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Solid-state anaerobic digestion of fungal pretreated *Miscanthus sinensis* harvested in two different seasons

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HIGHLIGHTS

- Solid-state anaerobic digestion of miscanthus harvested in fall and spring.
- Both miscanthus harvest seasons generated similar specific methane yield.
- Fungal pretreatment was effective for spring miscanthus, not for fall miscanthus.
- Fungal pretreatment increase specific methane yield by 25% for spring miscanthus.

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ABSTRACT

Solid-state anaerobic digestion of *Miscanthus sinensis* harvested in fall and spring was compared under different total solids contents and feedstock-to-inoculum ratios. The highest specific methane yields reached 170–175 L CH_4/kg volatile solids for both harvest seasons. Miscanthus harvested in fall generated a 6% higher methane yield in average than miscanthus harvested in spring. Fungal pretreatment with *Ceriporiopsis subvermispora* decreased the lignin content of miscanthus harvested in spring by 25.7%, but there was no significant delignification observed for miscanthus harvested in fall. Fungal pretreatment of miscanthus harvested in spring increased the specific methane yield by 25%, but fungal pretreatment caused a slight methane yield reduction for miscanthus harvested in fall. Methane yields for miscanthus were comparable with those from other energy crops.

1. Introduction

The search for alternative sources of energy, such as biomass, has been driven by the limited reserves of fossil fuels and concerns over greenhouse gas emissions caused by the burning of fossil fuels for energy use. Dedicated energy crops have the potential to provide a renewable, consistent, high volume feedstock supply for the bioenergy industry (U.S. Department of Energy, 2011). For the last three decades, some of the most studied perennial grasses were switchgrass and miscanthus, because they usually require less agronomic inputs than annual crops (Lewandowski et al., 2003a,b). Miscanthus is a tall perennial grass that can be harvested once per year. It generates high biomass yields, has a remarkable adaptability to different climate and soil conditions, and requires low fertilizer inputs (Jones and Walsh, 2001). Miscanthus has high water and nitrogen use efficiency (Jones and Walsh, 2001), can be grown on marginal land (Sanderson and Adler, 2008), and does not compete with food and feed production.

Miscanthus can be harvested from senescence until spring; however, late harvesting is most widely used (Jones and Walsh, 2001). When it is harvested in spring, the crop remains in the field during the winter, producing biomass with low moisture content, and allowing the complete translocation of nutrients back to the rhizomes, but also generating lower biomass yields since 30–50% of the dry matter can be lost, mainly because of the leaf fallen (Heaton et al., 2004; Lewandowski and Clifton-Brown, 2000). When harvested in fall, biomass losses can be reduced, but the product has higher moisture and nutrients contents (Heaton et al., 2004), increasing logistic expenses, such as drying prior to storage, and fertilizer requirements for next season (Amougou et al., 2011).

Miscanthus is typically combusted to generate electricity and heat (Jones and Walsh, 2001), but it can also be used as a feedstock for biological conversion such as anaerobic digestion (AD) for biogas production. AD is a simple and robust technique for

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the production of biogas from organic matter in oxygen-free conditions by a microbial consortium. For the transformation of lignocellulosic biomass such as miscanthus, solid-state anaerobic digestion (SS-AD) is preferred over the traditional liquid anaerobic digestion (L-AD) as SS-AD can solve the floating and stratification of fibrous material problems related to L-AD. SS-AD operates with a TS content of 15% or more, and therefore requires a smaller reactor volume for the same solids loading, less energy for heating and mixing, and generates an easier-to-handle digestate with a lower moisture content (Li et al., 2011). However, SS-AD usually has lower methane yields and needs a nitrogen supplement because the lignocellulosic feedstocks usually have a carbon-to-nitrogen (C/N) ratio higher than that recommended for anaerobic digestion (20–30) (Li et al., 2011).

Miscanthus is mainly composed of cellulose, hemicellulose, and lignin. At the initial stage of anaerobic digestion, hydrolytic microorganisms secrete enzymes to degrade the cellulose and hemicellulose into their monomers. But the high lignin content and the complex arrangement between cell wall components make miscanthus highly recalcitrant to hydrolysis (Le Ngoc Huyen et al., 2010). Several pretreatments have been developed to reduce the recalcitrance of lignocellulosic biomass and enhance accessibility for the hydrolytic enzymes. Some white rot fungi have been used for pretreatment of lignocellulosic biomass because of their ability to grow on it and reduce its recalcitrance (Wan and Li, 2011). *Ceriporiopsis subvermispora* is a white rot fungus that selectively degrades lignin over cellulose and has been proved to enhance enzymatic digestibility of several lignocellulosic feedstocks (Wan and Li, 2011).

