Waste Management 33 (2013) 2211–2218

Contents lists available at SciVerse ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Population dynamics during startup of thermophilic anaerobic digesters: The mixing factor

Sophia A. Ghanimeh [a], Pascal E. Saikaly [b,c], Dong Li [b], Mutasem El-Fadel [a,*]

[a] Department of Civil and Environmental Engineering, American University of Beirut, Lebanon
[b] Water Desalination and Reuse Center, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia
[c] Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia

ARTICLE INFO

Article history:
Received 28 February 2013
Accepted 6 June 2013
Available online 2 July 2013

Keywords:
Thermophilic anaerobic digestion
Mixing
Startup
Microbial dynamics
16S rRNA gene pyrosequencing

ABSTRACT

Two thermophilic digesters were inoculated with manure and started-up under mixed and stagnant conditions. The *Archaea* in the mixed digester (A) were dominated by hydrogenotrophic *Methanobateriaceae* (61%) with most of the methane being produced via syntrophic pathways. *Methanosarcinales* (35%) were the only acetoclastic methanogens present. Acetate dissipation seems to depend on balanced hydrogenotrophic-to-acetotrophic abundance, which in turn was statistically correlated to free ammonia levels. Relative abundance of bacterial community was associated with the loading rate. However, in the absence of mixing (digester B), the relationship between microbial composition and operating parameters was not discernible. This was attributed to the development of microenvironments where environmental conditions are significantly different from average measured parameters. The impact of microenvironments was accentuated by the use of a non-acclimated seed that lacks adequate propionate degraders. Failure to disperse the accumulated propionate, and other organics, created high concentration niches where competitive and inhibiting conditions developed and favored undesired genera, such as *Halobacteria* (65% in B). As a result, digester B experienced higher acid levels and lower allowable loading rate. Mixing was found necessary to dissipate potential inhibitors, and improve stability and loading capacity, particularly when a non-acclimated seed, often lacking balanced thermophilic microflora, is used.

1. Introduction

Anaerobic digestion (AD) is considered to be an attractive source of renewable energy and a viable method to treat various organic waste streams. In this context, the organic fraction of municipal waste (OFMSW) is known to be highly biodegradable resulting in fast VFA generation and high ammonia levels, which can inhibit the digestion process and induce instabilities in the system (Banks et al., 2008). This can be aggravated in thermophilic (55–60 °C) systems where the generation of metabolic intermediates is faster than at mesophilic (30–40 °C) temperatures. Furthermore, it is often difficult to acquire a sufficient amount of acclimated inocula to startup new thermophilic digesters because of the currently limited number of commercial thermophilic applications. As a result, thermophilic AD of OFMSW often faces operational difficulties and instability problems connected with poor startup and lack of acclimated seeds (Fdéz.-Güelfo et al., 2010; Suwannoppadol et al., 2011).

In this context, Stroot et al. (2001) recommended reduced mixing to enhance the stability of mesophilic AD systems. In contrast, others emphasized the importance of mixing to (1) ensure adequate contact between nutrients and the viable bacterial population in mesophilic digesters (Gomez et al., 2006; Ward et al., 2008); and (2) provide a uniform heat distribution and efficient dispersion of metabolic waste (Gerardi, 2003). In fact, the need for adequate mixing has been supported by many researchers (Elnekave et al., 2006; Halalsheh et al., 2011), while questioned by many others (Kaparaju and Angelidakia, 2008; Suwannoppadol et al., 2011). Accordingly, knowledge about the effect of mixing on the start-up of (1) AD in general is controversial and (2) thermophilic AD of OFMSW in the absence of an acclimated seed in particular is scarce.

On the other hand, the operating temperature plays a critical role in defining the major methanogenic population residing in the digester. Methane can be generated via direct cleavage of acetate by acetoclastic methanogens, whereby the methyl and carboxyl groups are split and converted into CH_4 and CO_2, respectively. Alternatively, methane can be generated by syntrophic pathways whereby both the methyl and carboxyl groups of acetate are oxidized by syntrophic bacteria, then, some of the CO_2 is reduced to CH_4 by hydrogenotrophic methanogens using

* Corresponding author. Address: American University of Beirut, Faculty of Engineering and Architecture Bliss Street, PO Box 11-0236, Beirut, Lebanon. Fax: +961 1 744 462.
E-mail address: mfadel@aub.edu.lb (M. El-Fadel).

0956-053X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.wasman.2013.06.011

H_2 (Zinder et al., 1984). Under thermophilic temperatures, the syntrophic pathway dominates (Krakat et al., 2010; Tang et al., 2011; Yabu et al., 2011), thus playing a significant role in system stability.

Based on the aforementioned studies, operational conditions (such as mixing and temperature) and seeding sources might affect the microbial community structure, which in turn will affect the digester's performance during startup. However, the literature reveals a gap in knowledge centered on understanding the correlation between microbial community composition/dynamics and digester's functional performance and stability (Goberna et al., 2009; Krakat et al., 2010). In particular, efforts targeting the role and activity of various methanogenic populations during anaerobic digestion of complex organic materials, such as OFMSW, are relatively scarce (Demirel and Scherer, 2008), with the ratio of acetoclastic-to-hydrogenotrophic methanogens often determined indirectly by measuring various parameters/ratios such as isotopic signature and CH_4/CO_2 ratio, among others (Qu et al., 2009; Boulanger et al., 2012; Schnürer and Nordberg, 2008).

