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## Effect of Lipid Content on Anaerobic Digestion Process and Microbial Community: Review Study

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### Abstract

The indiscriminate release of significant amounts of food waste, fat oil and grease, and sewage sludge (SS) into the environment causes severe contamination in many nations. There are numerous potential treatment methods to cope with organic wastes, but anaerobic digestion is currently widely accepted to handle different kinds of biological waste. One of the pillars supporting anaerobic digester biogas production increase in treatment plants is the use of fats in the wastewater. However, it has been claimed that high-fat wastes, particularly mono-digestion in the anaerobic reactor, inhibit acetoclastic and methanotrophic bacteria delay the formation of gas even more, and overtax the system. The aim of this review is to review several publications that dealt with the effect of LCFs and FOG on AD performance and associated methane production and microbial communities, as well as the mechanism of LCFA generation and its inhibitory effect on anaerobic digestion performance, and also addressed the improvement of system efficiency using co-digestion with lipid wastes.

**Keywords:** Anaerobic digestion; lipid content; microbial community;

anaerobic co-digestion; methane generation

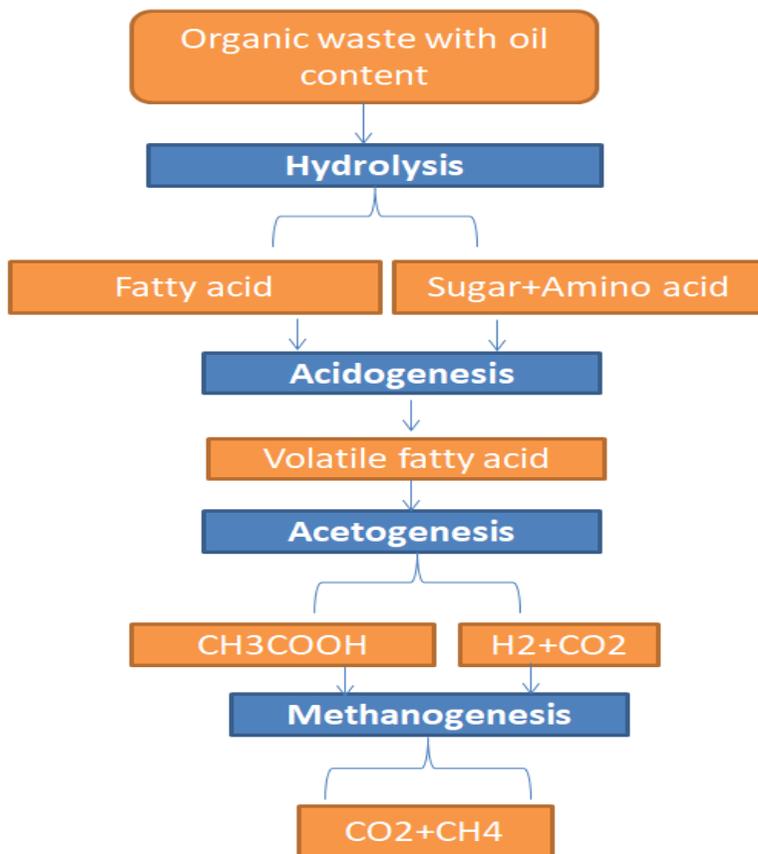
## Introduction

Fat in municipal wastewater comes from various places, including municipal garbage, industry (edible oil, food processing, and slaughterhouses), and trade (food trade). Lipids make up nearly 25% of the organic content in oily wastewater, which is derived from municipal wastewater (Chipasa et al., 2006). Fats, on the other hand, become a substantial contaminant in the effluents of palm oil factories (POME) at concentrations of more than 15,000 mg/L. While noted, the wastewater from the processing of meat and food contains a significant amount of lipid more than 35,000 mg/L (Ahmad et al., 2011; Nakhla et al., 2003; Quéméneur and Marty, 1994; Williams et al., 2012). The quantities of fats in municipal wastewater are classified as strong, medium, and weak, and their concentrations are 100, 90 and 40 mg/l, respectively. Additionally, there should be no more than 50 mg/L of fat and oil in industrial effluent that is released into public municipal sewers (Dehghani et al., 2014). Food waste (FW) is split into three categories: lipids, proteins, and carbs, with each having a different biodegradability or hydrolysis rate: (Lin et al., 2013; Sun et al., 2016) carbs > proteins > lipids, as a result, lipid breakdown is thought to be a rate-limiting stage in FW anaerobic digestion (AD) (Sun et al., 2016). In recent years, aside from their disposal, there has been a growth in interest in fat exploitation and the possibility for them to be used as a source of renewable energy, particularly in terms of waste recovery. The positive yield in biogas and methane production from high-fat wastewater has been widely reported (Davidsson et al., 2008; Palatsi et al., 2009). Benefiting from anaerobic digestion of lipid waste has become a potential source for energy production as the positive yield in biogas and methane production from high-fat wastewater has been widely reported (Luostarinen et al., 2009; Palatsi et al., 2009) as shown in Table 1.