Previous studies have employed L-AD to digest miscanthus under mesophilic conditions. Klimiuk et al. (2010) obtained a methane yield of 100 L/kg volatile solids (VS) for ensiled *Miscanthus* × *giganteus* harvested in October, while Theuretzbacher et al. (2014) attained a methane yield of 84 L/kg VS for *M.* × *giganteus* harvested in February, but increased it up to 345 L/kg VS by pretreating the miscanthus with steam explosion. A smaller increase of 25–27% methane yield was obtained with aqueous ammonia soaking pretreatment (Jurado et al., 2013). AD has been performed with other perennial grasses similar to miscanthus; giant reed had methane yields of 129.7 and 150.8 L/kg VS (Yang and Li, 2014), while switchgrass had methane yields of 116.9 and 111.0 L/kg VS (Brown et al., 2012), with SS-AD and L-AD, respectively.

Several researchers have studied the effect of harvest date on methane yield of energy crops other than miscanthus, sometimes showing inconsistent results (Lehtomäki et al., 2008; Seppälä et al., 2009). Moreover, most of the studies focused on harvest dates during the summer and fall, and excluded harvest in the following spring. Even though there are several studies of the effect of miscanthus harvest date on composition and transformation (Hayes, 2013; Le Ngoc Huyen et al., 2010; Lewandowski et al., 2003a), to our knowledge the effect of harvest date on the anaerobic digestion of miscanthus or on how fungal pretreatment can affect this process has not been reported so far.

It is well known that the harvest date of grasses can affect biochemical processes such as AD or solid-state fungal pretreatment (Lehtomäki et al., 2008), but it is unclear how the harvest date of *Miscanthus sinensis* will affect these processes, especially because of its unconventional harvest window between late fall and early spring. The objectives of this study were to (1) compare the SS-AD of *M. sinensis* harvested in two different seasons: fall and spring; (2) compare fungal pretreatment of *M. sinensis* harvested in the fall and spring seasons; and (3) evaluate the effect of fungal pretreatment of *M. sinensis* on biogas production by SS-AD.

2. Methods

2.1. Feedstock collection and preparation

M. sinensis (hereafter miscanthus) was harvested from a field located in The Ohio State University campus in Columbus, OH, USA. Harvesting was performed manually in November 2013 (fall harvest) and April 2014 (spring harvest). The initial TS content of the miscanthus harvested in fall and spring were 47.4% and 90.1%, respectively. Because of the high moisture content (low TS content), the fall harvested miscanthus was air dried to a TS content of 80.2% for storage. The spring harvested miscanthus was used directly. The whole dry plants (leaves and stems) were milled and passed through a 12 mm screen using a hammer mill (The C.S Bell Co., Tiffin, OH, USA) and stored at 4 °C.

2.2. Solid-state anaerobic digestion

The inoculum for anaerobic digestion was the effluent from an operating mesophilic anaerobic digester fed with sewage sludge (BK BioEnergy, Inc., Akron, OH, USA). Part of this inoculum was dewatered by centrifugation (Sorvall RC 6+ Centrifuge, Thermo Fisher Scientific, Inc, Waltham, MA, USA) to achieve the high TS values desired for the treatments. The raw and centrifuged inocula were kept at 4 °C and then incubated at 37 °C for 7 days to activate the microbial population before use.

SS-AD tests were performed following a full factorial design with two feedstock-to-inoculum (F/I) ratios (2 and 4, based on VS), three TS contents (20%, 25% and 30%), and two harvest times for the feedstock (fall and spring), with two replicates of each treatment. The inoculum and feedstock were mixed using a hand-mixer (Black & Decker, New Britain, CT, USA) and incorporated into 1 L flasks, leaving a uniform head space. Flasks were sealed with rubber stoppers, connected to Tedlar® bags (CEL-Scientific, Cerritos, CA, USA) and incubated at 37 °C for 60 days. A control reactor was set-up with only centrifuged effluent to estimate the amount of biogas produced by the effluent, which produced trial amount of biogas (23 L CH_4/kg VS) comparing to the treatments. The biogas composition and volume were measured every three days.

2.3. Fungal pretreatment

C. subvermispora (ATCC 96608) was obtained from the American Type Culture Collection (Manassas, VA, USA) and kept in 2% malt extract agar at 4 °C. For the inoculum preparation, one disc of agar (5 mm diameter) colonized with mycelium was transferred to a new malt extract agar plate and grown for 5 days at 28 °C. Then, one disc of this agar was transferred to 50 ml of liquid malt extract sterile medium in a 500 ml Erlenmeyer flask and incubated without agitation at 28 °C for 7 days. At the end of the incubation, fungal mycelium was harvested by filtering the liquid medium and resuspended in deionized water. The mixture of mycelium and water was gently homogenized with a hand blender (Hamilton Beach, Inc., Richmond, VA, USA) and used as inoculum for the fungal pretreatment.

About 65 g (dry matter) of miscanthus samples was added to 1 L bottles (reactors) with water to adjust the moisture to 60%. Reactors were sealed with cotton and sterilized by autoclaving (121 °C, 15 min). After cool down, reactors were inoculated with the prepared mycelia suspension and incubated at 28 °C under 50% humidity for 28 days. At the end, samples were taken out of the reactor and well mixed to allow homogenous sampling. Part of the finished material was dried at 40 °C in a convection oven for 24 h, milled and passed through a 1 mm screen (Model 4