment offset the increased glucose yield, resulting in decreased methane yields by AD. In summary, ensilage was found to be more suitable than fungal pretreatment for giant reed storage and its methane production via AD.

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1. Introduction

Renewable energy production from biomass has the potential to reduce our dependence on fossil fuels, and mitigate climate change due to greenhouse gas emissions (World Energy Council, 2013). Since the production of biomass is usually seasonal with a limited window for harvest, storage of biomass is crucial for maintaining a viable bioenergy supply chain. Dry storage is the most commonly used method for storing agricultural biomass, especially for low-moisture crops. In order to save costs, agricultural biomass is typically dried in the field without artificial drying; however, unpredictable weather conditions present serious challenges (Sultana and Kumar, 2011). Dry storage also has other drawbacks such as high dry matter losses during outdoor storage and risk of fire (Shinners et al., 2007). In contrast, wet storage with concurrent microbial pretreatment, such as ensilage and fungal pretreatment, have been proposed to reduce dry matter loss and/or reduce biomass recalcitrance for improved bioenergy production (Cui et al., 2012; Liu et al., 2015).

Ensilage, a traditional process for preserving green crops in the livestock industry, has been considered as an effective and reliable technology for biomass storage (Herrmann et al., 2011). Ensilage usually relies on naturally existing microorganisms, mainly lactic acid bacteria, to convert water soluble carbohydrates (WSC) in the biomass to organic acids, such as lactic acid, acetic acid, propionic acid, and butyric acid. The production of organic acids reduces pH values in the silage to below 4, which inhibits the growth of microorganisms and thus preserves biomass (Weiland, 2010). The dry matter loss can be as low as 1–5% after one year of ensilage (Herrmann et al., 2011), and the ensiled biomass was also found to be more digestible than that from dry storage (Cui et al., 2012). The

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improved degradability of biomass after ensilage might be associated with the conversion of non-structural carbohydrates to organic acids and ethanol (Oleskowicz-Popiel et al., 2011), as well as the degradation of hemicellulose and lignin that can reduce the recalcitrance of the biomass (Pakarinen et al., 2011; Yahaya et al., 2001). Ensilage was also found to increase the maximum daily methane production rate by 10%, and cumulative methane yield by 11%, compared to non-ensiled giant reed (Liu et al., 2015).

Fungal pretreatment reduces the recalcitrance of lignocellulosic biomass by degrading lignin with specific fungi, and has been considered an environmentally friendly pretreatment method as it is effective at ambient temperatures (Zheng et al., 2014). One of the drawbacks of fungal pretreatment is the long pretreatment time. However, this issue can be addressed by concurrently applying fungal pretreatment during wet storage (Wan and Li, 2011b). *Ceriporiopsis subvermispora* is one of the most effective fungal species that selectively degrade lignin over cellulose, although its selectivity has been found to vary with plant species and harvest time (Ge et al., 2014; Vasco-Correa and Li, 2015; Wan and Li, 2011b).

Both ensilage and fungal pretreatment have been studied for biomass preservation and pretreatment, however, to the best of the authors' knowledge, there have been no published studies that compared these two storage methods for bioenergy production from the same biomass feedstocks. Giant reed (*Arundo Donax L.*) is a perennial rhizomatous grass which exhibits several traits ideal for a bioenergy feedstock, including rapid growth, high productivity, minimal inputs for cultivation, and resistance to biotic and abiotic stresses (Ge et al., 2015). Anaerobic digestion (AD) has been studied for the conversion of giant reed to methane, due to its simple process, reliable performance, and low greenhouse gas emissions (Ge et al., 2015; Liu et al., 2015; Yang and Li, 2014). Most recently, ensilage has been found to be effective in preserving giant reed with low dry matter loss (about 1%) and improving the methane yield during subsequent methane production via AD (Liu et al., 2015). However, fungal pretreatment of giant reed by *C. subvermispora* has not been reported (Ge et al., 2015). Thus, it is still unknown whether or not fungal pretreatment is more effective than ensilage for storage and AD of giant reed.

The objective of this study was to conduct a side-by-side comparison of ensilage and fungal pretreatment of giant reed. Since giant reed has a long harvest window, and the harvest time may also affect the storage and AD process (Di Nasso et al., 2011; Wahid et al., 2015), giant reed harvested at different times was used in this study. Effects of ensilage and fungal pretreatment on giant reed degradation and organic compound production, sugar yield via enzymatic hydrolysis, and methanol production by AD were evaluated and compared. This study not only fills a knowledge gap for the performance of storage and pretreatment of giant reed harvested at different times, but also provides useful information for the management of this promising energy crop.