**Table 1.** Potential biogas production from different classes of components

Item	Methane production	Reference
Lipid	1000 mL/gVS	Awe et al., 2018
Protein	480 mL/gVS	
Carbohydrate	373 mL/gVS	
Lipid	1.452 L/g	Alves et al., 2009
Protein	0.830 L/g	
Carbohydrate	0.921 L/g	
Lipid	0.99 L CH <sub>4</sub> /g	Neves et al., 2009
Protein	0.63 L CH <sub>4</sub> /g	
Carbohydrate	0.42 L CH <sub>4</sub> /g	
Lipid	69.5%	He et al., 2016
Protein	50%	
Carbohydrate	68%	

In fact, anaerobic digestion is a complex process involving several groups of bacteria, both anaerobic and facultative, which flow through a series of stages and in the absence of oxygen mainly in the formation of methane and carbon dioxide. Anaerobic digestion can be divided into three main steps, i.e. hydrolysis, acidogenesis/acetate formation and methanogenesis as shown in figure 1 (He et al.,2016; Gujer et al 1983). FW, fats are a mixture of vegetable oils and fats. Due to the increased synthesis of long-chain fatty acids (LCFAs), the concentration of lipids in FW would disrupt the AD process. This has been shown to pose a risk to the anaerobic bacteria community (Chen et al., 2010; Chen et al., 2008). Although lipids are biodegradable through biological processes, the presence of intermediates known as LCFAs inhibits biodegradation and becomes a major source of process instability such as biomass clogging, foaming and flotation, especially when lipid residues are used as the sole carbon source in anaerobic fermentation (Pereira et al. al., 2004; Noutsopoulos et al., 2007). The hydrolysis of neutral lipids produces long-chain fatty acids (LCFAs), which are fatty acids with aliphatic tails of 13–21 carbon atoms. Palmitate, stearate, and oleate are three of the LCFAs that are primarily produced during anaerobic digestion((Hanaki et al.,1981). In AcoD manure-based systems, waste fats, oils, and grease (FOG) are frequently added as common substrates; they are mostly derived from the fish, food processing, and olive oil-producing industries. Due to the buildup of LCFAs, fat-rich wastes can be very inhibitive, especially to the  $\beta$ -oxidation processes and methanogenesis (Hanaki et al.,1981). The biogas generation in the anaerobic process can be improved by the co-digestion of co-lipids. This was most likely owing to oil's increased biodegradation rate (perhaps approaching 100%) when compared to SS (around 60 %). As a result, when comparing the combined digestion of food waste to the single anaerobic digestion FW, the combined digestion of food waste (FW) can be achieved a higher methane yield (Luostarinen et al., 2009; Wan et al., 2011). Theoretically, the methane potential of lipids is 1014 L/kg-VS, a value clearly higher than that of carbohydrates (e.g., 370 L/kg VS for glucose) (Labatut et al., 2018). This paper reviews the scientific literature on the anaerobic digestion of lipids, inhibiting agents, and microbial community, and will highlight future research needed to improve lipid-content methane production by anaerobic digestion with other waste biomass.



**Figure 1.** Schematic representation of the main conversion processes in anaerobic digestion (He et al.,2016; Gujer et al 1983)

### An Overview of LCFA in Anaerobic Digestion

In an anaerobic environment, the lipids are first degraded to provide glycerol and free long-chain fatty acids (LCFAs). The main lipid degradation intermediates are LCFAs, which are then further transformed into hydrogen and acetate by acetogenic bacteria ( $\beta$ -oxidation process), and then to methane by methanogenic archaea (Meng et al.,2015). Interspecies hydrogen transport among microorganisms in methanogenic settings is crucial for the breakdown of LCFAs. Obligate syntrophic communities of proton-reducing acetogenic bacteria degrade LCFA by converting them to acetate, hydrogen/formate, acetoclastic methanogenic archaea, and methanogenic archaea that consume hydrogen/formate. (Sousa et al.,2009). According to the study presented by He et al., it was found that FOG treatment is often impeded by anaerobic digestion due to the inhibitory effect of LCFAs. It was also found that Gram-positive microorganisms are more inhibited at lower LCFA concentrations than Gram-negative microorganisms, and also in UASB reactors, light-layer

adsorption of biomass LCFAs leads to sludge flotation and sludge leaching. (He et al. 2016). Musa and others reported in their study that large amounts of lipids composed of triglycerides and long-chain fatty acids are present in the majority of the organic part of the slaughterhouse (LCFA). But substances such as triglycerides are rapidly degraded to produce LCFA and glycerol, and high LCFA accumulation may be the reason for the inhibition of anaerobic digestion. Due to its toxicity, it may interfere with the regular operation of acetate and methane-producing bacteria (Musa et al., 2018). Major problems with using FOG as a co-substrate include the suppression of acetogens and methanogens at high concentrations of FOG, cell membrane damage, impaired mass transport, and increased cell permeability because of the buildup of long-chain fatty acids (LCFAs) (Kurade et al., 2019). In another study accumulation of long-chain fatty acids (LCFA) is thought to damage cell membranes, reduce nutrient transport, and decrease cell permeability affecting the cell's ability to regulate pH (Amha et al., 2017). According to Elsamadony et al., the LCFA-induced inhibition may be reversible, meaning that the activities of synthetic acetate or methanogens are not irreparably harmed. However, it was discovered that unsaturated LCFAs accumulated on microbial membranes at a faster rate than saturated species (Elsamadony et al., 2021). It has been noted that LCFAs are more soluble at higher temperatures than at mesophilic temperatures and that when present in large quantities, LCFAs bind to cell membranes and prevent the release of chemicals that can limit the action of bacteria (Jiang et al., 2018). Another study noted issues such as a prolonged lag phase, limited methane production, and the formation of volatile fatty acids (VFA), etc. during the shift from mesophilic to thermophilic settings (Zhang et al., 2020). Besides high temperatures, high concentrations of LCFAs were also found to inhibit acetogens and methanogens and the accumulation of LCFAs that damage cell membranes, reduce nutrient transport and create an acidic environment. Moreover, a high concentration of lipid waste (ie, FOG) reveals rapid inhibition of the AD process as a result of the accumulation of metabolites (ie hydrogen and acetate) (Elsamadony et al., 2021). Anaerobic digestion fails when LCFA concentrations are too high because LCFAs are thought to hinder anaerobic metabolism by attaching to cell walls and interfering with metabolic transport across membranes (He et al., 2016).