In order to analyze the microbial community structure in AD of OFMSW, PCR-based fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) of 16S rRNA gene fragment have been used (Bertin et al., 2012; Carballa et al., 2011; Martín-González et al., 2011). However, these methods can detect only the dominant members in a community. With the introduction of next-generation high throughput sequencing technology, in particular 16S rRNA gene pyrosequencing using the Roche 454 platform, it is now possible to characterize both the dominant and rare members within a community (Sogin et al., 2006). Characterizing the rare members in a community can be useful especially that they may play a key role in community flexibility and functional stability in methanogenic bioreactors (Fernandez et al., 2000).

This study aims to improve the startup of thermophilic AD of OFMSW seeded with a non-acclimated inocula (raw cattle manure), considering mixing as a system's determinant. Accordingly, the effect of mixing on population dynamics was examined and the latter was linked to performance and stability during the start-up phase. For this purpose, two digesters were started up under similar operating conditions but different mixing schemes, using manure for seeding. The microbial community structure and dynamics were monitored using 16S rRNA gene pyrosequencing, and the results were correlated with operational and environmental conditions (mixing, acetate concentration, free ammonia level) and performance parameters (methane generation, VFAs, VFA-to-alkalinity ratio, loading capacity).

2. Materials and methods

2.1. Feed preparation

A large (~50 kg) batch of OFMSW was collected from restaurants and food markets, then ground and stored at −20 °C and used to feed the digesters up to day 151. A second batch (prepared in a similar way to the first one and from the same sources) was needed to feed the digesters for the rest of the experiment. The physico-chemical characteristics of the consecutive batches were presented in Ghanimeh et al. (2012). Prior to use, the waste samples were thawed and diluted with distilled water to a total solids content of 7.5–8.0%.

2.2. Startup procedure

About 1.5 kg of cattle manure were diluted to 9 l and incubated anaerobically for one week at 50 °C then used to seed two 14 l digesters (9 l effective volume each). The digesters were started up under similar operating conditions but different mixing schemes. Digester A was operated under continuous slow mixing (100 rpm) while digester B was operated at mostly stagnant conditions. Both digesters were thoroughly mixed (for 1 min at 200 rpm) prior to sampling to homogenize their content and ensure representative samples; and right after feeding to distribute the substrate throughout the volume. Feeding was scheduled 3 times a week. The organic loading rate (OLR) was step-increased in both digesters with a concomitant decrease in HRT until overloading, which occurred on day 167 in digester A and day 191 in digester B. The maximum OLR achieved in digester B before reactor failure was 2.1 gVS/l/d (HRT = 46 days) compared to 2.5 gVS/l/d in digester A (HRT = 47 days). After overloading, feeding was stopped then resumed at low OLR. Details of the experimental procedures, loading conditions and digester's performance are presented in Ghanimeh et al. (2012).

2.3. Monitoring methods

2.3.1. Sample preparation

Samples were collected prior to feeding and after thorough mixing of the digester content. For testing of soluble components, samples were centrifuged and passed through 1.2 μm openings filters. For gas chromatography, samples were filtered in 0.2 μm openings syringe filters and acidified with phosphoric acid to pH of 2 then stored at −20 °C until testing.

2.3.2. Testing frequency and procedures

Temperature and pH were continuously monitored. Gas generation and composition were measured on a daily basis using the water displacement method and a dual wavelength infrared cell with reference channels (GEM-2000 monitor, Keison Products, UK). Physico-chemical analyses were performed weekly to determine: total and soluble solids using Standard Methods 2540B and 2540E procedures (APHA, AWWA and WPCF, 1998), total and soluble COD using COD kits (HACH Company, Loveland, Colorado), ammonia by spectrophotometry method and alkalinity by titration. Individual VFAs (acetate, propionate and butyrate) were measured, one to three times per week, using a gas chromatograph (Trace GC Ultra, Thermo Electron corporation) equipped with a flame ionization detector and a 30 m, 0.25 mm, 0.25 μm capillary column (TR-FFAP).

2.3.3. Microbial community analysis

The first samples were collected on day 15, when pH adjustment was not required anymore. Then, the digesters were sampled at every OLR, right before increasing to the next OLR (days 55–167 for A and 55–190 for B). Samples were taken also on day 214, i.e. after few weeks of reduced OLR due to overload. The samples were centrifuged at 13,400 rpm for 10 min. The supernatant was discarded and the settled solids stored at −20 °C for subsequent DNA extraction. Genomic DNA was extracted in duplicates from the samples using the PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions and pooled to reduce sample variability. Triplicate PCR reactions were performed for each sample in a 25-μl reaction volume using the HotStarTaq Plus Master Mix (QIAGEN, Valencia, CA), 0.5 μM of each primer and 100–200 ng of template DNA. Bacterial 16S rRNA genes were amplified using the bacterial-specific forward primer 515–532F (5′-Adaptor A-Barcode-CA Linker-GTGCCAGCAGCCGCGGTA-3′) and universal reverse primer 909–928R (5′-Adaptor B-TC linker-CCCCGYCAATTCMTTTRAGT-3′) (Wang and Qian, 2009). Archaeal 16S rRNA genes were amplified using the universal forward primer 519–537F (5′-Adaptor A-Barcode-CA Linker-CAGYMGCCRCGGKAAHACC-3′) and the archaeal-specific reverse primer 806–825R (5′-Adaptor B-TC