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Research article

Fat, oil, and grease (FOG) deposits yield higher methane than FOG in anaerobic co-digestion with waste activated sludge

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ABSTRACT

The formation of fat, oil, and grease (FOG) deposits in sewers is a global challenge for the maintenance of sewer collection systems. Tons of FOG deposits (FDs) are removed from sewer systems every year and present an opportunity for increased methane production via anaerobic co-digestion with waste activated sludge (WAS) at water resource recovery facilities with existing anaerobic digesters. We hypothesized that FDs have higher biomethane potential than that of FOG (e.g., FOG collected in grease interceptors), because of the reduction of inhibition of long chain fatty acids due to saponification. In this study, substantially enhanced methane production was found in anaerobic co-digestion of WAS with FDs within the substrate to inoculum (S/I) ratio range of 0.25–1.2, and the maximum ultimate methane production (685.7 ± 24.1 mL/gVS$_{added}$, at S/I = 0.5) was 4.0 times higher than in the control (with WAS only) after 42 days of incubation. Although the lag phase period was longer in FD co-digestion (S/I = 0.5) than in FOG co-digestion (S/I = 0.5) under the same organic loading (gVS) and two times the COD loading, the daily methane production rate became higher after Day 15 in FD co-digestion. Significantly higher cumulative methane production (10.2%, $p < 0.05$) was obtained in FD co-digestion than in FOG co-digestion after 42-days. Microbial community analysis revealed higher levels of *Geobacter* in FD co-digestion, possibly suggesting a role for direct interspecies electron transfer (DIET) between *Methanosaeta* and *Geobacter*. This work provides fundamental insights supporting anaerobic co-digestion of FDs with WAS, demonstrating the advantages of FDs compared to FOG as co-substrate for enhanced biomethane recovery.

1. Introduction

The increasing global consumption of fat, oil, and grease (FOG) has led to a large amounts of FOG discharged into sewers (Long et al., 2012; Williams et al., 2012), which react with other constituents in wastewater to form hardened and insoluble FOG deposits (FDs), and result in blockages in pipes and consequently sanitary sewer overflows (SSOs) (He et al., 2017). The annual blockage-related SSOs caused by FDs are 21%, 47%, 50%, 60% and 70% of the total annual SSOs in Australia, US, UK, Hong Kong, and Malaysia, respectively (Chan, 2010; Ducoste et al., 2008; Husain et al., 2014; Marlow et al., 2011; Williams et al., 2012). FDs are therefore becoming a global challenge for the maintenance and sustainability of sanitary sewer systems. There are no accurate estimates of how much FDs are removed from sanitary sewer systems every year, but big cities likely have tons of FDs: one notorious SSO incident in London involved “fatbergs”, with a total of 130 tons of FDs removed from sewer lines (Slotkin, 2017). The estimated annual cost for the removal of FDs was $25 billion and £15–50 million in the US and UK, respectively (Del Mundo and Sutheerawattananonda, 2017; Williams et al., 2012). The FDs that are collected are usually landfilled. FDs have high biomethane production potential since their major component are long chain fatty acids (LCFAs) (Keener et al., 2008; Sousa et al., 2009; Williams et al., 2012), and the anaerobic co-digestion of FDs is likely a missed opportunity for higher methane generation at water resource recovery facilities.

Anaerobic co-digestion of waste activated sludge (WAS) with FOG (e.g., FOG collected in grease interceptors) has been shown to significantly enhance biomethane yield (Beale et al., 2016; Mata-alvarez et al., 2014; Salama et al., 2019a; Wang et al., 2013; Ziels et al., 2016; Wang et al., 2019). The conversion of LCFAs (products of FOG hydrolysis) to methane was found to be the rate-limiting step for lipid degradation in anaerobic digesters (Davidsson et al., 2008; Sousa et al., 2009; Ziels

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et al., 2016), due to LCFA adsorption to the biomass, resulting in sludge flotation and washout (Sousa et al., 2009), and the inhibitory/toxic effect of LCFAs to key microorganisms (e.g., syntrophic β-oxidizing bacteria and methanogens) (Kurade et al., 2019; Ziels et al., 2016). The physical and chemical properties of FDs are different from FOG primarily because of the saponification between long chain fatty acids (LCFAs) and calcium (He et al., 2011, 2013), which may lead to a different level of methane production during anaerobic co-digestion. Previous studies revealed that saponification between metals (e.g., calcium) and lipid-rich waste/wastewater resulted in improved anaerobic biodegradability and reduced inhibition by LCFAs (Ahn et al., 2006; Battimelli et al., 2010; Hatamoto et al., 2007; Roy et al., 1985; Salama et al., 2019a). Thus, it is possible that anaerobic co-digestion of WAS with FDs may generate higher methane production than with FOG. However, no research has been conducted on the biomethane potential of FDs in anaerobic co-digestion with WAS.

Studies evaluating microbial community structure (using molecular biological techniques) and linking it with digester performance during co-digestion with lipid-rich waste (e.g., FOG) have shown a significant impact on phyla Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Chloroflexi, and Euryarchaeota (Amha et al., 2017; Ferguson et al., 2018; Kurade et al., 2019; Yang et al., 2016). Syntrophic LCFA-degrading bacteria *Syntrophomonas*, which are in partnership with hydrogen-consuming methanogens, have been shown to be a key microbial group and significantly correlated with methane production during FOG co-digestion (Amha et al., 2017; Wang et al., 2020; Ziels et al., 2016). Nevertheless, no study has been performed to investigate the microbial communities and their functional networks in FD co-digestion, and little is known about the differences in FOG and FD co-digestion microbial communities. Comparing the community structures and their interactive roles in anaerobic co-digestion of FOG and FDs would provide insights into the differences between FOG and FD co-digestion and methane production.

The main goal of this study was to investigate the anaerobic co-digestion of WAS with FDs, including determining a suitable range of substrate to inoculum (S/I) ratios for optimal ultimate methane production, evaluating co-digestion performance parameters through kinetic modeling, and elucidating the microbial community structure and their interactive roles using 16S rRNA gene sequencing of Bacteria and Archaea communities. Comparisons of methane production, kinetics, and microbial community composition between FOG and FD co-digestion were conducted. The results of the research are expected to provide valuable fundamental information for the beneficial use of FDs for increasing energy production at water resource recovery facilities and supporting FOG pretreatment by saponification with calcium.

2. Materials and methods

2.1. Substrate and inoculum

The WAS used for this study was obtained from Qilidian Wastewater Treatment Plant (WWTP) (Guilin, China), that uses a conventional activated sludge process at a design flow of 100,000 m^3/day with a hydraulic retention time of 0.5 day. The FD samples were collected from a grease interceptor that receives kitchen wastewater from the cafeteria at Guilin University of Technology (Guilin, China). The infrared spectrum indicating the occurrence of saponification between LCFAs and calcium in the FD samples is shown in Fig. S1. The inoculum was anaerobic digester sludge collected from a mesophilic continuously stirred tank reactor in a countryside field (Guilin, China) treating pig manure. Previous studies have used similar sludge as inoculum for lipid degradation (e.g., Meng et al., 2017). After collection, all samples were quickly transported to the laboratory and stored at 4 °C for no more than 3 days before use. Blending oil (Jinlongyu Co. Ltd, Shanghai, China), a mixture of 8 types of oil including peanut oil, soybean oil, canola oil, sunflower seed oil, rice bran oil, corn oil, sesame oil and flaxseed oil, was used as a pure oil substrate to demonstrate the difference in methane production between FOG and FDs. The characteristics of the substrates and inocula used in this study are shown in Table 1.

2.2. Experimental setup

Two sets of BMP tests were conducted in this study. In the first set, FDs were used as the single substrate to determine the optimum ratio of substrate (FD) to inoculum (S/I) on a total volatile solid basis. Five different S/I ratios (Table 2) were tested to delineate an ideal range for satisfactory methane generation and maximum ultimate methane production per unit mass of FDs. Inoculum without FD addition was used as blank in this set of experiments. Within the feasible S/I ratio range from the first set of BMP tests, the second set of BMP tests used FDs and WAS as co-substrates to investigate enhanced methane production compared to WAS substrate digestion. Anaerobic co-digestion of WAS with FOG (blending oil) was also performed to determine the difference in methane production of FOG and FD co-digestion under the same optimized S/I ratio. Inoculum without any substrate addition was used as blank, and inoculum with WAS was used as control in the second set of experiments (Table 2).

The FDs used in this study were first pressed into a thin layer and then cut into small particles to a size around 1 × 1 × 1 mm. In the BMP experiments, the mixtures of inocula and substrates were transferred to 200-mL serum bottles with a working volume of 150 mL and all reactions were performed in triplicate. To maintain anaerobic conditions, the bottles were flushed with N_2 gas for 5 min prior to being sealed. All bottles were then incubated in a temperature-controlled shaker (ZD-85, Jinyi instrument Co. Ltd, Changzhou, China) at 37 °C with a mixing speed of 150 rpm. The volume of biogas in each bottle was measured periodically by releasing the pressure in the bottle using a 10 mL gas-tight plastic syringe, and the composition of biogas was immediately analyzed. Liquid samples within all bottles were collected at the end of incubation for measurements of pH, chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS) and volatile fatty acids (VFAs). Digestion tests were run until the daily biogas production of each bottle decreased below 10 mL.

