Long-chain fatty acid feeding frequency in anaerobic codigestion impacts syntrophic community structure and biokinetics

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Abstract

This study investigated the impacts of long-chain fatty acid (LCFA) feeding frequencies on microbial community structure, bioconversion kinetics, and process stability during anaerobic codigestion. Parallel laboratory-scale anaerobic codigesters fed with dairy cattle manure were either pulse-fed every two days or continuously-fed daily, respectively, with oleate (C_{18:1}) in incremental step increases over 200 days up to 64% of the influent chemical oxygen demand (COD). The effluent acetate concentration exceeded 3,000 mg/L in the continuous-fed codigester at the highest oleate loading rate, but remained below 100 mg/L in the pulse-fed codigester at the end of its 48-hr oleate feed cycle. Maximum substrate conversion rates of oleate ($q_{max,oleate}$) and acetate ($q_{max,acetate}$) were significantly higher in the pulse-fed codigester compared to the continuous-fed codigester. 16S rRNA gene amplicon sequencing showed that *Bacteria* and *Archaea* community profiles diverged based on the codigester LCFA feeding pattern and loading rate. LCFA-degrading *Syntrophomonas* bacteria were significantly enriched in both LCFA codigesters relative to the control digester. The pulse-fed codigester had the highest community

fraction of *Syntrophomonas* 16S rRNA genes by the end of the experiment with 43% of *Bacteria* amplicon sequences. $q_{max,oleate}$ and $q_{max,acetate}$ values were both significantly correlated to absolute concentrations of *Syntrophomonas* and *Methanosaeta* 16S rRNA genes, respectively. Multiple-linear regression models based on the absolute abundance of *Syntrophomonas* and *Methanosaeta* taxa provided improved predictions of oleate and acetate bioconversion kinetics, respectively. These results collectively suggest that pulse feeding rather than continuous feeding LCFA during anaerobic codigestion selected for higher microbial bioconversion kinetics and functional stability, which were related to changes in the physiological diversity and adaptive capacity of syntrophic and methanogenic communities.

Keywords: Anaerobic digestion; biomethane; syntrophy; methanogen; long-chain fatty acids (LCFA); fats, oils, and grease (FOG); 16S rRNA

1. Introduction

Anaerobic digestion of agricultural livestock manure is an attractive solution to minimize carbon footprints by treating the organic waste through the recovery of biomethane. However, the low biomethane potential of animal manures (Labatut et al., 2011) can limit the economic viability of agricultural digesters (Bishop and Shumway, 2009). Codigesting high-strength organic wastes with manure can significantly improve process economics by increasing biomethane recovery as well as providing additional revenue from tipping fees (Bishop and Shumway, 2009). Fats, oils, and greases (FOG) are appealing substrates for codigestion because their biological methane yield per unit mass is 2.7-times greater than that for livestock manures (Labatut et al., 2011). When added to the digestion process, fats and oils are rapidly hydrolyzed into glycerol and long-chain fatty acids (LCFA) (Cirne et al., 2007; Hanaki et al., 1981), with over 90% of the original methane potential of FOG maintained within LCFA (Sousa et al., 2009).

The conversion of LCFA into methane is considered rate-limiting for the ultimate degradation of FOG in anaerobic digesters (Cirne et al., 2007; Hanaki et al., 1981). LCFA-degradation under methanogenic conditions involves an obligatory syntrophic partnership between acetogenic β -oxidizing bacteria and acetate- and hydrogen-utilizing methanogenic archaea (Schink, 1997; Sousa et al., 2009). The bacterial species known to β -oxidize LCFA in syntrophy with a hydrogen- or formate- consuming partner have so far been identified within only two families, *Syntrophomonadaceae* and *Syntrophaceae* (Sousa et al., 2009). The slower biodegradation of LCFA relative to lipid hydrolysis in anaerobic digesters can lead to LCFA accumulation and process instability, as LCFA are known inhibitors of hydrogenotrophic and aceticlastic methanogenic activity at high concentrations (Hanaki et al., 1981; Hwu and Lettinga, 1997; Silva

et al., 2016; Sousa et al., 2013). LCFA accumulation can also limit the kinetics of syntrophic β -oxidizing bacteria (Hanaki et al., 1981). Developing strategies to increase the robustness and stability of anaerobic digesters at high FOG loading rates is thus key for improving biomethane recovery from agricultural digesters.

The relationship between the LCFA-degrading community structure and LCFA bioconversion kinetics in anaerobic digesters has not been well characterized. Previous efforts have predicted LCFA conversion kinetics in anaerobic digesters using theoretical models, such as ADM1 (Palatsi et al., 2010; Zonta et al., 2013). However, those biokinetic models were based on predictions of biomass composition, instead of calibration with actual measurements of LCFA-degrading bacteria abundance. Such models have also represented LCFA-degrading bacteria as a single homogenous population, whereas it was previously shown that various LCFA-degrading taxa can coexist in anaerobic digester sludge (Sousa et al., 2007; Treu et al., 2016; Ziels et al., 2015) with potentially different functional roles (Kougias et al., 2016). Different methanogenic archaea species can also have varying degrees of tolerance to LCFA exposure (Silva et al., 2016; Sousa et al., 2013), suggesting that the digester methanogen community structure could be affected by FOG addition. A recent finding that effluent LCFA concentrations were inversely correlated to LCFA-degrading bacteria 16S rRNA gene concentrations during FOG codigestion (Ziels et al., 2016) implicated that the maximum LCFA bioconversion rate of syntrophic bacteria and methanogenic archaea could establish the allowable FOG loading rate possible before LCFA accumulation. However, no studies have examined whether different communities of syntrophic bacteria and methanogenic archaea with different LCFA bioconversion kinetics could be biologically selected during FOG codigestion. Additional understanding of the relationship

between LCFA conversion kinetics and the LCFA-degrading community structure is therefore needed to identify effective digester operating strategies that promote stable performance at high FOG loading rates.

