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# Reactor performance and microbial community dynamics during solid-state anaerobic digestion of corn stover at mesophilic and thermophilic conditions

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## HIGHLIGHTS

- Microbial community in solid state anaerobic digestion (SS-AD) of corn stover.
- Thermophilic SS-AD led to faster reductions of cellulose and hemicelluloses.
- Thermophilic SS-AD also led to higher accumulation of VFAs.
- Reactor performance correlated well with populations of microbes.
- Bacterial and archaeal communities underwent considerable successions in SS-AD.

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## ABSTRACT

Reactor performance and microbial community dynamics were investigated during solid state anaerobic digestion (SS-AD) of corn stover at mesophilic and thermophilic conditions. Thermophilic SS-AD led to faster and greater reductions of cellulose and hemicelluloses during the first 12 days compared to mesophilic SS-AD. However, accumulation of volatile fatty acids (VFAs) was 5-fold higher at thermophilic than mesophilic temperatures, resulting in a large pH drop during days 6–12 in the thermophilic reactors. Culture-based enumeration revealed 10–50 times greater populations of cellulolytic and xylanolytic microbes during thermophilic SS-AD than mesophilic SS-AD. DGGE analysis of PCR amplified 16S rRNA genes showed dynamic shifts, especially during the thermophilic SS-AD, of bacterial and archaeal communities over the 38 days of SS-AD as a result of acclimation of the initial seed microbial consortia to the lignocellulosic feedstock. The findings of this study can guide future studies to improve efficiency and stability of SS-AD.



## 1. Introduction

Anaerobic digestion (AD) is one of the few sustainable technologies that both produce energy and treat waste streams. Driven by a complex and diverse community of microbes (Yu and Schanbacher, 2010), AD is affected by a host of factors, many of which also affect the biodiversity and activity of the microbial community. Temperature is one of the key operational factors that affect the microbial diversity and biogas production in AD systems. While the majority of commercial AD systems in the US are operated at mesophilic temperatures (~20–40 °C), interest in thermophilic (50–60 °C) digestion has increased over the past several years. The advantages of thermophilic AD include a greater degree of pathogen reduction, decreased retention time, and a higher rate of biogas production compared to mesophilic AD (Ahring, 2003). Additionally, thermophilic conditions are favorable for AD of lignocellulosic feedstocks as the high operating temperature can facilitate degradation of recalcitrant cellulose (Frigon and Guiot, 2010; Li et al., 2011a). One of the challenges of thermophilic AD systems is a higher likelihood of system failure due to the accumulation of volatile fatty acids (VFAs), especially propionate, which causes a severe decrease in biogas production (Kim and Speece, 2002).

Depending on the total solid (TS) content in digesters, AD systems can be categorized as either liquid AD (L-AD), operated with 0.5–15% TS, or solid-state AD (SS-AD), generally operated with 15–40% TS. SS-AD systems have been developed for treating the organic fraction of municipal solid waste (OFMSW) over the past decades

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(Fantozzi and Buratti, 2011; Mata-Alvarez et al., 2000; Rapport et al., 2008) and recently gained attention due to the potential application of SS-AD to treat lignocellulosic biomass for energy production (Li et al., 2011b). Recent studies demonstrated that inoculating SS-AD with L-AD effluent improved biogas production from a variety of lignocellulosic biomass wastes (Li et al., 2011b; Liew et al., 2011). However, SS-AD of lignocellulosic biomass faces a few challenges, including slow start-up and acclimation of starting microbial communities to lignocellulosic feedstocks, and acidification at high organic loading (Liew et al., 2012; Xu et al., 2013). Recent studies showed that microbial activities and the chemical composition of the inoculum can greatly affect SS-AD performance, especially during start-up of SS-AD of lignocellulosic biomass (Griffin et al., 1998; Xu et al., 2013). Furthermore, the presence of lignin, crystalline cellulose, and surface availability significantly reduced the biodegradability of lignocellulosic biomass, making the hydrolysis step one of the bottlenecks that limit methane production (Liew et al., 2011; Frigon and Guiot, 2010). Slow degradation of lignocellulose and biogas production require larger reactor volumes and higher capital investments than otherwise required, a main barrier hindering commercial implementation of SS-AD. Moreover, prompt acclimation of the microbial community in the seed inoculum to recalcitrant lignocellulosic feedstocks to be digested is crucial for high biogas yield.

Although the microbiology of L-AD for wastewater treatment is well studied, knowledge of the microbial community underpinning SS-AD of plant biomass is limited. This knowledge gap hinders design and operation of SS-AD systems. The objective of this study was to investigate reactor performance and microbial community dynamics during SS-AD of corn stover at mesophilic and thermophilic conditions. Reduction of TS, volatile solids (VS), cellulose, and hemicelluloses were monitored during SS-AD and the relationship between biogas production and microbial communities was also explored. The acclimation of archaeal and bacterial communities to lignocellulosic feedstock at both mesophilic and thermophilic conditions was investigated using an integrated approach consisting of culture-based enumeration of cellulolytic microbes, xylanolytic microbes, and acetotrophic methanogens and denaturing gradient gel electrophoresis (DGGE) analysis of the archaeal and bacterial communities following PCR amplification of 16S rRNA.