2.3. Analytical methods

2.3.1. Chemical analysis

TS, VS, and alkalinity were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1999). The pH

Table 1
Inocula and substrate characteristics in the single-substrate and co-digestion experiments.

	Single-substrate digestion		Co-digestion			
	Inoculum	FDs	Inoculum	WAS	FDs	FOG
Density (g/mL)	0.93 ± 0.02	ND	1.02 ± 0.02	1.01 ± 0.01	ND	0.91 ± 0.03
pH	7.86 ± 0.04	ND	7.44 ± 0.01	7.02 ± 0.01	ND	ND
TS (g/L) or (mg/g)	47.4 ± 0.1	670.7 ± 15.1	61.2 ± 3.4	14.2 ± 0.3	662.0 ± 14.9	908.0 ± 31.0
VS (g/L) or (mg/g)	22.9 ± 0.5	655.3 ± 14.5	32.7 ± 1.7	9.8 ± 0.2	649.0 ± 15.2	907.8 ± 31.1
VS/TS (%)	48.3 ± 1.0	97.7 ± 0.1	53.4 ± 0.0	69.0 ± 0.4	98.0 ± 0.1	100.0 ± 0.0
COD (g/L) or (g/kg)	53.3 ± 2.7	ND	25.2 ± 4.0	12.7 ± 0.8	979.6 ± 51.5	2695.9 ± 135.0
Alkalinity (mg/L)	4277 ± 84	ND	4868 ± 139	325 ± 25	ND	ND

ND = not determined.

Table 2
Composition of inoculum and substrates in single-substrate and co-digestion experiment.

Single-substrate digestion				Co-digestion				
	Inoculum (mL)	FDs (g)	S/I ratio		Inoculum (mL)	WAS (mL)	FDs (g) or FOG (mL)	S/I ratio
Blank	20	0	0	Blank	20	0	0	0
FD-0.25	20	0.175	0.25	Control	20	5	0	0.075
FD-0.5	20	0.35	0.5	FDW-0.15	20	5	0.08	0.15
FD-1	20	0.7	1	FDW-0.25	20	5	0.18	0.25
FD-2	20	1.4	2	FDW-0.5	20	5	0.43	0.5
FD-4	20	2.8	4	FDW-1	20	5	0.93	1
				FDW-2	20	5	1.94	2
				FOGW-0.5	20	5	0.31	0.5

FD = FOG deposits, FDW = FOG deposits + WAS, FOGW = FOG + WAS.

was determined using a pH meter (IS128C, Shanghai Yimai, Shanghai, China). COD was analyzed using test kits (Ultra High Range, HACH, Loveland, CO, USA). Biogas production was recorded and normalized to STP conditions based on the local climatological data. The composition of biogas was analyzed using a gas chromatograph (GC126N, INESA, Shanghai, China) equipped with a thermal conductivity detector and a 2m*3 mm TDX-01 column (INESA, Shanghai, China). The volatile fatty acids (VFAs) were determined using the direct injection method as previously described (Mu et al., 2018). The composition of VFAs was characterized by GC-FID (6890B, Agilent Technologies, Palo Alto, CA, USA) using helium as carrier gas and a capillary column (DB-FFAP, 30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Palo Alto, CA, USA).

2.3.2. Kinetic analysis

A modified Gompertz model (Eq. (1)) (Li et al., 2011) was adopted to elucidate the kinetics of methane production during the anaerobic co-digestion experiment, where $B_{(t)}$ is the specific methane yield at a given time (mL/gVS_{added}); B_0 is the maximum methane potential (mL/gVS_{added}); t is the digestion time since the start of BMP tests (d); R_m is the maximum daily methane production rate (mL/gVS_{added}·d); λ is the lag-phase (d); e is 2.718.

$$B_{(t)} = B_0 exp\left\{ - exp\left[\frac{R_m e}{B_0}(\lambda - t) + 1\right]\right\}, \quad t \geq 0 \tag{1}$$

2.3.3. Microbial analysis

Mixed liquor samples from the anaerobic co-digestion experiments, including the initial inoculum, WAS, and the final solutions of the blank (inoculum only, at the end of incubation), FDW-0.25 (FDs + WAS, S/I = 0.25), FDW-0.5 (FDs + WAS, S/I = 0.5), and FOGW-0.5 (FOG + WAS, S/I = 0.5) were collected for microbial analysis. The samples were pelleted by centrifugation at 10,000×g for 10min. Biomass pellets were subjected to DNA extraction using CTAB method (Wirth et al., 2012). Ion torrent sequencing was conducted for the samples at the Sequencing Services Facility at the Novogene Co., Ltd (Beijing, China). Briefly, PCR amplification was performed using the primer set 341F/806R for Bacteria (Sundberg et al., 2013) and the primer set 519F/915R for Archaea (Fan and Xing, 2016). Amplicons of the PCR reactions were purified, pooled, and then sequenced using Ion S5^{TM}XL (Thermo Fisher Scientific, United State). Sequences were submitted to the NCBI Sequence Read Archive as BioProject PRJNA559039.

Raw reads of all samples were trimmed to remove primer sequences, and quality filtered using Cutadapt v1.9.1 (Martin, 2015). Chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011). The UPARSE pipeline (Edgar, 2013) was used to cluster sequences into operational taxonomic units (OTUs) based on 3% divergence. Taxonomy assignment to each cluster representative generated from OTU clustering was performed using Mothur (Schloss et al., 2009) based on the SILVA SSU Ref database (Pruesse et al., 2007). The compositional differences between microbiomes were analyzed by principal coordinate analysis (PCoA) using R Phyloseq package (McMurdie and Holmes, 2013) based on weighted and unweighted UniFrac distances (Lozupone and Knight, 2005).

2.4. Statistical analysis

The general linear model was used to compare the methane production values from different anaerobic co-digestion treatments. R was used to fit the models and calculate the statistical significance between the treatments. Pearson's correlation test was used to evaluate correlations among the different parameters. Differences between treatments were considered significant at a confidence level $p < 0.05$.

3. Results and discussion

3.1. Methane production in anaerobic digestion/co-digestion of FDs

3.1.1. FDs as single substrate: determining a suitable S/I ratio range

Since no previous studies have looked at anaerobic digestion of FDs, single substrate experiments were used to estimate a suitable S/I ratio range for the subsequent co-digestion experiments (Table 2). Different cumulative methane production values were obtained under five different S/I ratios (Fig. 1a). After 52 days of incubation, all digestions except for FD-4 (S/I = 4), produced higher cumulative methane volumes than the blank. The highest cumulative methane production (845.3 ± 33.2mL/gVS_{added}) was obtained from FD-0.5 (S/I = 0.5); relatively lower methane production values were observed in the FD-0.25 (S/I = 0.25) and FD-1 (S/I = 1) digestions, and much lower methane production was found in FD-2 (S/I = 2). The correlations of S/I ratio to ultimate methane production volume and ultimate methane percentage in biogas (Fig. 1b) showed that as the S/I ratio increased from 0.25 to 0.5, the ultimate methane production volume increased slightly and then decreased drastically as S/I increased from 0.5 to 4. On the other hand, the average methane percentage decreased slowly from 82% to 57% as S/I increased. The highest cumulative methane volume and percentage from anaerobic digestion of FDs appear to be at an S/I ratio between 0.25 and 1.0 (ideal S/I = 0.5). Compared to the reported optimal ranges of S/I for FOG digestion (between 0.25 and 0.75; ideal S/I = 0.5 (Li et al., 2011), or between 0.5 and 1 (Nazaitulshila et al., 2015)), the S/I range that results in high methane production and percentage in FD digestion is wider. This may be due to the compositional difference between FD and FOG, which results in different levels of methane production (see also below). A possible difference is the presence of calcium in FD, which is supported by previous finding that calcium could play a role in reducing LCFA inhibition when inoculum concentration was above a threshold level (Ma et al., 2015).

