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Research Article

Long chain fatty acid (LCFA) occurrence in primary and secondary sewage sludge fractions

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ABSTRACT

Sewage sludge produced in municipal wastewater treatment plants (WWTPs) is stabilized to produce methane/energy and a final stabilized biosolid suitable for land use using anaerobic digestion (AD) process. Fat, oil, and grease (FOG) matter present in the sewage sludge and their products, long chain fatty acids (LCFA), are not monitored qualitatively and quantitatively for their contribution or inhibition to methane production during the process. AD is designed and operated based on average volatile solid (VS) removal criteria. LCFA can be both present in the sewage sludge and produced as intermediate products during the hydrolysis of FOG. A 1.5-year monitoring of the primary sludge (PS) and secondary sludge (SS) fractions was conducted and evaluated in a timely base in a municipal WWTP. According to the results, the most common and highest presence belonged to palmitate (C16:0) and total LCFA occurred up to 11963 and 927 mg/L in PS and SS, respectively. Detected LCFA species were mostly saturated as laurate, myristate, palmitate, stearate, oleate and elaidate. The level in the mixed sludge feed complied with the inhibitory threshold values neglecting the accumulation in the anaerobic digesters.

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INTRODUCTION

Sewage sludge produced in municipal wastewater treatment plants (WWTPs) contains fatty matter from both municipal and industrial origins as fat, oil and grease (FOG) and long chain fatty acids (LCFA) accumulated during the treatment of municipal wastewater [1]. LCFA are the products of hydrolysis of FOG and exist in its presence. Oil and grease is a standardized parameter of FOG for wastewater discharges into the sewage collection system due to its clogging effect in the pipeline and toxicity towards the biological treatment units in the end of the sewage system. FOG tends to adsorption on the particulate matter and mostly settles out with the sewage sludge. Organic stabilization of the sewage sludge through anaerobic digestion (AD) converts 40–50% of volatile solids (VS) into methane and CO_2 gas providing a considerable recovery of energy in a 18–25 d of hydraulic retention time (HRT) reducing high operational costs in municipal WWTPs [2, 3]. FOG is converted with a higher yield to methane (1 m^3/kg) compared to proteins (0.63 m^3/kg) and carbohydrates (0.42 m^3/kg), therefore, it is a favorable waste type for AD [1]. Optimum process performance needs to be determined based on process parameters in relation to LCFA threshold values.

LCFA is not a routine pollutant/parameter monitored qualitatively and quantitatively in the sewage sludge fractions and effluent stabilized sludges. They are present in the raw sewage sludge and produced as intermediate products during the anaerobic hydrolysis of FOG. Scum

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formation was related to LCFA as an operational problem in anaerobic digesters [4]. Inhibition was mostly related to high C-number LCFA (>C10) as they accumulated in the sludge due to slow degradation. Inhibition due to oleate (C18:1) started at 0.5 g/L concentration with a higher degree than 50% on the unadapted granular sludge inoculum and palmitate (C16:0) was formed as the main product [5]. The reversibility of the inhibition was obtained in the batch study and revealed that adaptation to LCFA presence by the methanogens was advantageous in the digestion of lipids. A concentration range of 1.154–1.55 g/L for capric acid (C10) was reported as the biocidal threshold value for acetogens and methanogens with no adaptation ability of aceticlastic methanogens [6]. Pereira et al. [7] evidenced irreversibility of the inhibition on methanogens at LCFA concentration of 1–5 g COD-LCFA/g VSS as LCFA were biodegraded. Toxic effect of oleate (C18:1), linoleate (C18:2), palmitate (C16:0), and stearate followed the substrate inhibition pattern [8]. A low concentration range of 40–100 mg LCFA/L reported in the sewage sludge by Quémeneur and Marty was not representative as different types and levels of LCFA can be found depending on the urban activities (local food sector) and industrial wastewater intrusion as substantial sources for FOG/LCFA [9]. The types and concentrations of LCFA vary according to the type of industrial origins. LCFA that was detected at the highest amounts in wastewater and wastes were oleate (C18:1) and linoleate (C18:2) [4]. Oleate, linoleate, palmitate and stearate occurred in the proportions of 37, 13, 27 and 7%, respectively, in the composition of the dairy industry wastewater while other species were present only at the level of 16% in total. Palmitate and stearate were found in high concentrations in slaughterhouse wastewater and stearate in domestic wastewater from vegetable oil. Palmitic, palmitoleic and eicosapentanoic acids were found at a high level in palm and fish oils. As the chain length-C number of LCFA augments their solubility in water decreases and tendency to adsorption on solids/particles increases. In municipal WWTPs, LCFA are adsorbed on to the primary sludge (PS) in the pre-sedimentation unit and secondary (waste biological sludge) sludge (SS) in the biological treatment unit [1]. LCFA concentration is expected to rise through sludge thickening where PS and SS are mixed and thickened to increase the VS content in the feed to anaerobic digesters. The reduction in the methane yield as organic loading rate (OLR) increases without rise in the volatile fatty acids (VFA) was reported as a sign of LCFA accumulation and inhibition in the anaerobic digesters [10, 11].

