Acid-phase and Two-phase Codigestion of FOG in Municipal Wastewater

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Abstract

Acidogenic codigestion of fats, oils, and greases (FOG) was studied at 37°C using suspended sludge digesters operated as sequencing batch reactors (SBRs). Volatile fatty acid (VFA) production was found to increase with larger FOG loading rates, although this increase was insignificant compared the theoretical VFA production from FOG addition. Long chain fatty acids (LCFAs) were found to have accumulated in the reactor vessel in semi-solid balls that were primarily composed of saturated LCFAs.

Adding high FOG loadings to an APD not acclimated to LCFAs allowed for a mass balance calculation and resulted in near complete saturation of unsaturated LCFAs and significant accumulation of LCFA material in the digester, which was found to be mostly 16:0, 18:0, and 18:1. While 18:2 and 18:3 LCFAs were nearly completely removed, 18:0 and 14:0 LCFAs were produced, most likely from the degradation of 18:2 and 18:3 LCFAs. The APD pH was found to have a significant impact on the amount of accumulated LCFA material present, with higher pH levels resulting in less accumulated material.

Two-phase codigestion of FOG was also studied using an APD followed by gas-phase (GPD) digesters. The two-phase systems were compared by FOG addition to the APD versus GPD. FOG addition to the APD resulted in 88% destruction of LCFAs, whereas FOG addition to the GPD resulted in 95% destruction of LCFAs. Accumulated LCFAs in the APD receiving FOG were composed mostly of stearic acid (18:0). The low pH of the APD is likely the cause of LCFA accumulation due to saturation of unsaturated LCFAs.

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Introduction

Two-phase digestion (TPD) is an advanced anaerobic, biological treatment system for treating sludges from municipal wastewater treatment plants that is growing in popularity due to its ability to produce more biogas over a conventional digestion system. Codigestion of fats, oils, and grease (FOG) is also becoming more widely practiced because it can increase biogas production from anaerobic digestion with municipal wastewater sludges. The combination of these two practices is not as well characterized as each process individually.

The following literature review describes the research that has been conducted to better understand TPD and FOG codigestion processes. The major conclusions that were drawn are that FOG addition to the front end of a TPD system, the acid-phase digester (APD), will not result in degradation of the primary components in FOG, long chain fatty acids (LCFAs). The short SRT characteristic of an APD washes out the microbial communities responsible for the syntrophic interactions that make degradation of FOG thermodynamically feasible. However, the APD can serve to saturate any unsaturated LCFAs in the FOG and convert 18-carbon chain LCFAs to 16-carbon chain LCFAs. This reduces inhibition of methanogenesis because unsaturated LCFAs are more inhibitory to methanogens. In addition, shorter carbon chains are essentially one step further down the degradation pathway and will be degraded faster.

The first manuscript presented, Acidogenesis and Two-phase Codigestion of Fats, Oils, and Greases and Municipal Biosolids, studies the effect of FOG codigestion on APDs and a TPD system. While greater FOG loadings on the APD resulted in increased volatile fatty acid (VFA) production, this increase was insignificant compared to the theoretical VFA yield from the FOG addition. LCFAs were also found to accumulate into semi-solid balls that floated at the liquid

surface and were not removed through digester wasting. Two TPD systems were compared by adding FOG to the APD in one system and to the GPD in the other. This allowed a comparison of whether FOG should be added to the APD or GPD in a TPD system. Addition to the APD was found to produce more gas, while addition to the GPD resulted in higher destruction of LCFAs. Accumulated LCFA material was found in all digester receiving FOG, including the GPD being fed with the effluent from the APD receiving FOG. While gas production was greater in the system receiving FOG in the APD, significant accumulation of LCFA material in the APD would cause detrimental operational problems in a full scale wastewater facility.

The second manuscript presented, Degradation and Accumulation of LCFAs in Acid-phase Codigestion of Municipal Biosolids, explores the mechanisms of degradation and accumulation of LCFAs in APDs receiving FOG. A high loading rate of FOG was added to a digester previously not acclimated to LCFAs to perform a mass balance calculation on the system and better understand what processes are taking place in FOG codigestion in APD systems. LCFAs were again found to accumulate significantly and LCFAs with zero or one double-bond were found to preferentially attached to the accumulated LCFA mass floating in the digester, instead of passing through the system in the wasted effluent. This prompted a study into the role of pH in accumulation and degradation of LCFAs in the APD system. While pH did not affect degradation of LCFAs, it had a large impact on accumulation. Higher pH levels corresponded to much less accumulation of LCFAs in the digester and at a neutral pH, no accumulated LCFAs were found. Several theories are discussed that try to explain why this occurred.

Literature Review

Two-Phase Digestion

Two-phase digestion (TPD) is an advanced anaerobic, biological treatment system for treating sludges from municipal wastewater treatment plants. TPD splits the individual anaerobic microbial processes into two parts, allowing for each to operate more efficiently. This results in a smaller reactor footprint, a shorter overall retention time, and more control over the entire biosolids treatment process. TPD is considered an advanced digestion system because it is more technically complex, resulting in better performance than conventional digestions processes.

Anaerobic digestion is a two-step process that involves two distinct microbial communities which do not reach optimal performance when they coexist in the same environment (Ghosh et al. 1975). TPD spatially separates hydrolysis and acidogenesis from methanogenesis to achieve increased methane production and solids solubilization at half the detention time of a conventional single-stage anaerobic digester (Ghosh et al. 1975). The first digester in the TDP system is called the acid-phase, hydrolysis reactor, or fermenter and will be referred to here as acid-phase digester (APD). The APD is of more interest here since the characteristics of the second digester in which methanogenesis occurs, or gas-phase digester (GPD), are similar to a conventional single-phase digester where the desire is to optimize methane production.

Massey and Pohland (1978) found that near-complete inhibition of methanogenesis can be achieved in the APD with kinetic control via manipulation of the solids retention time (SRT), and that this allows for more stability and control when operating a TPD system, even with a complex substrate. In this environment, hydrolysis and acidogenesis are taking place simultaneously with the main products being volatile fatty acids (VFAs), mainly acetic and

propionic acid (Eastman and Ferguson 1981). In continuous and batch experiments degrading primary clarifier biosolids at 35°C, Eastman and Ferguson (1981) also found that hydrolysis is the rate limiting step, while both the hydrolysis and acidogenesis are significantly affected by pH. At an SRT of 36 hours, VFA production was 42% higher at pH 6.67 than at pH 5.1. In addition, Eastman and Ferguson (1981) found the degradation of carbohydrates and nitrogenous material to be 70% and 55%, respectively, while lipids were not degraded at all. More recent studies have found similar results (Elefsiniotis and Oldham 1994, Ghosh et al. 1995). The optimal parameters for APD operation have been widely studied and discussed, and summarized by Demirel and Yenigün (2002). Their study found that for complex substrates, HRT was often reported to be the most important parameter influencing VFA production and COD solubilization, with reported optimal values usually falling between 0.5 – 3 days. Demirel and Yenigün (2002) also found disagreement about the optimal pH for hydrolysis, but operating pHs have generally been reported as being best between 5.5 - 7.0. A more recent study by Wu et al. (2009) found that 5.0 was an optimal pH for COD solubilization over the range of pH 4.0 – 7.0, but that COD solubilization is equivalent at pHs 5.0 and 8.0, and the rate of hydrolysis further increases from pH 8.0 up to 10.0. Demirel and Yenigün (2002) also found that temperature was frequently concluded to be an important factor, with 30-35°C being reported most often as optimal.

FOG Addition

Mechanism of Degradation

Fats, oils, and greases (FOG) are a complex, high strength, organic waste material from a variety of sources such as food processing facilities and restaurants. The most notable

constituents of FOG are triglyceride esters, which are rapidly hydrolyzed to produce long chain fatty acids (LCFAs) during degradation (Heukelekian and Mueller 1958). An early study into anaerobic degradation of LCFAs was conducted by Novak and Carlson (1970). They found that the primary mechanism of degradation is β-oxidation, which produces a chain with *n*-2 carbons and an acetate molecule, as shown below (from Kim et al. (2004a)).

$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_{n-2}COOH + CH_3COOH + 2H_2$$

Longer chains were found to take longer to degrade, and unsaturated LCFAs degraded more quickly than the saturated versions. However, it was found that unsaturated LCFAs need to first be saturated before β-oxidation can take place.

More recent studies have found that saturation is not necessarily required for unsaturated LCFAs to undergo β-oxidation. Oleic acid (18:1) degrades up to 100 mg/l with palmitic (16:0) and myristic (14:0) acids being major by-products with no stearic acid (18:0) produced (Lalman and Bagley 2001). The study also found that 18:0 degrades with no byproducts in batch assays fed with glucose, but takes a significant amount of time to do so (roughly 40% degradation after 50 days). In a separate study, linoleic acid (18:2) was found to degrade completely, but forms 18:1 and palmitoleic acid (16:1) as transient products that were degraded further to 16:0 and 14:0 as by-products; 18:0 was not found as an intermediate (Lalman and Bagley 2000). These studies show that β-oxidation is not necessarily dependent on complete saturation because 18:0 was not found as a product of degradation of 18:1 or 18:2 while 16:0 and 14:0 were found to be by-products. This doesn't necessarily prove that 18:0 was not formed as a brief intermediate, but given the slow rate of degradation of 18:0, it is likely that 18:0 is not produced (Lalman and Bagley 2001). In addition, Pereira et al. (2002) found that 16:0 is the major intermediate of the degradation of 18:1, accounting for up to 80% of the LCFA intermediates. More recent studies

agree with these results (Pereira et al. 2005, Palatsi et al. 2012). A thermodynamic analysis of free energies (shown in Figure 1) agrees with the observed results presented in these studies, although it is important to note that the free energies presented were calculated at 25°C and a pH of 7 (Lalman and Bagley 2001).

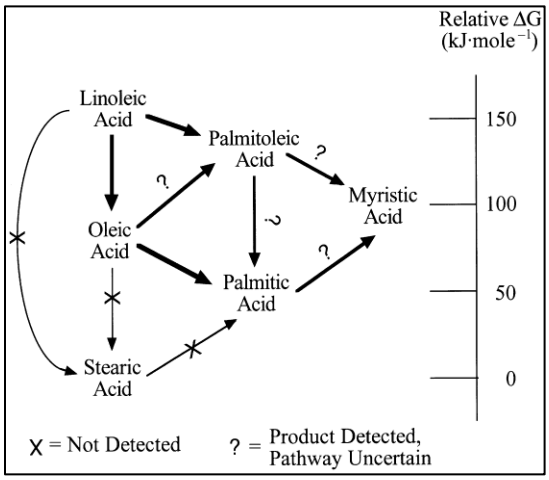

Figure 1 - Visual representation of thermodynamic free energy analysis with relative changes in free energies (Lalman and Bagley 2001), Used under fair use, 2013

Adsorption and Precipitation

FOG has been found to accumulate in a solid or semi-solid form in codigestion systems, eventually leading to digester failure by sludge flotation and washout of sludge granules if not removed from granular sludges (Hwu et al. 1998a, Jeganathan et al. 2006). This is significant because some studies have found LCFA accumulation to take place quickly, within 24 hours (Hanaki et al. 1981). Hwu et al. (1998a) also found that longer SRTs can decrease LCFA adsorption. This accumulation can occur due to entrapment of LCFAs in biomass flocs, adsorption onto microbe cells, or precipitation with divalent ions (Pereira et al. 2005). Accumulation is most likely due to some combination of each of these three mechanisms. Hwu et al. (1998b) determined that biosorption is significant and that it is required for degradation of 18:1 to occur. Pereira et al. (2005) found that precipitation occurred in inactivated (via autoclave)

sludge, but that biological activity accounts for 20-38% of the total COD accumulated, with the rest being due to precipitation by divalent cations. Several studies have determined 16:0 to be the main LCFA adsorbed to biomass in enhanced granular sludge bed (EGSB) digesters degrading 16:0 or 18:1 (Pereira et al. 2005, Palatsi et al. 2012, Jeganathan et al. 2006). It is important to note that these studies were all carried out using granular sludge in UASB or EGSB systems. Several other experiments were conducted using continuous or semi-continuous digesters that reported increased gas production from FOG addition with no mention of accumulated FOG in the digesters, although all of them saw a lag in methane production (Kabouris et al. 2008, Kabouris et al. 2009a, Davidsson et al. 2008, Luostarinen et al. 2009). This indicates the significance of adsorption when discussing inhibition of anaerobic digestion processes by LCFAs.