3.1.2. FDs and WAS as co-substrates

Based on the single-substrate study results, five S/I ratios between 0.15 and 2 were tested for the anaerobic co-digestion of WAS with FDs (Table 2). The S/I ratio was increased by the addition of increasing levels of FDs to a fixed amount of WAS (WAS as VS (w/w) decreased from 50% to 7.7%). The level of WAS/inoculum (gVS/gVS = 0.075), is comparable to the WAS/inoculum (gVS/gVS) in other studies (0.07 in Li et al., 2011;

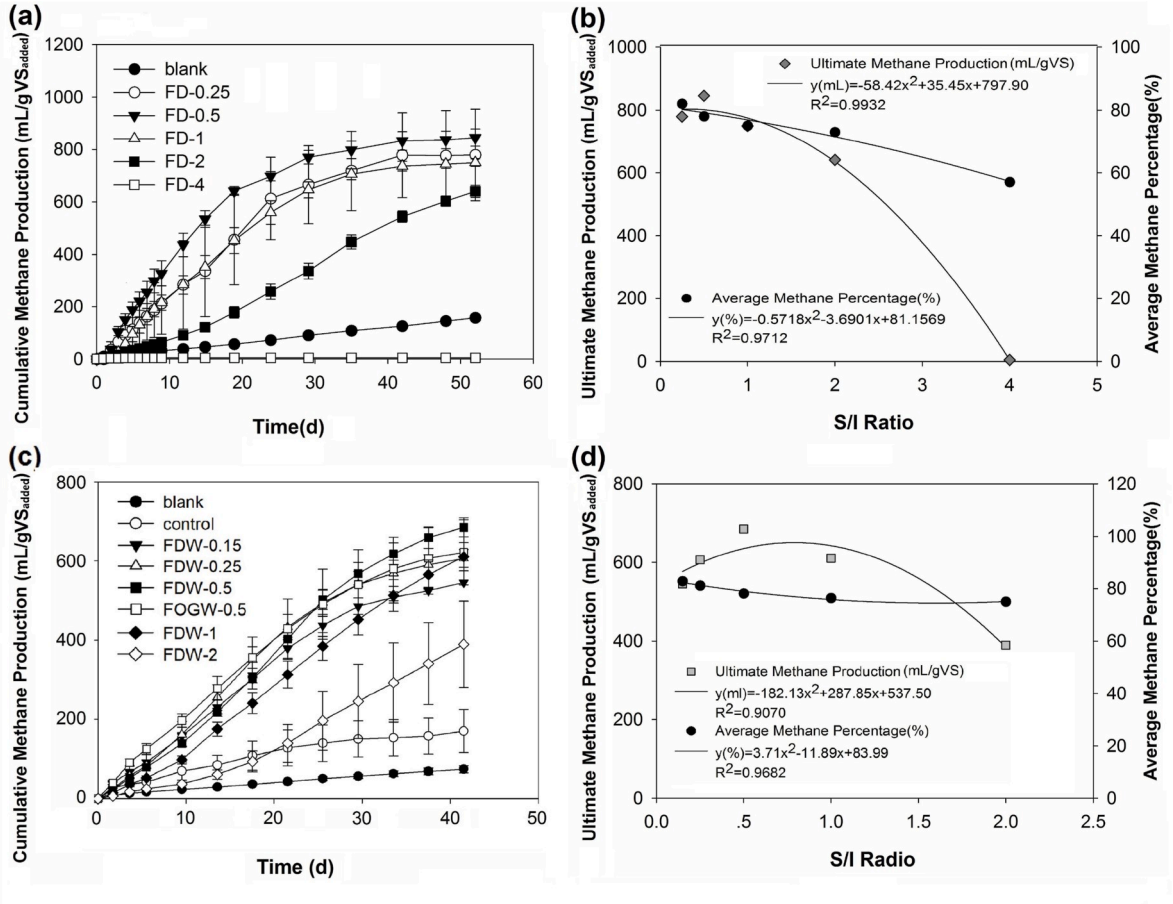

Fig. 1. Cumulative methane production from single-substrate digestion of FDs (a), from co-digestion of WAS with FDs (c); correlation of S/I ratios, ultimate methane production, and average methane percentage of biogas in single-substrate digestion of FDs (b) and co-digestion of WAS with FDs (d).

0.043 in Kabouris et al., 2009; 0.118 in Wang et al., 2013). Compared to the control (with WAS as only substrate), higher cumulative methane production values were found in all co-digestion treatments after 42 days of incubation (Fig. 1c). When the added FDs were increased from 0.08g to 0.43g as the FDs/total substrates (gVS/gVS) increased from 50% to 85%, the ultimate cumulative methane production increased from 545.6 ± 6.0 mL/gVS$_{added}$ to 685.7 ± 24.1 mL/gVS$_{added}$ (3.2 times and 4.0 times higher than the cumulative methane produced by WAS in the control, respectively), showing that addition of FDs could significantly enhance methane production (*t*-test, $p < 0.001$). Moreover, the conversion efficiency, determined by the ratio of measured ultimate methane production over theoretical maximum methane production (Nielfa et al., 2015), was increased from 28.7% (in control) to 78.9% (in FDW-0.5) (Table S1). At FDs addition higher than 0.43g (FDW-1 and FDW-2), the cumulative methane production levels per g VS were lower than in FDW-0.5, at conversion efficiencies of 86.4% and 64.6% in FDW-1 and FDW-2, respectively. Nevertheless, except for FDW-2, no accumulation of VFAs was observed in all reaction bottles (data not shown) and the pH in all co-digestions was in the range of 7.0–7.2 at the end of the incubation period.

The correlations between S/I ratio and ultimate methane production volume as well as ultimate methane percentage in biogas (Fig. 1d), show an acceptable S/I ratio between 0.25 and 1.2, which is a wider range than the optimal range reported for FOG co-digestion (Li et al., 2011). The ideal S/I ratio for FD was around 0.5, which is very close to the ideal S/I ratio of 0.46 reported for FOG (Li et al., 2011). A regression analysis of experimental data showed that the cumulative methane production fit well with the modified Gompertz model (Eq. (1)) with an R^2 value of 0.99 (Table 3). The regression analysis confirmed that the lag phase (λ) time was relatively shorter and the maximum daily methane production rate (R_m) was relatively higher for each tested S/I ratio within the optimal range of FD co-digestion. Taken together, these results indicate the importance of the S/I ratio (similar to F/M in wastewater treatment) in anaerobic co-digestion of FD and WAS, which would be useful in kinetic based mass balance equations that provide insights on appropriate design loading rates at pilot- or full-scale.

3.2. Comparison of FDs with FOG in anaerobic co-digestion

Considering the overlap of the optimal S/I ratio ranges for FDs and FOG, an S/I ratio of 0.5 was chosen and the same amounts of WAS and FDs/FOG (w, gVS) were used to investigate the difference between FDs and FOG in anaerobic co-digestions. The daily methane production in FDW-0.5 and FOGW-0.5 during 42 days of incubation (Fig. 2a) show two peaks of daily methane production in both FDW-0.5 and FOGW-0.5. In FOGW-0.5 the first peak was higher than the second peak while the reverse was observed in FDW-0.5. At the first peak on Day 4, the daily methane production in FOGW-0.5 was almost two times higher than that in FDW-0.5, which may be due to a higher COD loading of FOG, as the COD loading of FOG (1175.8 mgCOD$_{FOG}$/gVS$_{biomass}$, Table S2) was almost two times higher than that of FDs (597.7 mgCOD$_{FD}$/gVS$_{biomass}$, Table S2). The kinetic analysis showing a shorter lag phase in FOGW-0.5 than in FDW-0.5 (Table 3) also indicate a faster mass transfer of FOG to microorganisms that could rapidly utilize the substrate for growth. A drastic drop in daily methane production was observed in FOGW-0.5 after Day 4, while daily methane production in FDW-0.5 leveled off until Day 10. It is likely that the biodegradation of LCFAs was slower than FOG hydrolysis on Day 4, leading to a buildup of LCFAs that could

Table 3
Kinetic parameters estimated from the modified Gompertz model applied to co-digestion.

Parameters	Control	FDW-0.15	FDW-0.25	FDW-0.5	FDW-1	FDW-2	FOGW-0.5
Measured B (mL/gVS_{added})	170.4	545.6	606.7	685.7	610.7	389.8	622.2
Estimated B_0 (mL/gVS_{added})	172.6	583.9	635.5	783.2	750.1	661.7	667.2
λ (d)	0.0	1.9	3.0	4.3	5.3	10.8	1.0
R_m ($mL/gVS_{added}.d$)	6.1	20.1	24.6	23.9	19.3	13.0	22.2
R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99

B_0 = ultimate cumulative methane production; λ = lag phase time; R_m = maximum methane production rate.

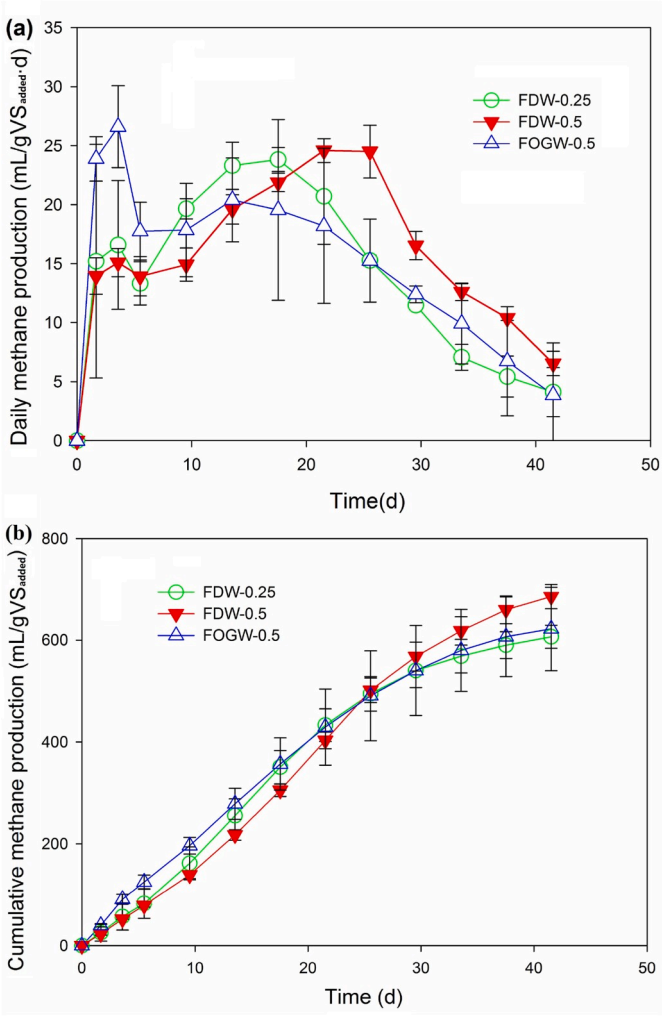

Fig. 2. Comparison of FDs with FOG in anaerobic co-digestion over experimental period: a) the change of daily methane production in FDW-0.25, FDW-0.5 and FOGW-0.5; b) cumulative methane production in FDW-0.25, FDW-0.5 and FOGW-0.5.

limit the reactions of syntrophic *β*-oxidizing bacteria and inhibit methanogenesis (Kurade et al., 2019; Sousa et al., 2009; Ziels et al., 2017, 2016), thereby reducing the methane production in FOGW-0.5. On the other hand, accumulation of LCFAs in FDW-0.5 may have been lower such that methanogenesis was less affected by LCFA inhibition.