### **Effect of LCFA Content on Biogas Production**

When fat, oil, and grease (FOG) from the food service industry are added directly to the anaerobic digester, it has been shown to improve biogas production by 30 % or more and may allow wastewater treatment plants to fulfill over 50 % of their electricity demand through on-site generation (Kabouris et al., 2008; Suto et al., 2006). Despite the claimed benefits of co-digestion, research into the anaerobic digestion of high-strength lipid wastes

has shown a slew of practical difficulties. Inhibition of acetoclastic and methanogenic bacteria, substrate and product transport limitations, sludge flotation, digester foaming, pipe and pump obstructions, and clogging of gas collection and handling systems are among the operational issues (Hanaki et al., 1981; Koster et al., 1987; Shea et al., 2010; Dasa et al., 2016). The LCFA<sub>s</sub> are the organic parts of FOG that are critical to methane production in anaerobic digestion. LCFA<sub>s</sub> with a C<sub>8</sub> to C<sub>20</sub> carbon chain and monounsaturated or polyunsaturated -carbonyls include caprylic acid (C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>), decanoic acid (C<sub>10</sub>H<sub>20</sub>O<sub>2</sub>), lauric acid (C<sub>12</sub>H<sub>24</sub>O<sub>2</sub>), and myristic acid (C<sub>14</sub>H<sub>28</sub>O<sub>2</sub>), palmitic acid (C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>), linoleic acid (C<sub>18</sub>H<sub>32</sub>O<sub>2</sub>), and oleic acid (C<sub>20</sub>H<sub>40</sub>O<sub>2</sub>). Theoretical calculations for LCFA<sub>s</sub> to methane conversion estimate that 1 gram of LCFA<sub>s</sub> can produce 1 liter of methane (Kim et al., 2004). However, the amount and components of FOG may cause digestive upset. When the anaerobic reactor is fed with high levels of different LCFA, it was observed to inhibit the formation of methane and cause toxicity to the system (Suto et al., 2006). It was observed that low amounts of the LCFAs oleate and stearate impeded all steps of the anaerobic thermophilic biogas process during the digestion of cattle manure (Angelidaki and Ahring, 1992). Also reported that the concentrations of oleate and stearate were 0.2 g/L and 0.5 g/L, respectively, the lag phase increased, but no growth was observed at 0.5 g/L for oleate and 1.0 g/L for stearate (Angelidaki and Ahring, 1992). Another investigation found that adding oil (5 % v/v) to the reactor at 2 g VS /L/day caused it to fail, whereas at 4.0 g VS/L/day, the reactor remained stable. The results of another investigation indicated that the reactor at 2.0 g VS L<sup>-1</sup> d<sup>-1</sup> failed following the addition of oil (5% v/v), whereas the reactor at 4.0 g VS L<sup>-1</sup> d<sup>-1</sup> was stable for 10 days prior to the buildup of VFAs, which decreased the generation of biogas and methane and lowered the pH. (Awe et al., 2018). Due to lipid inhibition produced by medium chain and LCFA<sub>s</sub> in desiccated coconut wastewater such as lauric acid and myristic acid, it was reported that the COD removal efficiency of anaerobic treatment sharply dropped from 90% to 30% (Samarasiri et al., 2016). Based on the study conducted by Usam et al., they found that high concentrations of FOG caused an increase in the lag phase before showing complete inhibition. Running AD with FOG levels in the 0.1-1.5% (v/v) increased bio-methane production by 2 to 19 times. Whereas, at the 2 - 3 % levels, large VFA accumulation (17-19 g/L) and low LCFA utilization (29 and 18%) were observed, respectively, and thus methane biosynthesis was permanently blocked (Usman et al., 2020). Table 2 shows the delay stages that occurred in the anaerobic system due to the presence of FOG.

