



Volume ratios between the thermophilic and the mesophilic digesters of a temperature-phased anaerobic digestion system affect their performance and microbial communities

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An experimental temperature-phased anaerobic digestion (TPAD) system, with the thermophilic digester operated at neutral pH and with a balanced acidogenesis and methanogenesis (referred to as NT-TPAD), was evaluated with respect to the microbial communities and population dynamics of methanogens when digesting dairy cattle manure at 15-day overall system hydraulic retention time (HRT). When fed a manure slurry of 10% total solid (TS), similar system performance, 36–38% volatile solid (VS) removal and 0.21–0.22 L methane g⁻¹ VS fed, was achieved between a 5-day and 7.5-day HRT for the thermophilic digester. However, the thermophilic digester achieved a greater volumetric biogas yield when operated at a 5-day RT than at a 7.5-day HRT (6.3 vs. 4.7 L/L/d), while the mesophilic digester had a stable volumetric biogas yield (about 1.0 L/L/d). Each of the digesters harbored distinct yet dynamic microbial populations, and some of the methanogens were significantly correlated with methane productions. *Methanosarcina* and *Methanosaeta* were the most important methanogenic genera in the thermophilic and the mesophilic digesters, respectively. The microbiological findings may help understand the metabolism that underpins the anaerobic processes within each of the two digesters of TPAD systems when fed dairy manure.

Introduction

Known and utilized for many decades, anaerobic digestion (AD) has received renewed interests recently in the pursuit of renewable energy and mitigation of greenhouse gas emissions. Indeed, AD is one of the few dual-purpose technologies that can simultaneously produce biogas from biomass wastes and reduce/prevent environmental pollutions [1,2]. While the main goal of AD was previously waste management, its current emphasis is on cost-effective production of biogas as renewable energy. New designs and operational strategies have been sought after to increase biogas yield while maintaining process stability [1,3]. A complex microbial community is responsible for the sequential steps (hydrolysis, acidogenesis, syntrophic acetogenesis, and methanogenesis) of AD process [4]. The microbial communities in digesters are also

dynamic and their composition and structure can be shaped by many factors including feedstock, design, and operation [5]. The complex and dynamic features of the microbial communities create many challenges to increase biogas yield and enhance process stability [4,6]. For example, the four major guilds of microbes, hydrolytic bacteria, acidogens, syntrophic acetogens, and methanogens, differ in several aspects relevant to AD, including growth rate and ability to survive and function at low pH and high ammonia concentration that are often observed during AD operation [7,8]. As a result, conditions and operations that meet the requirements of hydrolytic bacteria and acidogens often overwhelm the fastidious and exigent methanogens [9–11]. Thus, single-stage digesters often suffer from poor system performance [12] and susceptibility to high organic loading rates (OLR) [11], due to their inability to provide the conditions that are needed for optimal growth and function of each microbial guild.

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Two-stage anaerobic digester systems provide opportunities to create and maintain two separate intra-system environments in the two digesters. The advantages of two-stage digesters over single-stage digesters have been demonstrated in a numerous studies where two-stage anaerobic digester systems outperformed single-stage digesters when readily digestible feedstock were fed [9,13–17]. For livestock manure, including its co-digestion with a readily digestible feedstock, a few studies also showed that two-stage digesters achieved greater biogas yield and VS removal than single-stage digesters [17–19]. In most studies on two-stage digesters, both stages were operated at similar temperatures, about 35°C for mesophilic or 55°C for thermophilic operations. However, temperature-phased AD (TPAD) with the first stage operated at thermophilic temperature (typically about 55°C) and the second stage at mesophilic temperature (mostly about 35°C) is an emerging technology attracting much recent research interest. This is because the thermophilic digester of a TPAD system can enhance destruction of solid and pathogen [20,21], while the mesophilic digester facilitates stable biogas production [11,21].

A few studies have been reported on TPAD systems that digest cattle manure as the solely feedstock [22–24]. In these studies, TPAD systems achieved 36–40% VS removal and 0.21–0.22 L methane g⁻¹ VS fed when operated with a short HRT (14–15 days) and when fed a dairy cattle manure slurry containing about 10% TS. Sung and Santha [24] also showed that the final digestate met the criteria specified for Class A biosolids. In a recent study [23], comparing the performance of two TPAD systems with the thermophilic digester operated at either acidic or neutral pH (referred to as AT-TPAD and NT-TPAD, respectively), a NT-TPAD system was shown to outperform an AT-TPAD system. The objectives of the present study were to further evaluate NT-TPAD systems in terms of performance when operated at two different HRT by varying the volume ratio between the thermophilic and the mesophilic digesters and to investigate the microbial (bacterial and archaeal) communities and population dynamics of methanogens in each digester. Correlation between system performance and methanogen populations was also examined.