Mechanisms of Inhibition

While FOG addition can increase biogas production, it can also be inhibitory to acidogens (Lalman and Bagley 2004), acetoclastic methanogens (Lalman and Bagley 2000, Palatsi et al. 2012, Hanaki et al. 1981, Koster and Cramer 1987), and β-oxidizers (Lalman and Bagley 2000, Pereira et al. 2002, Kim et al. 2004b). Acetoclastic methanogens seem to be the most sensitive group to inhibition by LCFAs (Lalman and Bagley 2002). Hanaki et al. (1981) found that LCFAs caused a lag in gas production for acetoclastic methanogens, while hydrogenotrophic methanogens did not lag, but the latter experienced a lower gas production rate compared to the control receiving only acetate. It was also observed that most of the LCFAs adsorbed to the biomass solids, showing that solid, sorbed LCFAs are most likely inhibitory, although addition of $CaCl_2$ effectively mitigated this inhibition. Koster and Cramer (1987)

reported similar results and further found a mixture of LCFAs to be more toxic than one single LCFA. This was confirmed by Kim et al. (2004b), who found that unsaturated LCFAs are more inhibitory to acetoclastic methanogens and propionate degradation than saturated LCFAs. Lalman and Bagley (2002) found similar results and concluded that mixtures of LCFAs are more inhibitory to butyrate consumption than individual LCFAs. Pereira et al. (2002) concluded that the presence of 18:1 was inhibitory to the degradation of 16:0.

Koster and Cramer (1987) proposed that LCFA inhibition is caused by microbial toxicity, although more recent studies have found evidence to the contrary (Lalman and Bagley 2001, 2000, Pereira et al. 2005, Palatsi et al. 2012, Lalman and Bagley 2002, Pereira et al. 2004). Acetoclastic methanogens in a system fed with glucose can be completely inhibited by 18:1 or 18:2 LCFAs, while hydrogenotrophic methanogens in the same system show little to no inhibition (Lalman and Bagley 2001, 2000). This also means that β-oxidation of LCFAs is not likely to be inhibited by high partial pressures of hydrogen gas, since hydrogenotrophic methanogens are not inhibited and will remove hydrogen gas from the system (Beccari et al. 1998) These studies are in agreement with Hanaki et al. (1981) and suggest that the mechanism of inhibition is transport limitation through the cell wall caused by LCFA adsorption onto the biomass. Because the H_2 molecule is smaller than acetate, it more easily diffuses through the LCFA layer into the cell so methanogens utilizing hydrogen can still absorb substrate, while acetogenic methanogens starve because acetate is too large to diffuse through the adsorbed LCFA layer and into the cell (Pereira et al. 2004). This is further supported by the fact that methanogen inhibition is a reversible process and even though mineralization occurs, biodegradation can still take place (Pereira et al. 2005, Palatsi et al. 2012). Degradation of adsorbed 16:0 in an EGSB was inhibited by 18:1 LCFA, but was enhanced by stirring (Pereira et

al. 2002). This study also recommended suspended sludge digesters over granular sludge due to its better performance for LCFA accumulation and degradation.

FOG Addition to APD/Two-Phase/Fermenter

The key differences between an APD and a conventional anaerobic digester with respect to LCFA degradation are that the APD lacks methanogens and has a lower pH. Lipid degradation is only 10% in the APD at pH 5.6 (Ghosh et al. 1995). This is most likely because β-oxidation is not thermodynamically favorable (Lalman and Bagley 2001), so syntrophic microbial relationships are required to drive the reaction forward (Palatsi et al. 2012, Fox and Pohland 1994). The small amount of lipid degradation observed by some studies could be attributed to hydrogenotrophic methanogens, which are known to have faster growth rates than acetoclastic methanogens (Ghosh et al. 1995, Beccari et al. 1998). Therefore, the primary role of the two-phase digestion system for the anaerobic co-digestion of FOG is to use the APD as a method of saturating unsaturated LCFAs and converting 18-carbon chains to 16:0 in order to mitigate inhibition in the methanogenic digester where further β-oxidation takes place (Kim et al. 2004a, Beccari et al. 1998, Komatsu et al. 1991, Kim and Shin 2010).

Komatsu et al. (1991) added LCFAs to a suspended sludge two-phase digestion system and found that inhibition occurs in the APD, but that unsaturated 18-chain LCFAs became partially saturated. This was found to decrease inhibition of β-oxidation and methanogenesis in the methane reactor compared to a single-phase system. It was also determined that pH 6.0 was worse for acidification and β-oxidation in the APD than pH of 7.0. It was proposed that the low pH converted the LCFAs to a non-ionized form, making them less soluble. Similar experiments have been conducted, but achieved much more complete saturation of unsaturated LCFAs in the

APD, although this was probably due to the longer SRT used (3.7 days), allowing growth of hydrogenotrophic methanogens (Beccari et al. 1998). In these studies, as discussed previously, 18:1 and 18:2 LCFAs were added to APDs, but 16:0 was found in the effluent while 18:0 was absent. More recent studies using both suspended and granular sludge for the methane reactor have found similar results, although individual LCFAs were not quantified (Kim and Shin 2010, Kabouris et al. 2009b). Miron et al. (2000) conducted experiments with suspended sludge digesters at a variety of SRTs down to 2 days, with any below 8 days being only the acidogenic phase of digestion. The study found that the pH of digesters operating below an 8 day SRT was just below 5.0 and that saturated LCFAs with 12-, 14-, 16-, and 18-carbon chain lengths accumulated while unsaturated LCFAs with 16- and 18-carbon chain lengths were easily degraded. The accumulation of 18:0 could be due to high partial pressures of hydrogen gas (Miron et al. 2000) or high concentrations of hydrogen ions because the pH was significantly lower than the other studies mentioned here.

Summary

While both TPD and FOG addition can increase biogas production from anaerobic digestion processes, the combination of these practices is complex. Because LCFAs are nonpolar and require a long time to degrade, addition of FOG to the APD of a two-phase system theoretically doesn't result in any degradation of the FOG. If the APD is used as a pre-treatment system to saturate the more inhibitory unsaturated LCFAs, an increase in biogas production could be achieved. However, operational issues can occur due to this practice.

There is little agreement on the mechanisms of LCFA degradation or inhibition, particularly when FOG is added to a two-phase system. Because of the large theoretical benefit

of this practice, further study should focus on the mechanisms by which degradation, adsorption, precipitation, and inhibition occur so that we may better understand how to improve current TPD and FOG addition practices.

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Acidogenesis and Two-phase Codigestion of Fats, Oils, and Greases and Municipal Biosolids

Abstract

Acidogenic codigestion of fats, oils, and greases (FOG) was studied using suspended sludge digesters operated as sequencing batch reactors (SBRs). The digesters were maintained at a 2-day retention time and at 37°C. Volatile fatty acid (VFA) production was found to increase with larger FOG loading rates. This increase was insignificant compared to the theoretical VFA production from FOG addition due to inhibition. Long chain fatty acids (LCFAs) were found to have accumulated in the reactor vessel in semi-solid balls that were primarily composed of saturated LCFAs. Two-phase codigestion of FOG was studied at 37°C using SBRs as acid-phase (APD) followed by gas-phase (GPD) digesters operated with 2-day and 15-day retention times, respectively. The two-phase systems were compared by FOG addition to the APD versus GPD. FOG addition to the APD resulted in 11.18 l-day^{-1} of biogas production and 88% destruction of LCFAs, whereas FOG addition to the GPD resulted in 9.87 l-day^{-1} of gas production and 95% destruction of LCFAs. Accumulated LCFAs were again found in the APD receiving FOG and were composed mostly of stearic acid (18:0).

Introduction

Two-phase digestion (TPD) is an advanced anaerobic, biological treatment system for treating sludges from municipal wastewater treatment plants that is growing in popularity due to its ability to produce more biogas over a conventional digestion system. Codigestion of fats, oils, and greases (FOG) is also becoming more widely practiced because it can increase biogas production from anaerobic digestion with municipal wastewater sludges. The combination of these two practices is not as well characterized as each process individually.

TPD spatially separates the individual anaerobic microbial processes into two phases, acidogenesis followed by methanogenesis, allowing for each to operate more efficiently. This results in a smaller reactor footprint, a shorter overall retention time, and more control over the

entire biosolids treatment process. TPD is considered an advanced digestion system because it is more technically complex, resulting in better solids destruction and biogas production than conventional digestions processes. The acid-phase digester (APD) is the major difference between TPD and conventional digestions systems. The APD utilizes a short solids retention time (SRT) to kinetically inhibit methanogens so that hydrolysis and acidogenesis can take place at a lower pH that is more conducive to these reactions.

FOG is a complex, high strength, organic waste material from a variety of sources such as food processing facilities and restaurants. The most notable constituents of FOG are triglyceride esters, which are rapidly hydrolyzed to produce long chain fatty acids (LCFAs) during degradation (Heukelekian and Mueller 1958). LCFAs are commonly denoted by the number of carbon atoms in the chain followed by the number of double bonds in the chain. For example, oleic acid has an 18-carbon chain and one double bond so it is denoted as 18:1. An early study into anaerobic degradation of LCFAs was conducted by Novak and Carlson (1970). They found that the primary mechanism of degradation is β-oxidation and that unsaturated LCFAs degraded more quickly than the saturated versions, although unsaturated LCFAs need to first become saturated before β-oxidation can take place.

More recent studies have found that saturation is not necessarily required for unsaturated LCFAs to undergo β-oxidation. Studies have found that palmitic (16:0) and myristic (14:0) acids are the major by-products of oleic acid (18:1) degradation (Lalman and Bagley 2001), and that 18:1 and palmitoleic acid (16:1) are transient products that were degraded further to 16:0 and 14:0 in the degradation of linoleic acid (18:2) (Lalman and Bagley 2000).

FOG has been found to accumulate in a solid or semi-solid form in codigestion systems, eventually leading to digester failure by sludge flotation and washout of sludge granules if not

removed from granular sludges (Hwu et al. 1998). Several studies have determined 16:0 to be the main LCFA adsorbed to biomass in enhanced granular sludge bed (EGSB) digesters degrading 16:0 or 18:1 (Palatsi et al. 2012, Pereira et al. 2005). Other studies have found using continuous or semi-continuous digesters resulted in increased gas production from FOG addition with no mention of accumulated FOG in the digesters, although all of them saw a lag in methane production (Kabouris et al. 2008, Kabouris et al. 2009, Davidsson et al. 2008, Luostarinen et al. 2009). This indicates the significance of adsorption when discussing inhibition of anaerobic digestion processes by LCFAs.

While FOG addition can increase biogas production, it can also be inhibitory to acidogens (Lalman and Bagley 2004), acetoclastic methanogens (Lalman and Bagley 2000, Palatsi et al. 2012), and β-oxidizers (Lalman and Bagley 2000, Kim et al. 2004, Pereira et al. 2002). Some studies suggest that the mechanism of inhibition is transport limitation caused by LCFA adsorption onto the biomass (Pereira et al. 2004) and that this is a reversible process, so biodegradation can still take place even though mineralization occurs, (Palatsi et al. 2012, Pereira et al. 2005). The short SRT characteristic of an APD washes out the microbial communities responsible for the syntrophic interactions that make degradation of FOG thermodynamically feasible (Palatsi et al. 2012). The primary role of the APD in a two-phase system codigesting FOG is to saturate the unsaturated LCFAs and convert 18-carbon chains to 16:0 in order to mitigate inhibition in the methanogenic digester where further β-oxidation takes place (Komatsu et al. 1991, Kim and Shin 2010).

Materials and Methods

Feed Characteristics

All municipal sludges were obtained from the nearby Christiansburg, VA Wastewater Treatment plant. Seed sludge was taken from an anaerobic digester degrading combined biosolids from the primary clarifier and thickened waste activated sludge (WAS). The feed sludge used in the studies was a mixture of 70/30 v/v mixture of primary and thickened WAS that was diluted with tap water to 2.5% total solids. FOG was shipped to the lab from the Hampton Roads Sanitation District wastewater treatment plant in Williamsburg, VA. The FOG was from a single source and sampled from a truck before mixing with FOG from other sources. This ensured homogeneity of the FOG used in the study. The characteristics of the feed components for the digesters are shown in Table 1.