Though the methanogens recovered from earlier inhibition and showed an enhanced methane production after Day 10 in FOGW-0.5, the daily methane production of the second peak was lower than that of the first peak (Fig. 2a). However, in FD co-digestions, the daily methane production of FDW-0.5 continuously increased, exceeded the daily methane production of FOGW-0.5 on Day 15, and achieved its maximum level on Day 21. Moreover, the cumulative methane production became higher in FDW-0.5 than in FOGW-0.5 by Day 26 (Fig. 2b), and the ultimate methane production of FDW-0.5 was 10.2% higher than that of FOGW-0.5 at the end of incubation despite the similar concentrations and profiles of LCFAs in both FDW-0.5 and FOGW-0.5 (both liquid and solid phases) (Table S3). The conversion efficiency (based on methane produced) of FDW-0.5 (78.9%) was also higher than that of FOGW-0.5 (51.7%). There was a significant difference ($p = 0.001$) in cumulative methane production between FDW-0.5 and FOGW-0.5 after 42 days of incubation. Based on the analysis of maximum methane potential (B_0) of FDW-0.5 and FOGW-0.5 (Table 3), a bigger difference (17.4%) in ultimate methane production could be expected if a longer incubation time was allowed. It is worth noting that the cumulative methane production between FDW-0.25 and FOGW-0.5 (Fig. 2b) was not significantly different ($p = 0.32$) during the 42 days of incubation. This shows that using a lower amount (w, gVS) of the FDs (when the COD loading of FDs (246.5 $mgCOD_{FD}/gVS_{biomass}$) was almost one quarter of that of FOG) can result in the same cumulative methane production. Analysis of daily methane production, kinetic characteristics, and cumulative methane production showed that compared to FOG, FDs could be a better option in terms of reduced substrate addition and enhanced methane production in anaerobic co-digestion with WAS.

The increase in methane production due to the addition of FOG containing co-substrates may vary drastically depending on the source of sludge, the % FOG load, temperature, reactor configuration, solid retention time (SRT), and other variables. Comparison of the maximum methane yield enhancement with other studies (Table 4) shows that, with similar or even lower methane yield of WAS during co-digestion, the 220% increase in methane yield in FDW-0.15 (50% FD load, w/w as VS) is higher than the 67–182% increase reported for 46–60% FOG or grease trap waste load (w/w as VS) (Davidsson et al., 2008; Kabouris et al., 2009; Luostarinen et al., 2009; Yalcinkaya and Malina, 2015), and even higher than for 70% FOG load (w/w as VS) under hyper-thermophilic/thermophilic co-digestion (145% increase) (Alqarallah et al., 2016). At the higher FD load in this study (85% as VS, FDW-0.5), the enhanced methane yield (302% increase) is slightly lower than that of grease interceptor waste load (65% as VS, 318% increase) (Wang et al., 2013), although the COD loading of FDW-0.5 (597.7 $mgCOD/gVS_{biomass}$) is much lower than that used by Wang et al. (2013) (~900 $mgCOD/gVS_{biomass}$). These comparisons suggest that FDs could be a better option than FOG in terms of methane yield enhancement during anaerobic co-digestion. The amount of FDs in sewer systems around the world recovered annually has not been accurately estimated, but the pretreatment of FOG with calcium to form FDs may lead to a significantly increased methane yield at water resource recovery facilities with existing anaerobic digesters. This is supported by a recent study that showed that addition of 0.5% calcium to FOG resulted in a 6-fold increase in biomethane production during anaerobic co-digestion of FOG with sludge (Salama et al., 2019b).

There are two possible explanations for the higher methane yield of FDs compared to FOG. The first concerns the bond between calcium and LCFAs in FDs, which may prevent LCFAs from being released quickly and inhibiting the methanogens. This is supported by the observation that no drastic drop in daily methane production was observed in FDW-0.5 and FDW-0.25 (Fig. 2a). The other possible explanation is the aggregation of microorganisms on the surface of FDs, which may enable a more efficient arrangement of diverse microorganisms leading to faster

Table 4
Comparison of the maximum methane yield enhancement.

	Source of sludge	Source of FOG/FD	Methane production from sludge	Maximum methane production	FOG/FD load at max methane production	% increase[a]	Reactor configuration	Temp.[b]	SRT
FDW-0.15 (present study)	Thickened WAS from WWTP in Guilin, China	FDs from a grease interceptor	170.4 mL/ gVS_{added}	545.6 mL/ gVS_{added}	50% VS	220	Batch BMP tests	M	–
Kabouris et al. (2009)	Primary sludge + thickened WAS from WWTP in Pinellas county, FL	Polymer dewatered FOG	159 mL/ gVS_{added}	449 mL/ gVS_{added}	48% VS	182	CSTR, 4L, semi-continuous feeding	M	12 days
Kabouris et al. (2009)	Primary sludge + thickened WAS from WWTP in Pinellas county, FL	Polymer dewatered FOG	197 mL/ gVS_{added}	512 mL/ gVS_{added}	48% VS	160	CSTR, 4L, semi-continuous feeding	T	12 days
Luostarinen et al. (2009)	Sewage sludge from a WWTP in Mikkeli, Finland	Grease trap sludge from a meat processing plant	278 mL/ gVS_{added}	463 mL/ gVS_{added}	46% VS	67	CSTR, 5L, fed once a day	M	16 days
Yalcinkaya and Malina, 2015	Municipal wastewater sludge from WWTP in Austin, TX	Un-dewatered grease trap waste	384 mL/ gVS_{added}	641 mL/ gVS_{added}	46% VS	67	CSTR, 6L, semi-continuous feeding	M	15 days
Davidsson et al. (2008)	Primary sludge + WAS from WWTP in Malmo, Sweden	Grease trap sludge	325 mL/ gVS_{added}	681 mL/ gVS_{added}	60% VS	110	Batch BMP tests	M	–
Wang et al. (2013)	Thickened WAS from WWTP in Durham, NC	Grease interceptor waste	180 mL/ gVS_{added}	752 mL/ gVS_{added}	65% VS	318	CSTR, 8L, semi-continuous feeding	M	20 days
Alqaralleh et al. (2018)	Thickened WAS from WWTP in Gloucester, Canada	FOG from organic resources management Inc.	235.4 mL/ gVS_{added}	576.5 mL/ gVS_{added}	70% VS	145	Dual stage CSTR, 2.5L, semi-continuous feeding	Hyper T/T	15 days
FDW-0.5 (present study)	Thickened WAS from WWTP in Guilin, China	FDs from a grease interceptor	170.4 mL/ gVS_{added}	685.7 mL/ gVS_{added}	85% VS	302	Batch BMP tests	M	–

[a] % increase = (methane production with FOG or FD addition)/(methane production with sludge)*100%.
[b] M, mesophilic (~35 °C); T, thermophilic (~55 °C).

mass and electron transfer that accelerate the biodegradation of LCFAs to generate methane. This may be responsible for the faster and longer increase in daily methane production in FDW-0.25 and FDW-0.5 than in FOGW-0.5 after Day 6 (Fig. 2a). The higher cumulative methane production in FDW-0.5 compared to that in FOGW-0.5 is in agreement with the results of previous studies showing that the addition of calcium to FOG could prevent LCFAs from upsetting an anaerobic digestion system (Ahn et al., 2006; Hatamoto et al., 2007; Roy et al., 1985; Salama et al., 2019b).

3.3. Microbial community composition during anaerobic co-digestion

A total of 623,448 quality-filtered and chimera-free sequences were generated for 18 samples, with an average of 2518 OTUs per sample.