The stability of the anaerobic digestion process during disturbances has been attributed to the resilience, resistance, and redundancy of the various microbial trophic guilds of the digester food web (Carballa et al., 2015; Werner et al., 2011). *Resistance* refers to the degree in which a community composition remains unchanged during a disturbance; *resilience* indicates the rate at which the microbial community returns to its original composition following a disturbance; and *functional redundancy* refers to the potential for various microbial taxa to process the same substrate (Allison and Martiny, 2008). In anaerobic digesters, hydrolytic bacteria are thought to have a high degree of functional redundancy (Carballa et al., 2015; Vanwonterghem et al., 2014), whereas syntrophic bacteria are thought to be more metabolically specialized (Briones and Raskin, 2003; McInerney et al., 2009) and less likely to undergo competition (Werner et al., 2011). However, if different syntrophic and methanogenic consortia have different kinetics of LCFA bioconversion, then selective pressures could be incorporated into FOG codigester operating strategies to favorably select for a LCFA-degrading community with higher bioconversion kinetics. It was observed that four distinct clades of syntrophic propionate oxidizing bacteria (POB) were enriched in an anaerobic digester operated in fill-and-draw mode, and the presence of multiple syntrophic POB groups was attributed to periodic changes in propionate concentrations with batch feeding along with different biokinetic characteristics of the POB clades (Ariesyady et al., 2007). Distinct syntrophic POB populations with different biokinetic parameters were also detected in the first and second phases of a thermophilic phased-

digester based on differences in organic loading (Zamanzadeh et al., 2013). The digester feeding strategy was also found to impact the methanogen community structure (Conklin et al., 2006) and digester robustness during overloading events (Conklin et al., 2006; De Vrieze et al., 2013; Mulat et al., 2016). With regards to LCFA degradation, pulse-feeding LCFA during codigester startup was suggested to promote greater stability at higher loading rates (Cavaleiro et al., 2009); however, the resulting LCFA-degrading community structure and biokinetics were not compared to a continuous-fed codigester in parallel. Thus, the impact of LCFA feeding frequency on the bioconversion kinetics and community composition of LCFA-degrading syntrophic bacteria and methanogenic archaea during the codigestion of LCFA-rich wastes remains unknown.

In this study, anaerobic codigesters fed with dairy manure and oleate were operated with either a pulse or semi-continuous feed regime. The objective of this research was to investigate the effects of LCFA feeding frequency during codigestion on: (1) LCFA bioconversion kinetics, (2) LCFA codigester stability and performance, and (3) the composition of syntrophic LCFA-degrading consortia. An additional goal was to develop a quantitative model to predict LCFA-conversion kinetics based on syntrophic LCFA-degrading bacteria community structure and abundance.

2. Materials and Methods

2.1 Anaerobic Digester Description and Operation

The three anaerobic digesters operated in this study consisted of 5.5 L glass semi-continuous stirred tank reactors with a 4.0 L working volume (Supplemental Figure S1). The digesters were intermittently mixed for 2 minutes every 10 minutes with an axial impeller mixer (5VB, Cleveland Mixer), and temperature was controlled at 35°C in an environmental chamber.

The digesters were inoculated with effluent sludge from a mesophilic anaerobic digester treating dairy manure at a nearby farm. Fresh cow dairy manure feedstock was collected from the influent mixing tank of the same dairy farm digester. The manure feedstock was blended for 1 minute before storing ~1L aliquots at -20°C. Single manure aliquots were thawed at 4°C daily before use. Three batches of manure were collected throughout the entire experimental period. The manure feed was manually added once daily after withdrawing 200 mL of sludge. The total feed volume (manure + oleate) was maintained at 200 mL/day to maintain a 20-day hydraulic and solids retention time (HRT and SRT). Daily biogas production was measured with wet tip gas meters (Wet Tip Gas Meter Co., Nashville), and the methane composition of the headspace biogas was measured at least twice weekly at the end of the daily feeding cycle. Total COD (TCOD), total solids (TS), and volatile solids (VS) of the manure feed and digester effluents were measured 3-times weekly. Effluent volatile fatty acids (VFA) were measured 3-7 times weekly, and effluent long-chain fatty acids (LCFA) were measured weekly.

Digester operation conditions were varied under three experimental phases: (1) start-up period with manure-only feeding (days -50 to 0); (2) Phase I of oleate codigestion with manure (days 1

to 85); and (3) Phase II of oleate codigestion with manure (days 86 to 204). The manure feed VS loading was maintained at 1.3 ± 0.2 g VS/L-d during all experimental phases.

During Phase I, two of the digesters received an aqueous solution of sodium oleate (>97%, Tokyo Chemical Industry Co.) at 10% of the daily influent feed volume for codigestion, while the third digester was fed only manure as the control. Sodium oleate was used as a model substrate for LCFA in this study because it has a good solubility (Alves et al., 2001), and is the most abundant LCFA found in domestic and dairy wastewaters (Alves et al., 2009). Concentrations given for the oleate feed in this study have been corrected for the mass of sodium, so that they are in terms of the oleate ion. The oleate feed organic loading rate (OLR) for the two codigesters was equal, and was increased over time by increasing the oleate concentration. The oleate feeding frequency was different for the two codigesters. One codigester received the oleate feed every 6-hrs daily, and is designated as the *continuous-fed codigester* (CF codigester). The other codigester received the oleate feed once every 2-days, and is designated as the *pulse-fed codigester* (PF codigester). The oleate OLR was nearly doubled about every 20-days (equal to one SRT), accounting for up to 27% of the influent feed total COD by the end of Phase I on day 85 (Table 1). On day 85, the PF codigester operation was terminated due to accidental damage to the reactor vessel.

During Phase II, two oleate-fed digesters were operated in parallel: (1) the operation of the CF codigester from Phase I was continued; and (2) the control digester from Phase I was converted to a PF codigester by feeding sodium oleate in pulse-feed mode as described in Phase I operation. The PF digester in Phase II is designated as the *pulse-fed codigester #2* (PF2

codigester). The oleate OLR to the PF2 codigester in Phase II was increased nearly every 10 days and the step increases to the CF codigester remained nearly every 20 days, such that the oleate accounted for 64% of the total influent COD to both codigesters by day 150 (Table 1). Due to acetate accumulation in the CF codigester at an oleate OLR of 3.8 g COD/L-d, the oleate OLR was reduced to 3.2 g COD/L-d on day 184 (Table 1).

