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Reactor performance and microbial community dynamics during anaerobic co-digestion of municipal wastewater sludge with restaurant grease waste at steady state and overloading stages

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HIGHLIGHTS

- Linkage between reactor performance and bacterial and archaeal community dynamics.
- GTW co-digestion with MWS at steady state and overloading conditions.
- Two 10 L bench-scale reactors operated at mesophilic and 20 days SRT.
- Pyrosequencing determined the sequence abundance of bacterial and archaeal communities.
- The CCA revealed the linkage between microbial dynamics and environmental variables.

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ABSTRACT

Linkage between reactor performance and microbial community dynamics was investigated during mesophilic anaerobic co-digestion of restaurant grease waste (GTW) with municipal wastewater sludge (MWS) using 10 L completely mixed reactors and a 20 day SRT. Test reactors received a mixture of GTW and MWS while control reactors received only MWS. Addition of GTW to the test reactors enhanced the biogas production and methane yield by up to 65% and 120%, respectively. Pyrosequencing revealed that *Methanosaeta* and *Methanomicrobium* were the dominant acetoclastic and hydrogenotrophic methanogen genera, respectively, during stable reactor operation. The number of *Methanosarcina* and *Methanomicrobium* sequences increased and that of *Methanosaeta* declined when the proportion of GTW in the feed was increased to cause an overload condition. Under this overload condition, the pH, alkalinity and methane production decreased and VFA concentrations increased dramatically. *Candidatus cloacamonas*, affiliated within phylum *Spirochaetes*, were the dominant bacterial genus at all reactor loadings.

1. Introduction

Disposal and sustainable management of grease trap waste (GTW) have been a challenge for years due to a variety of operational issues and municipal disposal limitations. GTW is a lipid-rich organic material collected from the waste streams of restaurant and food service establishments. Direct disposal of this waste into the environment is no longer permitted by most municipalities (Long et al., 2012). Anaerobic digestion as a robust alternative technology is widely applied to stabilize municipal wastewater sludge (MWS) and many organic wastes economically and effectively. However, due to some intrinsic limitations of using this technology for the treatment of MWS, various pretreatments are required to improve its efficiency. GTW anaerobic co-digestion (ACD) with MWS has become a valuable alternative to improve nutrient balance in mixed substrates and enhance buffer capacity, biogas production and reactor performance (Zhu et al., 2011).

Despite all the reported benefits of ACD systems, previous studies have indicated that the performance and stability of such systems are dependent on reactor design as well as many operational and physico-chemical parameters such as substrate characteristics, organic loading rates, temperature, and pH among others (Razaviarani et al., 2013a,b; Zhu et al., 2011). Anaerobic digestion, as a syntrophic biological process, also is reliant on microorganisms' activities via the four major stages of hydrolysis, acidogenesis, acetogenesis and methanogenesis. A deeper understanding and resolution of the linkage between microbial community dynamics and process stability can provide invaluable information to predict the reactor performance. Yet, the microbial

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dynamics and their interactions still remained uncertain primarily due to the complexity of the microbial activities within the interrelated biological reactions in these systems (Supaphol et al., 2011). Several studies were conducted over the last decade to investigate the microbial population structure in anaerobic digestion of lipid-rich waste (Palatsi et al., 2010; Pereira et al., 2002) with a focus mostly on the LCFA inhibition effects. Nevertheless, to the authors' knowledge, microbial studies linked to reactor performance of the anaerobic co-digestion of GTW with MWS have not yet been conducted.

Among the available microbial fingerprinting techniques, denaturing gradient gel electrophoresis (DGGE) and clone library are the most popular methods used to evaluate the microbial populations (Lee et al., 2010). However, using the DGGE method for investigation of complex microbial populations is intricate due to drawbacks which include the identification of limited bands and co-migration of sequences. Also, the cloning technique and its data analysis are laborious and uneconomical. The development of 454 Pyrosequencing, as a new generation of sequencing techniques, facilitates the investigation of microbial community dynamics in various environments by identifying a larger number of sequences more quickly (Guo et al., 2014).

The objective of this study was to investigate the reactor performance linked the microbial community dynamics of mesophilic ACD of GTW and MWS at (1) steady-state conditions conducted at two different runs with different organic loadings and (2) during reactor overload conditions. For this purpose, physico-chemical analysis along with the Pyrosequencing microbial technique was performed and reactors' stabilities and performance were monitored accordingly with the associated microbial population dynamics.