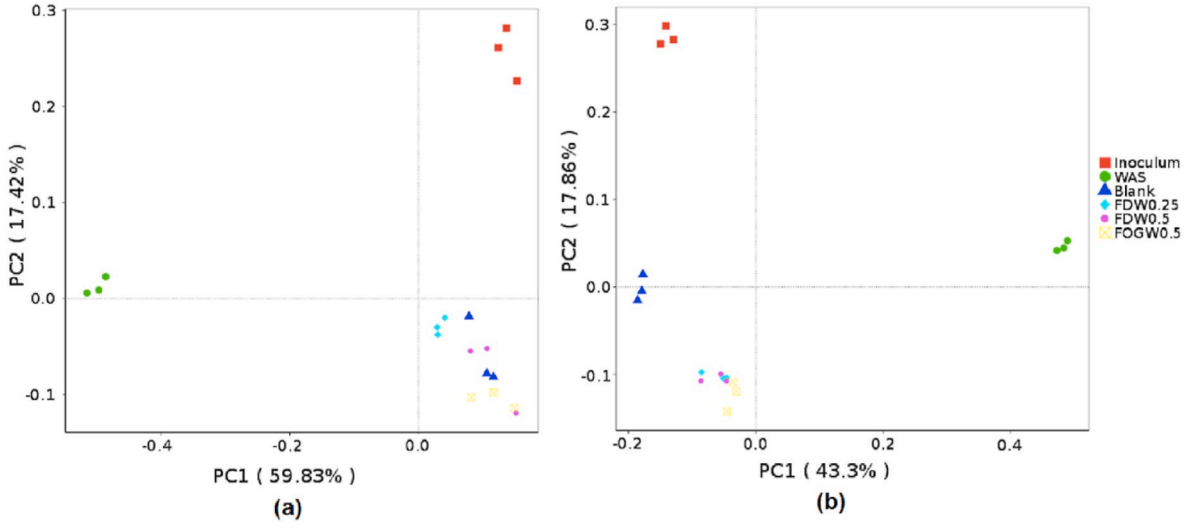

Fig. 3. Bacterial community dissimilarity of inoculum, WAS, and biomass samples from co-digestions applying principal coordinates analysis (PCoA) based on weighted (a) and unweighted (b) UniFrac distances. Inoculum and WAS were samples collected before anaerobic co-digestion. Blank (inoculum after incubation), FDW-0.25, FDW-0.5, and FOGW-0.5 were all collected at the end of incubation. Triplicates are shown in the same color. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

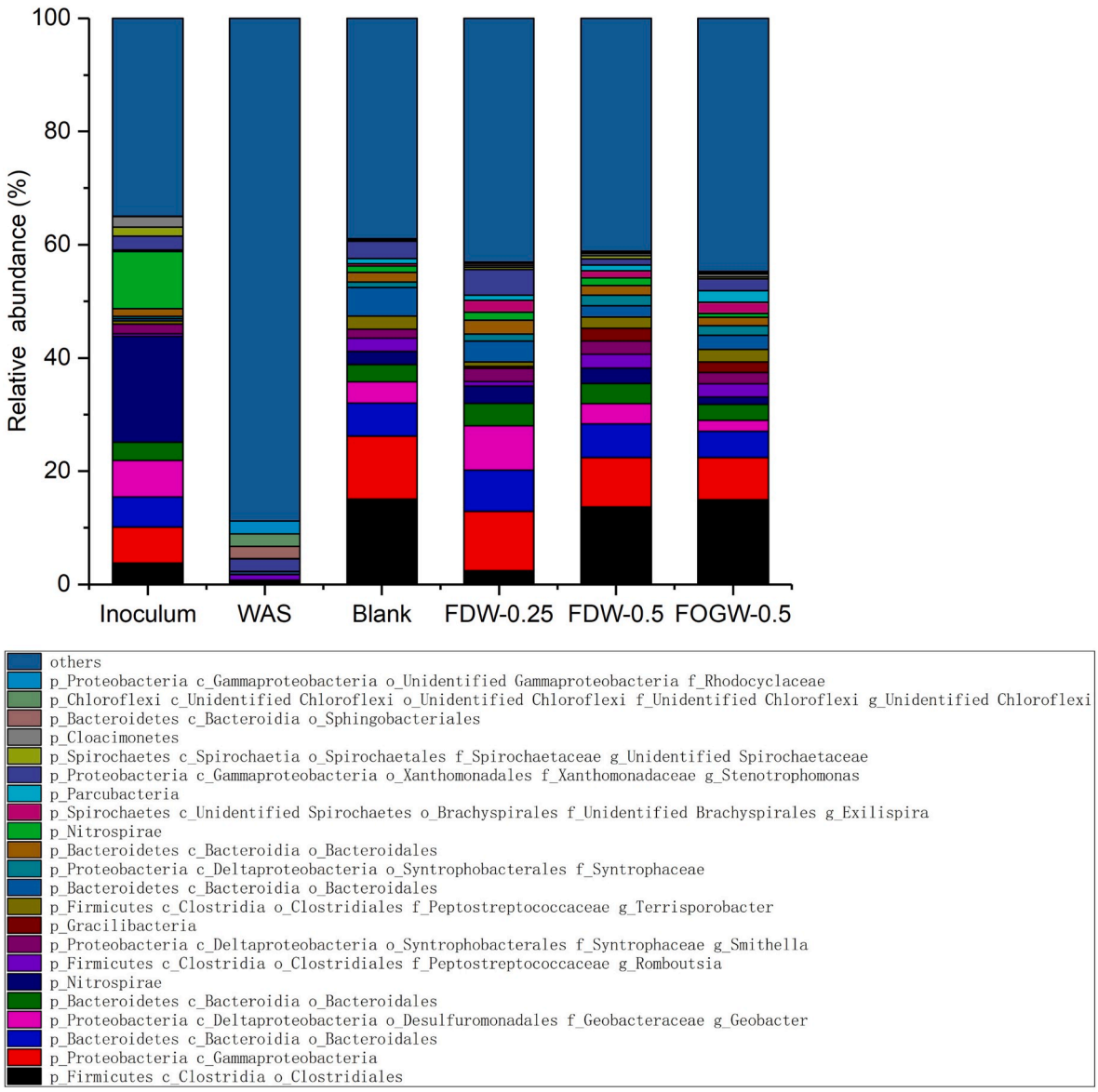

Fig. 4. Bacterial community structures of inoculum, WAS, and biomass samples collected from the blank, FDW-0.25, FDW-0.5 and FOGW-0.5 at the end of experimental period.

Overall dissimilarities of the bacterial communities are shown in principal coordinate analysis (Fig. 3), and show high reproducibility in triplicate samples. Apart from the inoculum and WAS, bacterial communities of the blank (inoculum only, at the end of incubation), FDW-0.25, FDW-0.5, and FOGW-0.5 were clustered together (Fig. 3a), showing a not significant dissimilarity among them based on the quantitative measure of β diversity using weighed UniFrac. Based on the qualitative β diversity measure, high similarity was found among the communities of FDW-0.25, FDW-0.5, and FOGW-0.5, while the community of the blank was significantly different (Fig. 3b), suggesting that the addition of WAS was the cause of the dissimilarity of bacterial communities in the blank and the FOG/FD co-digestions. The results (Fig. 3a and b) indicate that the addition of co-substrates did not lead to a significant change in the bacterial community after 42 days of co-digestion, though new species were introduced by the addition of WAS.

Twenty of the most abundant OTUs, with relative abundance ≥1.5% in all tested samples, were detected in bacterial community analysis (Fig. 4). At the phylum level, Proteobacteria, Firmicutes and Bacteroidetes were dominant in the blank, FDW-0.25, FDW-0.5 and FOGW-0.5, while the inoculum was dominated by Nitrospirae, Proteobacteria and Bacteroidetes, and the WAS was dominated by Bacteroidetes, Proteobacteria and Chloroflexi. Firmicutes were at a higher relative abundance in FOGW-0.5 (19.4%) and FDW-0.5 (18.2%) than in FDW-0.25 (4.0%). The most abundant OTU in FOGW-0.5 and FDW-0.5 was assigned to order Clostridiales belonging to Firmicutes, which have been found widely in anaerobic digesters, involved in diverse fermentation pathways (Wirth et al., 2012), and contributing to the anaerobic biodegradation of LCFAs (Ma et al., 2015; Sousa et al., 2009). *Romboustsia* and *Terrisporobacter*, as the identified genera in Firmicutes that may participate in converting short-chain fatty acids to acetate (Gerritsen et al., 2014; McIlroy et al., 2017), were also at a higher abundance in FOGW-0.5 and FDW-0.5 than in FDW-0.25. Bacteroidetes were enriched the most in FDW-0.25 (17.3%), followed by FDW-0.5 (13.2%), and FOGW-0.5 (11.5%), which can synthesize various lytic enzymes to degrade many types of organic compounds and produce acetate under anaerobic conditions (Kurade et al., 2019). Proteobacteria, which include fermenting species (e.g., Gammaproteobacteria), syntrophic species (e.g., *Syntrophaceae*) and exoelectrogenic species (e.g., *Geobacter*), were highly enriched in FDW-0.25 (26.5%) and FDW-0.5 (19.0%). Members in class Gammaproteobacteria can degrade

complex organic matter and utilize several VFAs (Kurade et al., 2019; Yang et al., 2014) and species in *Stenotrophomonas* are responsible for the hydrolysis and fermentation of organic compounds (Ma et al., 2015). Although a low abundance of fermenters affiliated with Firmicutes was found in FDW-0.25, the fermentation of LCFAs may not have been impeded due to the higher relative abundance of OTUs assigned to order Bacteroidales affiliated with Bacteroidetes, class Gammaproteobacteria and *Stenotrophomonas*.