2.2 Oleate Bioconversion Kinetics

Oleate bioconversion kinetics were measured for each codigester sludge in a series of batch tests on days 0, 41, and 84 during Phase I, and on days 129, 148, 170, and 204 for Phase II. The 30-mL glass serum batch test bottles were purged with N₂:CO₂ (80:20) before adding 15 mL digester effluent (digestate), sealed with butyl rubber septa and purged again with N₂:CO₂ (80:20). A 100 g/L aqueous solution of sodium oleate was added to the batch test bottles via syringe for initial oleate concentrations of 100, 175, 350, 750, 1800, and 4500 mg/L. A control without any added oleate was also observed. The pH was adjusted to 7.2 with 1 N HCl. Triplicate test bottles were maintained at 35°C on an orbital shaker at 150 rpm. Gas production was measured using a digital manometer (Series 490, Dwyer Instruments, Michigan City) approximately 7 times over 8 hrs. The headspace methane concentration was sampled with each pressure measurement. The background methane production from the control was subtracted from each substrate-amended treatment, and the slope of methane production was determined via linear regression in R version 3.2.1. Methane production rates were adjusted to standard temperature and pressure (STP), and were normalized to the amount of VS added to the vials initially. Batch tests for the PF codigesters were conducted at the end of the 48-hr pulse-feeding cycles to minimize background substrate concentrations.

2.3 Oleate Inhibition of Aceticlastic Methanogenic Activity

To test inhibitory effects of oleate on methanogenic activity, aceticlastic maximum methane production rates ($q_{max,acetate}$) were measured in batch tests with different initial oleate concentrations. The aceticlastic maximum methane production rate was measured according to the procedure described by Conklin et al. (2006). Batch test bottle preparation was conducted as described above in Section 2.2. A series of triplicate bottles received 50 mM sodium acetate in addition to sodium oleate at initial concentrations of 0, 1.5, 4, 8, 12, and 16 g/L. One set of triplicate vials without any added substrate was incubated in parallel to measure background digestate methane production. Methane production was monitored 8-times over a 6-hr period based on headspace pressure and composition. The $q_{max,acetate}$ was calculated based on linear regression of methane production curves as described by Conklin et al. (2006). Aceticlastic inhibition tests were conducted within 3-days of oleate bioconversion kinetic tests.

2.4 Analytical Methods

TCOD was measured with High-Range COD digestion vials (Hach Co., Loveland, CO) according to Hach Method 8000. TS and VS were measured according to Standard Methods (Eaton and Franson, 2005). Prior to daily wasting and feeding of the digesters, pH was measured (EcoSens pH100A, YSI, Yellow Springs, Ohio) immediately after withdrawing samples.

Effluent sludge samples for volatile fatty acid (VFA) analysis were initially centrifuged at 14,000 g for 10 minutes. The supernatant was serial filtered with 0.45 and 0.22 μm polyethersulfone (PES) syringe filters, and 100 μL of 10% formic acid was then added to 1 mL of filtrate to acidify the sample. VFA were measured with a GC-FID (Shimadzu GC-2010) equipped with a

BP-21 column (30 m×0.32 mm I.D., 0.25 µm film thickness, SGE). LCFA were extracted from digester effluent according to the protocol described by Ziels et al. (2015), and were analyzed by GC-FID with a BP-21 column (30 m×0.32 mm I.D., 0.25 µm film thickness; SGE). Biogas samples (~0.5-1 mL) were collected with a syringe equipped with a Teflon Mininert valve, and the methane composition was analyzed using a SRI 8610C GC-FID with a Supelco Alumina Sulfate Plot column (50 m, 0.53 mm i.d.).

2.5 DNA Extraction and Quantification

DNA was extracted in triplicate from digester sludge samples collected on the same days as the batch oleate bioconversion kinetic assays. Briefly, ~15 mL of digester sludge was collected via catheter syringe and transferred to pre-purged, O₂-free centrifuge tubes on ice. The digester sludge was aliquoted into 2 mL microcentrifuge tubes on ice in an anaerobic glove chamber. The 2 mL samples were then centrifuged at 14,000 g for 10 min at 4°C, immediately decanted, and stored at -80°C. DNA was extracted from the pellet using the PowerMicrobiome™ kit (MO BIO Laboratories Inc., Carlsbad, CA) with phenol:chloroform:isoamyl during bead-beating, according to the manufacturer's instructions. Extracted DNA was quantified with the Quant-iT HS DNA kit using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA).

2.6 Quantitative PCR

Quantitative PCR (qPCR) was conducted in accordance to Ziels et al. (2015) targeting 16S rRNA gene fragments of the syntrophic β -oxidizing bacterial genus of *Syntrophomonas*, as well as the domains *Bacteria* and *Archaea* (Yu et al., 2005). More detailed descriptions of the reaction conditions, primers and probes, and calibration standards are given in the Supplemental

Information. qPCR was conducted on triplicate DNA extractions from each sampling point, and gene copy numbers were normalized per g VS of digester biomass.

2.7 16S rRNA Gene Amplicon Sequencing

Amplicon sequencing of 16S rRNA genes was conducted on triplicate DNA extractions from each sampling point. Briefly, 16S rRNA gene fragments from all samples were amplified with barcoded and indexed universal prokaryotic V4-V5 primers 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926R (5'-CCGYCAATTYMTTTRAGTTT) (Parada et al., 2015), and the products were pooled and sequenced on a 2x300bp Illumina MiSeq run by the United States Department of Energy Joint Genome Institute. The raw sequence data is available through the JGI Portal (<http://jgi.doe.gov>) under project ID number 1105525.

2.8 Bioinformatics

To obtain a high resolution of the community composition, amplicon reads were denoised into their exact sequences using the DADA2 pipeline (Callahan et al., 2016). Reads were first quality filtered by trimming the first 10 bp from all sequences, truncating forward reads at 265 bp, truncating reverse reads at 180 bp, and filtering all reads based on a maximum expected error of 2 using DADA2. Parameters for the DADA2 error model were initially calculated on dereplicated reads of a random subset of 20 samples, and the estimated error parameters were subsequently used to denoise all dereplicated sample reads into exact sequences. Forward and reverse sequences were then merged with DADA2 using a minimum overlap of 20 bp and zero allowed mismatches. Merged denoised sequences were truncated to 390 bp and clustered into 99.5% operational taxonomic units (OTUs) following chimera removal with UPARSE (Edgar,

2013), resulting in 5014 non-singleton OTUs. Representative sequences of the 99.5% OTUs were classified against the SILVA SSU Ref NR dataset (v.123) using the RDP classifier (Wang et al., 2007). By denoising and error-correcting the sequences with DADA2 before OTU clustering, a smaller clustering radius could be used in this study without generating spurious or erroneous OTUs (Callahan et al., 2016). This higher resolution allowed for fine-scale changes in microbial taxa to be investigated within the codigesters.