As sewage sludge is formed mostly in two fractions, the LCFA's qualitative and quantitative contents require investigation and were determined and evaluated in the PS and SS samples collected in a municipal WWTP serving a metropolitan area for a 1.5-year period in this study. The characterization was aimed to indicate seasonal changes and accumulation pattern, and enable a comparison to threshold values for inhibitory effect as well as the difference in contents in the actual sewage sludge samples.

Table 1. Characterization of the sewage sludge samples

Sludge	pH	TS (g/L)	VS (g/L)	Oil and grease (%VS)
PS	6.3–7.0	40–60	25–35	66–75
SS	7.2–8.0	7–10	6–8	29-35

MATERIALS AND METHOD

The samples were taken from the primary settling tank's sludge outlet and the sludge recycle of the biological treatment unit for PS and SS, respectively, from January 2018 to the end of April 2019 in Konya municipal WWTP which served an equivalent population of 1.2 million. The WWTP had an average flow rate of 160000 m^3/d receiving wastewaters both from domestic (major source) and industrial sources (minor source mostly dairy, slaughterhouse and animal husbandry). The suspended solid concentration of the inlet wastewater ranged at 450–650 mg/L. The biological treatment unit consisted of a Bardenpho process with partial nitrogen removal operated at a sludge age between 14–17 d. The samples contained a high level of oil and grease as presented in the Table 1. Standard methods for VS (2540 E) and oil and grease (5520-E soxhlet extraction) were applied to PS and SS samples [12]. PS contained fatty matter at a 70 (+/-5)% of its VS content more than double the level for SS. Drying and esterification procedures were carried out for LCFA analysis in the sludge samples. The sludge drying method was developed by Neves et al. [13] and was applied at 85 °C for 12 h. According to Sönnichsen and Müller, LCFA esterification was carried out on a 20 mg of dry sludge sampled which was weighed and mixed directly with the methanolic HCl, methanol and hexane and the esterification process was performed for 1 h at 100 °C [14]. After the addition of hexane and water, the upper filtrate phase of 1 ml was directed to GC/FID analysis (Shimadzu 17A VP3 and Agilent FFAP 30 m x 0.250 mm x 0.25 μm capillary column). The temperature program started at 100 °C (5 mn) and increased to 240 °C with a rate of 4 °C/mn. The injection mode was 1:2 split. The injector and detector temperature was adjusted to 240 °C. Nitrogen was the carrier gas. F.A.M.E. (Free Acis Methyl ester) Mix, C8 - C22, certified reference material, (ampule of 100 mg (Neat)) from Supelco (CRM18920; Foreign Trade Commodity Code: 38229000000) and Supelco 37 Component FAME Mix TraceCERT® (in dichloromethane (varied conc.), ampule of 1 mL; CRM47885; Foreign Trade Commodity Code: 38229000000) standards were used for the identification and confirmation of the LCFA.

RESULTS AND DISCUSSION

LCFA are low in water solubility as 7.2 and 2.9 mg/L for palmitic and stearic acids, respectively at 20 °C [15]. Unsaturated LCFA are relatively more soluble in water (linoleic acid at 160 mg/L (6.7 °C). This property makes LCFA adsorp onto the particles, raw and microbial cells, and limit their degradation by bacterial species. That is the reason for LCFA

accumulation at a high level onto the PS during the transportation through sewerage systems. Floating particles that escape primary settlers enters the biological treatment units and settle out with the bacterial flocs, undegraded through the activated sludge process. Once fed into the anaerobic digesters, LCFA undergo beta-oxidation releasing H_2 and acetate (and propionate for odd-numbered C-LCFA) at each cleavage stage [16]. Syntrophic acetogens (*Syntrophomonadaceae*) carry out the reaction which proceeds by the H_2 and acetate conversion to methane by hydrogenotrophic and aceticlastic methanogens, respectively, where H_2 utilization is faster and promotes the overall reaction. Acetate conversion is more prone to inhibition due to the responsible archae's sensitivity [17]. Completely mixed reactor configuration such as anaerobic sludge digesters is the advantageous reactor type due to high dilution and HRT to overcome the inhibitory effects of LCFA which will tend to accumulate onto the biomass. The importance of monitoring LCFA in the feed and effluent of ADs lies in the determination of the optimum HRT and OLR that will allow the maximum conversion to methane, especially in the case of thermophilic ADs which are subject to accumulation of VFAs as intermediate products, pH drop and instability due to fastened hydrolysis, higher solubility of LCFA and beta-oxidation resulting in propionate and acetate accumulation [18]. Inhibition by LCFA affects directly acetogens and methanogens but may not be reflected as VFA accumulation but lowered methane production. The amount of LCFA present in the feed sludge needs to be determined especially before the design of the HRT value for thermophilic digesters and any consideration for shortened retention in the ADs.