2. Methods

2.1. Giant reed

Giant reed was harvested from the Ohio State University research farm in Columbus, OH, USA, in 2014 on August 26, October 3, November 6, and December 10, respectively. The giant reed feedstock was ground to pass through a 12 mm sieve using a shredder-chipper (Mighty Mac, Mackissic Inc., Parker Ford, PA, USA), and subjected to ensilage or fungal pretreatment on the same day. Characteristics of the giant reed are presented in Table 1.

2.2. Ensilage of giant reed

Giant reed, except that harvested in early fall, was supplemented with water to reach a total solids (TS) content of around 40%, and packed into 1-gallon-size zipper bags (Ziploc Vacuum Freezer System, SC Johnson Inc., Racine, WI, USA) with 1 kg of wet giant reed in each bag. The bags were vacuumed to minimize the presence of oxygen and placed at room temperature (25 ± 3 °C). Ensilage was conducted in triplicate for giant reed harvested on each of the four dates. After 60 days of ensilage, all silage samples from the three replicates of each harvest date were taken out and mixed thoroughly. After sampling for compositional analysis, the remaining silage samples were stored at −20 °C for AD and enzymatic hydrolysis tests.

2.3. Fungal pretreatment

The fungus species *C. subvermispora* (ATCC 96608) was purchased from American Type Culture Collection (Manassas, VA, USA). The inoculum used for fungal pretreatment was prepared by activating the fungus on 2% malt extract agar plates at 28 °C for 7 days. Then the liquid inoculum was prepared by inoculating 10 pieces (around 1 cm in diameter per piece) of the agar medium that contained fungus mycelium into 50 mL of 2% malt extract liquid medium in a 500-mL Erlenmeyer flask (4 flasks in total). The flasks were then sealed with cotton plugs, and statically cultivated at 28 °C for 7 days. Mycelia floating on the surface of the liquid cultures were transferred to a sterilized beaker, washed twice with 100 mL of sterilized deionized (DI) water, suspended in 200 mL of sterilized DI water, and then homogenized aseptically with a blender.

Table 1
Properties of giant reed biomass harvested at different times and inoculum for AD.

Material	Giant reed harvested at different times				Inoculum for AD
	August	October	November	December	
TS, %	32.26 ± 0.03	43.53 ± 0.55	42.94 ± 0.61	50.02 ± 1.29	6.12 ± 0.03
VS, %TS	90.98 ± 0.12	92.19 ± 0.10	92.68 ± 0.27	93.54 ± 0.20	63.95 ± 0.30
Ash, %TS	9.02 ± 0.12	7.81 ± 0.10	7.32 ± 0.27	6.46 ± 0.20	36.05 ± 0.30
Extractives, %TS	19.58 ± 0.39	22.32 ± 0.38	23.44 ± 0.28	20.40 ± 0.28	13.14 ± 1.49
Cellulose, %TS	31.04 ± 0.61	29.00 ± 1.01	28.84 ± 0.37	30.28 ± 0.18	1.16 ± 0.09
Hemicellulose, %TS	16.13 ± 0.52	15.36 ± 0.55	14.42 ± 0.04	15.27 ± 0.21	ND
Lignin, %TS	15.97 ± 0.38	16.42 ± 0.26	16.94 ± 0.42	17.81 ± 0.21	NA
Crude protein, %TS	6.97 ± 0.85	4.05 ± 0.66	3.09 ± 0.09	2.29 ± 0.04	17.86 ± 0.56
WSC, %TS	2.80 ± 0.16	4.94 ± 0.25	5.14 ± 0.08	5.96 ± 0.16	0.48 ± 0.08
N, %TS	0.86 ± 0.01	0.77 ± 0.03	0.56 ± 0.03	0.44 ± 0.01	3.86 ± 0.05
C, %TS	49.61 ± 0.21	49.98 ± 0.03	45.97 ± 0.44	45.97 ± 0.38	39.10 ± 0.32
C/N	57.66 ± 1.14	64.55 ± 2.47	82.63 ± 4.91	103.99 ± 2.66	10.24 ± 0.10
pH	5.42 ± 0.03	5.40 ± 0.12	5.67 ± 0.03	5.43 ± 0.03	8.16 ± 0.04

WSC: water soluble carbohydrates; ND: not detectable; NA: not applicable.

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