### **Effect of LCFA on the Microbial of Anaerobic Digestion**

Introducing substrates with a high-fat content into the AD may immediately result in process failure since these substrates have a long-lasting harmful effect on acetogenic bacteria and methanogenic archaea. Alves et al. reported In their study, that anaerobic digestion of wastes with high-fat content led to sludge flotation and biomass washing due to lipid/LCFA adsorption on biomass, inhibiting acetate-causing bacteria and methanogenic archaea by LCFA. It directly leads to process failure, due to the permanent toxic effect of these compounds on acetogenic bacteria and methanogenic archaea (Alves et al., 2009). In contrast, it was found in the research on methanogenic activity that the addition of more than 1 g COD/L of LCFAs linearly decreased the activity of methanogens. When operating a large-scale continuous system, the potential for unsaturated LCFA accumulation in the reactor should be taken into account (Cho et al., 2013). Another study found that the total number of archaea in the control sample peaked on the first day of incubation and then slightly increased on the final day. In addition, the number of archaea was slightly reduced by the inclusion of 5 % (w/w) phospholine gum, (a byproduct of the refining of crude palm oil). On the other hand, the addition of 50% (w/w) phospholine gum decreased the overall amount of archaea on day two of fermentation and dramatically decreased it on the last day ( $6.1 \times 10^7$  to  $3.3 \times 10^4$ , respectively) (Mustapha et al., 2017). Adding (5 % v/v) from the oil is probably going to influence the makeup of the microbial community, which frequently has an impact on its dynamics and abundance (Awe et al., 2018). Another study found that oleic acid increases with increasing lipid concentration as the oleic acid concentrations were 1403 and 3207 mg L for lipid concentrations of 18% and 60% respectively, which resulted in a 50% reduction in methanogenic activities where the results indicated that oleic acid one of the most toxic long-chain fatty acids (Sun et al., 2014). A prior study found that high organic loading led to reactor failure and bacterial methane inhibition after lipid deposition on biomass, which was primarily recognized as C16:0 (>60 percent), whereas the supplied LCFA included 30 percent C16:0 and 50 percent C18:0 (Neves et al., 2009). A decrease in the production of biogas was seen when the OLR of lipids was raised from 2 to 2.5 g COD/(L.day). Additionally, at an HRT of 1.5 days, a poor biogas output of 0.3 L/g injected COD was recorded. The impact of the elevated LCFA concentrations on the anaerobic microbes can be used to explain this decline. Table 3 shows the review study conducted by (Long et al., 2012) on the effect of LCFA concentrations on methanogenic activity.

**Table 2.** The delay stages that occurred in the anaerobic process

Type of substrate	Lag phase (d)	Effect on the digestion	Reference
FOG	5	With the highest FOG loading produced very little methane after which they noticed an exponential rise.	Kabouris et al., 2008
grease feed on anaerobic sludge	20	Grease trap sludge additions of 55% and 71% of feed VS resulted in increased VS and COD <sub>sol</sub> in digested material and decreased methane production indicating overloading and LCFA inhibition. Despite the high methane production potential, methane production from grease trap sludge started slowly most likely due to LCFA inhibition	Luostarinen et al., 2009
FOG and Organic Fraction of Municipal Solid Wastes (OFMSW) grease waste (GW)	2	FOG and OFMSW, 35% FOG-VS in feed resulted in a 2-d lag phase	Martínez et al., 2016
	5	With a lag phase of 5 days, samples with 699 GW/kg-VS exhibited the longest lag phase. This inhibition was caused by the accumulation of VFAs over the first eight days, as well as hydrogen accumulation.	Silvestre et al., 2011
Lipid-rich waste	6-10	In the beginning, all testing showed a lag phase that lasted between 6 and 10 days. For tests with 5 percent, 10 percent, and 18 percent lipid, the rate of methane production was comparable. A greater inhibition was noticed for lipid concentrations of 31%, 40%, and 47%.	Cirne et al., 2007

COD<sub>sol</sub>: soluble chemical oxygen demand**Table 3.** Effect of lipid content on methanogenic activity (Longe et al., 2012)

LCFA- Component name	Value	Effect on Methanogenic activity
C8:0 - Caprylic acid	10 mM	Loss of 50% of the acetoclastic methanogenic activity
C10:0-Capric acid	5.9 mM	
C12:0-Lauric acid	4.3 mM	
C14:0-Myristic acid	4.8 mM	
C18:1-Oleic acid	4.35 mM	

C10:0-Capric acid	6.7 mM	Methanogenic and acetogenic populations are decimated
C18:0-Stearate	1.0 g/L	No growth of Methanogenic
C18:1-Oleic acid	2g COD/g VSS synthetic waste based on oleic acid	Maximum capacity for anaerobic sludge (beyond which concentration methanogenic activity ceased)

### **Anaerobic Co-Digestion for Improvement the Performance of the System**

Over the past few decades, lipid inhibition in anaerobic wastewater treatment has been thoroughly investigated by using a variety of techniques to increase the biological activity of anaerobic microbes against lipid inhibition. Numerous methods have been developed and put into practice to enhance the anaerobic digestion of various oily effluents, including (operating temperature, feeding sequence, saponification, enzymatic pre-treatment, absorbent addition, and anaerobic co-digestion) (Long et al., 2012; Samarasiri et al., 2016). Oil and grease are preferred substrates for co-digestion due to the higher theoretical yield of methane ( $1.0 \text{ m}^3 \text{ CH}_4/\text{kg}$ ) compared to protein and carbohydrates ( $0.63 \text{ m}^3 \text{ CH}_4/\text{kg}$ ,  $0.42 \text{ m}^3 \text{ CH}_4/\text{kg}$  respectively) (Alves et al., 2009; Awe et al., 2018). In a prior study, co-digestion with lipid (30 % w/w) and FW (70 % w/w) resulted in an ideal methane output of  $0.8 \text{ m}^3/\text{kg}$  (Chowdhury et al., 2019). During the anaerobic digestion of primary sludge and active waste, the addition of solid waste raised  $\text{CH}_4$  output by 18.4%, while the addition of FOG and FW increased it by 21.1 %. The co-digestion of FW with FOG at  $1.0 \text{ kg m}^{-3} \text{ day}^{-1}$  fat loading rate significantly improved daily biogas production to 13% in co-reactors compared to the mono-reactors of food wastes (Iskander et al., 2021). The researchers discovered in another investigation a cattle waste anaerobic reactor was supplemented with fish lipids (total concentration of 5%), which resulted in a 25–50  $\text{m}^3$  of increase in methane production(). The performance of AD may therefore be improved by combining lipids with other substrates. The determined LCFA concentration for the anaerobic co-digestion of the synthetic medium containing various concentrations of carbohydrates, proteins, and fats was 4.8 g/L which was significantly higher than the typical maximum inhibitory concentrations (1-5 g/L) (Samarasiri et al., 2016). As a result, greater production of bio-methane and successful treatment are both aided by anaerobic co-digestion. In Table 4,  $\text{CH}_4$  production from the combined digestion of FW and FOG from several substrates is shown.