LCFA species obtained in the raw sludge samples were determined at a very high level in PS samples compared to SS in terms of concentration and number of species (Fig. 1a, b). Total number of 16 species in PS outnumbered 11 of SS also in concentration. Total LCFA occurred up to 11963 and 927 mg/L in PS and SS, respectively. Palmitate was the most common and highest concentration LCFA found in both PS and SS samples reaching 40–55% of the total LCFA concentration. It is likely that high levels of the unsaturated C-18 LCFAs in the FOG material were converted to palmitate as a result of hydrolysis and hydrogen saturation reactions in wastewater and PS samples, and/or use of palm oil dominated the food sector in the metropolitan area. Palmitate was reported as the main product from oleate degradation with a tendency to accumulate on the biomass [19]. Stearate and oleate were second and third, respectively, in the quantity and frequency which were higher in the months of summer compared to other seasons indicating trends in food consumption. Palmitate ranged between 1800–4400 mg/L in PS samples whereas stearate occurred mostly under 1000 mg/L with some peaks reaching 2000 mg/L in the summer months and oleate ranged below 250 mg/L with peaks reaching 1000 mg/L in the summer time. The other common LCFA were myristate (6–11%), palmitoleate (1–9%), elaidate (3–4%), laurate (2–3%), linoleate (1–5%) and pentadecanoate (%1–2). Two heavy LCFAs with C22 and 24 were detected at 1500–2000 mg/L and 500 mg/L occasionally, in the summer months, indicating mineral oil intrusions into the sewage line. Detected LCFA species indicated a high degree of consumption of vegetable oils and animal fat reflecting a current profile resulting from urban activities. Oleate was reported as the most toxic LCFA and palmitate was found accumulated on the cell surface as the first product of its beta-oxidation due to low degradation caused by the inhibited acetogens and methanogens [19]. Saturated species formed the most of the total LCFA indicating an overall slow degradation rate for the subsequent AD.

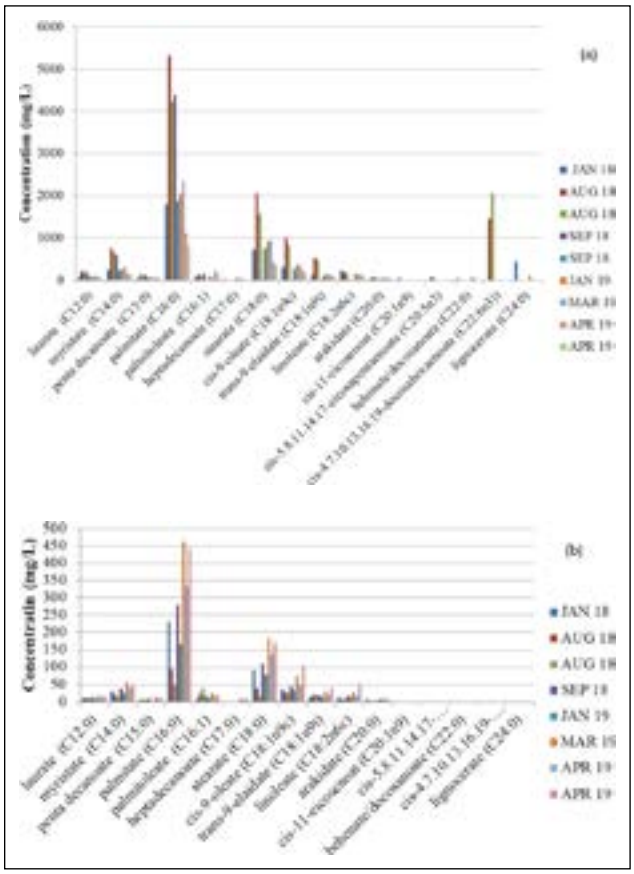

Figure 1. LCFA detected in **(a)** PS and **(b)** SS samples as concentration.

