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Impact of different ratios of feedstock to liquid anaerobic digestion effluent on the performance and microbiome of solid-state anaerobic digesters digesting corn stover

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HIGHLIGHTS

- Ratio of feedstock:effluent of L-AD digester affected stability of SS-AD process.
- Ratio of feedstock:effluent of liquid digester affected microbiome successions.
- Groups of bacterial OTUs were correlated to SS-AD digester performance.
- *Methanomassiliicoccus* abundance was positively correlated to daily biogas yield.
- Alkalinity of effluent of liquid digesters contributes to SS-AD process stability.

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ABSTRACT

The objective of this study was to understand how the non-microbial factors of L-AD effluent affected the microbiome composition and successions in the SS-AD digesters using both Illumina sequencing and qPCR quantification of major genera of methanogens. The SS-AD digesters started with a feedstock/total effluent (F/Et) ratio 2.2 (half of the effluent was autoclaved) performed stably, while the SS-AD digesters started with a 4.4 F/Et ratio (no autoclaved effluent) suffered from digester acidification, accumulation of volatile fatty acids, and ceased biogas production two weeks after startup. Some bacteria and methanogens were affected by non-microbial factors of the L-AD fluent. Alkalinity, the main difference between the two F/Et ratios, may be the crucial factor when SS-AD digesters were started using L-AD effluent.

1. Introduction

The non-hazardous waste management hierarchy of the US EPA calls for reduce, reuse, and recycle (or Three R's) of wastes (http://www.epa.gov/waste/nonhaz/municipal/hierarchy.htm). Anaerobic digestion (AD) is one of the most attractive and practical technologies to achieve the three R's. Liquid anaerobic digestion (L-AD), which is suited for feedstock containing less than 15% total solid (TS), is the traditional technology, while solid-state AD (SS-AD) is relatively new (Kothari et al., 2014; Li et al., 2011). Operated at TS content greater than 20%, SS-AD is the technology primarily used to digest feedstock of low moisture content, such as crop residues and the organic fraction of municipal solid waste (OFMSW). Compared to L-AD, SS-AD has several advantages over L-AD, including smaller digester volumes required for a given amount of feedstock, less capital to build, less energy requirement for operation such as heating and mixing, and easier to transport and use of the final digestate (Kothari et al., 2014; Li et al., 2011). Over the past decade, more SS-AD systems than L-AD systems were built in Europe to meet the demand for bioenergy. However, it has always been challenging and time-consuming to start up a SS-AD system. The limited or lack of mixing also retards mass transfer

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and translocation of microbial cells within the solid digestate matrix.

Several studies have demonstrated that effluent from L-AD reactors can expedite startup of SS-AD reactors and improve biogas production by providing the inoculum microbes, nitrogen, phosphorus, minerals, moisture, and buffering capacity needed for AD (Li et al., 2011). However, the ratio between feedstock and L-AD effluent (F/E) greatly affects digester performance, especially length of the lag time before active biogas production and process stability (Kusch et al., 2012; Xu and Li, 2012). A recent study showed that, by including different portions of autoclaved L-AD effluent into the total effluent, non-microbial factors present in L-AD effluent also affect the SS-AD process with respect to pH, VFA concentration, biogas production, and process stability (Shi et al., 2014). Community (both archaeal and bacterial) profiling using DGGE also revealed considerable effects on the microbial populations by different portions of active and autoclaved L-AD effluent. Canonical correspondence analysis (CCA) of the DGGE profiles showed that some DGGE bands were correlated to SS-AD performance. However, the DGGE profiling did not identify the specific bacterial or archaeal populations that were affected by the treatments or correlated with digester performance. In the present study, thus, Illumina sequencing of 16S rRNA amplicons was used to further examine the microbiome dynamics in two SS-AD reactors in response to microbial and non-microbial factors present in L-AD effluent. Population shifts of total methanogens and individual genera of methanogens were also evaluated using quantitative PCR (qPCR). Plausible correlations between microbial populations and the abiotic environmental factors in the SS-AD reactors were assessed using CCA. The results of this study provide new insights into the microbiological underpinning of SS-AD process and stability and may be helpful to future research on SS-AD.