The conversion of LCFAs to methane involves a syntrophic partnership between acetogenic β-oxidizing bacteria and methanogenic archaea (Sousa et al., 2009; Ziels et al., 2017). All of the isolated bacterial species known to β-oxidize LCFAs syntrophically belong to two families of *Syntrophomonadaceae* and *Syntrophaceae* (Sousa et al., 2009; Ziels et al., 2017). In this study, only two OTUs assigned to the family *Syntrophaceae* were detected with a relative abundance greater than 1.5%. However, the total relative abundances of OTUs within the family *Syntrophomonadaceae* were 0.15%, 0.52%, 2.34% and 3.23% in the blank, FDW-0.25, FDW-0.5 and FOGW-0.5, respectively. For the typical β-oxidizing genus *Syntrophomonas* belonging to *Syntrophomonadaceae*, the total relative abundances were 0.12%, 0.37%, 2.12%, and 1.97% in the blank,

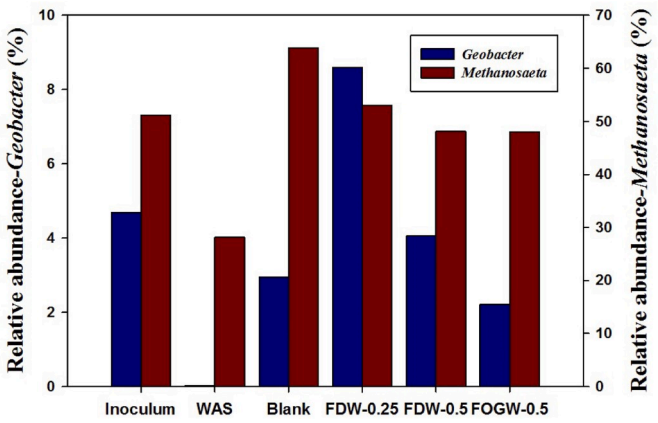

Fig. 6. Relative abundance of *Geobacter* in bacterial community and *Methanosaeta* in archaeal community in inoculum, WAS, blank, FDW-0.25, FDW-0.5, and FOGW-0.5.

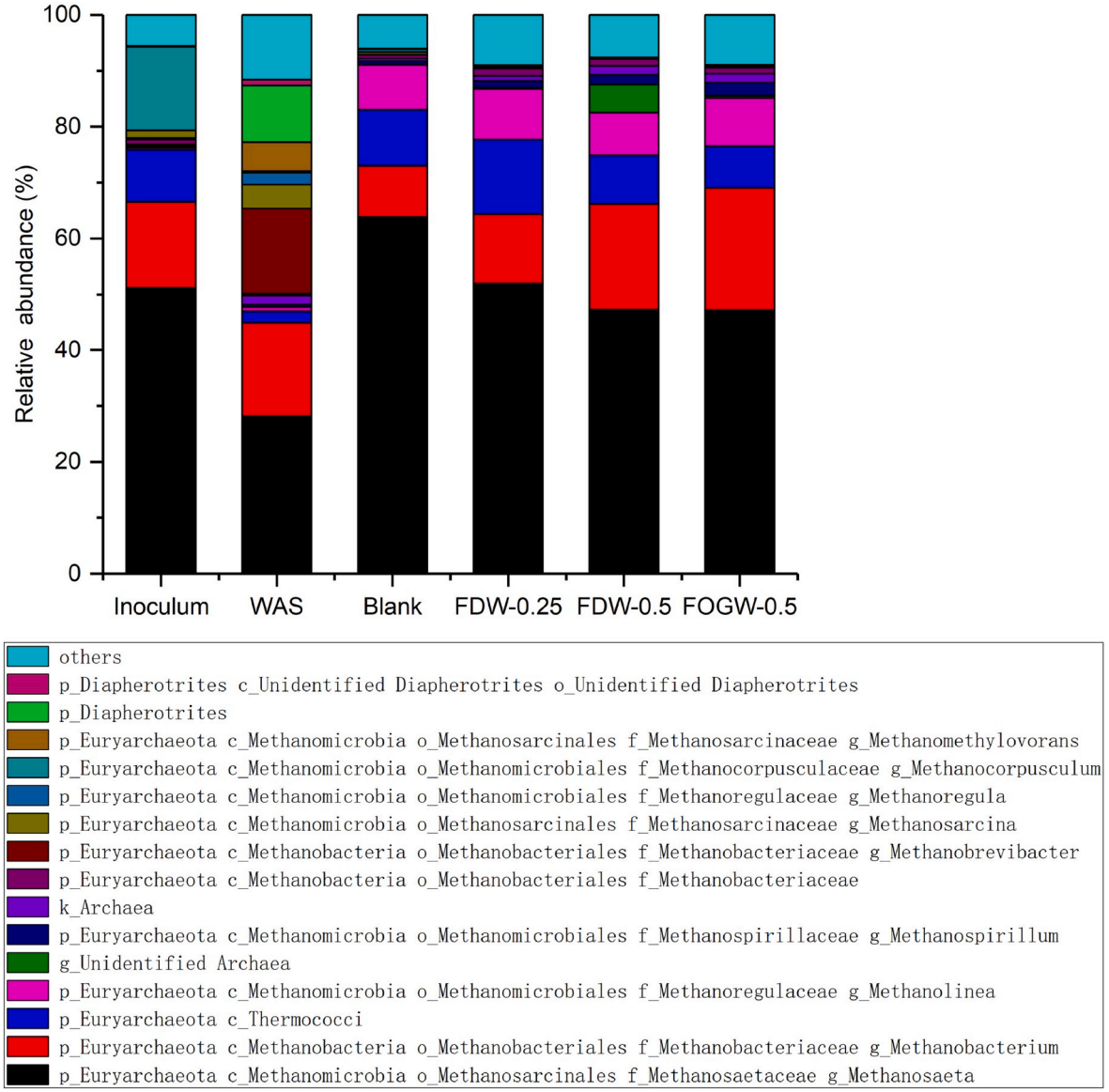

Fig. 5. Archaeal community structures of inoculum, WAS, and biomass samples collected from the blank, FDW-0.25, FDW-0.5 and FOGW-0.5 at the end of experimental period.

FDW-0.25, FDW-0.5 and FOGW-0.5, respectively. Significantly higher relative abundances of *Syntrophomonas* were observed in FDW-0.5 and FOGW-0.5 than in the blank. Though the relative abundance of *Syntrophomonas* in FDW-0.25 was higher than that in the blank, it was significantly lower than that in FDW-0.5, which may be due to the 50% lower initial loading of FDs (gVS) and a lower amount of FDs left in FDW-0.25 than in FDW-0.5 at the end of incubation. This suggests a positive correlation between the amount of FDs and the growth of *Syntrophomonas*. With respect to *Syntrophaceae*, the scenario is different. The total relative abundances of OTUs affiliated with the family *Syntrophaceae* were 6.57%, 7.17%, 6.78%, and 5.85% in the blank, FDW-0.25, FDW-0.5 and FOGW-0.5, respectively. For the typical β-oxidizing genus *Syntrophus*, the total relative abundances were 0.32%, 0.27%, 0.32% and 0.31% in the blank, FDW-0.25, FDW-0.5 and FOGW-0.5, respectively. No significant difference was observed between the blank and co-digestions in terms of the relative abundance of *Syntrophus/Syntrophaceae*. These results are consistent with the previous observation that FOG co-digestion resulted in significant growth of *Syntrophomonas* but limited change in levels of *Syntrophus* (Ziels et al., 2016).

Geobacter, a participant in direct interspecies electron transfer (DIET), was highly enriched in FDW-0.25, followed by FDW-0.5 and FOGW-0.5. DIET as an alternative to interspecies H_2/formate transfer in anaerobic digestion has been suggested by the finding that *Methanosaeta* can make direct electrical connections with *Geobacter*, accepting electrons to reduce carbon dioxide for methane production (Lovley, 2017; Rotaru et al., 2014). The possible occurrence of DIET in FDW-0.25, FDW-0.5 and FOGW-0.5 is supported by three observations: 1) The relative abundance of *Methanosaeta* was significantly higher than that of other methanogens in the archaeal community analysis (Fig. 5); 2) The relative abundance of *Geobacter* was positively correlated with that of *Methanosaeta* (Pearson's coefficient = 0.97; Fig. 6); 3) A higher relative abundance of *Geobacter* and *Methanosaeta* in the microbial community resulted in higher percentage of methane in the biogas as the average methane percentages of biogas were 81%, 78% and 76% in FDW-0.25, FDW-0.5 and FOGW-0.5, respectively. In addition to *Methanosaeta*, hydrogenotrophic *Methanobacterium*, *Methanolinea*, and *Methanospirillum* were found abundantly in FDW-0.25 (22.6%), FDW-0.5 (28.3%) and FOGW-0.5 (32.8%) (Fig. 5), indicating the occurrence of H_2-consuming methanogenesis in FOG/FD co-digestions. Nevertheless, the lower abundance of hydrogenotrophic methanogens in FDW-0.5 than in FOGW-0.5 suggested that the enhanced methane production in FDW-0.5 might not be all due to H_2-consuming methanogenesis. In addition, *Methanobacterium* and *Methanospirillum*, as the syntrophic partners with *Syntrophomonas* species for LCFA degradation (Sousa et al., 2009), were slightly lower in FDW-0.5 (20.6%) than in FOGW-0.5 (24.3%), suggesting that the partnership between syntrophic bacteria and hydrogenotrophic archaea for LCFA degradation might not be the major reason for the enhanced methane production in FDW-0.5 compared to FOGW-0.5. Comparing FDW-0.25 and FOGW-0.5 also results in the same conclusion, that hydrogenotrophic methanogenesis and the partnership between syntrophic bacteria and hydrogenotrophic archaea for LCFA degradation do not fully explain the similar cumulative methane production. It is possible that the higher relative abundance of *Geobacter* led to a higher level of DIET, explaining the higher methane production in FDW-0.5 than in FOGW-0.5. DIET may also explain why there was a similar methane production in FDW-0.25 and FOGW-0.5, even though the COD loading was lower in FDW-0.25. This is in line with a previous finding that the bioaugmentation of *Geobacter* species accelerated methane production significantly in a system with acetate as substrate and *Methanosaetaceae* as dominant archaea (Zhang et al., 2018). Without conductive materials, the growth of mixed-species cellular aggregates promoted DIET in upflow anaerobic sludge blanket digesters (Lovley, 2017; Morita et al., 2011; Rotaru et al., 2014; Shrestha et al., 2014); this raises the possibility that the aggregation of microorganisms on FDs enhanced electron transfer through electrically conductive pili of *Geobacter* in the aggregates. These lines of evidence are indirect and preliminary, and the possible role of DIET in these systems need to be studied further. Additional research on the role of *Geobacter* in FD and FOG co-digestion is needed to understand the possible significance and impact of DIET. The limitations of DNA-based analysis in inferring function from phylogeny are well known and the results should be interpreted with caution. Nevertheless, the results provide an emerging picture of the advantages of, and possible mechanisms explaining, FD co-digestion leading to enhanced methane production rate and ultimate methane production.