**Table 4.** CH<sub>4</sub> production in the AD process from the combined digestion of FW and FOG by using various substrates

Co-substrate	Loading rate	HRT/SRT (day)	Remark of CH <sub>4</sub> production	Reference
Primary sludge:lipid+FW	1.9-3.5 Kg/VS m <sup>3</sup> /day	15 HRT	452-700 m <sup>3</sup> /tonVS <sub>added</sub>	Noutsopoulos et al., 2013
Sewage sludge and GW	3 Kg/VS m <sup>3</sup> /day	20 HRT	CH <sub>4</sub> increased to 123%	Silvestre et al., 2011
Primary sludge:lipid+FW	2.4-3 Kg/VS m <sup>3</sup> /day	13 HRT	0.68-1.08 m <sup>3</sup> CH <sub>4</sub> /kg, CH <sub>4</sub> content increased from 65-71%	Davidsson et al., 2008
Scum+sewage sludge	7 g COD eq/(L.day)	80 SRT	50 L CH <sub>4</sub> /kg improves biogas yields while a 29% increase in specific CH <sub>4</sub>	Alanya et al., 2013
Thickened waste sludge(TWS) + FOG	2.3-3.4 g VS/L/day	15 HRT	598-614 L/kgVS <sub>added</sub> , CH <sub>4</sub> content 66.8-67.5%	Wan et al., 2011
Sludge +FOG	2.2-3.7 Kg/m <sup>3</sup> /day	13.3 HRT	588-2240 mL CH <sub>4</sub> , CH <sub>4</sub> content 65-70%	Kabouris et al., 2008
Sewage sludge+GW	1.67-3.46 Kg/m <sup>3</sup> /day	16 HRT	376-463 L/kg VS <sub>added</sub>	Luostarinen et al., 2009
Waste activated sludge +FW(lipid rich waste)	1.19-2.93 Kg/m <sup>3</sup> /day	46 10 HRT	192-339 L/kg VS <sub>added</sub>	Heo et al., 2003
FOG+TWS	1.24-1.58 Kg/m <sup>3</sup> /day	20 SRT	0.180-502 L CH <sub>4</sub> /gVS <sub>added</sub> , CH <sub>4</sub> content 60.2-68.2%	Wang et al., 2013
FOG and kitchen waste	2.56 Kg/m <sup>3</sup> /day	30 SRT	0.32- 0.63 m <sup>3</sup> /kg VS	Li et al., 2011
FW+FOG+Meat waste	0.7-1.8 Kg/m <sup>3</sup> /day	30-56 SRT	0.18-0.52 m <sup>3</sup> /kg VS	Sethi, 2018
Fat+SS	0.8 gVS/L.day	12 SRT	80 L/Kg <sub>VS</sub> ,CH <sub>4</sub> content 55%	Martínez et al.,2016
	1.3 gVS/L.day	17 SRT	293 L/KgVS CH <sub>4</sub> content 62%	
	1.2 gVS/L.day	58 SRT	520 L/KgVS CH <sub>4</sub> content 61%	

HRT: Hydraulic retention time; SRT: Sludge retention time; tVS: total volatile solid

## Conclusion

Provided the appropriate technology is utilized and the right feeding strategy is followed, lipids can be effectively converted to methane by

syntrophic consortia of acetogenic bacteria and methanogenic archaea. However, when applying fats in high concentrations and above the specified load, it can cause damage to the cell membrane, impaired mass transport, and increased cell permeability due to the accumulation of long-chain fatty acids, thus impairing the cell's pH regulation. Not only high concentrations but also high temperature causes high solubility of LCFAs and the appearance of inhibitory acids such as oleic and thus leading to an increase in the lag phase and limiting the production of methane. As acetogens and methanogens are sensitive to high LCFA concentrations and thus showed low abundance in such an environment. Therefore anaerobic co-digestion offers benefits such as increased degradation of organic waste and dilution of inhibitor compounds compared to mono-digestion as the biggest advantage of using fats is that improved biogas production can be achieved in anaerobic co-digestion. Furthermore, several studies reported higher methane yield in lipid co-digesters compared to mono-digesters, this was likely due to the higher biodegradation of lipids. Finally, in order to implement a successful anaerobic system for lipids wastes sludge, several factors must be focused on as mentioned by (Long et al.,2012): FOG% as volatile solids, concentrations of long-chain fatty acids, reactor temperature, pH, hydraulic residence time, reactor size and feeding approach ( continuous or batch).