## 2. Methods

### 2.1. Feedstock and inoculum

Corn stover was collected in October 2009 from a research farm operated by the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH, USA (40°48′33″N, 81°56′14″W). Upon receipt, corn stover was dried to a moisture content of less than 10% and then ground to pass a 9 mm sieve (Mighty Mac, MacKissic Inc., Parker Ford, PA, USA). Effluent from a mesophilic liquid anaerobic digester fed with municipal sludge and food wastes (operated by quasar energy group, Cleveland, OH, USA) was obtained and used as the inoculum for the SS-AD. The effluent was dewatered by centrifugation to increase its TS content from 6.3% to 9.6%. Characteristics of the corn stover and the concentrated effluent are shown in Table 1. One aliquot of the effluent was incubated anaerobically at 36 ± 1 °C for 1 week while another aliquot was incubated anaerobically at 55 ± 1 °C for 2 weeks before being inoculated into SS-AD reactors at mesophilic and thermophilic conditions, respectively.

### 2.2. Solid-state anaerobic digestion

SS-AD reactors (1 L working volume) were loaded with a mixture of corn stover and the effluent, at a feedstock-to-effluent (F/E) ratio of 2 (based on VS) to obtain a TS content of 20%. Each reactor was sealed with a rubber stopper and placed in a 36 ± 1 °C or a 55 ± 1 °C incubator for mesophilic or thermophilic digestion, respectively, for a duration of 45 days. As a negligible amount of biogas was produced from day 38–45, data for the last week are not shown in figures that follow. A 5-L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA, USA) was attached to the outlet of each reactor to collect the biogas produced. Composition and volume of the biogas were measured every 2–3 days during the 38-day period. At predetermined time intervals (day 0, 2, 4, 6, 8, 10, 12, and 38), all the content in each reactor was taken out of the reactor and mixed thoroughly by a hand-held homogenizer prior to sampling for composition and microbial analysis. An aliquot of each digestate sample was stored at −80 °C for PCR-DGGE analysis. All tests were conducted in duplicate. Reactors that received only the effluent were run in parallel as controls.

### 2.3. Analytical methods

Samples of the feedstock, effluents, and reactor contents collected during the SS-AD process were characterized. The TS and VS contents were determined according to the Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2005). Total carbon and nitrogen contents were determined by an elemental analyzer (Vario Max CNS, Elementar Americas, Mt. Laurel, NJ, USA) and were used to calculate the carbon-to-nitrogen (C/N) ratio. Total ammonia nitrogen was determined by the AmVer™ Salicylate Test 'N Tube™ using a DR 2800™ Portable Spectrophotometer (Loveland, CO, USA) as described by Hach Method 10031 (Hach, 2012). The samples for VFA measurement were prepared by suspending 10 g of digestate in 10 mL of distilled water, thoroughly mixing it, and then separating the solids by centrifugation (8000 rpm, 5 min). The supernatant was acidified to pH 2–3 by adding hydrochloric acid and then filtered through a syringe filter with 0.2 μm porosity for GC analysis of individual VFAs, including acetic, propionic, iso-butyric, and butyric acids (Li et al., 2011a). Total VFAs and alkalinity were measured using an auto-titrator (Mettler Toledo, DL22 Food & Beverage Analyzer, Columbus, OH, USA) following a titration procedure modified from McGhee (1968).

Lignin, cellulose, and hemicellulose contents in the corn stover and the reactor contents were determined using a two-step acid hydrolysis process according to the NREL Laboratory Analytical Procedure (Sluiter et al., 2008). Monomeric sugars (glucose, xylose, galactose, arabinose, and mannose) and cellobiose were measured using HPLC (Shimadzu LC-20AB, Columbia, MD, USA) equipped with a Biorad Aminex HPX-87P column and a refractive index detector (RID) using deionized water as the mobile phase at a flow rate of 0.6 mL/min.

The volume of biogas collected in the Tedlar bags was measured by liquid displacement (Park and Li, 2012) and the composition of the biogas ($CO_2$, $CH_4$, $N_2$, and $O_2$) was analyzed by gas chromatograph (GC) (Agilent Technologies, HP 6890, Wilmington, DE, USA) equipped with a 30 m × 0.53 mm × 10 μm alumina/KCl deactivation column and a thermal conductivity detector (TCD) using helium at a flow rate of 5.2 mL/min as a carrier gas. The temperatures of the injector and detector were kept at 150 and 200 °C, respectively.

### 2.4. Microbial analysis

Cellulolytic microbes (CM), xylanolytic microbes (XM), and acetotrophic methanogens (AM) in the effluent and the reactor contents were quantified by the most-probable-number (MPN) method in triplicate (Balch et al., 1979) using the enumeration medium (EM) medium prepared under anaerobic conditions (Champion et al., 1988). The EM medium contains (in 1 L): 2.0 g trypticase, 1.0 g yeast