Relative abundance values were calculated based on normalized sequence counts obtained with DESeq2 (Love et al., 2014), which accounted for different sequencing depths among samples. Diversity estimates were based on Hill diversity numbers (qD) of orders $q = 0, 1$, and 2 (Jost, 2006) using DESeq2 normalized sequence counts.

2.9 Regression Analysis

A stepwise regression approach was used to identify a subset of OTUs that significantly explained ($p < 0.05$) maximum methane production rates from oleate and acetate. Predictor variables (before stepwise feature selection) for the $q_{max,oleate}$ regression model included the inferred absolute abundances of 13 dominant *Syntrophomonas* OTUs, and the predictor variables for the $q_{max,acetate}$ regression model consisted of inferred absolute abundances of 3 dominant *Methanosaeta* OTUs. Inferred absolute abundances were calculated by multiplying each OTU relative abundance value by the target group 16S rRNA gene copies per g VS obtained by qPCR. The triplicate amplicon sequence profiles for each sampling point were included individually in the model development and validation. Prior to model fitting, multicollinearity between OTU abundances was examined using Pearson's correlation, and OTUs with correlation coefficients

greater than 0.7 were removed from the dataset. Stepwise feature selection was conducted with the remaining OTU abundance datasets, and model selection was cross-validated based on 10 sub-samplings repeated 10 times using the caret package in R (Kuhn, 2008).

3. Results

3.1 Effect of LCFA OLR and feeding frequency on codigester performance

Impacts of LCFA (oleate) feeding frequency on codigester performance and bioconversion kinetics were investigated by increasing the oleate loading in two operational phases over 204 days (Table 1). There was no significant effect of the LCFA OLR on codigester effluent VS concentrations, indicating that LCFA addition with both feeding strategies did not impair the manure VS destruction efficiency and that most of the LCFA fed was degraded (Supplemental Tables S1 and S2). The pH of the codigesters was on average 7.2 and 7.5 by the end of Phase I and II, respectively (Supplemental Tables S1 and S2), indicating sufficient buffering capacity in the codigesters.

During Phase I, the average daily methane production rate was similar for the two codigesters, and increased with each incremental increase in the feed LCFA concentration (Figure 1A). By the end of Phase I, the daily methane production for the two codigesters was on average 70% higher than that for the manure-fed control (1700 ± 400 mL CH₄/d versus 1000 ± 100 mL CH₄/d). The specific methane yield in terms of mass of VS fed was 45% higher for the two codigesters than the control at the end of Phase I (290 ± 40 mL CH₄/g VS_{fed} versus 200 ± 20 mL g VS_{fed}). Similar increases in methane production during Phase II were observed with each incremental increase in LCFA loading to the codigesters (Figure 1B). At the highest LCFA loading of 64% of the influent COD during Phase II, the daily methane production of both codigesters was on average 440% higher than that for the Phase I manure-fed control (5500 ± 700 mL CH₄/d versus 1000 ± 100 mL CH₄/d), and the codigester specific methane yields were 160% higher than the control (520 ± 40 mL CH₄/g VS_{fed} versus 200 ± 20 mL g VS_{fed}). The

average specific methane yield was 8% higher for the PF2 codigester relative to the CF codigester at the highest LCFA loading during Phase II, but the difference was not statistically significant due to the large daily variation in gas production with the pulse-feeding regime (Supplemental Table S2).

Acetate was the only VFA that accumulated above 140 mg/L during LCFA codigestion in this study (Supplemental Figure S2). Previous studies have also reported acetate as the primary VFA intermediate during oleate degradation in anaerobic digestion (Lalman and Bagley, 2001; Novak and Carlson, 1970). In Phase I, acetate was always below 40 mg/L (Figure 1C), and no other VFA were detected. In Phase II, effluent acetate persisted after the oleate OLR was increased to 64% of the total influent COD ($3.8 \text{ g COD}_{\text{oleate}}/\text{L-d}$) on day 150 (Figure 1D). After day 180, the effluent acetate concentration in the CF codigester steadily increased to about 3,000 mg/L by the end of the experiment on day 204. From day 160-204, the PF2 codigester effluent acetate concentration was elevated to about 900-1200 mg/L at 24 hrs after LCFA feeding, but it was consumed to below 100 mg/L before the next oleate feeding every 48-hrs (Figure 1D).

3.2 Impacts of LCFA OLR and feeding frequency on LCFA and acetate bioconversion kinetics

After the addition of oleate to the codigesters, their $q_{\text{max,acetate}}$ and $q_{\text{max,oleate}}$ values increased with increasing oleate OLRs up to day 170. At the end of Phase I, the CF and PF codigesters had similar $q_{\text{max,acetate}}$ values, but the $q_{\text{max,oleate}}$ value for the PF codigester was 16% higher (Table 2). During Phase II, the oleate OLR was increased for the CF codigester from 2.8 to $3.8 \text{ g COD}/\text{L-d}$ by day 170 (Table 1). The PF2 codigester had the same oleate OLR of $3.8 \text{ g COD}/\text{L-d}$ by day 170, but its OLR had been increased at a faster rate starting from day 104. On day 170, the PF2

codigester had a higher $q_{max,oleate}$ than the CF codigester by 17%, and a higher $q_{max,acetate}$ by 28% (Table 2). The $q_{max,acetate}$ and $q_{max,oleate}$ values for both codigesters decreased between days 170 and 204 in Phase II, but the biokinetics of the PF2 codigester remained faster with 35% and 33% higher $q_{max,acetate}$ and $q_{max,oleate}$ values than that of the CF codigester on day 204, respectively (Table 2). These results were confirmed by substrate inhibition kinetic modeling (Aiba et al., 1968) described in the Supplemental Information, which showed that the PF and PF2 codigesters had 65% and 27% higher $q_{max,oleate}$ values than the CF codigester at the end of Phase I and II, respectively (Supplemental Table S7). Pulse-feeding oleate during codigestion therefore selected for higher oleate and acetate utilization kinetics.

Tests were conducted to examine the effect of oleate concentration on the maximum acetate utilization rate ($q_{max,acetate}$), and showed that $q_{max,acetate}$ values were not inhibited by oleate concentrations below 4 g/L until day 204 (Supplemental Table S3). On day 204, the $q_{max,acetate}$ of both Phase II codigesters was not inhibited by an oleate concentration of 0.8 g/L, but at 4 g/L oleate it was inhibited by over 60% (Supplemental Table S3). Effluent oleate concentrations for both Phases I and II were below 150 mg/L (Supplemental Tables C4 and C5), which were below these inhibitory concentrations for aceticlastic methanogenesis. Yet, a decreased aceticlastic tolerance to oleate in both codigesters on day 204 was indicated by lower total $q_{max,acetate}$ values (Table 2), as well as acetate accumulation within the CF codigester (Figure 1D). The decrease in aceticlastic methanogenesis rates at higher oleate concentrations was not likely caused by sodium inhibition, as the maximum sodium concentration in the batch tests of 57 mM at the highest oleate level (16 g/L) was well below the 50% inhibition concentration of sodium (440 mM) observed by Rinzema et al. (1988).