### References:

1. Ahmad, A., Ghufran, R., & Wahid, Z. A. (2011). Bioenergy from anaerobic degradation of lipids in palm oil mill effluent. *Reviews in Environmental Science and Bio/Technology*, 10(4), 353-376. <https://doi.org/10.1007/s11157-011-9253-8>.
2. Alves, M. M., Pereira, M. A., Sousa, D. Z., Cavaleiro, A. J., Picavet, M., Smidt, H., & Stams, A. J. (2009). Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA). *Microbial biotechnology*, 2(5), 538-550. <https://doi.org/10.1111/j.1751-7915.2009.00100.x>.
3. 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 research*, 123, 277-289. <https://doi.org/10.1016/j.watres.2017.06.065>.
4. Angelidaki, I., & Ahring, B. K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Applied microbiology and biotechnology*, 37(6), 808-812.
5. Awe, O. W., Lu, J., Wu, S., Zhao, Y., Nzihou, A., Lyczko, N., & Minh, D. P. (2018). Effect of oil content on biogas production, process performance and stability of food waste anaerobic digestion. *Waste*

- and biomass valorization*, 9(12), 2295-2306.  
<https://doi.org/10.1007/s12649-017-0179-4>.
6. Chen, X., Romano, R. T., & Zhang, R. (2010). Anaerobic digestion of food wastes for biogas production. *International Journal of Agricultural and Biological Engineering*, 3(4), 61-72.. <https://doi.org/10.3965/j.issn.1934-6344.2010.04.0-0>.
  7. Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. *Bioresource technology*, 99(10), 4044-4064.  
<https://doi.org/10.1016/j.biortech.2007.01.057>.
  8. Chipasa, K. B., & Mędrzycka, K. (2006). Behavior of lipids in biological wastewater treatment processes. *Journal of industrial microbiology and biotechnology*, 33(8), 635-645. [10.1007/s10295-006-0099-y](https://doi.org/10.1007/s10295-006-0099-y).
  9. Cho, H. S., Moon, H. S., Lim, J. Y., & Kim, J. Y. (2013). Effect of long chain fatty acids removal as a pretreatment on the anaerobic digestion of food waste. *Journal of Material Cycles and Waste Management*, 15(1), 82-89. Click to copy the URI to your clipboard. <https://doi.org/10.1007/s10163-012-0092-7>
  10. Chowdhury, B., Lin, L., Dhar, B. R., Islam, M. N., McCartney, D., & Kumar, A. (2019). Enhanced biomethane recovery from fat, oil, and grease through co-digestion with food waste and addition of conductive materials. *Chemosphere*, 236, 124362.  
<https://doi.org/10.1016/j.chemosphere.2019.124362>
  11. Cirne, D. G., Paloumet, X., Björnsson, L., Alves, M. M., & Mattiasson, B. (2007). Anaerobic digestion of lipid-rich waste—effects of lipid concentration. *Renewable energy*, 32(6), 965-975.  
<https://doi.org/10.1016/j.renene.2006.04.003>.
  12. Dasa, K. T., Westman, S. Y., Millati, R., Cahyanto, M. N., Taherzadeh, M. J., & Niklasson, C. (2016). Inhibitory effect of long-chain fatty acids on biogas production and the protective effect of membrane bioreactor. *BioMed Research International*, 2016;2016:7263974.  
<https://doi.org/10.1155/2016/7263974>.
  13. Davidsson, Å., Löfstedt, C., la Cour Jansen, J., Gruvberger, C., & Aspegren, H. (2008). Co-digestion of grease trap sludge and sewage sludge. *Waste Management*, 28(6), 986-992. <https://doi.org/10.1016/j.wasman.2007.03.024>.
  14. Dehghani, M., Sadatjo, H., Maleknia, H., & Shamsedini, N. (2014). A survey on the removal efficiency of fat, oil and grease in Shiraz Municipal wastewater treatment plant. *Jentashapir Journal of Health Research*, 5(6). <https://doi.org/10.17795/jjhr-26651>.

