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Evaluation of system performances and microbial communities of two temperature-phased anaerobic digestion systems treating dairy manure

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HIGHLIGHTS

- The NT-TPAD system achieved better overall performance than the AT-TPAD system.
- Both digesters of each TPAD system had different roles between two TPAD systems.
- Each digester harbored distinctive microbial populations.
- Certain microbial groups were significantly correlated with system performance.
- *Methanosarcina* was important in both systems but *Methanosaeta* only in NT-TPAD.

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ABSTRACT

Two temperature-phased anaerobic digestion (TPAD) systems, with the thermophilic digesters acidified by acidogenesis products (AT-TPAD) or operated at neutral pH and balanced hydrolysis/acidogenesis and methanogenesis (NT-TPAD), were evaluated to treat high-strength dairy cattle manure. Despite similar methane productions (about 0.22 L/g VS fed), the NT-TPAD system removed significantly more VS (36%) than the AT-TPAD system (31%) and needed no pH adjustments. The thermophilic digester of the NT-TPAD system dominated the system performance and performed significantly better than that of the AT-TPAD system. The opposite held true for the mesophilic digesters. Differences of the thermophilic digesters between two TPAD systems affected the microbial communities of both local and downstream digesters. Each digester harbored distinctive microbial populations, some of which were significantly correlated with system performance. *Methanosarcina* was the most important methanogenic genus in both TPAD systems, while *Methanosaeta* only in the NT-TPAD system. Their populations were inversely related to VFA concentrations.

1. Introduction

Compared to mesophilic single-stage digesters that are commonly used in full scale (De Baere, 2000), temperature-phased anaerobic digestion (TPAD) systems are considered one of the most promising approaches that improve both efficiency and reliability of the anaerobic digestion (AD) process in renewable energy production and biomass waste management (Lv et al., 2010; Mata-Alvarez et al., 2000). Previous studies suggested that TPAD systems could achieve improved pathogen control, solid removal, and methane production compared to mesophilic single-stage digesters (Riau et al., 2010; Santha et al., 2006), and other AD systems (Kim et al., 2004; Riau et al., 2010).

A TPAD system consists of a first stage thermophilic digester and a second stage mesophilic digester. The thermophilic digester can enhance hydrolysis and acidogenesis due to reduced feedstock recalcitrance and increase microbial metabolism at an elevated temperature, while the mesophilic digester provides lenient conditions supporting efficient and stable syntrophic acetogenesis and methanogenesis due to reduced inhibitor toxicity at a lower temperature (Lv et al., 2010). Depending on the pH of the thermophilic digester, a TPAD system can be categorized as either an AT-TPAD system, where the thermophilic digester is operated at acidic pH (Youn and Shin, 2005), or a NT-TPAD system, where the thermophilic digester is operated at neutral pH (Sung and Santha, 2003). The acidic pH of the thermophilic digester in an AT-TPAD system has been shown to favor hydrolysis and acidogenesis at the expense of syntrophic acetogenesis and methanogenesis (Chyi and

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Dague, 1994; Angelidaki et al., 2002), while the neutral pH of the thermophilic digester in a NT-TPAD system is aimed at reaching a dynamic balance between hydrolysis/acidogenesis and methanogenesis. Schmit and Ellis (2001) reported that a NT-TPAD system had either better or similar performances in solid removal and methane production compared to an AT-TPAD system, depending on the feedstock composition.

Feedstock composition can significantly affect the performances of AT-TPAD and NT-TPAD systems (Lv et al., 2010; Schmit and Ellis, 2001). However, these two systems have not been compared in treating dairy manure, one of the most important biomass wastes for renewable energy production (Yu et al., 2009). A comparative study between these two types of TPAD systems in digesting high-strength dairy manure slurry is necessary. In addition, little is known about microbial population dynamics and interactions important to the AD process (Hofman-Bang et al., 2003; O'Flaherty et al., 2006), and the understanding of how the conditions within a thermophilic digester affect the system performance and the microbial community of the downstream mesophilic digester and the entire TPAD system is limited. Thus, the objective of this study was to evaluate and compare both system performances and microbial communities between an AT-TPAD system and a NT-TPAD system in order to understand the microbial populations underpinning system performance.

2. Methods

2.1. Experimental setup

Both the thermophilic and the mesophilic digesters were made from two Nalgene polypropylene wide-mouth bottles of 4.3 L capacity with 100 mm screw caps (Fisher Scientific, PA). Each digester had a feeding port, a sampling port, and a gas outlet. The feeding port and the sampling port were sealed by rubber stoppers to keep the digesters airtight. The biogas produced was collected and measured by water displacement. The first stage thermophilic digester and the second stage mesophilic digester had working volumes of 1 L and 2 L, respectively. The two digesters were placed in two water baths to maintain their temperatures (50 °C for the thermophilic digester and 35 °C for the mesophilic digester).