Until day 84 in Phase I, the rate of oleate bioconversion in the batch tests with both codigester sludges was not inhibited below 350 mg/L oleate, as indicated by lower methane production rates at higher oleate concentrations (Supplemental Table S6). On day 84 in Phase I, the rate of oleate conversion in PF codigester batch tests was not inhibited up to 1,800 mg/L oleate, whereas the conversion rate in the CF codigester batch tests was inhibited at 350 mg/L (Supplemental Table S6). During Phase II, the inhibitory oleate concentration increased for both codigesters batch tests toward ~1,800 mg/L until day 204 (Supplemental Table S6). On day 204, oleate bioconversion rates in CF codigester batch tests were inhibited at an oleate concentration of 875 mg/L, whereas the rates in the PF2 codigester batch tests were not inhibited up to 1,800 mg/L. Thus, pulse-feeding LCFA during codigestion led to a higher resistance to oleate inhibition during its biodegradation in comparison to continuous-feeding.

3.3 Microbial community structure and diversity was altered by the codigester feeding pattern

In Phase I, the absolute and relative concentrations of syntrophic bacteria and methanogenic archaea increased over time with increases in the oleate OLR. 16S rRNA gene sequences of β -oxidizing *Syntrophomonas* increased to $9\% \pm 0.2$ and $4\% \pm 0.1$ of bacterial reads in the PF and CF codigesters by day 84, respectively, which were both significantly higher ($p < 1e-3$) than the control at $0.5\% \pm 0.04$ (Figure 2A). While the fraction of *Syntrophomonas* in the PF codigester was almost double that of the CF codigester by day 84 ($p < 0.001$; Figure 2A), the CF codigester had significantly higher absolute concentrations of total *Bacteria* 16S rRNA genes at the end of Phase I ($p < 0.01$; Figure 3A). Consequentially, the absolute concentration of *Syntrophomonas* 16S rRNA genes measured by qPCR was similar in the PF and CF codigesters on day 84 (Figure 3C). Likewise, the PF codigester had a higher community fraction of aceticlastic *Methanosaeta*

on day 84 in Phase I (Figure 2B), but the CF codigester had a higher total *Archaea* 16S rRNA concentration (Figure 3B) resulting in similar absolute concentrations of *Methanosaeta* in the two codigesters (Supplemental Figure S3). The absolute concentration of *Methanosaeta* was significantly higher in both codigesters relative to the control by the end of Phase I ($p<0.001$; Supplemental Figure S3)

Both the structure of the microbial community and the absolute concentration of different populations were altered by the LCFA feeding strategy during Phase II. Correspondence analysis revealed that the *Bacteria* and *Archaea* OTU read abundance profiles diverged between the PF2 codigester and CF codigester beginning on day 129 onward (Figure 4). On day 170, the PF2 codigester *Archaea* population had significantly higher fractions of *Methanosaeta*, *Methanoculleus*, and *Methanospirillum* sequences compared to the CF codigester ($p<0.001$; Figure 5B), whereas the CF codigester had a significantly higher fraction of *Methanobacterium* ($p<0.001$). By day 204, the total *Archaea* 16S rRNA gene concentration was similar in both codigesters (Figure 3E), indicating that the absolute concentrations of *Methanosaeta*, *Methanoculleus*, and *Methanospirillum* was higher in the PF2 codigester (Supplemental Figure S3). The *Bacteria* community of the PF2 codigester had a significantly higher fraction of β -oxidizing *Syntrophomonas* compared to the CF codigester by day 170 ($p<0.001$; Figure 5A). On day 204, the fraction of *Syntrophomonas* sequences in the PF2 *Bacteria* sequence library increased to $43\% \pm 2$, which was almost double that of the CF codigester at $22\% \pm 0.4$ (Figure 5A). The total *Bacteria* 16S rRNA gene concentration reached similar levels in both codigesters by day 204 (Figure 3D), indicating that the absolute concentration of *Syntrophomonas* was higher in the PF2 codigester due to its higher community fraction (Figure 5A). This result was confirmed with qPCR on total *Syntrophomonas* 16S rRNA genes, which showed a higher

concentration in the PF2 codigester relative to the CF codigester on day 204 ($p < 0.001$; Figure 3F).

In order to obtain a high resolution of digester microbial community compositions, amplicon reads were denoised into their exact sequences with DADA2 (Callahan et al., 2016) prior to clustering into OTUs at a 99.5% similarity. Such high-resolution analysis showed that at least 85% of *Syntrophomonas*-classified sequences were distributed among 13 OTUs (Figure 6). Time-series abundance profiles of *Syntrophomonas* OTUs showed divergence based on codigester LCFA feeding patterns by the end of Phases I and II (Figure 6). Correspondence analysis of *Syntrophomonas* OTU sequence counts revealed that the genus composition differed based on the codigester LCFA feeding frequency and OLR (Supplemental Figure S4). These results indicate that the fine-scale community structure of LCFA β -oxidizing bacteria in the codigesters was impacted by the LCFA feeding frequency. In contrast, over 95% of the sequences classified within the aceticlastic genus of *Methanosaeta* were comprised by only 3 OTUs, which had similar relative abundance profiles between the two codigesters (Supplemental Figure S5). The hydrogen-utilizing genus *Methanobacterium* was represented at over 90% by a single OTU. The biological selective pressure from LCFA feeding frequency therefore had larger impacts on the composition of syntrophic bacteria than selection for different dominant taxa of aceticlastic and hydrogenotrophic methanogens.

