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Microbial community adaptation influences long-chain fatty acid conversion during anaerobic codigestion of fats, oils, and grease with municipal sludge

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ABSTRACT

Codigesting fats, oils, and greases with municipal wastewater sludge can greatly improve biomethane recovery at wastewater treatment facilities. Process loading rates of fats, oils, and greases have been previously tested with little knowledge of the digester microbial community structure, and high transient fat loadings have led to long chain fatty acid (LCFA) accumulation and digester upsets. This study utilized recently-developed quantitative PCR assays for syntrophic LCFA-degrading bacteria along with 16S amplicon sequencing to relate changes in microbial community structure to LCFA accumulation during transient loading increases to an anaerobic codigester receiving waste restaurant oil and municipal wastewater sludge. The 16S rRNA gene concentration of the syntrophic β-oxidizing genus *Syntrophomonas* increased to ~15% of the *Bacteria* community in the codigester, but stayed below 3% in the control digester that was fed only wastewater sludge. *Methanosaeta* and *Methanospirillum* were the dominant methanogenic genera enriched in the codigester, and together comprised over 80% of the *Archaea* community by the end of the experimental period. Constrained ordination showed that changes in the codigester *Bacteria* and *Archaea* community structures were related to measures of digester performance. Notably, the effluent LCFA concentration in the codigester was positively correlated to the specific loading rate of waste oil normalized to the *Syntrophomonas* 16S rRNA concentration. Specific loading rates of 0–1.5 × 10^{-12} g VS oil/16S gene copies-day resulted in LCFA concentrations below 30 mg/g TS, whereas LCFA accumulated up to 104 mg/g TS at higher transient loading rates. Based on the community-dependent loading limitations found, enhanced biomethane production from high loadings of fats, oils and greases can be achieved by promoting a higher biomass of slow-growing syntrophic consortia, such as with longer digester solids retention times. This work also demonstrates the potential for controlling the loading rate of fats, oils, and greases based on the analysis of the codigester community structure, such as with quantitative PCR measurements of syntrophic LCFA-degrading bacteria abundance.

1. Introduction

Anaerobic digestion is commonly used at municipal wastewater treatment plants (WWTPs) to process waste sludge and recover renewable energy as biomethane. Fats, oils, and greases (FOG) are desirable substrates for enhancing biomethane recovery through codigestion because they have a methane yield potential per g VS that is 250%–350% greater than the wastewater sludge typically fed to municipal digesters (Davidsson et al., 2008; Girault et al., 2012; Luostarinen et al., 2009). Reported increases in digester methane production from 140% to 620% during FOG codigestion with wastewater sludge (Wan et al., 2011; Wang et al., 2013) have demonstrated the potential to significantly improve economics and

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reduce energy footprints of municipal WWTPs with FOG codigestion.

When fats and oils are added to the anaerobic digestion process, they are rapidly hydrolyzed into their major constituents of glycerol and long-chain fatty acids (LCFA) (Hanaki et al., 1981). After lipid hydrolysis, most of the energy content resides in LCFA, which can comprise over 90% of the chemical oxygen demand of the original lipid molecule (Sousa et al., 2009). The formation of methane from LCFA involves a syntrophic partnership of proton-reducing acetogenic bacteria, which utilize the β-oxidation pathway to convert LCFA into acetate and formate/hydrogen, along with aceticlastic and hydrogenotrophic methanogenic archaea (Schink, 1997; Sousa et al., 2009; Weng and Jeris, 1976). All of the isolated bacterial species known to β-oxidize LCFA syntrophically belong to two families, *Syntrophomonadaceae* and *Syntrophaceae* (Hatamoto et al., 2007; Jackson et al., 1999; McInerney, 1992; Sousa et al., 2007b; Wu et al., 2007). Generally, the conversion of LCFA into methane is considered the rate-limiting step for lipid degradation in anaerobic digesters (Angelidaki and Ahring, 1992; Cirne et al., 2007; Hanaki et al., 1981).

Process failures observed at elevated FOG loading rates have impeded the ability to fully exploit higher biomethane production during FOG codigestion with municipal wastewater sludge (Davidsson et al., 2008; Girault et al., 2012; Luostarinen et al., 2009; Noutsopoulos et al., 2013; Wan et al., 2011; Wang et al., 2013). Specifically, the LCFA released during lipid hydrolysis can inhibit anaerobic microorganisms at high concentrations (Angelidaki and Ahring, 1992; Koster and Cramer, 1987; Lalman and Bagley, 2000; Rinzema et al., 1994), thereby limiting their bioconversion into methane. Causes of inhibition have been attributed to LCFA adsorption onto cell surfaces, which can lead to direct toxicity (Hanaki et al., 1981; Rinzema et al., 1994) and/or substrate transport limitations (Pereira et al., 2005). While aceticlastic methanogens are believed to be the most sensitive group to LCFA toxicity (Koster and Cramer, 1987; Lalman and Bagley, 2000, 2001; Rinzema et al., 1994), the inhibition of hydrogenotrophic methanogens and syntrophic bacteria by LCFA has also been suggested (Hanaki et al., 1981; Lalman and Bagley, 2002; Pereira et al., 2005; Roy et al., 1985). Reported threshold values for FOG loading that led to decreased methane yields during codigestion with municipal wastewater sludge ranged from ~0.4 to 2.1 g VS/L-d (Girault et al., 2012; Luostarinen et al., 2009; Noutsopoulos et al., 2013; Silvestre et al., 2011; Wan et al., 2011; Wang et al., 2013). However, these empirical FOG loading thresholds do not account for digester microbial populations and their role in LCFA conversion, and are thus of limited use for predicting the response of a digester following transient increases in FOG loading. An improved understanding of the relationship between the digester biomass composition and LCFA accumulation is needed to develop strategies for stable codigester operation with increased FOG loadings and enhanced methane recovery.