Table 1 - Characteristics of Digester Feed Components

Component	Feed Sludge	FOG
COD (g/l)	37.8 (± 0.77)[a]	1,560 (± 577)
Total Solids (% of total mass)	2.48 (± 0.44)	57.2 (± 3.14)
Volatile Solids (% of total mass)	2.03 (± 0.44)	57.0 (± 3.06)
Lipids/LCFAs (% of total mass)	N.D.	63.7
LCFAs (g/l COD)	1	1,453

[a] Bracketed values show 95% confidence interval

Acid-Phase Codigestion of FOG

Four 2-liter acid-phase anaerobic digesters were constructed and operated as completely mixed Sequencing Batch Reactors (SBRs). Each digester was sealed to be airtight and kept at 37°C. Continuous mixing was accomplished using headspace gas recirculation via a peristaltic pump. A 2-day SRT was maintained by manual feeding and wasting, daily. Great care was taken

to limit the amount of air exposure so as to mitigate any oxygen toxicity effects. In two separate sampling campaigns, various FOG loadings (ranging from 10 to 44% of the total COD in the feed) were applied to the digesters by mixing the FOG with the feed sludge, plus a control that received no FOG. The reactor vessels were emptied and cleaned between the two sampling campaigns. Samples were taken daily from the wasted effluent for VFA and pH analysis.

Two-Phase Codigestion of FOG

Two separate two-phase digestion systems were constructed using two 2-liter acid-phase digesters (APD) and two gas-phase methane digesters (GPD). The APDs were operated as specified above with additional mixing just before sampling to ensure an accurate representation of digester contents. The GPDs were 11.25 liters and operated at a 15 day SRT with the same temperature and mixing parameters as the acid-phase digesters. 750ml of the APD effluent was used each day to manually feed and waste the GPD.

A FOG loading comprising 20% of the total feed COD was applied to each system. In one system, the FOG was added to the acid-phase by mixing it into the feed sludge. In the other system, the FOG was added to the methane digesters by mixing a proportional weight into the acid-phase effluent before feeding. This ensured that each two-phase system was treating the same quantity of FOG. Samples of wasted effluent were analyzed for VFA, LCFA, pH, and solids. Gas production was recorded and for the methane digesters, also analyzed for CH_4 and CO_2 concentrations.

Analytical Methods

Total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) were determined by Standard Methods 2540B, 2540B, and 5220C respectively (Clesceri et al. 1998); pH was measured with a pH probe and meter. Biogas was collected in sampling bags and when full, pumped out via peristaltic pump at a known rate for a measured time to determine quantity. CH_4 and CO_2 were quantified using thermal conductivity detection (TCD) on a Shimadzu GC-14A gas chromatograph equipped with a Haysept D (6ft) column. The isocratic program was set up with helium as the carrier gas, column (initial), injector, and TCD temperatures of 40, 70, and 110°C, respectively, and a detector current of 150mA.

The weight percentage for the lipids/LCFA component of the FOG was determined using a hexane extraction technique based on Standard Method 5520D (Clesceri et al. 1998). Instead of repeated washing in a separatory funnel, the samples were centrifuged after adding the hexane and MTBE mixture in order to separate the hexane and aqueous layers. The hexane was allowed to evaporate, leaving the lipid/LCFA material behind for quantification.

LCFA samples were prepared for GC analysis by conversion to Fatty Acid Methyl Esters (FAMEs). This was achieved by an esterification process after extracting the lipids/LCFAs as described above. A transesterification fluid containing mostly methanol was added to the extracted lipid/LCFA sample in excess and heated to 90°C for 30 minutes. This replaces the carboxylic acid with a methane group. The esterification process also severs the glycerin head from the triglyceride molecule so that the individual LCFAs are represented in the analysis, irrespective of the parent triglyceride. An HP 5890 GC using a flame ionization detector (FID) was equipped with a Supelcowax 10 fused silica capillary column (30m x 0.32mm ID x 1.00 µm

film thickness) for the analysis. Helium was the carrier gas and the program ran from 140°C to 260°C over 30 minutes.

VFAs were also analyzed with FID on a HP 5890 GC. A Nukol column (15m x 0.53mm x 0.5µm film thickness) was used with helium as the carrier gas and a program from 80°C to 140°C over 10 minutes.

Results

Acid-Phase Codigestion of FOG

Four acid phase digesters were operated as SBRs with a 2-day SRT. Two separate sampling campaigns were conducted under identical operating conditions, except for the amount of FOG addition. After steady state was established, as determined by stability of the effluent pH after 3 SRTs, the 10-day sampling campaign began. Samples were taken daily and the averages are shown in Table 2.

Table 2 – Average of total VFA concentration and pH in the digester effluent for each FOG loading

FOG (% of Total Feed COD)	Campaign	Average Total VFA Concentration (mg/l)	Average pH
0	2	2390	4.84
10	2	2531	4.83
17	1	2248	5.62
20	2	2830	4.79
28	1	2974	5.01
30	2	3085	4.73
37	1	3401	4.92
44	1	4220	4.71

VFA production was found to increase with higher FOG loadings (see Figure 2), but was significantly lower than theoretical VFA production, as calculated from the theoretical oxygen demand of the LCFAs represented as acetic acid. An inhibitory effect is most likely the cause of

this poor COD conversion to VFAs. The pH decreased with increased FOG loadings (see Figure 2), as expected due to the acid production, although the trend is much less consistent between campaigns than VFA production. It is possible that the feed sludge used for campaign 1 had less alkalinity to buffer the VFA production, although alkalinity was not quantified.

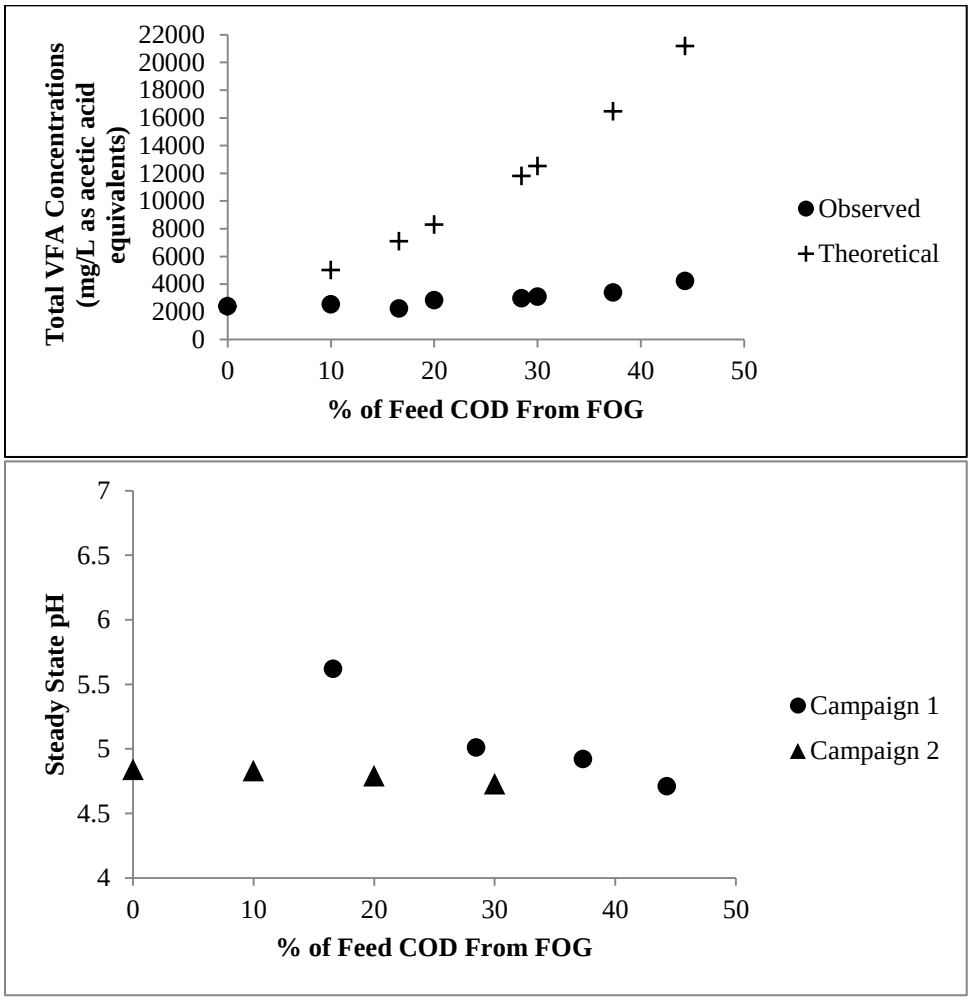

Figure 2 - Total experimental and theoretical VFA production (top) and pH (bottom) as a function of FOG addition

Large amounts of accumulated LCFAs were observed when the reactor vessels were opened for cleaning at the end of each sampling campaign, and were generally observed to be in proportion to the amount of FOG added. These solid, accumulated LCFAs were likely not proportionally removed by wasting because the LCFAs floated near the top, whereas the effluent

valve was located near the bottom of the liquid layer. Therefore, sludge was preferentially wasted from digester areas with low concentrations of accumulated LCFAs. Regardless of mixing considerations, some accumulated LCFA material (small grease balls) were found to have a diameter greater than the effluent valve size and often got stuck in the effluent valve towards the end of each campaign. Some of this accumulated LCFA material is shown in Figure 3.

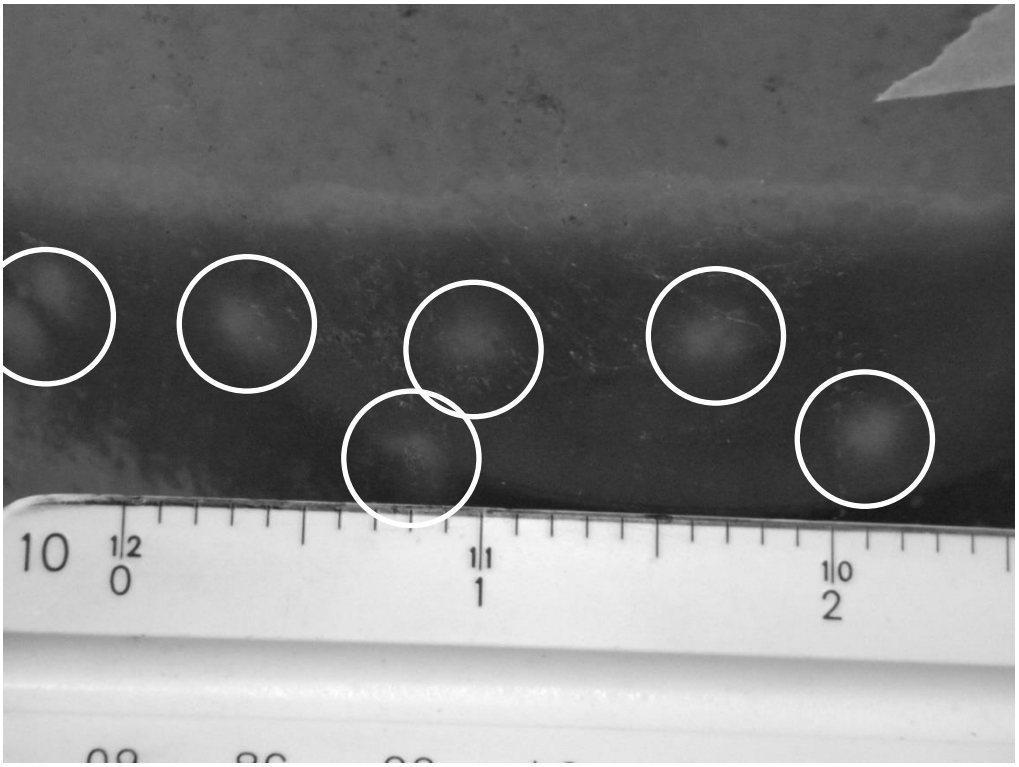

Figure 3 - Accumulated LCFAs (circled) seen floating near the liquid surface of a digester receiving FOG; ruler shown in inches

Two-Phase Codigestion of FOG

A summary of the two two-phase digestion systems is shown in Figure 4. FOG was mixed with sludge when added to the FOG to Acid System (FTA), and was mixed with APD effluent in the FOG to Gas System (FTG). All digesters were operated for at least 3 SRTs and

the pH of each digester effluent was found to be stable before sampling began. Sampling occurred 3 times per week from the wasted digester effluents.