The presence of LCFA as a co-substrate in the feed resulted in lower *Bacteria* diversity in both codigesters, while pulse-feeding LCFA during codigestion resulted in higher *Archaea* community diversity. Diversity of the *Bacteria* community at all orders of Hill diversity numbers

(qD) assessed ($q = 0, 1$, and 2) decreased over time for both codigesters during Phases I and II (Supplemental Figure S6). In contrast, *Bacteria* Hill diversity numbers for the Phase I control digester increased over time (Supplemental Figure S6). Despite their decreasing trend in both codigesters during Phase II, *Bacteria* Hill diversity numbers of the orders of 1D and 2D were higher in the PF codigester relative to the CF codigester until day 170, after which 1D and 2D were lower in the PF2 codigester (Supplemental Figure S6). Similarly, diversity numbers of orders 1D and 2D for the *Archaea* community were significantly higher ($p < 0.01$) in the PF codigester relative to the CF codigester at the end of Phase I, and stayed significantly higher ($p < 0.01$) in the PF2 codigester during Phase II (Supplemental Figure S7). Richness (i.e. 0D) values of the *Archaea* communities were similar between all digesters in Phase I, but were significantly higher ($p < 0.01$) in the PF2 codigester relative to the CF codigester starting at day 170 in Phase II (Supplemental Figure S7).

3.4 Oleate and acetate bioconversion kinetics were correlated to syntrophic and methanogenic community abundance

A linear regression model was established to examine the relationship between maximum oleate conversion rates ($q_{max,oleate}$) measured during batch kinetic tests with all digester sludges (16 tests total) and the 16S rRNA gene concentration of total *Syntrophomonas* in the sludges (Figure 7A). The total *Syntrophomonas* 16S rRNA gene concentration was a significant predictor of $q_{max,oleate}$ values ($p < 0.001$). The specific slope of the linear regression was 8.6×10^{-10} mL CH_4 /16S rRNA gene-day, and the overall model fit had an R^2 of 0.73.

To examine the importance of syntrophic bacteria community structure, a multiple linear regression model was developed to predict $q_{max,oleate}$ values based on absolute concentrations of a

subset of *Syntrophomonas* OTUs (Figure 7B). Feature selection and cross-validation showed that the linear model provided the greatest R^2 by incorporating the absolute concentrations of six of the thirteen most abundant *Syntrophomonas* OTUs. All of the six selected *Syntrophomonas* OTUs were significant predictors of $q_{max,oleate}$ ($p<0.05$; Supplemental Table S8). The individual OTU correlation coefficients ranged from -7.0×10^{-8} to 2.8×10^{-8} mL CH₄/16S rRNA gene-day (Supplemental Table S8). The negative slope contribution found for one OTU suggests that it had an antagonistic effect on oleate conversion. The resulting multiple linear regression model had an improved R^2 of 0.92 in comparison to the linear regression that was dependent only on the total *Syntrophomonas* 16S rRNA gene concentration (Figure 7). A phylogenetic comparison of the six selected *Syntrophomonas* OTUs showed that they were distributed throughout the genus (Supplemental Figure S8).

A similar approach showed that the absolute concentration of total *Methanosaeta* 16S rRNA genes was a significant predictor of the acetoclastic $q_{max,acetate}$ values ($p=0.001$), although the R^2 of the correlation was only 0.51 (Supplemental Figure S9A). A multiple-regression model was developed to predict $q_{max,acetate}$ values based on the absolute 16S rRNA gene concentrations of two *Methanosaeta* OTUs, which resulted in an R^2 of 0.62 (Supplemental Figure S9B). Thus, oleate and acetate conversion kinetics of the codigester biomasses were both predicted more precisely by incorporating fine-scale syntrophic and methanogenic community structures.

4. Discussion

4.1 Codigester stability and bioconversion kinetics were impacted by LCFA feeding frequency

The results of this study showed that pulse-feeding oleate during codigestion selected for higher oleate and acetate bioconversion kinetics, as well as more stable digester performance at high LCFA loadings. The improved tolerance to high LCFA loadings was apparent during Phase II, when acetate accumulated to above 3,000 mg/L in the CF codigester but was completely degraded between feedings in the PF2 codigester. Even though the maximum acetate conversion rates ($q_{max,acetate}$) decreased in both codigesters with high LCFA loadings by the end Phase II, the PF2 codigester had a greater aceticlastic tolerance by maintaining a higher $q_{max,acetate}$ than the CF codigester. The higher acetate conversion kinetics in the pulse-fed system likely prevented acetate accumulation at the high LCFA OLRs. These findings agree with previous work showing that a once-daily acetate feeding improved acetate utilization kinetics and digester tolerance to organic overloading in comparison to hourly acetate feeding (Conklin et al., 2006). Pulse-feeding also provided a greater tolerance to organic overloading and ammonia toxicity in comparison to a continuous-fed digester during anaerobic treatment of synthetic wastewater (De Vrieze et al., 2013). Periodic pulse-feeding of LCFA has been suggested as a strategy to enable efficient digester start-up by providing sufficient batch degradation time to consume inhibitory LCFA and stimulate microbial activity (Cavaleiro et al., 2009; Nielsen and Ahring, 2006). However, our study is the first to operate a continuous-fed codigester in parallel with a pulse-fed codigester for a comparison of stability and LCFA bioconversion kinetics during LCFA codigestion. The mechanism of biological selection imposed by LCFA pulse feeding could have been attributed to transiently high acetate and LCFA concentrations, which could select for more resistant methanogenic archaea and syntrophic bacteria populations, as well as promote populations with

higher maximum substrate utilization kinetics (Conklin et al., 2006). Thus, pulse-feeding LCFA-rich substrates, such as FOG, may allow for more stable codigester performance at higher average daily loading rates relative to continuous-fed codigestion systems.

4.2 Codigester feeding frequency and loading drove biological selection of syntrophic bacteria and methanogen population structures

Our results showed that LCFA codigestion promoted the growth of *Syntrophomonas* bacteria as the dominant LCFA β -oxidizing group, with different LCFA feeding frequencies resulting in divergent *Syntrophomonas* community compositions and absolute concentrations. *Syntrophomonas* have been identified by previous studies to play an important role in LCFA β -oxidation in anaerobic digestion systems (Sousa et al., 2007, 2009, Ziels et al., 2015, 2016). The maximum relative fraction of *Syntrophomonas* observed in the PF2 codigester of 43% was the largest *Syntrophomonas* community fraction reported for an anaerobic digestion community thus far (Stams et al., 2012; Ziels et al., 2015, 2016). Previous work has suggested that syntrophic bacteria populations are stable and resilient, and are unlikely to undergo competition with different syntrophs that have similar metabolic functions (Werner et al., 2011). The high-resolution 16S rRNA gene amplicon analysis conducted in this study revealed that various *Syntrophomonas* taxa were capable of occupying the same ecological niche within the codigesters, and the resulting *Syntrophomonas* community structure was affected by the digester feeding pattern. Thus, the highly-specialized metabolic role of syntrophic fatty-acid oxidation (McInerney et al., 2009) may have a level of physiological diversity in anaerobic digesters, with multiple syntrophic species/strains capable of contributing to LCFA-degradation. The dynamic LCFA concentrations from pulse-feeding could have possibly promoted competition and/or

cooperation among syntrophic taxa with different affinities and/or maximum substrate conversion rates, as was reported for syntrophic propionate oxidation in fill-and-draw anaerobic reactors (Ariesyady et al., 2007).