The importance of biomass adaptation for stable FOG digestion has been indicated by previous studies. Silvestre et al. (2011) observed that stepwise increases in FOG loading led to the development of biomass with higher LCFA β-oxidation and methanogenic activities during codigestion with municipal sludge. Alves et al. (2001) found that both the tolerance to LCFA toxicity as well as the LCFA-biodegradation activity increased with long-term exposure to lipids in an anaerobic fixed-bed bioreactor. Similarly, long-term acclimation was identified as a key factor influencing the resilience to LCFA toxicity in a series of digester sludges exposed to skim milk and oleate based wastewaters (Silva et al., 2014). While these results collectively indicated that biomass adaptation could affect the efficiency of FOG conversion, the microbial community structures of these digester sludges were not assessed. The dynamics of LCFA-degrading microbial communities have been previously studied using highly enriched systems with LCFA as the primary carbon source (Salvador et al., 2013; Shigematsu et al., 2006; Sousa et al., 2007a; Ziels et al., 2015). However, the relationship between the LCFA conversion efficiency and microbial community structure during FOG codigestion with municipal wastewater sludge has received little attention. The changes in LCFA-degrading community structure caused by transient increases in FOG loading therefore need further study to enable better predictions of acceptable FOG loadings during codigestion.

The main objective of this study was to elucidate the relationship between digester biomass composition and LCFA conversion rates and removal efficiency during FOG codigestion with municipal wastewater treatment sludge. Quantitative PCR targeting LCFA-degrading syntrophic bacteria and methanogenic archaea was conducted along with Illumina 16S rRNA gene amplicon sequencing of *Bacteria* and *Archaea* communities to monitor microbial population structure changes in a FOG codigester and control digester treating municipal wastewater solids. The specific goals were to: (1) determine the effects of FOG addition on the digester microbial community structure, and (2) examine relationships between microbial community structure and LCFA removal following transient variations in FOG loading.

2. Materials and methods

2.1. Digester operation

Two semi-continuous complete-mix anaerobic digesters (4 L working volume) were operated at 37 °C with a 20-day hydraulic retention time (HRT) for 198 days. The digesters were mixed with axial flow impellers at 275–325 rpm. They were started with anaerobic digester sludge collected from Henriksdal WWTP in Stockholm, Sweden, and were fed with a mixture of waste primary sludge (WPS) and waste activated sludge (WAS) collected from the same plant throughout the experiment. The WAS+WPS was collected biweekly and stored at 4 °C. The digesters were manually fed once daily by withdrawing the volume of reactor liquid corresponding to the volume of the feed prior to addition. The average feed WPS+WAS volatile solids (VS) concentration was 28 ± 2 g VS/L and the feed sludge VS loading rate (VSLR) for both digesters averaged 1.4 ± 0.1 g VS/L-day over the course of the experiment. After an initial startup period of 53 days of only feeding WPS+WAS, waste cooking oil (hereby referred to as FOG) from a nearby restaurant was added to one of the digesters for codigestion. The start of FOG codigestion was defined as day 1 of the experimental period (Table 1). The FOG VS content was ~99%, and its addition to the codigester was increased in a stepwise manner over time to 1.5 g VS/L-d (52% of the total feed VS) by day 94 (Table 1).

Digester performance was monitored with daily biogas production, methane content, pH, effluent volatile fatty acids (VFA), effluent LCFA, total solids (TS), and VS. Biogas production was measured with tipping bucket displacement gas meters (Milli-Gascounters, Ritter, Germany). The biogas composition was analyzed weekly for methane, carbon dioxide, oxygen, and hydrogen sulfide using a portable gas analyzer (Biogas Check, Geotech, UK). All measured gas volumes are reported at standard temperature and pressure (1 atm pressure and 0 °C). The TS and VS contents of the sludge were determined according to Swedish Standard Method SS028311. The pH of the digesters was measured using an Inolab pH 7310 meter (InoLab, Wissenschaftlich-Technische Werkstätten, Germany) immediately after withdrawing sludge from the reactors. VFA (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, capronate and iso-capronate) were analyzed by GC-FID (HP 6890, Hewlett Packard),