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Fungal pretreatment of unsterilized yard trimmings for enhanced methane production by solid-state anaerobic digestion

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HIGHLIGHTS

- Unsterilized yard trimmings (YT) successfully pretreated by white rot fungus.
- Pre-colonized YT used as inoculum for fungal pretreatment.
- Solid-state anaerobic digestion of fungal pretreated YT for biogas production.
- Methane yield was comparable to traditional fungal pretreatment with sterilization.
- Fungal pretreatment resulted in two-fold increase in net biogas energy production.

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ABSTRACT

Fungal pretreatment is an environmentally friendly process that has been widely studied to improve the digestibility of lignocellulosic biomass. However, sterilization of feedstocks, a costly process, is generally required prior to the fungal pretreatment. In this study, fungal pretreatment of unsterilized yard trimmings using yard trimmings pre-colonized with *Ceriporiopsis subvermispora* as an inoculum was investigated. Degradation of lignin, cellulose, hemicellulose, and dry matter in yard trimmings during 30 days of fungal pretreatment using different inoculum/substrate ratios (1:19, 1:9 and 1:4) was 14.8–20.2%, 8.1–15.4%, 20.7–27.8%, and 9.8–16.2%, respectively. Methane yields of 34.9–44.6 L/kg volatile solids were achieved during solid-state anaerobic digestion (SS-AD) of the pretreated yard trimmings, which were comparable to those obtained by using the traditional method requiring feedstock sterilization. The technology developed in this study can save about 501–789 kJ/kg of dry yard trimmings processed, which is about half of the total biogas energy produced by SS-AD.

1. Introduction

Solid-state anaerobic digestion (SS-AD) is a simple and robust technology for biogas production from abundant and diverse solid wastes, including forestry and agricultural residues, and municipal solid waste (MSW). Yard trimmings, one type of MSW, are rich in carbohydrates and have a high potential for biogas production by SS-AD. The lignocellulosic structures of yard trimmings are recalcitrant to digestion. Fungal pretreatment has been reported to reduce the recalcitrance of yard trimmings and increase methane yield of SS-AD by 2.5-fold (Zhao et al., 2014). Various pretreatment technologies, such as alkali, wet oxidation, and fungal pretreatments, have been investigated to increase the digestibility of lignocellulose. Compared with physical or chemical pretreatment methods, fungal pretreatment is attractive as it is an environmentally friendly process (Shi et al., 2012; Wan and Li, 2012).

Fungal pretreatment is conducted with selective lignin-degrading fungi (mainly white rot fungi) with the aim of degrading lignin over cellulose, since lignin is one of the main contributors to lignocellulosic recalcitrance (Wan and Li, 2012). Sterilization of feedstocks is routinely used before inoculation in order to kill indigenous microbial communities that may outcompete the introduced fungi (Keller et al., 2003; Ma et al., 2010; Reid, 1989a,b; Salvachúa et al., 2011; Yu et al., 2009). Either pressurized steam (Akhtar et al., 1998) or chemicals (Akhtar et al., 1995) have been studied to sterilize or partially sterilize lignocellulosic biomass. However, these processes are not feasible for commercial-scale biogas production by SS-AD due to the significant increase in operating and/or capital costs of the process (Akhtar et al., 1998).

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There are few publications on directly inoculating white rot fungi to unsterilized feedstock for pretreatment. Delignification of unsterilized aspen wood was unsuccessful using mycelium of white rot fungus grown in liquid medium as inoculum due to the competition from the indigenous microorganisms (Reid, 1989a). Fungal pre-colonized feedstock is another option for inoculation, which was used to inoculate sterilized aspen wood, but overlooked for inoculating unsterilized feedstock in Reid's study (1989a). Akin et al. (1995) inoculated white rot fungi grown on Bermuda grass agar to unsterilized Bermuda grass, and observed that indigenous microorganisms did not alter the improvement in grass biodegradability by the white rot fungi. This inoculation method has not been studied on the fungal pretreatment of other unsterilized feedstock.

In this research, yard trimmings pre-colonized by a white rot fungus, *Ceriporiopsis subvermispora*, were used as an inoculum for pretreating unsterilized yard trimmings at different inoculum/substrate ratios. Degradation of the lignin, hemicellulose, and cellulose in yard trimmings during the fungal pretreatment and methane production from SS-AD of the pretreated yard trimmings were determined. Energy savings from this technology were also estimated.

2. Methods

2.1. Preparation and characterization of feedstock

Yard trimmings were collected from the Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio. After being air-dried to a moisture content of less than 10%, yard trimmings were milled to pass through a sieve with 12.7 mm openings, and stored in bags at room temperature. The total solids (TS), volatile solids (VS), and chemical composition of the feedstock were determined before pretreatment, and are shown in Table 1.