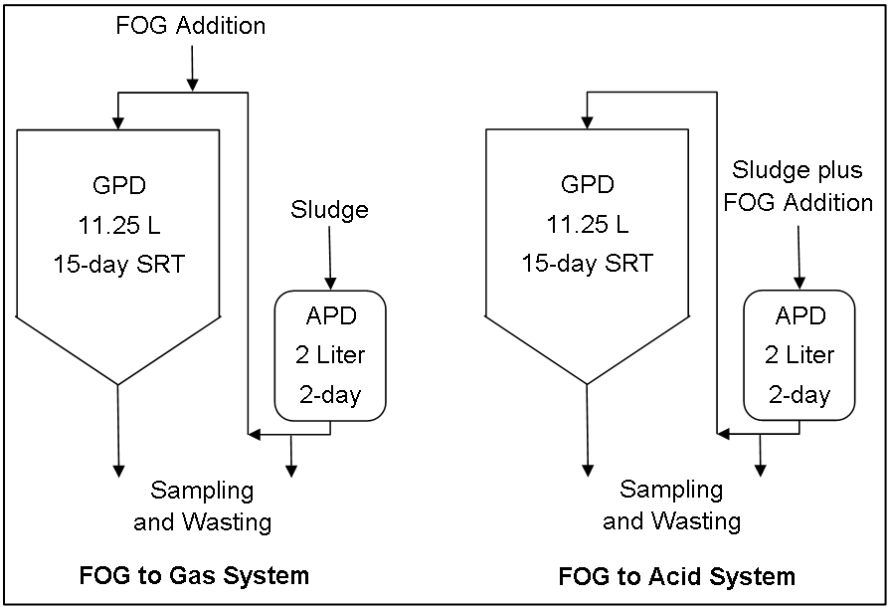

Figure 4 - Schematic representation of the two-phase digestions systems receiving FOG addition to the APD (right) and to the GPD (left)

The pH remained stable at 7.0 for the GPDs and 4.9 for the APDs of each system. The pH of the APDs was similar to that observed in Campaign 2 of the acid-phase codigestion study. The average volatile solids reduction (VSR) was 48% and 49% for the FTA and FTG systems, respectively, and was not statistically different between the two systems. Daily gas production for both phases of the each system is shown in Figure 5. Gas production from the APDs was insignificant, as expected in an anaerobic environment dominated by hydrolysis and acidogenesis reactions. The average daily gas production from the FTA system GPD was 11.18 l-day^{-1} while that of the FTG system GPD was 9.87 l-day^{-1}. Gas production in the FTA system was 13% greater, but this is most likely not significant due to the high variability in gas measurements.

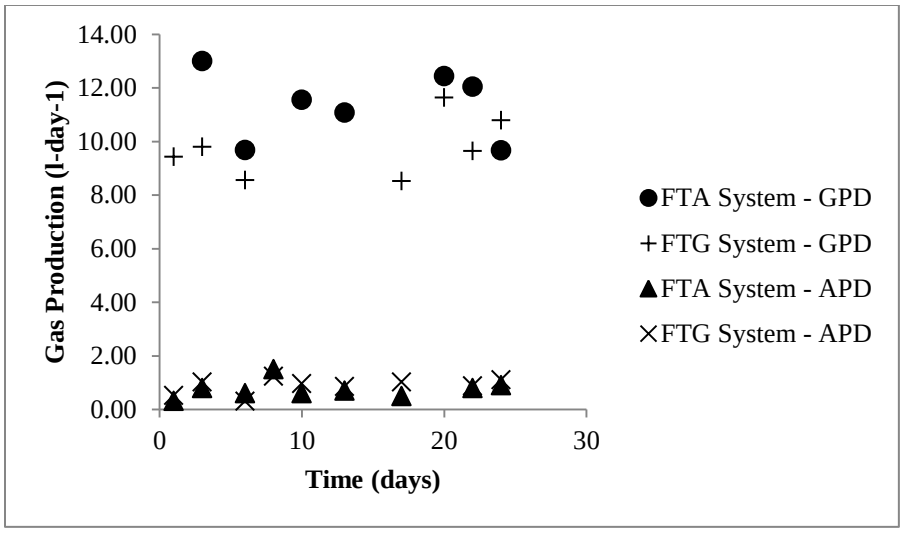

Figure 5 - Daily gas production in two-phase system

Table 3 shows the LCFA content of the feed and effluent for each digester of the two-phase systems. The FTA System Feed was calculated by adding the LCFA content of the FOG addition to the LCFA content of the sludge. The FTG System APD + FOG addition was calculated by adding the LCFA content of the FOG addition to the average LCFA content of the FTG System APD Effluent. It is important to note the limitations of the extraction procedure used for analysis of LCFAs. Assuming no degradation of LCFAs in the APD, as evidenced in the Acid-phase Codigestion of FOG study, recovery of LCFAs from the effluent was only 24%. This is essentially due to flotation of sludge solids in the extraction procedure. This material was discarded, but likely contained LCFAs because it floated above the aqueous layer. These LCFAs were probably adsorbed onto the biomass surface and were most likely 16:0, as found in previous studies (Palatsi et al. 2012, Pereira et al. 2005, Jeganathan et al. 2006). The LCFA content of the FTA feed and 'APD Effluent + FOG Addition' are accurate, because nearly all LCFA content comes from FOG addition; analysis of LCFAs in FOG was not affected by extraction errors.

The majority of LCFAs in the FOG were unsaturated 18-carbon chains, while most LCFAs in all digester effluents were saturated. Degradation of the LCFAs occurred primarily in the methane phase of digestion, although significant removal of the unsaturated 18-carbon chains did occur in the acid phase. It is also important to note that stearic acid (18:0) increased in both APDs and palmitic acid (16:0) increased in the APD of the FTG System.

Table 3 - LCFA content of the feed, average APD effluent, and average GPD effluent for both systems (mg/l COD)

System	Component	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3
FTA	Feed (sludge + FOG)	58	1	1197	25	468	1478	6994	1692
	APD Effluent	55	0	732	7	650	323	176	18
	GPD Effluent	8	0	152	1	83	71	9	0
FTG	Feed	51	0	445	19	202	388	55	2
	APD Effluent	49	0	613	6	379	177	26	1
	APD Effluent + FOG Addition	107	1	1811	31	847	1655	7020	1694
	GPD Effluent	20	0	250	1	75	107	17	1

A comparison of LCFA removal on a total and percentage COD basis is shown in Figure 6. This figure accounts for accumulated LCFAs in the digesters at the end of the sampling campaign as non-degraded material. The FTG System was found to have better removal efficiency (95%) compared to the FTA System (88%). The bottom portion of Figure 6 shows that the FTA System did not remove the 16:0 and 18:0 LCFAs as well as the FTG System. This is due to adsorption of the LCFAs onto sludge solids in the FTA System APD, where the saturated acids were entrapped and not degraded because they weren't transferred to the GPD.

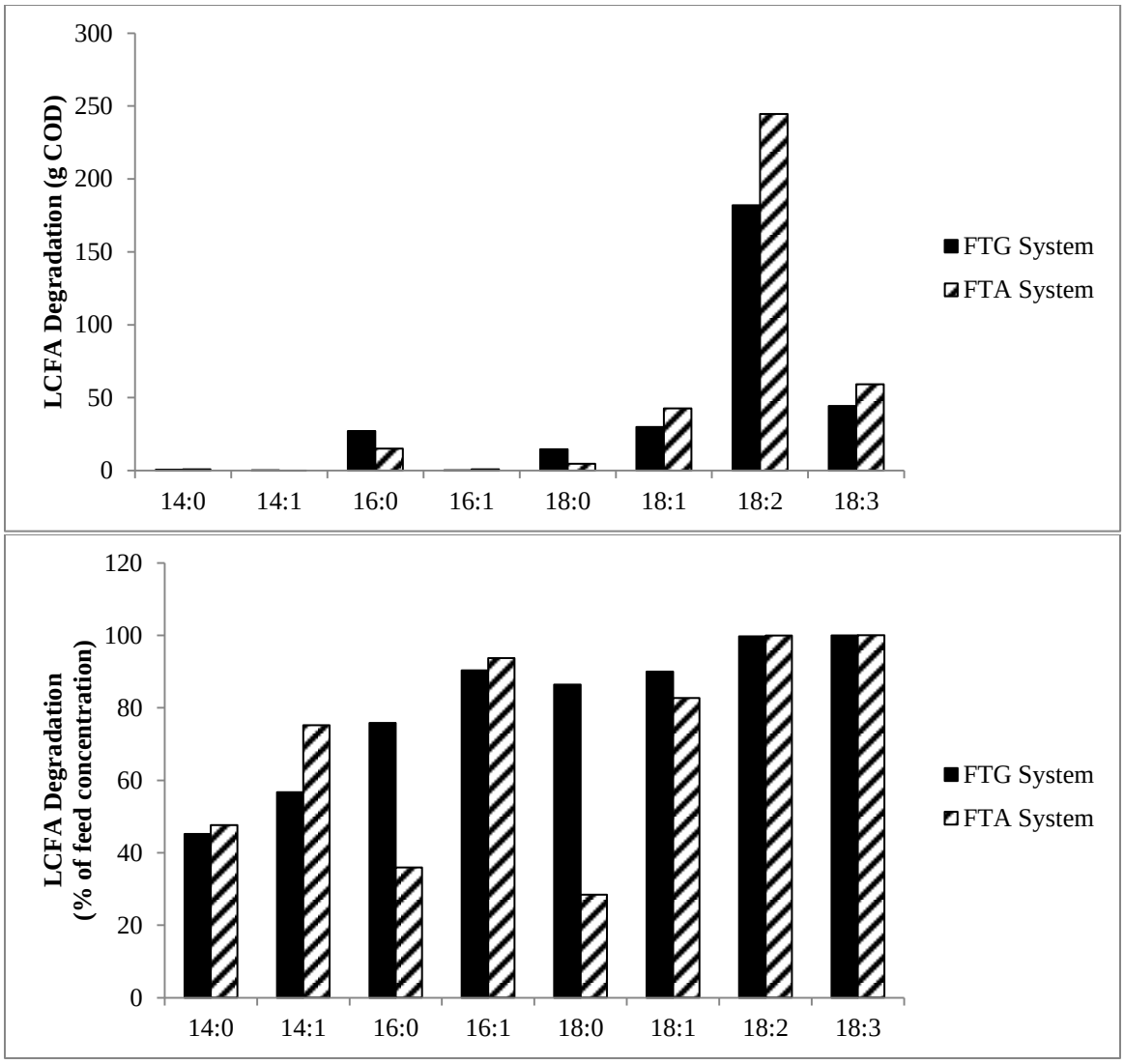

Figure 6 - LCFA degradation in both two-phase systems as COD (top) and % of COD concentration in feed (bottom)

Total accumulation of LCFAs in the acid-phase digester of the FTA System was 34.8 g, which accounts for 8% of the LCFAs added to the system. Accumulation also occurred in the GPDs in both the FTA System (9.6 g) and the FTG System (7.4 g), although to a much lesser extent. Figure 7 shows the accumulated LCFAs from all digesters except the FTG System APD, which received no FOG and had no accumulated LCFAs. The accumulated LCFAs shown in Figure 7 were selected as a qualitative representation of the median of the size distribution. The accumulated LCFAs from the FTA System APD are similar in size to those observed in the acid-

phase codigestion study. Because these are greater than ¼", they were not able to be removed even after the pre-sampling rapid mix procedure was implemented. Therefore, the shape of the accumulated material in the FTA system GPD may be influenced by accumulation on a microscopic level in the APD. This implies that once accumulation begins it is difficult to reverse, even by transferring to a GPD. It is interesting to note the appearance of the accumulated LCFAs from the FTG System GPD because it appears to show adsorption of the LCFAs to particles in the sludge. This indicates that solids must be present in significant amounts in order to support accumulation of LCFAs.