2.2. Seed sludge

A thermophilic (55 °C) and a mesophilic (37 °C) bench-scale reactors had been maintained separately for 6 months digesting dairy cattle manure. The contents from these two reactors were used as seed sludge for the thermophilic and the mesophilic digesters of the AT-TPAD system in a respective manner. The contents of the thermophilic and the mesophilic reactors contained 8.80% and 9.00% total solid (TS), respectively, and about 6.40% volatile solid (VS).

After the AT-TPAD system was finished in the study, its thermophilic and mesophilic digester contents were then used as seed sludge for the NT-TPAD system. The thermophilic and the mesophilic seed sludge contents contained 11.52% and 9.33% TS, and 9.67% and 7.44% VS, respectively.

2.3. Feedstock

Dairy manure (including feces and urine) from cattle was collected on a daily basis from the Waterman Dairy Center, The Ohio State University. Based on dry matter (DM), the ration fed to the cattle during this study was primarily composed of 50.00% corn silage, 4.50% alfalfa hay, 21.00% co-product of corn wet milling, 9.05% ground corn, 4.64% soybean meal, 1.30% Aminoplus®, 1.30% soyhulls, 0.38% fat, 2.01% vitamin and minerals. The average TS and VS contents of the collected manure were 14.61% (w/v) and 12.81% (w/v), respectively. Prior to use, the manure was diluted to desired TS and VS contents using tap water and mixed thoroughly into slurry. This was done to reduce potential clogging in digesters (Sung and Santha, 2003) and improve the substrate accessibility to the microbial community (Angelidaki and Ahring, 2000).

2.4. Start-up, operation, and sampling

In the AT-TPAD system, the thermophilic and the mesophilic digesters were seeded with 100 mL and 200 mL of the corresponding seed sludge, respectively, and then filled to their working volumes with pre-warmed manure slurry. After maintained at desired temperatures for 4 days to allow temperature equilibration and microbial activity recovery, the AT-TPAD system was then fed with the same manure slurry of 11.25% TS and 9.86% VS. In the NT-TPAD system, both digesters were filled to their working volumes with seed sludge: the thermophilic digester was filled with a mixture of the thermophilic and the mesophilic seed sludge (0.5 L each), while the mesophilic digester was filled with only the mesophilic seed sludge (2 L). The NT-TPAD system was then fed with manure slurry of 13.56% TS and 11.90% VS.

Both TPAD systems were operated in a fed-batch mode on a daily basis: the effluent was discharged from the mesophilic digester, then the same volume of the content from the thermophilic digester was transferred to the mesophilic digester, and finally the same volume of manure slurry was fed to the thermophilic digester. Daily biogas production and effluent pH from each digester were recorded daily before feeding. The content of each digester was manually mixed before and after feeding, and the pH of the thermophilic digester of the AT-TPAD system was adjusted to 6.5 using 10 M NaOH solution when necessary. During the start-up of the NT-TPAD system, an extra volume of sludge (the same volume as the feeding slurry) was recycled once daily between the two digesters of the NT-TPAD system in case of acidification (indicated by pH decreases) in the thermophilic digester until the thermophilic digester reached balance between hydrolysis/acidogenesis and methanogenesis (indicated by stable and neutral pH).

The operation of each TPAD system lasted 144 days and was summarized in Table 1. Both systems were operated until they reached their steady states when the variation of daily biogas production from each digester was less than 10% for five consecutive days without any upward or downward trend (Wen et al., 2007). Biogas and sludge samples from each TPAD system were collected multiple times during steady-state operation. The sludge samples were aliquoted and stored at −80 °C until being analyzed.

2.5. Analysis

2.5.1. System performance analysis

Contents of CH_4 in biogas samples were analyzed by gas chromatography (GC) as described previously (Patra and Yu, 2012), and methane yields were calculated from corresponding biogas productions and methane contents. Sludge samples were centrifuged to collect their supernatants, prepared per the same protocol used by Oelker et al. (2009), and subjected to VFA analysis using GC. Concentrations of TS and VS of each sludge sample were determined following the standard methods (American Public Health Association et al., 2005), and solid removal was calculated for each sludge sample.

2.5.2. Microbial community analysis

Community DNA was extracted from each sludge sample using the RBB + C method, which allows efficient extraction of microbial