15. Elsamadony, M., Mostafa, A., Fujii, M., Tawfik, A., & Pant, D. (2021). Advances towards understanding long chain fatty acids-induced inhibition and overcoming strategies for efficient anaerobic digestion process. *Water Research*, *190*, 116732. <https://doi.org/10.1016/j.watres.2020.116732>.
16. Gujer, W., & Zehnder, A. J. (1983). Conversion processes in anaerobic digestion. *Water science and technology*, *15*(8-9), 127-167.
17. Hanaki, K., Matsuo, T., & Nagase, M. (1981). Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotechnology and bioengineering*, *23*(7), 1591-1610. <https://doi.org/10.1002/bit.260230717>.
18. He, J., Deng, Y., Li, X., Zhang, Y., Zhu, N., & Yin, X. (2016). A review of process limitations and microbial community in anaerobic digestion of fat, oil, and grease (fog). *Res Rev J Microbiol Biotechnol*, *5*, 39-44.
19. Heo, N. H., Park, S. C., Lee, J. S., Kang, H., & Park, D. H. (2003). Single-stage anaerobic codigestion for mixture wastes of simulated Korean food waste and waste activated sludge. In *Biotechnology for Fuels and Chemicals* (pp. 567-579). Humana Press, Totowa, NJ. <https://doi.org/10.1385/abab:107:1-3:567>.
20. Iskander, S. M., Amha, Y. M., Wang, P., Dong, Q., Liu, J., Corbett, M., & Smith, A. L. (2021). Investigation of Fats, Oils, and Grease Codigestion With Food Waste in Anaerobic Membrane Bioreactors and the Associated Microbial Community Using MinION Sequencing. *Frontiers in bioengineering and biotechnology*, *9*, 613626. <https://doi.org/10.3389/fbioe.2021.613626>.
21. Jiang, J., Li, L., Cui, M., Zhang, F., Liu, Y., Liu, Y., ... & Guo, Y. (2018). Anaerobic digestion of kitchen waste: the effects of source, concentration, and temperature. *Biochemical Engineering Journal*, *135*, 91-97. <https://doi.org/10.1016/j.bej.2018.04.004>.
22. Kabouris, J. C., Tezel, U., Pavlostathis, S. G., Engelmann, M., Todd, A. C., & Gillette, R. A. (2008). The anaerobic biodegradability of municipal sludge and fat, oil, and grease at mesophilic conditions. *Water Environment Research*, *80*(3), 212-221. <https://doi.org/10.2175/10.2175https://doi.org/106143007X220699>.
23. Kim, S. H., Han, S. K., & Shin, H. S. (2004). Kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation and  $\beta$ -oxidation. *Journal of Environmental Science and Health, Part A*, *39*(4), 1025-1037. <https://doi.org/10.1081/ese-120028411>.
24. Koster, I. W., & Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied and*

- environmental microbiology*, 53(2), 403-409.  
<https://doi.org/10.1128/aem.53.2.403-409.1987>.
25. 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. *Bioresource technology*, 272, 351-359. <https://doi.org/10.1016/j.biortech.2018.10.047>.
  26. Labatut, R. A., & Pronto, J. L. (2018). Sustainable waste-to-energy technologies: Anaerobic digestion. In *Sustainable food waste-to-energy systems* (pp. 47-67). Academic Press. <https://doi.org/10.1016/B978-0-12-811157-4.00004-8>.
  27. 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. *Bioresource technology*, 102(20), 9471-9480. <https://doi.org/10.1016/j.biortech.2011.07.103>.
  28. Lin, C. S. K., Pfaltzgraff, L. A., Herrero-Davila, L., Mubofu, E. B., Abderrahim, S., Clark, J. H., & Luque, R. (2013). Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy & Environmental Science*, 6(2), 426-464. <https://doi.org/10.1039/c2ee23440h>.
  29. Long, J. H., Aziz, T. N., Francis III, L., & Ducoste, J. J. (2012). Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Safety and Environmental Protection*, 90(3), 231-245. <https://doi.org/10.1016/j.psep.2011.10.001>.
  30. 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. *Bioresource technology*, 100(1), 79-85. <https://doi.org/10.1016/j.biortech.2008.06.029>.
  31. Martínez, E. J., Gil, M. V., Fernandez, C., Rosas, J. G., & Gómez, X. (2016). Anaerobic codigestion of sludge: addition of butcher's fat waste as a cosubstrate for increasing biogas production. *PLoS One*, 11(4), e0153139. <https://doi.org/10.1371/journal.pone.0153139>.
  32. Meng, Y., Li, S., Yuan, H., Zou, D., Liu, Y., Zhu, B., & Li, X. (2015). Effect of lipase addition on hydrolysis and biomethane production of Chinese food waste. *Bioresource technology*, 179, 452-459. <https://doi.org/10.1016/j.biortech.2014.12.015>.
  33. Musa, M. A., Idrus, S., Hasfalina, C. M., & Daud, N. N. N. (2018). Effect of organic loading rate on anaerobic digestion performance of mesophilic (UASB) reactor using cattle slaughterhouse wastewater as