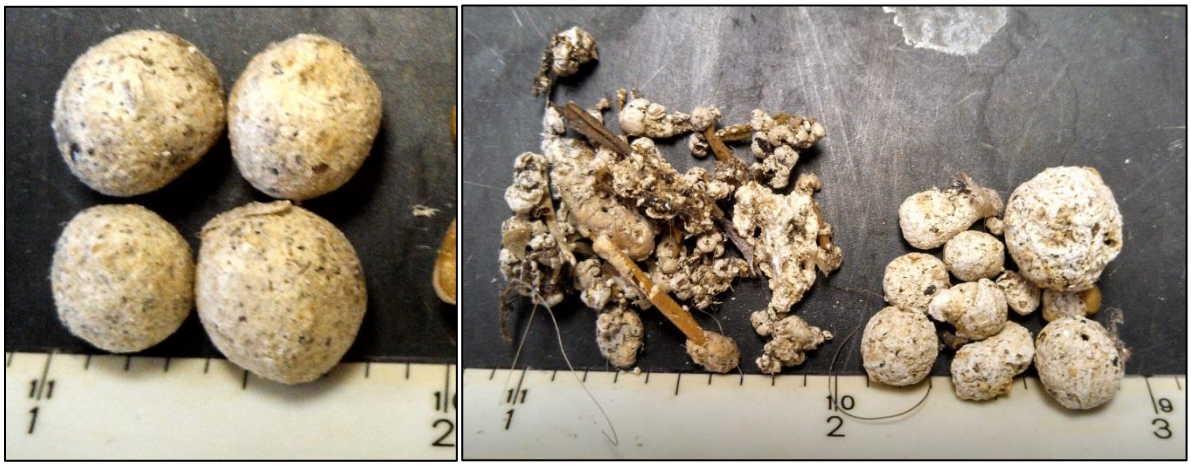

Figure 7 - Accumulated LCFAs after drying at 37°C from the FTA System APD (left), the FTG System GPD (middle), and the FTA System GPD (right) (ruler shown in inches)

An analysis of non-degraded LCFAs from both systems is shown in Figure 8. Non-degraded LCFAs passed through the systems and were measured in the GPD effluent, or accumulated in the digesters and collected at the end of the sampling campaign, then analyzed for LCFA content. Figure 8 shows that the GPD of each system is similar, with the accumulated and effluent LCFAs being mostly saturated and somewhat equal. The major difference in the two systems is the APD receiving FOG in FTA System. Because of the high level of accumulation leading to poor degradation, a large portion of LCFAs are non-degraded because they never

reach the gas-phase. This figure also shows that the loss of 16:0 between the feed and effluent of the FTA System APD is caused by accumulation and not degradation.

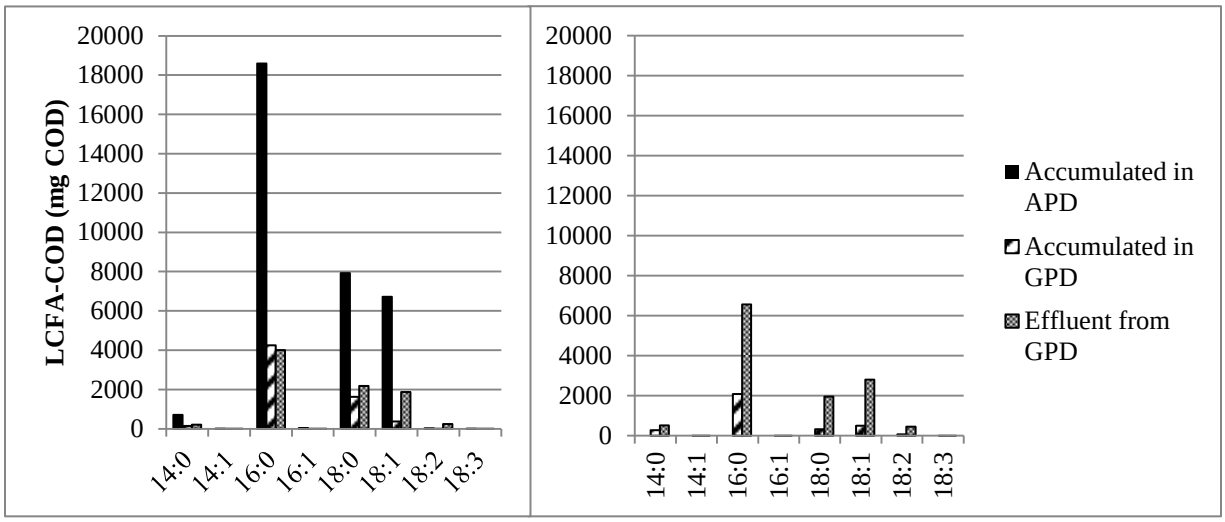

Figure 8 - Fate of non-degraded LCFAs in the FTA System (left) and the FTG System (right)

Discussion

Table 3 shows that while the majority of LCFAs in the FOG are unsaturated 18-carbon chains, the main LCFA in all digester effluents and accumulated material is 16:0. This is in agreement with Pereira et al. (2005) and Palatsi et al. (2012) , although they found there to be no other by-products, while the 18:0 LCFA and oleic acid (18:1) were found in significant quantities in this research. The single double-bonded 18:1 LCFA was present in the feed in significant quantities and may not have been completely degraded. While all LCFAs were found to be degraded at least partially in the two-phase system, the FTA System APD receiving FOG showed production of saturated LCFAs. This is shown in Figure 9 by negative values, which indicate that the sum of the LCFA in the effluent and accumulated material from the digester is greater than what was in the influent.

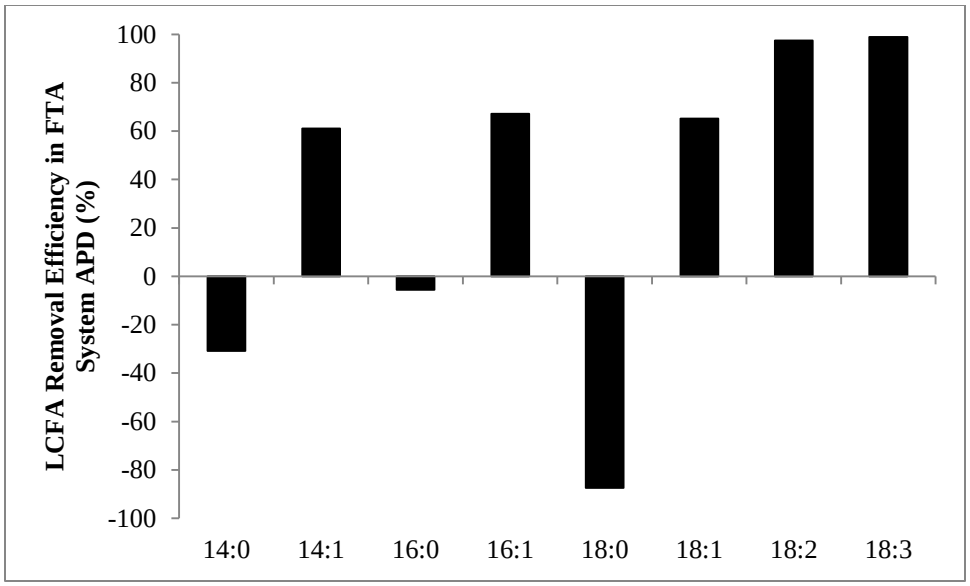

Figure 9 - LCFA removal efficiency in the FTA System APD as a percentage of the individual LCFA in the feed; negative values indicate production and accumulation

It is possible that the low pH of the acid-phase environment caused saturation of unsaturated 18-carbon chains where they would normally be β-oxidized to the 16:0 LCFA and further to myristic acid (14:0). The driving force behind this would be the concentration of H^+, which is 100 times greater at an acid-phase pH of 5.0 compared to a conventional or gas-phase environment pH near 7.0. A thermodynamic analysis of β-oxidation reactions was performed by Lalman and Bagley (2001), and shows that while conversion of 18:2 to 16:0 is most favorable, the reaction also produces an H^+ ion. The high H^+ ion concentration in the APD digester could make this reaction unfavorable so that 18:2 is converted to 18:1 instead, with no H^+ ion as a product. The same principle applies to β-oxidation of 18:1. Conversion of 18:1 to 16:1 or 16:0 produces an H^+ ion, so the low pH level of an APD would make these reactions less favorable, forcing 18:1 to be converted to 18:0 without any H^+ as a product.

It is still unclear why so much LCFA was accumulated into large balls in the FTA system APD instead of adsorbing to solids as smaller particles and being dispersed evenly through the solution. The high rate of saturation results in removal of double bonds and causes the molecules

to become less flexible. This could cause accumulation similar to the greater buildup of saturated fats compared to unsaturated fats in human arteries, where inflexibility leads to LCFA molecules becoming stuck to each other instead of flowing past.

Conclusions

These studies conclude that volatile fatty acid (VFA) production from acid-phase digestion of municipal sludge increases with fats, oils, and grease (FOG) addition. Degradation of long chain fatty acids (LCFAs) from FOG is low, however, and VFA production increases only marginally when compared to the theoretical VFA yield. LCFAs also accumulate into large balls that float at the digester liquid surface. This makes removal through wasting difficult and has the potential to cause significant operational problems in a full scale digester.

In two-phase systems, near-complete degradation of FOG was achieved with satisfactory gas production and volatile solids reduction. Adding FOG to the acid-phase digester (APD) results in significant accumulation and could cause severe operational issues. The APD removes a large fraction of unsaturated LCFAs, but produces saturated LCFAs in greater amounts than the amount added. Further work is necessary to identify the role of pH during acid-phase degradation and accumulation of LCFAs.

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Degradation and Accumulation of Long Chain Fatty Acids in Acid-phase Codigestion of Municipal Biosolids

Abstract

The degradation and accumulation of long chain fatty acids (LCFAs) was studied in acidogenic codigestion of wastewater sludge and fats, oils, and grease (FOG). The acid-phase digesters (APDs) were operated as sequencing batch reactors (SBRs) at two or three day solids retention times (SRTs) at 37°C. Adding high FOG loadings to an APD not acclimated to LCFAs resulted in near complete saturation of unsaturated LCFAs and significant accumulation of LCFA material in the digester, which was found to be mostly saturated. Non-degraded LCFAs with zero or one double-bond were found to preferentially accumulate rather than pass through the system through wasting. While 18:2 and 18:3 LCFAs were nearly completely removed, 18:0 and 14:0 LCFAs were produced, most likely from the degradation of 18:2 and 18:3 LCFAs. The APD pH was found to have a significant impact on the amount of accumulated LCFA material present. While degradation was unaffected, the amount of accumulated LCFAs in the digester at a pH of 4.9, 5.7, and 6.9 was 34.8 g, 14.4 g, and 0.0 g, respectively. Digester pH and sludge alkalinity are important parameters in codigestion with FOG in a two-phase digestion system.

Introduction

Two-phase digestion (TPD) is an advanced anaerobic, biological treatment system for treating sludges from municipal wastewater treatment plants that is growing in popularity due to its ability to produce more biogas over a conventional digestion system. TPD spatially separates the individual anaerobic microbial processes into two phases, acidogenesis followed by methanogenesis, allowing for each to operate more efficiently. The acid-phase digester (APD) utilizes a short solids retention time (SRT) to kinetically inhibit methanogens so that hydrolysis and acidogenesis can take place at a lower pH that is more conducive to these reactions. Fat, oil, and grease (FOG) codigestion is also becomingly more widely used because it can increase biogas production from anaerobic digestion of sludges. However, the short SRT of an APD

washes out the microbial communities responsible for the syntrophic interactions that make degradation of FOG thermodynamically feasible (Palatsi et al. 2012).

FOG is a complex, high strength, organic waste material from a variety of sources such as food processing facilities and restaurants. The most notable constituents of FOG are triglyceride esters, which are rapidly hydrolyzed to produce long chain fatty acids (LCFAs) during degradation (Heukelekian and Mueller 1958). LCFAs are commonly denoted by the number of carbon atoms in the chain followed by the number of double bonds in the chain. For example, oleic acid has an 18-carbon chain and one double bond so it is denoted as 18:1.An early study into anaerobic degradation of LCFAs was conducted by Novak and Carlson (1970). They found that the primary mechanism of degradation is β-oxidation and that unsaturated LCFAs degraded more quickly than the saturated versions, although unsaturated LCFAs need to first become saturated before β-oxidation can take place.