Materials and methods

Experimental setup, seed sludge, and feedstock

Two bench-scale digesters were made from two Nalgene polypropylene wide-mouth bottles of 4.3-L capacity (Fisher Scientific, PA) and used as the thermophilic and the mesophilic digesters of the NT-TPAD system [22]. Each of the digesters had a feeding port, a sampling port, and a biogas outlet. The feeding port and the sampling port were each sealed by a rubber stopper to keep both digesters airtight except during feeding and sampling. The biogas produced by each digester was collected and measured by water displacement using an inverted graduated cylinder, which was maintained at room temperature. The first stage thermophilic digester and the second stage mesophilic digester were started with working volumes of 1 and 2 L, respectively. The two digesters were placed in two adjacent water baths to maintain their respective temperatures (50°C for the thermophilic digester and 35°C for the mesophilic digester).

The seed sludge for the thermophilic digester (pH about 6.0) and mesophilic digester (pH about 7.5) was the content of two digesters of an AT-TPAD system that had been operated using dairy cattle

manure slurry for 144 days in a previous study [22]. Briefly, this AT-TPAD system had a thermophilic digester operated at 50°C and acidic pH mainly for hydrolysis/acidogenesis and a mesophilic digester operated at 35°C for balanced hydrolysis/acidogenesis and methanogenesis. The content from these two digesters was used as the seed sludge for the two digesters of the NT-TPAD system. The thermophilic and the mesophilic seed sludge contained 11.52% and 9.33% total solid (TS), and 9.67% and 7.44% volatile solid (VS), respectively.

The feedstock was dairy manure (including both feces and urine) slurry that was prepared as described previously [22]. Briefly, fresh dairy manure was collected on a daily basis from the Waterman Dairy Center, The Ohio State University, where Jersey cattle were fed the same total mixed ration (TMR, based on dry matter, 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% soy hulls, 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), with variations less than 1.01% and 0.74%, respectively. Prior to use, manure was diluted to desired TS and VS contents using tap water and mixed thoroughly into slurry with a 10% TS to reduce potential clogging in digesters [24] and improve the substrate accessibility to the microbial community [25].

Start-up, operation, and sampling

Both digesters of the NT-TPAD system were filled to their working volumes with the seed sludge as done previously [22]. Briefly, the thermophilic digester received a mixture of the thermophilic and the mesophilic seed sludge (0.5 L each), while the mesophilic digester received only the mesophilic seed sludge (2 L). The thermophilic digester was also inoculated with the mesophilic seed sludge to augment the methanogenesis activity. The NT-TPAD system was then operated in a feed-batch mode on a daily basis [23]. Contents of both digesters were manually mixed before and after feeding. Biogas production and effluent pH from each digester were recorded daily before feeding. During the startup, about 150 ml of sludge was recycled between the two digesters when acidification (as indicated by pH decrease below 6.5) was observed in the thermophilic digester. The sludge recycling ended when the thermophilic digester reached and maintained neutral pH without any recycling.

The operation of the NT-TPAD system was essentially the same as described in the previous study [23] and was separated into three sequential periods: startup period, period 1, and period 2 (Fig. 1). As defined in a previous study [26], the NT-TPAD system was considered to have reached steady state in each period when the variation of daily biogas production by both digester was less than 10% for five consecutive days without any upward or downward trend. During the startup period, the HRT (also solid retention time SRT because of mixing of digester content) was 33% longer than that applied during periods 1 and 2, while the OLR was 75% of that applied during periods 1 and 2. The overall HRT/SRT was maintained at 15 days for all the three operation periods. Two volume ratios of thermophilic over mesophilic digesters were used during the study: 1:2 during the startup period and period 1, and 1:1 during period 2. Six biogas samples and six sludge samples were collected from each digester at its stable state during each of these three periods of operation: at days 24, 25, 28, 29, 32, and 33 during the startup; at days 77, 78, 82, 86, 87, and 92 of period 1; at days

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