- substrate. *International journal of environmental research and public health*, 15(10), 2220. <https://doi.org/10.3390/ijerph15102220>.
34. Mustapha, N. A., Sharuddin, S. S., Zainudin, M. H. M., Ramli, N., Shirai, Y., & Maeda, T. (2017). Inhibition of methane production by the palm oil industrial waste phospholine gum in a mimic enteric fermentation. *Journal of Cleaner Production*, 165, 621-629. <https://doi.org/10.1016/j.jclepro.2017.07.129>.
  35. Nakhla, G., Al-Sabawi, M., Bassi, A., & Liu, V. (2003). Anaerobic treatability of high oil and grease rendering wastewater. *Journal of Hazardous Materials*, 102(2-3), 243-255. [https://doi.org/10.1016/s0304-3894\(03\)00210-3](https://doi.org/10.1016/s0304-3894(03)00210-3).
  36. Neves, L., Oliveira, R., & Alves, M. M. (2009). Fate of LCFA in the co-digestion of cow manure, food waste and discontinuous addition of oil. *Water research*, 43(20), 5142-5150. <https://doi.org/10.1016/j.watres.2009.08.013>.
  37. Noutsopoulos, C., Andreadakis, A., Mamais, D., & Gavalakis, E. (2007). Identification of type and causes of filamentous bulking under Mediterranean conditions. *Environmental technology*, 28(1), 115-122. <https://doi.org/10.1080/09593332808618771>.
  38. Noutsopoulos, C., Mamais, D., Antoniou, K., Avramides, C., Oikonomopoulos, P., & Fountoulakis, I. (2013). Anaerobic co-digestion of grease sludge and sewage sludge: The effect of organic loading and grease sludge content. *Bioresource technology*, 131, 452-459. <https://doi.org/10.1016/j.biortech.2012.12.193>.
  39. Palatsi, J., Laureni, M., Andrés, M. V., Flotats, X., Nielsen, H. B., & Angelidaki, I. (2009). Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. *Bioresource technology*, 100(20), 4588-4596. <https://doi.org/10.1016/j.biortech.2009.04.046>.
  40. Pereira, M. A., Sousa, D. Z., Mota, M., & Alves, M. M. (2004). Mineralization of LCFA associated with anaerobic sludge: kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnology and bioengineering*, 88(4), 502-511. <https://doi.org/10.1002/bit.20278>.
  41. Quéméneur, M., & Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Research*, 28(5), 1217-1226. [https://doi.org/10.1016/0043-1354\(94\)90210-0](https://doi.org/10.1016/0043-1354(94)90210-0).
  42. Samarasiri, B. K. T., Mihiranga, P. A. D., & Rathnasiri, P. G. (2016). Effect of lipid inhibition in anaerobic wastewater treatment: a case study using desiccated coconut wastewater. Annual Session of the Institution of Engineers, Sri Lanka, 1-10. <https://doi.org/10.13140/RG.2.2.36760.80646>.

43. Sethi, R. (2018). *Biogas Production from Organic Waste, Meat and Fog by Anaerobic Digestion and Ultimate Sludge Digestibility* (Doctoral dissertation, Florida Atlantic University).
44. Shea, T., Johnson, T. D., Gabel, D., & Forbes, B. (2010). Introducing FOG to sludge—a sticky proposition. *Proceedings of the Water Environment Federation*, 2010(14), 2688-2700. <https://doi.org/10.2175/193864710798170513>.
45. Silvestre, G., Rodríguez-Abalde, A., Fernández, B., Flotats, X., & Bonmatí, A. (2011). Biomass adaptation over anaerobic co-digestion of sewage sludge and trapped grease waste. *Bioresource technology*, 102(13), 6830-6836. <https://doi.org/10.1016/j.biortech.2011.04.019>.
46. Sousa, D. Z., Smidt, H., Alves, M. M., & Stams, A. J. (2009). Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. *FEMS microbiology ecology*, 68(3), 257-272. <https://doi.org/10.1111/j.1574-6941.2009.00680.x>.
47. Sun, H., Wu, S., & Dong, R. (2016). Monitoring volatile fatty acids and carbonate alkalinity in anaerobic digestion: titration methodologies. *Chemical Engineering & Technology*, 39(4), 599-610. <https://doi.org/10.1002/ceat.201500293>.
48. Sun, Y., Wang, D., Yan, J., Qiao, W., Wang, W., & Zhu, T. (2014). Effects of lipid concentration on anaerobic co-digestion of municipal biomass wastes. *Waste Management*, 34(6), 1025-1034. <https://doi.org/10.1016/j.wasman.2013.07.018>.
49. Suto, P., Gray, D., Larsen, E., & Hake, J. (2006). Innovative anaerobic digestion investigation of fats, oils, and grease. *Proceedings of the Water Environment Federation*, 2006(2), 858-879. <https://doi.org/10.2175/193864706783796853>.
50. Usman, M., Salama, E. S., Arif, M., Jeon, B. H., & Li, X. (2020). Determination of the inhibitory concentration level of fat, oil, and grease (FOG) towards bacterial and archaeal communities in anaerobic digestion. *Renewable and Sustainable Energy Reviews*, 131, 110032. <https://doi.org/10.1016/j.rser.2020.110032>.
51. Wan, C., Zhou, Q., Fu, G., & Li, Y. (2011). Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. *Waste management*, 31(8), 1752-1758. <https://doi.org/10.1016/j.wasman.2011.03.025>.
52. Wang, L., Aziz, T. N., & Francis, L. (2013). Determining the limits of anaerobic co-digestion of thickened waste activated sludge with grease

- interceptor waste. *Water research*, 47(11), 3835-3844. <https://doi.org/10.1016/j.watres.2013.04.003>.
53. Williams, J. B., Clarkson, C., Mant, C., Drinkwater, A., & May, E. (2012). Fat, oil and grease deposits in sewers: Characterization of deposits and formation mechanisms. *Water research*, 46(19), 6319-6328. <https://doi.org/10.1016/j.watres.2012.09.002>.
54. Xue, S., Zhao, N., Song, J., & Wang, X. (2019). Interactive effects of chemical composition of food waste during anaerobic co-digestion under thermophilic temperature. *Sustainability*, 11(10), 2933. <https://doi:10.3390/su11102933>.
55. Zhang, J., Tian, H., Wang, X., & Tong, Y. W. (2020). Effects of activated carbon on mesophilic and thermophilic anaerobic digestion of food waste: Process performance and life cycle assessment. *Chemical Engineering Journal*, 399, 125757. <https://doi.org/10.1016/j.cej.2020.125757>.