More recent studies have found that saturation is not necessarily required for unsaturated LCFAs to undergo β-oxidation. Studies have found that palmitic (16:0) and myristic (14:0) acids are the major by-products of oleic acid (18:1) degradation (Lalman and Bagley 2001), and that 18:1 and palmitoleic acid (16:1) are transient products that were degraded further to 16:0 and 14:0 in the degradation of linoleic acid (18:2) (Lalman and Bagley 2000).

FOG has been found to accumulate in a solid or semi-solid form in codigestion systems, eventually leading to digester failure by sludge flotation and washout of sludge granules if not removed from granular sludges (Hwu et al. 1998a). Several studies have determined 16:0 to be the main LCFA adsorbed to biomass in enhanced granular sludge bed (EGSB) digesters degrading 16:0 or 18:1 (Palatsi et al. 2012, Pereira et al. 2005). Other studies have found that using continuous or semi-continuous digesters resulted in increased gas production from FOG

addition with no mention of accumulated FOG in the digesters (Kabouris et al. 2008, Kabouris et al. 2009, Davidsson et al. 2008, Luostarinen et al. 2009).

Miron et al. (2000) found that the pH of digesters operating below an 8 day SRT was just below 5.0 and that saturated LCFAs with 12-, 14-, 16-, and 18-carbon chain lengths accumulated while unsaturated LCFAs with 16- and 18-carbon chain lengths were easily degraded. It was suggested that the accumulation of 18:0 could be due to high partial pressures of hydrogen gas (Miron et al. 2000) or high concentrations of hydrogen ions because the pH was significantly lower than neutral.

Materials and Methods

Feed Characteristics

All municipal sludges were obtained from the nearby Christiansburg, VA Wastewater Treatment plant. Seed sludge was taken from an anaerobic digester degrading combined biosolids from the primary clarifier and thickened waste activated sludge (WAS). The feed sludge used in the studies was a mixture of 70/30 v/v primary and thickened WAS that was diluted with tap water to 2.5% total solids. FOG was shipped to the lab from the Hampton Roads Sanitation District wastewater treatment plant in Williamsburg, VA. The FOG was from a single source and sampled from a truck before mixing with FOG from other sources. This ensured homogeneity of the FOG used in the study. The characteristics of the feed components for the digesters are shown in Table 4.

Table 4 - Characteristics of Digester Feed Components

Component	Feed Sludge	FOG
COD (g/l)	37.8 (± 0.77)[a]	1,560 (± 577)
Total Solids (% of total mass)	2.48 (± 0.44)	57.2 (± 3.14)
Volatile Solids (% of total mass)	2.03 (± 0.44)	57.0 (± 3.06)
Lipids/LCFAs (% of total mass)	N.D.	63.7
LCFAs (g/l COD)	1	1,453

[a] Bracketed values show 95% confidence interval

High FOG Loading on a Non-acclimated Digester

Two two-liter acid-phase anaerobic digesters were constructed and operated as completely mixed Sequencing Batch Reactors (SBRs). Each digester was sealed to be airtight and kept at 37°C. Continuous mixing was accomplished using headspace gas recirculation via a peristaltic pump. A 3-day SRT was maintained by manual feeding and wasting, daily. Great care was taken to limit the amount of air exposure so as to mitigate any oxygen toxicity effects. Because the digester had previously received a low concentration of FOG, none was added for 27 days to allow for all β-oxidizing microbes to wash out. After this period, a high loading of FOG was applied to the experimental digester so that 60% of the total COD in the feed was from the FOG addition. Samples were taken daily from the wasted effluent for VFA, LCFA, and pH analysis.

FOG Digestion at Controlled pHs

Four 2-liter acid-phase digesters were operated as completely mixed SBRs at an SRT of 2 days and temperature of 37°C using headspace gas recirculation for mixing. As shown in Table 5, one digester served as a control and received no FOG, one digester received 20% FOG addition but no buffer (pH 4.9), two received 20% FOG addition and buffer to control for a higher pH level (pH 5.7 and pH 6.9).

Table 5 - Operating parameters for FOG codigestion at controlled pH study

FOG Addition (% of total COD)	Steady-state pH	Buffer Addition ($g\text{-}day^{-1}$)
0	4.9	None
20	4.9	None
20	5.7	5.00
20	6.9	10.00

The buffer, anhydrous dibasic potassium phosphate (K_2HPO4), and FOG were mixed into the feed sludge before the feed/waste procedure. FOG addition began at startup for the experimental digesters, which were operated for 30 days before sampling began. Samples were taken daily for VFA, LCFA, and pH analysis.

Analytical Methods

Total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) were determined by Standard Methods 2540B, 2540B, and 5220C respectively (Clesceri et al. 1998); pH was measured with a pH probe and meter. Gas production was determined for all digesters, but it was determined to be negligible (<1% of COD in feed) and therefore not included here.

The weight percentage for the lipids/LCFA component of the FOG was determined using a hexane extraction procedure based on Standard Method 5520D (Clesceri et al. 1998). Instead of repeated washing in a separatory funnel, the samples were centrifuged after adding the hexane and MTBE mixture in order to separate the hexane and aqueous layers. The hexane was allowed to evaporate, leaving the lipid/LCFA material behind for quantification.

LCFA samples were prepared for gas chromatograph (GC) analysis by conversion to Fatty Acid Methyl Esters (FAMEs). This was achieved by an esterification process after extracting the lipids/LCFAs as described above. A transesterification fluid containing mostly methanol was added to the extracted lipid/LCFA sample in excess and heated to 90°C for 30

minutes. This replaces the carboxylic acid with a methane group. The esterification process also severs the glycerin head from the triglyceride molecule so that the individual LCFAs are represented in the analysis, irrespective of the parent triglyceride. An HP 5890 GC using a flame ionization detector (FID) was equipped with a Supelcowax 10 fused silica capillary column (30m x 0.32mm ID x 1.00 µm film thickness) for the analysis. Helium was the carrier gas and the program ran from 140°C to 260°C over 30 minutes.

VFAs were also analyzed with FID on a HP 5890 GC. A Nukol column (15m x 0.53mm x 0.5µm film thickness) was used with helium as the carrier gas and a program from 80°C to 140°C over 10 minutes.

Results

High FOG Loading on a Non-acclimated Digester

In order to easily track LCFA degradation and accumulation in a completely mixed SBR digester system, a high loading rate of FOG was added on non-acclimated sludge. Because the digester was not acclimated to FOG, initial adsorption of LCFAs onto sludge solids was included in the mass balance calculation. An SRT of three days was used to allow for manual feeding once per day, which reduced the chance for floating, accumulated LCFA material to escape through wasting. Accumulated LCFAs in the waste stream were not accounted for in the mass balance calculation, but was negligible. Daily FOG loading equal to 60% of the total COD began for the experimental digester at Day 0 and continued through the entire sampling campaign while the digesters continued to operate as completely mixed SBRs. The digesters had not been previously acclimated to FOG or LCFAs.

An increase in VFA concentration and drop in pH was observed, as shown in Figure 10, but it is most likely that this is due to changes in the feed sludge composition and not the high loading of FOG. The high loading of FOG did not significantly affect digester pH or VFA concentration compared to the control digester.

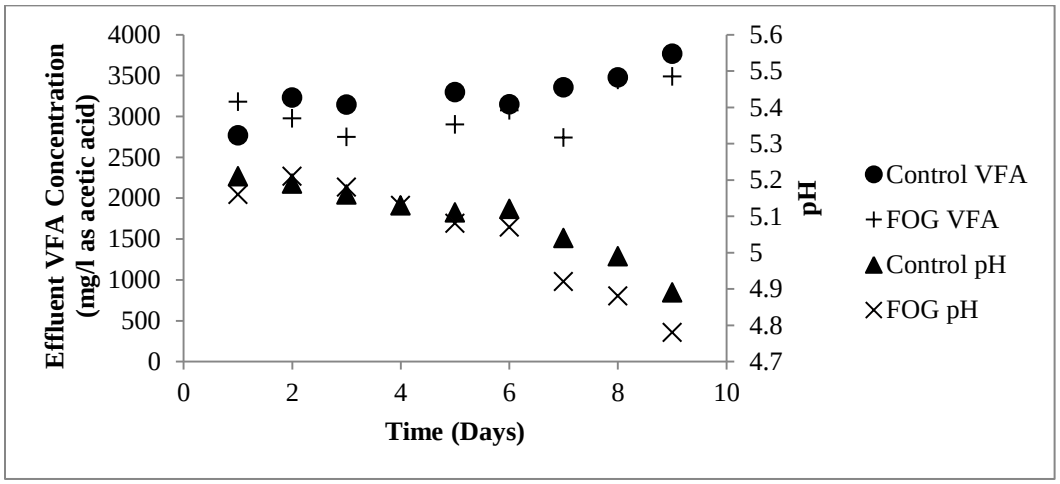

Figure 10 - pH and total VFA concentration in the effluent of both digesters during the high FOG loading on a non-acclimated digester study

The major component of the FOG used in this study was linoleic acid (18:2) and represented 61% of the LCFA-COD in the feed receiving 60% FOG, as shown in Table 6. This means 18:2 accounted for 37% of the total COD in the feed receiving 60% FOG. It is important to note the limitations of the extraction procedure used for analysis of LCFAs. Assuming no degradation of LCFAs in the APD, as evidenced by no difference in VFA production between the control digester and the one receiving FOG, recovery of LCFAs from the effluent was only 44%. This is essentially due to flotation of sludge solids in the extraction procedure. This material was discarded, but likely contained LCFAs because it floated above the aqueous layer. These LCFAs were probably adsorbed onto the biomass surface and were most likely 16:0, as found in previous studies (Palatsi et al. 2012, Pereira et al. 2005, Jeganathan et al. 2006). The

LCFA content of the '60% FOG Feed' is accurate, because nearly all LCFA content comes from FOG addition; analysis of LCFAs in FOG was not affected by extraction errors.

Degradation was low in the control digester compared to the digester receiving FOG, although the same degradation pattern was observed in each; unsaturated 18-carbon LCFAs were significantly removed while shorter chain and saturated LCFAs were degraded to less of an extent. An accumulation of 31.2 g of LCFA material was collected at the end of the sampling campaign from the reactor vessel and analyzed for LCFA content, as shown in Table 6. Because this material was collected at the end of the sampling campaign, it was normalized to the sum of the feed volume over the entire sampling campaign.

Table 6 - LCFA content (mg/l COD) in feed components, digester effluents, and accumulated LCFA material (normalized to feed volume) for 60% FOG loading on a non-acclimated digester study (effluent as average of all samples)

	14:0	**14:1**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**	**Total**
Control Feed	105	0	625	88	723	258	144	28	1971
60% FOG Feed	125	1	2867	107	1514	3504	20819	5064	34000
Control Effluent	63	0	426	23	614	110	74	13	1324
FOG Effluent	78	0	617	34	886	670	2354	602	5243
Accumulated FOG	276	1	2244	41	2779	2113	5	3	7462

A mass balance of the digester receiving FOG was conducted and the data are shown in Figure 11. This approach accounts for all LCFAs added as FOG and all LCFAs in the digester effluent and accumulated material throughout the sampling campaign. Figure 11 shows positive values for degradation and negative values for production for each of the individual LCFAs analyzed. While the unsaturated 18-carbon LCFAs were degraded, the saturated LCFAs were produced faster than they were removed from the system. Almost all LCFA-COD was lost from the removal of the 18:2 and linolenic acid (18:3) LCFAs, while nearly all LCFA-COD gained came from production of stearic acid (18:0). The bottom portion of Figure 11 shows that the 18:0

LCFA found in the effluent and accumulated LCFAs combined is nearly twice the amount that was added to the digester as FOG. Therefore, the 18:0 LCFA must have been a product of the degradation of unsaturated 18-carbon LCFAs.