4. Conclusions

Substantial enhancement of methane production was achieved by anaerobic co-digestion of WAS with FDs at an optimal S/I ratio range between 0.25 and 1.2, which is a wider range than that reported for FOG co-digestion. Under the same organic loading (gVS, S/I = 0.5) when the COD loading of FOG was 2 times higher than that of FDs, different patterns of daily methane production were observed and significantly higher cumulative methane production (10.2%) was obtained in FD co-digestion than in FOG co-digestion at the end of 42 days of incubation. When the organic loading (gVS) in FD co-digestion was reduced to half (S/I = 0.25) of that in FOG co-digestion (S/I = 0.5), no significant difference in ultimate methane production was observed after 42 days of incubation. Although the dissimilarity of bacterial communities of the biomass samples collected from FD co-digestion and FOG co-digestion was not significant, the relative abundance of *Geobacter* species was significantly higher in FD co-digestion, indicating the possible role of DIET in FD co-digestion. It is possible that the bond between calcium and LCFAs in FDs prevented LCFAs from releasing and causing inhibition of the methanogens, resulting in a wider loading range possible for FDs than for FOG. Further investigation on the nature and mechanisms of the release of LCFAs from FDs and the aggregation of microorganisms and possible DIET on FDs is needed to understand FD co-digestion, and provide insights on FOG pretreatment with calcium addition to improve anaerobic co-digestion.

Declaration of competing interest

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jiahou Hao: Investigation, Formal analysis, Data curation, Software, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.110708.

References

Ahn, J.H., Do, T.H., Kim, S.D., Hwang, S., 2006. The effect of calcium on the anaerobic digestion treating swine wastewater. Biochem. Eng. J. 30, 33–38. https://doi.org/10.1016/j.bej.2006.01.014.

Alqaralleh, R.M., Kennedy, K., Delatolla, R., Sartaj, M., 2016. Thermophilic and hyperthermophilic co-digestion of waste activated sludge and fat , oil and grease:

evaluating and modeling methane production. J. Environ. Manag. 183, 551–561. https://doi.org/10.1016/j.jenvman.2016.09.003.
Amha, Y.M., Sinha, P., Lagman, J., Gregori, M., Smith, A.L., 2017. Elucidating microbial community adaptation to anaerobic co-digestion of fats , oils , and grease and food waste. Water Res. 123, 277–289. https://doi.org/10.1016/j.watres.2017.06.065.
APHA, 1999. Standard Methods for Examination of Water and Wastewater. American Public Health Association, American Water Works Association and Water Environmental Federation, Washington, DC, USA, ISBN 9780875532356.
Battimelli, A., Torrijos, M., Moletta, R., Delgenès, J.P., 2010. Slaughterhouse fatty waste saponification to increase biogas yield. Bioresour. Technol. 101, 3388–3393. https://doi.org/10.1016/j.biortech.2009.12.043.
Beale, D.J., Karpe, A.V., Mcleod, J.D., Gondalia, S.V., Muster, T.H., Othman, M.Z., Palombo, E.A., Joshi, D., 2016. An ' omics ' approach towards the characterisation of laboratory scale anaerobic digesters treating municipal sewage sludge. Water Res. 88, 346–357. https://doi.org/10.1016/j.watres.2015.10.029.
Chan, H., 2010. Removal and recycling of pollutants from Hong Kong restaurant wastewaters. Bioresour. Technol. 101, 6859–6867. https://doi.org/10.1016/j.biortech.2010.03.104.
Davidsson, A., Lovstedt, C., la Cour Jansen, J., Gruvberger, C., Aspegren, H., 2008. Co-digestion of grease trap sludge and sewage sludge. Waste Manag. 28, 986–992. https://doi.org/10.1016/j.wasman.2007.03.024.
Del Mundo, D.M.N., Sutheerawattananonda, M., 2017. Influence of fat and oil type on the yield, physico-chemical properties, and microstructure of fat, oil, and grease (FOG) deposits. Water Res. 124, 308–319. https://doi.org/10.1016/j.watres.2017.07.047.
Ducoste, J.J., Keener, K.M., Groninger, J.W., Holt, L.M., 2008. Fats, Roots, Oils and Grease (FROG) in Centralized and Decentralized Systems. Water Environment Research Foundation, London.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.
Fan, X., Xing, P., 2016. Differences in the composition of archaeal communities in sediments from contrasting zones of Lake Taihu. Front. Microbiol. 7, 1–11. https://doi.org/10.3389/fmicb.2016.01510.
Ferguson, R.M.W., Coulon, F., Villa, R., 2018. Understanding microbial ecology can help improve biogas production in AD. Sci. Total Environ. 642, 754–763. https://doi.org/10.1016/j.scitotenv.2018.06.007.
Gerritsen, J., Fuentes, S., Grievink, W., van Niftrik, L., Tindall, B.J., Timmerman, H.M., Rijkers, G.T., Smidt, H., 2014. Characterization of Romboutsia ilealis gen. nov., sp. nov., isolated from the gastro-intestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus Clostridium into the genera Romboutsia gen. nov. Intestinib. Int. J. Syst. Evol. Microbiol. 64, 1600–1616. https://doi.org/10.1099/ijs.0.059543-0.
Hatamoto, M., Imachi, H., Yashiro, Y., Ohashi, A., Harada, H., 2007. Diversity of anaerobic microorganisms involved in long-chain fatty acid degradation in methanogenic sludges as revealed by RNA-based stable isotope probing. Appl. Environ. Microbiol. 73, 4119–4127. https://doi.org/10.1128/AEM.00362-07.
He, X., de los Reyes, F.L., Ducoste, J.J., 2017. A critical review of fat, oil, and grease (FOG) in sewer collection systems: challenges and control. Crit. Rev. Environ. Sci. Technol. 47, 1191–1217. https://doi.org/10.1080/10643389.2017.1382282.
He, Xia, de los Reyes III, Francis L., Leming, Michael L., Dean, Lisa O., Lappi, Simon E., Ducoste, Joel J., 2013. Mechanisms of fat, oil and grease (FOG) deposit formation in sewer lines, 47, pp. 4451–4459. https://doi.org/10.1016/j.watres.2013.05.002.
He, X., Iasmin, M., Dean, L.O., Lappi, S.E., Ducoste, J.J., de los Reyes, F.L., 2011. Evidence for fat, oil, and grease (FOG) deposit formation mechanisms in sewer lines. Environ. Sci. Technol. 45, 4385–4391. https://doi.org/10.1021/es2001997.
Husain, I., Alkhatib, M., Jammi, M., Mirghani, M., Zainuddin, Z., Hoda, A., 2014. Problems, control, and treatment of fat, oil, and grease (FOG): a review. J. Oleo Sci. 752, 747–752. https://doi.org/10.5650/jos.ess13182.
Kabouris, J.C., Tezel, U., Pavlostathis, S.G., Engelmann, M., Dulaney, J., Gillette, R.A., Todd, A.C., 2009. Methane recovery from the anaerobic codigestion of municipal sludge and FOG. Bioresour. Technol. 100, 3701–3705. https://doi.org/10.1016/j.biortech.2009.02.024.
Keener, K.M., Ducoste, J.J., Holt, L.M., 2008. Properties influencing fat, oil, and grease deposit formation. Water Environ. Res. 80, 2241–2246. https://doi.org/10.2175/193864708X267441.
Kurade, M.B., Saha, S., Salama, E.S., Patil, S.M., Govindwar, S.P., Jeon, B.H., 2019. Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats, oil and grease for enhanced production of methane. Bioresour. Technol. 272, 351–359. https://doi.org/10.1016/j.biortech.2018.10.047.
Li, C., Champagne, P., Anderson, B.C., 2011. Evaluating and modeling biogas production from municipal fat , oil , and grease and synthetic kitchen waste in anaerobic co-digestions. Bioresour. Technol. 102, 9471–9480. https://doi.org/10.1016/j.biortech.2011.07.103.
Long, J.H., Aziz, T.N., Reyes, F.L.D.L., Ducoste, J.J., 2012. Anaerobic co-digestion of fat, oil, and grease (FOG): a review of gas production and process limitations. Process Saf. Environ. Protect. 90, 231–245. https://doi.org/10.1016/j.psep.2011.10.001.
Lovley, D.R., 2017. Syntrophy goes electric: direct interspecies electron transfer. Annu. Rev. Microbiol. 71, 643–664. https://doi.org/10.1146/annurev-micro-030117-020420.
Lozupone, C.A., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235. https://doi.org/10.1128/AEM.71.12.8228.
Luostarinen, S., Luste, S., Sillanpää, M., 2009. Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. Bioresour. Technol. 100, 79–85. https://doi.org/10.1016/j.biortech.2008.06.029.
Ma, J., Zhao, Q.B., Laurens, L.L.M., Jarvis, E.E., Nagle, N.J., Chen, S., Frear, C.S., 2015. Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. Biotechnol. Biofuels 8, 1–12. https://doi.org/10.1186/s13068-015-0322-z.
Marlow, D.R., Boulaire, F., Beale, D.J., Grundy, C., Moglia, M., 2011. Sewer performance reporting: factors that influence blockages. J. Infrastruct. Syst. 17, 42–51. https://doi.org/10.1061/(ASCE)IS.1943-555X.0000041.
Martin, M., 2015. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17, 10–12.
Mata-alvarez, J., Dosta, J., Romero-güiza, M.S., Fonoll, X., Peces, M., Astals, S., 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sustain. Energy Rev. 36, 412–427. https://doi.org/10.1016/j.rser.2014.04.039.
McIlroy, S.J., Kirkegaard, R.H., McIlroy, B., Nierychlo, M., Kristensen, J.M., Karst, S.M., Albertsen, M., Nielsen, P.H., 2017. MiDAS 2.0: an ecosystem-specific taxonomy and online database for the organisms of wastewater treatment systems expanded for anaerobic digester groups. Database 2017, 1–9. https://doi.org/10.1093/database/bax016.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8. https://doi.org/10.1371/journal.pone.0061217.
Meng, Y., Luan, F., Yuan, H., Chen, X., Li, X., 2017. Enhancing anaerobic digestion performance of crude lipid in food waste by enzymatic pretreatment. Bioresour. Technol. 224, 48–55.
Morita, M., Malvankar, N., Franks, A., Summers, Z., Giloteaux, L., Rotaru, A., Rotaru, C., Lovley, D., 2011. Potential for direct interspecies electron transfer in methanogenic. mBio 2, 5–7. https://doi.org/10.1128/mBio.00159-11.
Mu, L., Zhang, L., Zhu, K., Ma, J., Li, A., 2018. Semi-continuous anaerobic digestion of extruded OFMSW: process performance and energetics evaluation. Bioresour. Technol. 247, 103–115. https://doi.org/10.1016/j.biortech.2017.09.085.
Nazaitulshila, R., Idris, A., Harun, R., Wan Azlina, W.A.K.G., 2015. The influence of inoculum to substrate ratio on the biochemical methane potential of fat, oil, and grease in batch anaerobic assays. Energy Sources, Part A Recover. Util. Environ. Eff. 37, 590–597. https://doi.org/10.1080/15567036.2014.907374.
Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Rep. 5, 14–21. https://doi.org/10.1016/j.btre.2014.10.005.
Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864.
Rotaru, A.E., Shrestha, P.M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler, K., Wardman, C., Nevin, K.P., Lovley, D.R., 2014. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy Environ. Sci. 7, 408–415. https://doi.org/10.1039/c3ee42189a.
Roy, F., Albagnac, G., Samain, E., 1985. Influence of calcium addition on growth of highly purified syntrophic cultures degrading long-chain fatty acids. Appl. Environ. Microbiol. 49, 702–705.
Salama, E., Jeon, B., Kurade, M.B., Patil, S.M., Usman, M., Li, X., Lim, H., 2019b. Enhanced anaerobic co-digestion of fat, oil, and grease by calcium addition: boost of biomethane production and microbial community shift. Bioresour. Technol. 296, 122353. https://doi.org/10.1016/j.biortech.2019.122353.
Salama, E.-S., Saha, S., Kurade, M.B., Dev, S., Chang, S.W., Jeon, B.-H., 2019a. Recent trends in anaerobic co-digestion: fat, oil, and grease (FOG) for enhanced biomethanation. Prog. Energy Combust. Sci. 70, 22–42. https://doi.org/10.1016/j.pecs.2018.08.002.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Shrestha, P.M., Malvankar, N.S., Werner, J.J., Franks, A.E., Elena-Rotaru, A., Shrestha, M., Liu, F., Nevin, K.P., Angenent, L.T., Lovley, D.R., 2014. Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. Bioresour. Technol. 174, 306–310. https://doi.org/10.1016/j.biortech.2014.10.004.
Slotkin, J., 2017. Behold the Fatberg: London's 130-Ton, "Rock-Solid" Sewer Blockage [WWW Document]. npr. URL. https://www.npr.org/sections/thetwo-way/2017/09/12/550465000/behold-the-fatberg-london-s-130-ton-rock-solid-sewer-blockage.
Sousa, D.Z., Smidt, H., Alves, M.M., Stams, A.J.M., 2009. Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. FEMS Microbiol. Ecol. 68, 257–272. https://doi.org/10.1111/j.1574-6941.2009.00680.x.
Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., Sørensen, S.J., Karlsson, A., 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol. Ecol. 85, 612–626. https://doi.org/10.1111/1574-6941.12148.
Wang, L., Aziz, T.N., De los Reyes, F.L., 2013. Determining the limits of anaerobic co-digestion of thickened waste activated sludge with grease interceptor waste. Water Res. 47, 3835–3844. https://doi.org/10.1016/j.watres.2013.04.003.

Wang, L., Hossen, E.H., Aziz, T.N., Ducoste, J.J., de los Reyes, F.L., 2020. Increased loading stress leads to convergence of microbial communities and high methane yields in adapted anaerobic co-digesters. Water Res. 169, 115155. https://doi.org/10.1016/j.watres.2019.115155.

Williams, J.B., Clarkson, C., Mant, C., Drinkwater, A., May, E., 2012. Fat, oil and grease deposits in sewers: characterisation of deposits and formation mechanisms. Water Res. 46, 6319–6328. https://doi.org/10.1016/j.watres.2012.09.002.

Wirth, R., Kovács, E., Maróti, G., Bagi, Z., Rákhely, G., Kovács, K.L., 2012. Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. Biotechnol. Biofuels 5, 41. https://doi.org/10.1186/1754-6834-5-41.

Yalcinkaya, S., Malina, J.F., 2015. Anaerobic co-digestion of municipal wastewater sludge and un-dewatered grease trap waste for assessing direct feed of grease trap waste in municipal digesters. Int. Biodeterior. Biodegrad. 104, 490–497. https://doi.org/10.1016/j.ibiod.2015.08.007.

Yang, Y., Yu, K., Xia, Y., Lau, F.T.K., Tang, D.T.W., Fung, W.C., Fang, H.H.P., Zhang, T., 2014. Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants. Appl. Microbiol. Biotechnol. 98, 5709–5718. https://doi.org/10.1007/s00253-014-5648-0.

Yang, Z., Xu, R., Zheng, Y., Chen, T., Zhao, L., Li, M., 2016. Characterization of extracellular polymeric substances and microbial diversity in anaerobic co-digestion reactor treated sewage sludge with fat , oil , grease. Bioresour. Technol. 212, 164–173. https://doi.org/10.1016/j.biortech.2016.04.046.

Zhang, S., Chang, J., Liu, W., Pan, Y., Cui, K., Chen, X., Liang, P., Zhang, X., Wu, Q., Qiu, Y., Huang, X., 2018. A novel bioaugmentation strategy to accelerate methanogenesis via adding Geobacter sulfurreducens PCA in anaerobic digestion system. Sci. Total Environ. 642, 322–326. https://doi.org/10.1016/j.scitotenv.2018.06.043.

Ziels, R.M., Karlsson, A., Beck, D.A.C., Ejlertsson, J., Yekta, S.S., Bjorn, A., Stensel, H.D., Svensson, B.H., 2016. Microbial community adaptation influences long-chain fatty acid conversion during anaerobic codigestion of fats, oils, and grease with municipal sludge. Water Res. 103, 372–382. https://doi.org/10.1016/j.watres.2016.07.043.

Ziels, R.M., Sousa, D.Z., Stensel, H.D., Beck, D.A.C., 2017. DNA-SIP based genome-centric metagenomics identifies key long-chain fatty acid-degrading populations in anaerobic digesters with different feeding frequencies. ISME J. 12, 112–123. https://doi.org/10.1038/ismej.2017.143.