2. Methods

2.1. Inoculum and substrates

Municipal wastewater sludge (MWS) consisting of a 4:1 (v/v) mixture of primary sludge (PS) and thickened waste activated sludge (WAS), was collected from a wastewater treatment plant (WWTP) in Edmonton, Alberta, Canada. Primary sludge is mixed with thickened waste activated sludge in a 4:1 volumetric ratio at this WWTP before it is pumped to the on-site anaerobic digesters. The MWS used in the study was collected from a pipeline that conveys this 4:1 mixture to the on-site digesters. GTW was received from a local waste collection company in Edmonton, Alberta. Samples were stored at 4 °C until their utilization. The GTW from restaurants and food services typically has some settable solids which are not degraded during the anaerobic digestion and collect at the bottom of the reactor. Pre-treatment should be practiced at WWTPs to remove these solids and water which can be treated more efficiently in other process units. Therefore, before adding the GTW to the MWS, it was brought to room temperature and then the top layer of grease, fats and oils (FOG) was separated from the settable solids and water layers. This top layer of FOG was then blended with the MWS in the desired proportion. Digested effluent from a full-scale mesophilic anaerobic reactor at the same Edmonton WWTP was used as the inoculum (biomass) for the start-up of the reactors. The characteristics of substrates and inoculum are shown in Table 1.

2.2. Reactor operation and loading protocol

The experiment was conducted at two separate stages, as shown in Table 2, with respect to the collection of MWS at different times. During each stage, two identical 10 L (8 L working volume) reactors were mixed by magnetic stirrers and operated at 37 ± 0.5 °C and a 20-day solids retention time (SRT). The reactors were sustained at desired temperature using heating tape wrapped around the reactors and the temperatures were monitored and controlled by Type K thermocouples and digital temperature controllers. An insulating jacket was also applied around each reactor to minimize heat loss.

Each reactor was initially filled with 8 L of inoculum and then the reactors' headspace was purged with nitrogen gas. Each day, 0.4 L of digested material was withdrawn from each reactor and replaced with the same volume of substrate to provide a 20 day SRT. Reactor 1 served as a control (C-1) and received only MWS, while reactor 2 was operated as the test digester (T-1) and was fed a mixture of MWS and GTW based on a percentage of the control reactor COD loading. Initially, for the first run of the experiment, C-1 and T-1 received an equal amount of MWS_1 to establish their baseline performance for a period of 30 days (1.5 SRT). When the equivalence of the reactors' performance was established, the COD loading of T-1 reactor was increased with the addition of a known volume of GTW to the MWS_1 to obtain the desired COD loading of 150% relative to the control reactor (C-1). This operating mode was continued for 3 SRT (60 days) to reach the steady-state conditions and then for another 10 day period during which daily sampling was conducted. Similarly, for the second stage, the control (C-2) and test (T-2) reactors were fed with same volume of MWS_2 for 30 days to establish the baseline performance. Then the test reactor (T-2) was fed with a mixture

Table 1
Characteristics of substrates and inoculum.

Parameter	Mean value ± standard deviation of 4 samples			Inoculum[e]
	MWS_1[b]	MWS_2[c]	GTW	
COD (g/L)	75.3 ± 1.2	40.9 ± 0.9	2697.8 ± 52.5	22.9
TS (g/L)	63.9 ± 1.0	22.9 ± 0.8	776.8 ± 16.7	22.3
VS (g/L)	35.7 ± 0.9	18.3 ± 0.5	776.2 ± 13.8	12.2
TSS (g/L)	61.5 ± 1.3	19.2 ± 0.8	47.7 ± 1.0	20.9
VSS (g/L)	34.8 ± 1.4	15.6 ± 0.5	47.6 ± 0.9	11.3
VFA (mg/L)	2983 ± 39	1640 ± 28	nm[d]	8
TKN[e] (mgN/L)	804	794	nm	2140
TAN[e] (mgN/L)	346	332	nm	755
Alkalinity[a] (mg/L)	1850 ± 15	2000 ± 18	nm	nm
pH	5.8 ± 0.1	5.7 ± 0.1	5.0 ± 0.1	7.3

[a] Alkalinity represented as mg/L $CaCO_3$.
[b] Municipal wastewater sludge used in Stage 1.
[c] Municipal wastewater sludge used in Stage 2.
[d] Non-measured.
[e] 1 sample (no sample standard deviation).