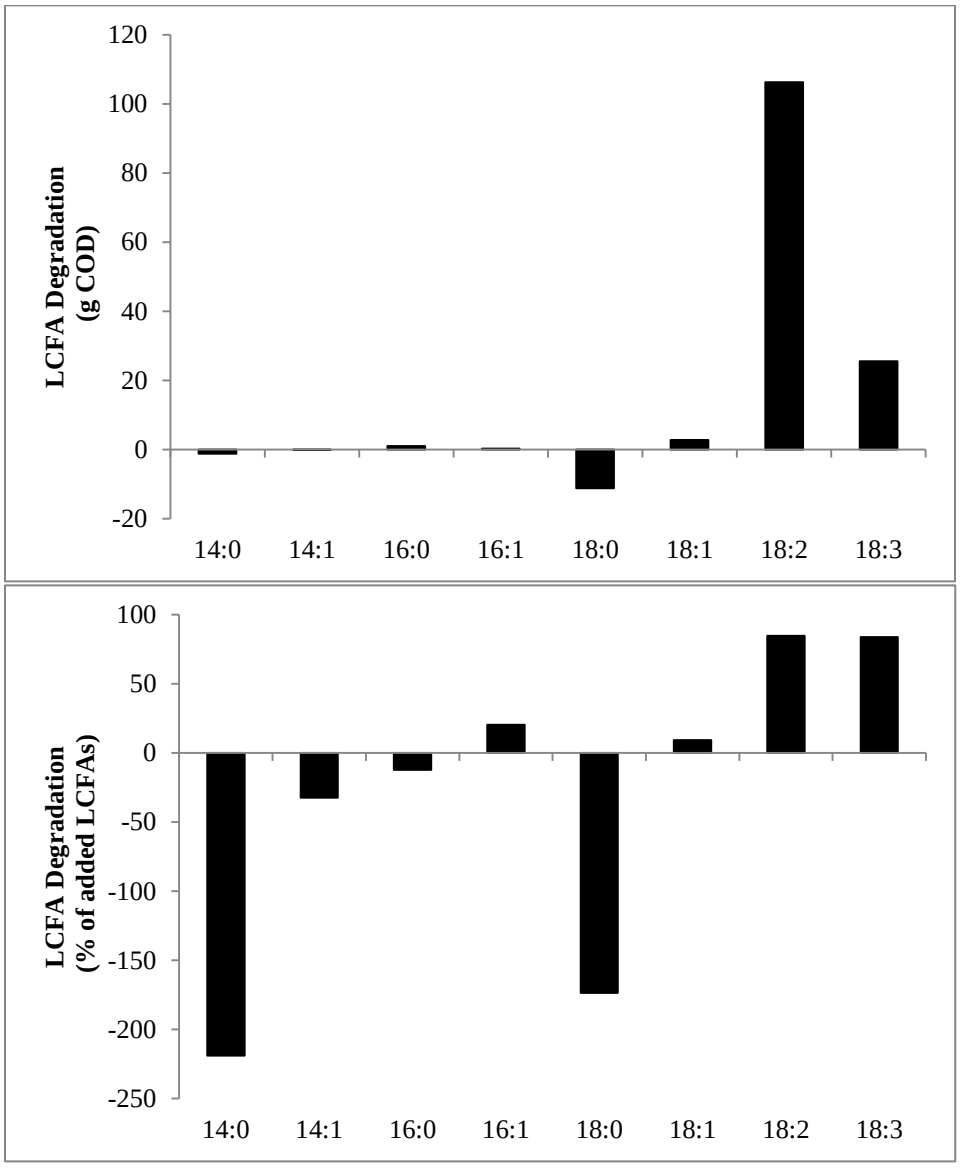

Figure 11 - Degradation of LCFAs in g COD (top) and % of individual LCFA added (bottom) with negative numbers indicating production

The production of 18:0 is surprising because it is not a known primary intermediate of the degradation of unsaturated 18-carbon chains. The presence of 18:0 could be a degradation

intermediate of longer chain LCFAs (longer than 18 carbons) that were not analyzed here. A more probable explanation is that that high H^+ ion concentration or high partial pressure of H_2 gas causes saturation of unsaturated 18-carbon LCFAs and accumulation of 18:0, as suggested by Miron et al. (2000).

The sum of accumulated LCFAs from the digesters and the sum of LCFAs in the effluent are shown as non-degraded LCFAs in Figure 12. The 18:2 and 18:3 LCFAs are more soluble than shorter and more saturated LCFAs, and pass through the system through wasting. But LCFAs with one or zero double bonds preferentially adsorb to sludge solids or other LCFAs and end up in the accumulated LCFA matter found floating at the liquid surface. The presence of unsaturated LCFAs in this accumulated material might be an indication that the LCFA was adsorbed onto the sludge solids but was not degraded due to the short detention time used. This is in agreement with previous studies that have found adsorption of unsaturated LCFAs onto sludge solids to be the first step in degradation (Hwu et al. 1998b), although they are inhibitory to β-oxidation of LCFAs (Pereira et al. 2002).

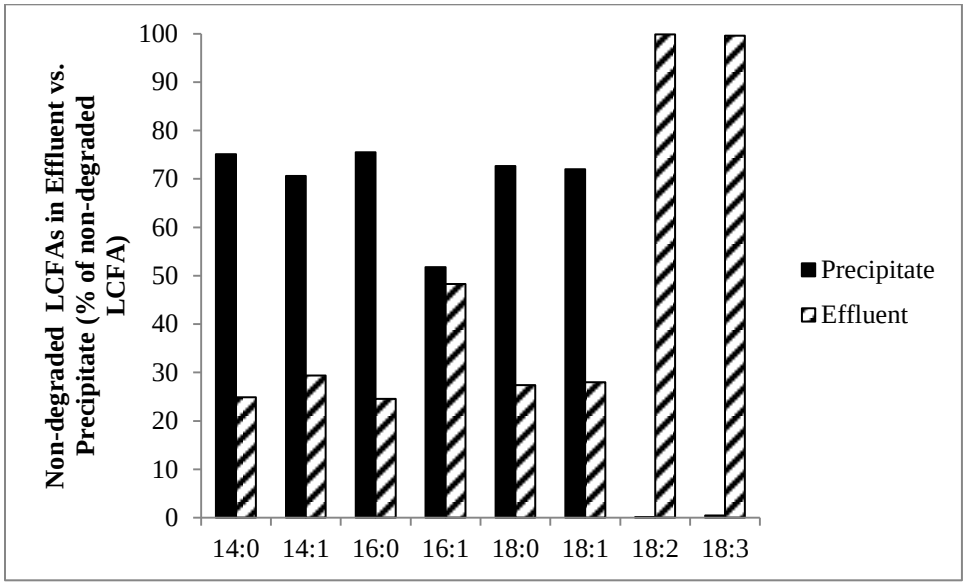

Figure 12 - Fate of non-degraded LCFAs in 60% FOG digester (sum over entire sampling campaign)

FOG Digestion at Controlled pHs

Four acid-phase digesters were operated simultaneously where the control received no FOG, one digester received 20% FOG addition but no buffer (pH 4.9), and two received 20% FOG addition and buffer to control for a higher pH level (pH 5.7 and pH 6.9). The digesters reached steady state, as determined by stability of the pH of the effluent, before sampling began and a constant FOG addition continued through the entire sampling campaign. Total VFA and LCFA concentrations, as well as the mass of accumulated LCFA material, from each digester is shown in Table 7.

Higher pH levels were accompanied by higher total VFA and LCFA concentrations in the digester effluents and a lower mass of accumulated LCFAs in the digester. However, Table 7 shows that the VFA concentrations are highly variable across the sampling campaign, and are not necessarily significant. The increase in total LCFA concentration in the effluent was larger than the increase in total VFA concentration. This means the decrease in accumulated LCFAs is probably due to higher pH levels causing LCFAs to be more soluble, or at least less likely to adsorb to sludge solids.

Table 7 - Total VFA concentrations and pH from digester effluent, average of all 10 samples

	pH	Total VFA Concentration (mg/l)	Total LCFAs in Effluent (mg/l)	Total Accumulated LCFAs (g)
Control	4.88 (±0.21) [a]	2472 (±1163)	1055 (±304)	0.0
20% FOG, pH 4.9	4.87 (±0.11)	2556 (±906)	2233 (±506)	34.8
20% FOG, pH 5.7	5.67 (±0.15)	3039 (±1099)	2745 (±1089)	14.4
20% FOG, pH 6.9	6.91 (±0.16)	3092 (±511)	3592 (±1471)	0.0

[a]Bracketed values indicate 95% confidence interval

The composition of the digester feed and effluents are shown in Table 8. The same pattern of degradation occurred as with the mass balance study. Unsaturated LCFAs were nearly completely removed while saturated LCFAs were produced or not well degraded. Unlike the

mass balance study, the palmitic acid (16:0) was removed, although to a much smaller extent than the unsaturated acids. All LCFAs were found in higher concentrations in the effluent at the higher pH levels of the digesters. The same issues that affected LCFA extraction from sludge also apply in this study. Due to LCFA adsorption onto biomass, only 30% of LCFAs were recovered, with the remaining 70% presumably being adsorbed to biomass that passed through the effluent. This assumes no degradation of LCFAs in the digester, but given the high variability of the VFA production, it is likely that this is an appropriate assumption. VFA production was not significantly different in each digester and therefore LCFAs were likely not degraded.

Table 8 - LCFA content in feed components and digester effluents (mg/l COD) for pH study (effluent as average of all samples)

	14:0	**14:1**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**
Control Feed	51	0	445	19	202	388	55	2
20% FOG Feed	58	1	1197	25	468	1478	6994	1692
Control	34	0	446	5	397	140	28	4
20% FOG, pH 4.9	50	0	720	6	700	418	270	29
20% FOG, pH 5.7	53	0	835	2	1451	293	113	14
20% FOG, pH 6.9	76	0	1076	3	1511	629	305	35

The sum of individual LCFAs in the digester effluents over the sampling campaign is shown in Figure 13 along with an analysis of the accumulated LCFAs collected from the digesters at the end of the sampling campaign. This agrees with the mass balance from the high FOG loading on a non-acclimated digester study in that more saturated LCFAs are likely to be found in the non-degraded, accumulated LCFA material floating at the liquid surface of the digester. Higher pH levels correlate to less LCFA accumulation.

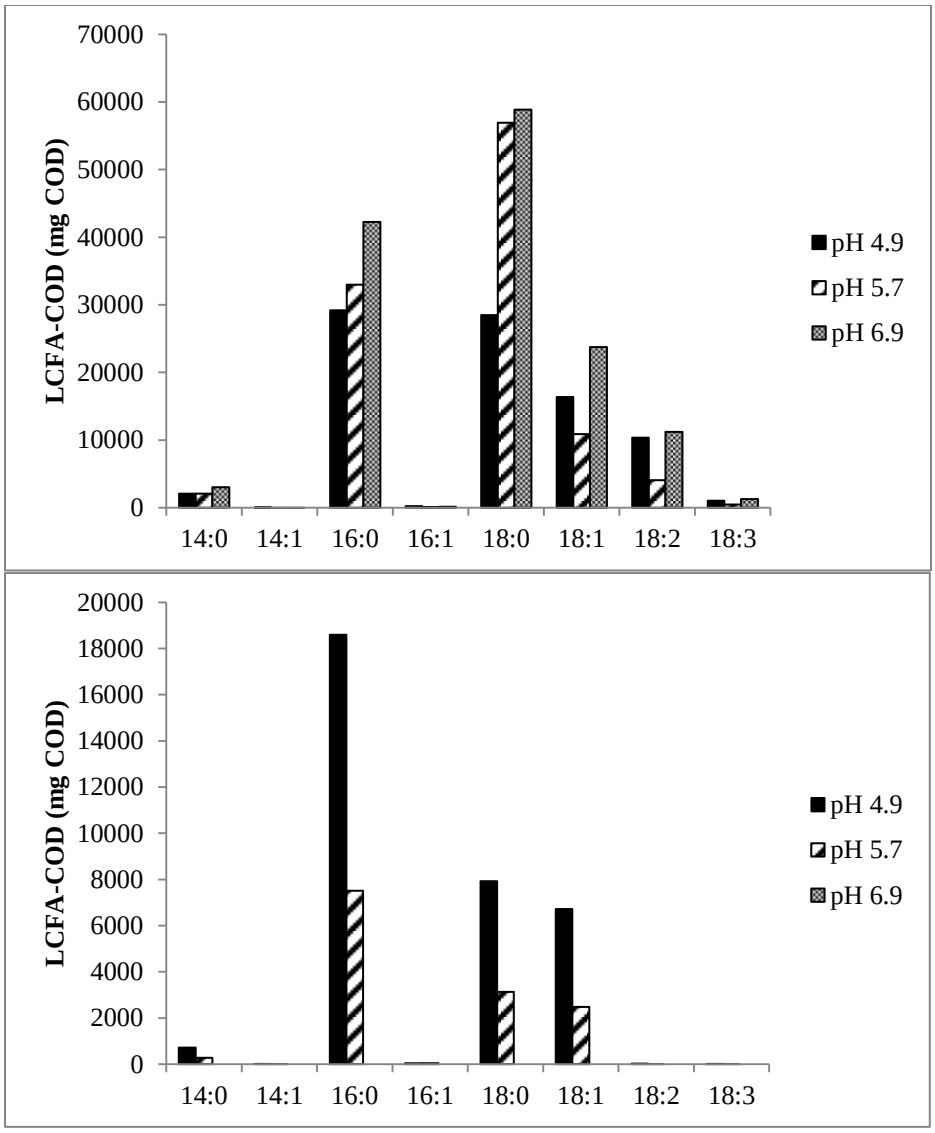

Figure 13 - Non-degraded LCFAs in the effluents (top) and accumulated FOG (bottom) from digesters receiving 20% FOG (mg COD)

