



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

*Ali Alhraishawi*

Department of Civil Engineering, College of Engineering, Misan University,  
Iraq

*Sukru Aslan*

Sivas Cumhuriyet University Department of Environmental Engineering,  
58140, Sivas, Türkiye

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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 the 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 wastewaters. However, it has been claimed that high-fat wastes, particularly mono-digestion in the anaerobic reactor, inhibits acetoclastic and methanotrophic bacteria, delays the formation of gas even more, and overtaxes the system. This paper examines the research on the impact of lipids on biogas enhancement, reactor inhibition, impact on the microbial communities, and co-digestion with lipids in the anaerobic digestion process.

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**Keywords:** Anaerobic digestion, lipid content, microbial community, anaerobic co-digestion, methane generation

## Introduction

Fat in the municipal wastewater comes from a variety of places, including municipal garbage, industry (edible oil, food processing, and slaughterhouses), and trade (food trade). Lipids make nearly 25% of the organic content in the oily wastewater, which is derived from municipal wastewater. 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., 2011; Quéméneur et al., 1994; Williams et al., 2012). A municipal wastewater, defined as strong, medium and weak pollution, is considered to contain approximately 100, 90 and 40 mg/L of oil, 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). Lipid is well known that benefiting from AD of lipid waste has become a potential source of 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	

The FW, lipids are a blend of vegetable oils and fats. Due to the increased synthesis of long chain fatty acids (LCFA<sub>s</sub>), the lipid concentration in FW will disrupt the AD process. This has been shown to be hazardous to

the community of anaerobic bacteria (Chen et al., 2010; Chen et al., 2008). Although lipids are biodegradable through biological processes, the presence of intermediates known as LCFA<sub>s</sub> inhibits biodegradation and becomes a major source of process instability as a biomass blockage, foaming, and flotation, especially when lipid residues are used as the sole carbon source in anaerobic fermentation (Pereira et al., 2004; Noutsopoulos et al., 2009). The biogas generation in the anaerobic process can be improved by co-digestion of 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).

### **Effect of high oil content on the biogas production in anaerobic digestion**

When fat, oil, and grease (FOG) from the food service industry is 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 ole (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 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. For 10 days prior to volatile fatty acids (VFA<sub>s</sub>) accumulation, resulting in a lower pH, lowering biogas and methane production (Awe et al., 2018). Due to lipid inhibition produced by medium chain and LCFA<sub>s</sub> in a 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). It was reported that increased levels of FOG lengthened the lag phase of anaerobic process and eventually resulted in full inhibition. Due to the high amount of VFA accumulation (17–19 g/L) and low level reduction of LCFA (29% and 18%) compared to the 1 % fat content, the addition of more than 1% fat into the AD reactor, irreversibly prevented the generation of bio-methane was reported by Usman et al. (2020). Table 2 shows the delay stages that occurred in the anaerobic system due to the presence of FOG.

### **Effect of oil content 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 acetogenes bacteria and methanogenic archaea. In other words, methanogenic archaea are affected by LCFA in a bactericidal manner. Based on the discovery that acetoclastic methanogens do not adapt to LCFA either after lengthy exposure to non-lethal doses or after repeated exposure to toxic concentrations (Alves et al., 2009). In contrast, it was found in a research of 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 amount 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 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). Sun et al. (2014) also reported that oleic acid increased sharply when the lipid concentration was increased from 8% to 60%. This led to a 50% decrease in the activities of methanogens when the oleic acid concentration was raised from 50 to 200

mg as oleic acid was the more toxic LCFA (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 lipid was raised from 2 to 2.5 g COD/(L.day). Additionally, at a 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 (Longe et al., 2012) on the effect of lipid content methanogenic activity inhibition concentration 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 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)	2	FOG and OFMSW, 35% FOG-VS in feed resulted in a 2-d lag phase	Martínez et al., 2016
grease waste (GW)	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 %. In a different investigation, the researchers discovered that when FW and FOG were digested together at a rate of  $1.0 \text{ kg m}^3 /\text{day}$ , the rate of biogas production was higher ( $p < 0.05$ ) than when FW was digested alone. By 13.2% after switching from mono-digestion to co-digestion under the  $1.0 \text{ kg m}^3 /\text{day}$  feeding condition (Iskander et al., 2021). 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, CH<sub>4</sub> production from the combined digestion of FW and FOG from several substrates are 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	Referen ce
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>	Noutsop oulos 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 Sludge +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
	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 etal., 2008
Sewage sludge+GW	1.67-3.46 Kg/m <sup>3</sup> /day	16 HRT	376-463 L/kg VS <sub>added</sub>	Luostari nen et al., 2009
Waste activated sludge +FW(lipid rich waste) FOG+TWS	1.19-2.93 Kg/m <sup>3</sup> /day	46 HRT	192-339 L/kg VS <sub>added</sub>	Heo et al., 2003
	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	58 SRT	520 L/KgVS CH <sub>4</sub> content 61%	

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

## Conclusion

There is an increasing demand for sustainable energy sources that can offer basic electricity generation capability due to the rising expense of traditional energy supplies and concerns over climate change. Even as the

world's population has grown in recent years, the available energy has decreased. As a result, the hunt for energy from renewable sources is intensive. One of the most crucial substrates for increasing biogas production efficiency, particularly in the combined digestion, is fat. Previous studies indicated that there is a chance to expand biogas output, particularly from the biogas plant housed in wastewater treatment plants, when sludge is jointly digested with FW and FOG. More research is required to establish the ideal operating parameters, particularly the organic load, which can be utilized to establish the ideal volume of organic waste namely waste with a high fat content, to prevent system failure and inhibition.

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