In addition to the kinetic characteristics of the acetogenic bacteria, the structure of syntrophic communities likely can also be influenced by the kinetics of the methanogenic partners, since the metabolism of these communities exists delicately near thermodynamic equilibrium (Stams and Plugge, 2009). The rate of fatty-acid oxidation by syntrophic bacteria was shown to limit fatty-acid conversion when the methanogenic partner maintained low concentrations of acetogenesis products (Junicke et al., 2016), whereas methanogens have also been found to limit syntrophic conversion rates (Warikoo et al., 1996). In our study, higher $q_{max,oleate}$ values were observed in the PF codigesters, which also had higher values of $q_{max,acetate}$. It is plausible that the higher acetate consumption kinetics drove more rapid oleate conversion, since the thermodynamics of oleate conversion into methane relies upon the intermediate acetate concentration (Schink, 1997). Differences in acetate utilization kinetics with pulse- versus continuous-feeding patterns have been attributed to divergent aceticlastic populations, since *Methanosaeta* species have higher affinities for acetate but lower maximum utilization rates in comparison to *Methanosarcina* species (Conklin et al., 2006). In this study, *Methanosaeta* was the dominant aceticlastic group in both codigesters, despite acetate accumulation to over 3,000 mg/L in the CF codigester and periodic acetate accumulation to 500-1,000 mg/L in the PF2 codigester. A higher tolerance to LCFA was reported for pure cultures of *Methanosaeta concilii* in comparison to *Methanosarcina mazei* (Silva et al., 2016), which could explain the presence of *Methanosaeta* rather than *Methanosarcina* in our study codigesters.

The PF2 codigester had almost twice the *Syntrophomonas* concentration as the CF codigester by the end of Phase II despite similar levels of influent LCFA removal in the two systems, indicating that the LCFA feeding frequency may have altered physiological growth parameters. Growth yields under anaerobic conditions have been related to thermodynamics, with greater Gibbs free energies potentially supporting higher yields (Kleerebezem and Van Loosdrecht, 2010). High initial oleate concentrations coupled with low digester acetate levels could have promoted more free energy for growth of *Syntrophomonas* in the PF2 codigester (Schink, 1997). In addition to lower acetate, a lower hydrogen concentration would also provide more free-energy for growth via LCFA β -oxidation (Schink, 1997; Stams and Plugge, 2009). Lower hydrogen levels could have been promoted in the PF2 codigester, which had higher concentrations of hydrogenotrophic *Methanoculleus* that have higher affinities for hydrogen in comparison to *Methanobacterium* (Chong et al., 2002; Lovley, 1985) that were more abundant in the CF codigester. A more detailed thermodynamic analysis would be needed to elucidate how the LCFA feeding strategy impacted the fluxes of intermediate metabolites during syntrophic conversion, but such work is warranted to improve our understanding of codigester LCFA conversion kinetics and syntrophic community structure.

The OLR has also been previously related to the anaerobic digester microbial community structure (De Vrieze et al., 2015; Hao et al., 2016). Correspondence analysis showed larger divergences between the CF and PF2 codigester community structures at higher oleate OLRs after day 129. While the OLR to the PF2 codigester was ramped up at twice the frequency than the CF codigester from day 104 to 150 during Phase II, the communities did not converge

between days 150 and 204 when the codigester OLRs were identical. The oleate feeding frequency may thus have impacted the community structure to a greater extent than the rate of oleate OLR increase to the codigesters.

4.3 Relating bioconversion kinetics to microbial community structure

This study demonstrated that codigester biokinetics were correlated to measurements of community composition. While the $q_{max,oleate}$ and $q_{max,acetate}$ both correlated significantly with the absolute concentrations of total *Syntrophomonas* and *Methanosaeta* 16S rRNA genes, respectively, improved model accuracy was obtained by incorporating measurements of fine-scale community composition and absolute concentration measurements. Bocher and colleagues (2015) demonstrated the promising use of multiple-linear regression to predict specific methanogenic activities from glucose and propionate based on methanogen community descriptors. Yet, the models developed in the latter study utilized relative DGGE band intensities as predictor variables, while the multiple-linear regression models developed in our study utilized absolute concentrations of fine-scale OTUs as predictor variables. Absolute quantification of taxa through the combination of high-throughput 16S amplicon sequencing and quantitative enumeration methods was recently suggested to provide a more comprehensive view of microbial compositional dynamics (Props et al., 2016). Since reactor bioconversion kinetics are dependent on biomass concentrations, we suggest that our predictive modeling approach based on absolute quantification of taxa can be used to identify certain OTUs that contribute to higher bioconversion rates for substrates of interest, such as LCFA and acetate in anaerobic digesters. From a practical perspective, identifying such key microbial taxa could enable more

focused monitoring efforts for predicting codigester performance and capacities under dynamic FOG loading situations, leading to improved digester stability and methane recovery.

5. Conclusions

This study assessed the impacts of codigester LCFA feeding pattern on microbial community structure and bioconversion kinetics. The following can be concluded from the results of this study:

- LCFA feeding frequency and OLR were biological selective pressures that influenced the *Bacteria* and *Archaea* community compositions as well as biokinetics in the study codigesters.
- The codigester LCFA feeding frequency impacted the composition of the syntrophic and methanogenic communities. Higher relative and absolute concentrations of *Syntrophomonas* and *Methanosaeta* 16S rRNA genes were observed in the PF2 codigester than the CF codigester in Phase II at high LCFA loadings.
- A 48-hr LCFA pulse-feeding frequency during codigestion resulted in higher microbial bioconversion kinetics for acetate and oleate.
- Both $q_{max,oleate}$ and $q_{max,acetate}$ correlated significantly with the concentrations of total *Syntrophomonas* and *Methanosaeta* 16S rRNA genes, respectively.
- Improved predictive accuracy for $q_{max,oleate}$ and $q_{max,acetate}$ was obtained through multiple-linear regression based on the absolute concentration of syntrophic and methanogenic taxa.