A mass balance of the digesters receiving FOG was conducted and the data are shown in Figure 14. This approach accounts for all LCFAs added as FOG and all LCFAs in the digester effluents and accumulated material throughout the sampling campaign. Figure 14 shows positive values for degradation and negative values for production for each of the individual LCFAs analyzed. While the unsaturated LCFAs were degraded, the saturated LCFAs were produced faster than they were removed from the system. Almost all (>70%) LCFA-COD was lost from

the removal of the 18:2 LCFA, while nearly all LCFA-COD gained came from production of 18:0. The bottom portion of Figure 14 shows that the 18:0 LCFA found in the effluent and accumulated LCFAs combined is nearly twice the amount that was added to the digester as FOG. Therefore, the 18:0 LCFA must have been a product of the degradation of unsaturated 18-carbon LCFAs.

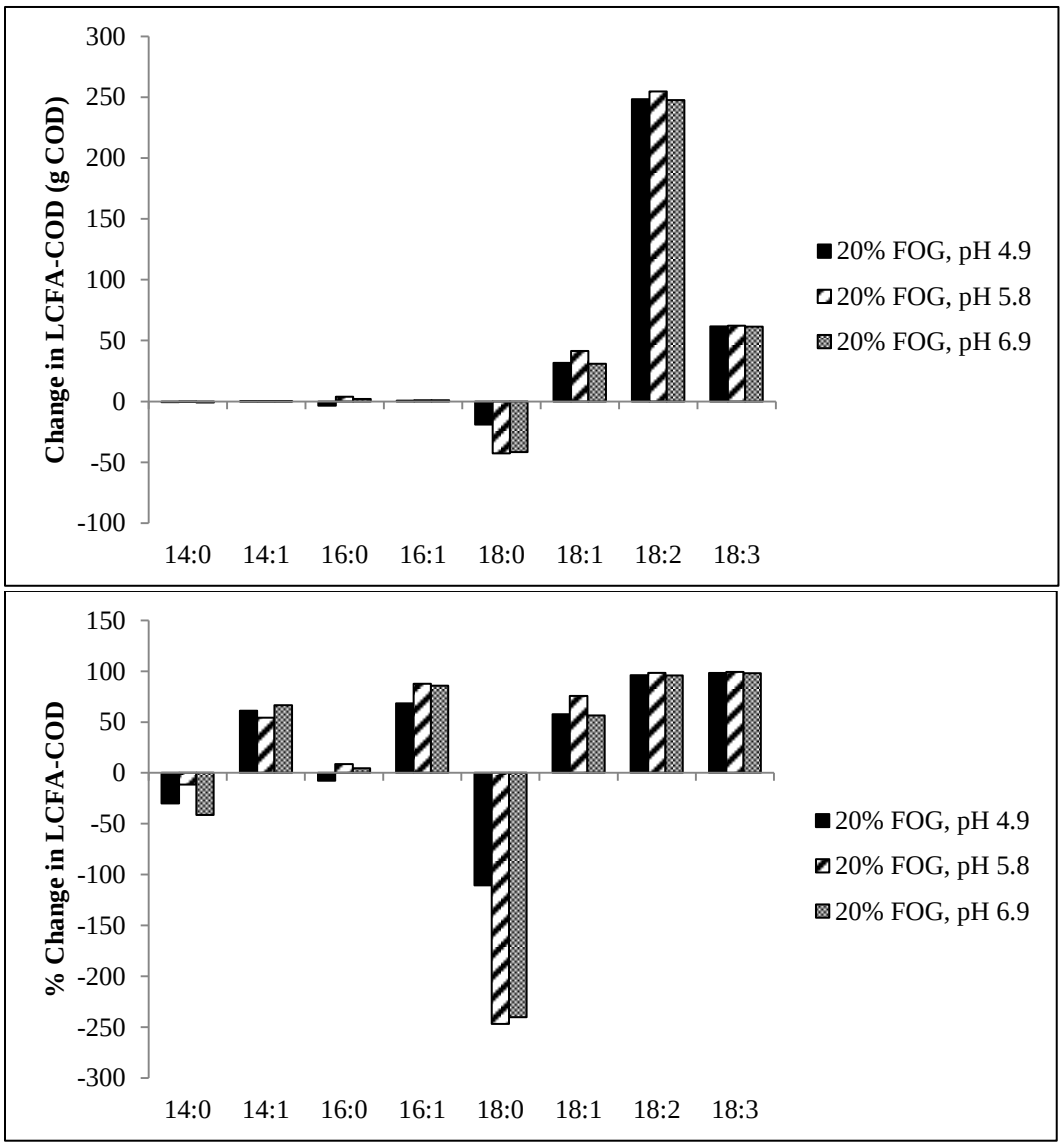

Figure 14 - Degradation of LCFAs in g COD (top) and % of individual LCFA added (bottom) with negative numbers indicating production

It is important to note that the digester had the same degradation pattern. This implies that pH does not affect the degradation of LCFAs in the digester. This is shown again in Figure 15, which shows that each digester had approximately the same amount of non-degraded FOG, but that higher pH levels correlate to less accumulated LCFA material.

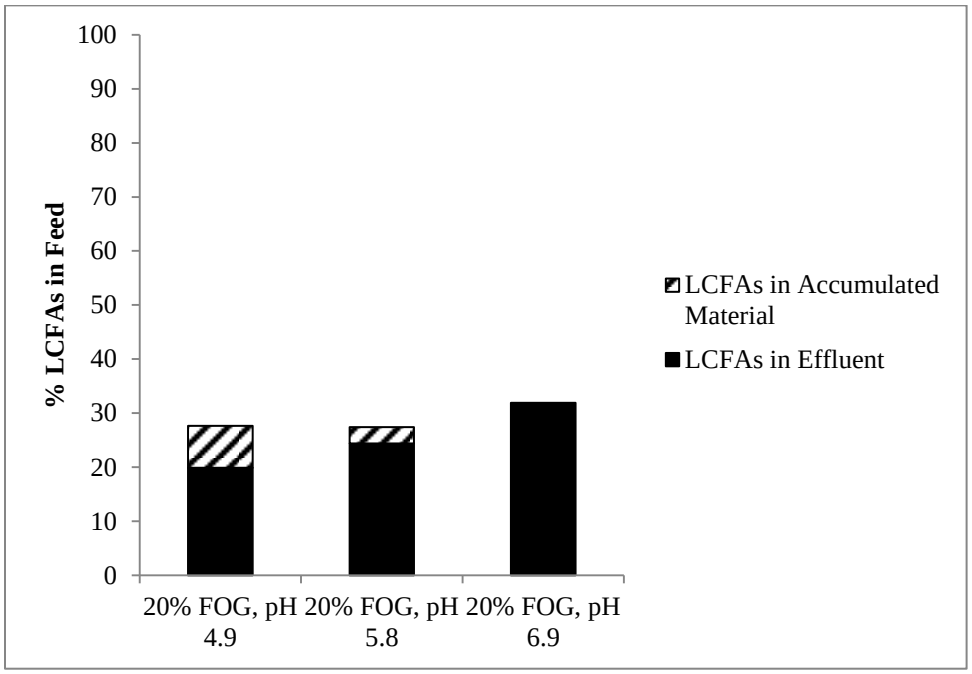

Figure 15 - Fate of non-degraded LCFAs in the digesters receiving FOG

Discussion

While pH doesn't seem to affect production of intermediates, it does significantly affect the accumulation of LCFAs. Accumulation of LCFAs can occur due to precipitation with divalent cations (Pereira et al. 2005). Because the buffer used in this study was dibasic potassium phosphate (K_2HPO_4), a large amount of monovalent K^+ ions were present in solution. This could have decreased the ratio of divalent to monovalent ions in solution and made precipitation of LCFAs with divalent ions less favorable. While precipitation is likely the cause of LCFA accumulation, adsorption probably accounts for the LCFAs that were not detected in the analysis procedures. These LCFAs were adsorbed to the cell walls of biomass and escaped in the effluent.

Several studies have suggested that FOG addition to acidogenic digesters is an effective way to minimize inhibition of acetoclastic methanogens in a subsequent methanogenic digester (Komatsu et al. 1991, Kim et al. 2004, Kim and Shin 2010, Beccari et al. 1998). However, the minimum operating pH of any of the digesters in these studies was 6.0, and none of these studies mention accumulation of LCFAs in any significant amount. The digesters in this research maintained a pH of 4.9 without the addition of buffer and showed significant accumulation of LCFA material. This suggests that alkalinity is an important parameter to consider for FOG codigestion with municipal biosolids in practice. While addition of FOG to the acidogenic digester of a two-phase digestion system can increase biogas production, sufficient alkalinity must be present to maintain an approximately neutral pH and prevent the accumulation of LCFA material.

These studies suggest that 18:0 is an intermediate in the degradation pathway of unsaturated 18-carbon LCFAs. It is possible that the low pH of the acid-phase environment caused saturation of unsaturated 18-carbon chains where they would normally be β-oxidized to the 16:0 LCFA and further to myristic acid (14:0). The driving force behind this would be the concentration of H^+, which is 100 times greater at an acid-phase pH of 5.0 compared to a conventional or gas-phase environment pH near 7.0. A thermodynamic analysis of β-oxidation reactions was performed by Lalman and Bagley (2001), and shows that while conversion of 18:2 to 16:0 is most favorable, the reaction also produces an H^+ ion. The high H^+ ion concentration in the APD digester could make this reaction unfavorable so that 18:2 is converted to 18:1 instead, with no H^+ ion as a product. The same principle applies to β-oxidation of 18:1. Conversion of 18:1 to 16:1 or 16:0 produces an H^+ ion, so the low pH level of an APD would make these reactions less favorable, forcing 18:1 to be converted to 18:0 without any H^+ ion as a product.

This is evidenced by lower production of 18:0 at pH 6.9 compared to pH 4.9 or 5.7, as shown in Figure 14.

A high partial pressure of hydrogen gas could also cause saturation of unsaturated 18-carbon LCFAs. The short SRTs used (2 days for the controlled pH study and 3 days for the high FOG loading on a non-acclimated digester study) prevent hydrogenotrophic methanogens from becoming established, which could result in a buildup of H_2 gas (Beccari et al. 1998). A higher partial pressure of H_2 gas could cause saturation of unsaturated 18-carbon LCFAs during β-oxidation. The effect of higher partial pressures of H_2 could be exacerbated by the gas recirculation system used for mixing, which creates more liquid-gas interaction area than a conventional mechanically mixed digester.

Conclusion

These studies show that acid-phase codigestion of fat, oil, and grease (FOG) leads to production and subsequent accumulation of saturated long chain fatty acids (LCFAs) in the form of solid, accumulated LCFA material which floats at the liquid surface of the digester. While the 18:0 and 14:0 LCFAs are the major intermediates produced, all non-degraded LCFAs with zero or one double bonds will become trapped in the digester instead of passing through the system in the effluent. The low degradation is most likely due to the short retention time used for acid-phase digestion.

The pH significantly affects accumulation of LCFAs. Higher pH levels result in less accumulated LCFA material, but increase the concentration of LCFAs in the digester effluent. Future work should focus on controlled pH acid-phase digestion followed by methane phase digestion to assess effects on methane production and LCFA destruction.

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