2. Methods

2.1. Sample information

The samples have been analyzed in a previous study (Shi et al., 2014). Briefly, two sets of batch SS-AD reactors (1.0-liter working volume each) were fed the same ground corn stover and inoculated with the same amount of non-autoclaved L-AD effluent to achieve the same feed/alive effluent (F/Ea) ratio of 4.4 (based on volatile solid content). Corn stover obtained from a farm operated by the Ohio Agriculture Research and Development Center in Wooster, OH was air dried to a moisture content of less than 10% and ground to pass through a 9 mm sieve. The L-AD effluent was collected from a mesophilic L-AD fed a mixture of municipal sewage sludge and food wastes (operated by the Quasar Energy Group, Cleveland, OH, USA). In addition to the non-autoclaved L-AD effluent, one set of the SS-AD reactors received an additional autoclaved L-AD effluent to achieve a feed/total effluent (F/Et) ratio of 2.2 (referred to as LFE digesters, while the other set of SS-AD reactors didn't received the additional autoclaved effluent but sterile water, resulting in a F/Et ratio of 4.4 (referred to as HFE digesters). As a result, the two sets of reactors had the same F/Ea ratio of 4.4 but different F/Et ratios (4.4 vs. 2.2). In other word, the two sets of SS-AD reactors started with the same inoculum (or seed), but differed in non-microbial factors, such as pH, C/N ratio, alkalinity, and micronutrients (Shi et al., 2014). Water was added when needed to remain the TS content of 20%. All the batch SS-AD rectors were incubated at 36 °C for 38 days. At predetermined time points (day 0, 2, 4, 6, 8, 10, 12, and 38), two replicate reactors from each set were terminated, and the content of each reactor was mixed thoroughly using a hand-held mixer and sampled for chemical and microbial analyses (Shi et al., 2014). In the present study, samples taken at day 0, 8, 12, and 38 for the LFE digesters (designated as samples LFE-t1, LFE-t2, LFE-t3, and LFE-t4, respectively) and at day 0, 4, 10, and 38 for the HFE digesters (designated as samples HFE-t1, HFE-t2, HFE-t3, and HFE-t4, respectively) were analyzed to examine microbial population shifts during the SS-AD process. More samples at the early stage of the SS-AD process were collected because of the dynamic changes in VFA concentration, pH, and biogas production rates during that period.

2.2. DNA extraction and 16S Illumina sequencing

Total metagenomic DNA was extracted from each sample using the repeated bead-beating plum column purification method (Yu and Morrison, 2004). The DNA quality was verified using agarose gel (1.0%) electrophoresis, and DNA concentrations were determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The diversity and composition of the microbiome of the SS-ADs samples were examined by sequencing and analyzing the V4 hypervariable region of 16S rRNA gene (Caporaso et al., 2012). Briefly, the V4 region was first amplified with the primer set 515F and 806R targeting both bacteria and archaea. This primer set has been validated and used in the standard protocol of the Earth Microbiome Project (EMP) (http://www.earthmicrobiome.org/emp-standard-protocols/16s/). The 806R reverse primer also contained a barcode sequence unique for each sample for multiplexing. The amplicon libraries from all the samples were pooled at equal molar ratio and sequenced on an Illumina MiSeq system using the pair-ended 2 × 250 bp protocol at University of Connecticut.

2.3. Sequence data analysis

The Illumina sequencing data was processed and analyzed using QIIME (v 1.7) (Caporaso et al., 2010) following the protocol developed by Nelson et al. (2014). Briefly, the paired reads were merged to form single sequences using SeqPrep (https://github.com/jstjohn/SeqPrep), followed by removal of the phiX sequences. Sequences shorter than 300 bp were filtered out. After demultiplexing, the reads were assigned to species-equivalent OTUs (at 97% sequence similarity) in a two-step process involving reference-based and *de novo* OTU clustering. Chimera checking was performed on the representative sequences of *de novo* OTUs using ChimeraSlayer (Haas et al., 2011) against the Greengenes database (gg_13_08) (DeSantis et al., 2006). OTUs representing less than 0.005% of the total sequences were filtered out prior to further analysis (Bokulich et al., 2013). Taxonomic classification of the remaining OTUs and calculation of alpha and beta diversity indices or metrics were carried out as described by Nelson et al. (2014). Briefly, the sequences of each sample were rarefied before the diversity analysis. Alpha diversity measurements, including Shannon and Simpson indices, phylogenetic distance, and observed number of OTUs, were calculated for each sample. Beta diversity was calculated using the distance matrices generated using both the phylogenetic-based method UniFrac (Lozupone and Knight, 2005) and the non-phylogenic-based methods Ochiai and Bray-Curtis, and then visualized using principal coordinates analysis (PCoA).

Canonical correspondence analysis (CCA) was performed using the Vegan Community Ecology package of R (http://cran.r-project.org/web/packages/vegan/) to examine correlations between OTUs and measures of reactor environmental factors important to AD, including pH, VFA concentration, and daily biogas production. Relative abundance of the major OTUs (each accounting for $\geqslant$0.1% of total sequences) and the 13 archaeal OTUs identified was used in the CCA analysis. Furthermore, the distribution of the major OTUs were further visualized using the generalized