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Table 1

Table 1 Summary of oleate feed and total feed characteristics to the codigesters during Phase I and II. Values in parentheses represent one standard deviation.

Step # LCFA increase	Days	Oleate Feed				Total Feed (Manure + Oleate)			
		VS (g/L)	VSLR (g VS/L-d)	COD (g/L)	OLR (g COD/L-d)	VSLR (g VS/L-d)	OLR (g COD/L-d)	% VS as LCFA	% COD as LCFA
Phase I: Pulse-fed and Continuous-Fed Codigesters									
0	-50 - 0	0	0	0	0	1.3 (0.1)	2.4 (0.7)	0%	0%
1	1 - 20	0.7	0.04	2.1	0.1	1.3 (0.1)	2.2 (0.3)	3%	5%
2	21-45	1.5	0.08	4.5	0.2	1.3 (0.1)	3.0 (0.4)	6%	7%
3	46-65	2.5	0.1	7.5	0.4	1.3 (0.1)	2.3 (0.4)	10%	16%
4	66-85	4.8	0.2	14	0.7	1.5 (0.2)	2.7 (0.2)	16%	27%
Phase II: Continuous-Fed Codigester									
5	86 - 106	7.5	0.4	22	1.1	1.7 (0.2)	3.5 (0.2)	22%	32%
6	107 - 126	11.6	0.6	34	1.7	1.8 (0.2)	3.7 (0.3)	32%	47%
7	127 - 149	18.9	0.9	56	2.8	2.1 (0.2)	5.2 (0.3)	45%	54%
8	150 - 183	25.6	1.3	76	3.8	2.6 (0.2)	5.9 (0.3)	50%	64%
9	184 - 204	21.8	1.1	65	3.2	2.3 (0.1)	5.2 (0.4)	48%	62%
Phase II: Pulse-Fed Codigester									
1	104-116	1.5	0.1	4.6	0.2	1.4 (0.2)	2.3 (0.1)	5%	10%
2	117-126	3.5	0.2	10	0.5	1.3 (0.2)	2.4 (0.4)	13%	22%
3	127-137	6	0.3	18	0.9	1.7 (0.3)	3.0 (0.3)	18%	30%
4	138-149	13.6	0.7	40	2.0	1.9 (0.2)	3.9 (0.3)	36%	52%
5	150-183	25.6	1.3	76	3.8	2.6 (0.2)	5.9 (0.4)	50%	64%
6	184-204	21.8	1.1	65	3.2	2.3 (0.1)	5.2 (0.4)	48%	62%

VSLR = volatile solids loading rate; OLR = COD loading rate

Table 2

Table 2 Maximum conversion rates for oleate ($q_{max,oleate}$) and acetate ($q_{max,acetate}$) observed in the batch kinetic assays with digester sludges collected throughout Phases I and II. Values in parentheses represent one standard deviation.

Day	Oleate q_{max} (mL CH ₄ /g VS-d)			Acetate q_{max} (mL CH ₄ /g VS-d)		
	Control	CF Codigester	PF Codigester	Control	CF Codigester	PF Codigester
<i>Phase I Digesters</i>						
0	24 (2)	27 (2)	25 (4)	45 (4)	47 (5)	37 (3)
41	11 (0)	34 (1)	23 (1)	35 (4)	40 (3)	32 (2)
84	15 (1)	56 (4)	65 (2)	39 (1)	64 (6)	61 (8)
<i>Phase II Digesters</i>						
129	-	167 (0)	49 (1)	-	137 (11)	88 (8)
148	-	-	124 (9)	-	-	104 (19)
170	-	144 (5)	168 (4)	-	111 (13)	142 (9)
204	-	81 (2)	108 (1)	-	52 (5)	70 (8)

Figure 2

Figure 3

16S rRNA Gene Conc. (copies/g VS)

Figure 4

Figure 5

Figure 6

Figure 7

Figure 1 Comparison of (A) daily methane production and organic loading rate (OLR) for Phase I experimental digesters; (B) daily methane production and OLR for Phase II experimental digesters; (C) digester effluent acetate concentrations during Phase I; and (D) digester effluent acetate concentrations during Phase II. Error bars represent one standard deviation with $n=4$.

Figure 2 Relative read fractions of the (A) 12 most abundant *Bacteria* genera, and (B) 10 most abundant *Archaea* genera in the 16S rRNA amplicon sequence libraries for the digesters during Phase I. Genus abundance profiles from triplicate DNA extractions are shown for each time point.

Figure 3 Concentration of 16S rRNA genes in the Phase I digesters for (A) *Bacteria*, (B) *Archaea*, and (C) *Syntrophomonas*, and in the Phase II digesters for (D) *Bacteria*, (E) *Archaea*, and (F) *Syntrophomonas*. Error bars represent one standard deviation based on triplicate DNA extractions and duplicate qPCR technical replicates.

Figure 4 Correspondence analysis (CA) plots based on (A) *Bacteria* and (B) *Archaea* normalized OTU sequence counts in the codigester and control digester samples collected throughout Phases I and II. The color of each marker represents the sample collection day and the shape of each marker represents the digester sludge source. The triplicate samples shown for each sample day are from replicate DNA extractions. The percentage shown along each axis represents the fraction of variance explained by that CA axis.

Figure 5 Relative read fractions of the (A) 12 most abundant *Bacteria* genera, and (B) 10 most abundant *Archaea* genera in the 16S rRNA amplicon sequence libraries for the digesters during Phase II. Genus abundance profiles from triplicate DNA extractions are shown for each time point.

Figure 6 Relative read fractions of the 13 most abundant OTUs classified within the *Syntrophomonas* genus of the digesters during Phases I and II based on 16S rRNA amplicon sequencing. OTU abundance profiles from triplicate DNA extractions are shown at each time point.

Figure 7 Linear regression of observed $q_{max,oleate}$ values versus (A) the total *Syntrophomonas* 16S rRNA gene concentration; and (B) predicted $q_{max,oleate}$ values from a multiple-linear regression model based on inferred 16S rRNA gene concentrations of 6 selected *Syntrophomonas* OTUs. Inferred absolute abundances were calculated by multiplying the relative fraction of each OTU within total *Syntrophomonas* read counts by the total *Syntrophomonas* 16S rRNA gene concentration. Error bars represent one standard deviation.

Graphical Abstract

Different syntrophic taxa and LCFA bioconversion kinetics