2.2. Preparation of mycelium grown in liquid medium

C. subvermispora (ATCC 96608) was purchased from American Type Culture Collection (Manassas, VA, USA). *C. subvermispora* was activated on 2% malt extract agar plates at 28 °C for 7 days. After that, three pieces of agar medium (around 1 cm in diameter) with fungus mycelium were transferred into 20 mL of 2% malt extract liquid medium in an Erlenmeyer flask sealed with a cotton plug, and cultivated at 28 °C for 6 days (2 flasks in total). The liquid broth was dumped and all the mycelia were combined in a sterilized beaker, re-suspended with 40 mL of sterilized deionized (DI) water, and homogenized aseptically in a blender.

2.3. Preparation of pre-colonized yard trimmings

Sterilized yard trimmings pretreated by *C. subvermispora* were used as the pre-colonized yard trimmings for further experiments.

Table 1
Composition of yard trimmings (%, w/w).[a]

Components	Original	Pre-colonized
Cellulose	30.8 ± 0.9	31.1 ± 0.7
Hemicellulose	15.9 ± 0.5	7.2 ± 1.8
Acid insoluble lignin	32.3 ± 0.1	28.7 ± 0.9
Acid soluble lignin	0.6 ± 0.1	0.7 ± 0.0
Extractives	9.6 ± 0.3	N/A
Volatile solids	98.9 ± 0.2	98.5 ± 0.0
Total solids[b]	94.3 ± 0.1	33.5 ± 0.6

[a] Average ± standard deviation of triplicates, dry weight basis.
[b] Wet weight basis.

The pretreatment, which was established previously (Zhao et al., 2014), is briefly described as follows. About 100 g of the pre-processed yard trimmings (Section 2.1) were loaded into a 1 L glass bottle. A designated amount of DI water was added into to the bottle in order to obtain a moisture content of 60% (w/w, wet basis), taking into account the moisture from the inoculum. The bottles containing yard trimmings and DI water were autoclaved at 121 °C for 30 min followed by cooling down to room temperature. Twenty mL of homogenized liquid mycelium (Section 2.2) was used to inoculate 100 g of yard trimmings (dry weight). The bottle was sealed with a cotton plug and incubated at 28 °C for 30 days, after which fungal mycelia were well developed on the yard trimmings. These pre-colonized yard trimmings were mixed and sampled for determination of TS, VS, lignin, hemicellulose, and cellulose contents. Composition of pre-colonized yard trimmings is shown in Table 1.

2.4. Fungal pretreatment of unsterilized yard trimmings

Pre-colonized yard trimmings (Section 2.3) or mycelium grown in liquid medium (Section 2.2) were inoculated to unsterilized yard trimmings in 1 L glass bottles. For the trials using pre-colonized yard trimmings, three different inoculum/substrate ratios, 1:19, 1:9 and 1:4 (dry matter basis), were tested. For those using mycelium grown in liquid medium, 14 mL of mycelium suspension (Section 2.2) were inoculated to 70 g of unsterilized yard trimmings. DI water was added to obtain a moisture content of 60% (w/w, wet basis). The bottles were sealed with cotton plugs and incubated at 28 °C for 30 days. Each pretreatment trial was performed in duplicate. The pretreated yard trimmings were mixed and sampled for determination of TS, VS, and chemical composition. The dry matter loss and degradation of cellulose, hemicellulose, and lignin during the pretreatment were calculated using the following equations:

$$\text{Dry matter loss} = \frac{TS_{Initial} - TS_{Final}}{TS_{Initial}} \times 100\% \quad (1)$$

$$\text{Biomass component degradation} = \frac{Mass_{Initial} - Mass_{Final}}{Mass_{Initial}} \times 100\% \quad (2)$$

2.5. Solid-state anaerobic digestion of pretreated yard trimmings

The inoculum used for SS-AD was an effluent collected from a mesophilic anaerobic digester, which was fed mainly with municipal wastewater in Akron, OH, and operated by *KB Compost Services Inc.* The effluent was stored at 4 °C in a walk-in cooler until use. Before the inoculation, designated amounts of effluent were taken and incubated at 37 °C for 1 week to reactivate microbes. The TS and VS of the effluent were determined to be 10.1% and 60.6% (dry basis), respectively.

The pretreated yard trimmings or original yard trimmings (no pretreatment) were mixed with the effluent at a feedstock/effluent (F/E) (VS basis) ratio of 4 based on results of a previous report (Zhao et al., 2014). The TS of the mixture was adjusted to about 20% using DI water. After mixing, the mixture was loaded into a 1 L glass bottle reactor (about 50 g VS) plugged with a robber stopper and connected to a 5 L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA) for biogas collection. The reactors were incubated in a walk-in incubator at 37 ± 1 °C for 28 days. Biogas composition and volume were measured during the SS-AD process. All SS-AD tests were performed in duplicate.