Palmitate, stearate and oleate ranged at 100–450, 80–170 and 44–100 mg/L in SS samples independently from time of the year (Fig. 1b). Palmitate was obtained below 1/10th compared to PS making 26–51% of the total LCFA. All LCFA except palmitoleate were at low concentration ranges in the summer months. Similar percentages were obtained for LCFA except palmitoleate, oleate, elaidate. The descending order in concentration was as palmitate, stearate, oleate, myristate, palmitoleate, elaidate and linoleate (40–57 mg/L), laurate, hepta- and pentadecanoate. The highest number of C was 20 as arachidate at a very low concentration detected occasionally. Total LCFA concentration range (186–927 mg/L) was large but in competence with anaerobic biomass tolerance. The pattern of LCFA occurrence was similar to PS at a lower level in concentration and number and also significant in that LCFA were transported to the biological activated sludge unit in the WWTP. The floating ability of

Table 2. LCFA concentrations (mg/L) calculated in the mixed sludge after thickening

LCFA	Jan 18	Aug 18	Aug 18	Sep 18	Jan 19	Mar 19	Apr 19	Apr 19
Laurate (C12:0)	57	134	131	104	42	60	61	46
Myristate (C14:0)	158	458	400	377	143	188	197	118
Penta decanoate (C15:0)	31	80	66	68	30	41	37	36
Palmitate (C16:0)	1174	3237	2561	2749	1198	1415	1535	844
Palmitoleate (C16:1)	42	82	69	91	4	64	43	140
Heptadecanoate (C17:0)	21				24	31	29	7
Steareta (C18:0)	474	1256	940	48	480	616	624	331
cis-9-oleate (C18:1n9c)	199	617	505	21	165	246	242	186
trans-9-elaidate (C18:1n9t)	83	323	289	11	62	90	91	73
Linoleate (C18:2n6c)	137	120	91	10	6	106	84	97
Arachidate (C20:0)	33	46	39	4	29	28	39	29
cis-11-eicosenoate (C20:1n9)	37							
cis-5.8.11.14.17-eicosapentaenoate (C20:5n3)		47	28					
Behenate/docosanoate (C20:0)	24					30	30	
cis-4.7.10.13.16.19-docosahexaenoate (C22:6n3)		875	1237					
Lignocerate (C24:0)	268					69		
Total	2738	7275	6356	3483	2183	2984	3012	1907

the FOG/LCFA may have been the possible path for their transportation and intrusion into the biological sludge. Another observation was that palmitate's highest presence was opposite as in summer in PS and winter in SS.

When PS and SS are mixed in the sludge thickeners the LCFA concentration sum up to a range of 1907–7275 mg/L for which a conventional dilution rate (1/HRT) of 0.05 (+/- 0.005) complies with the threshold values if their accumulation and synergistic effect in the digester is neglected (Table 2). However, slow degradation of high-C number LCFA will result in accumulation to some degree in the anaerobic digesters. Saturated C16:0 and C14:0 LCFA were reported to accumulate from the degradation of Linoleic acid (C18:2) [20]. Also, individual LCFA concentrations are still higher than reported values in previous inhibition studies on aceticlastic methanogens: linoleic acid (C18:2) that is commonly found in vegetable oils fully deactivated acetate conversion to methane at a 30 mg/L level and IC_{50} was increased up to 137 mg/L for oleic acid after an adaptation was developed on methanogens [20, 21]. Optimization studies on mono- and co-digestion of PS and SS indicated that an optimum OLR allowed a maximum conversion to methane with the highest methane yield outcome, higher OLRs induced an inhibitory effect with the absence of VFA accumulation and temperature increase did not enhance methane yield but VS removal, all outcome in competence with LCFA inhibition being noticeable above a threshold OLR or accumulation degree [10, 11]. As Cavaleiro et al. [22] reported higher degradation rates for unsaturated LCFA and slower rates for saturated LCFA as products, anaerobic digestion of PS and SS necessitated high HRT due to expected slow degradation rates of the products and their efficient conversion to methane. Regarding the lower LCFA content in the SS, higher HRT comes out as a necessity in PS digestion for which mono-digestion may offer a better solution in municipal WWTPs. Alternatively as thermophilic sludge digestion, the profile and level of the LCFA detected especially in PS samples pointed out to a potential hazard in case of a possible conversion to thermophilic operation for which LCFA intolerance was reported at a much higher degree [23, 24]. Oleate was reported less inhibitory than free oleic acid meaning that lower pH than 6.0 presents a toxic environment leading to conversion to free acid forms and necessity for pH or OLR control in thermophilic sludge digestion [23].

CONCLUSIONS

The study reported an actual characterization of LCFA in a long time base in a real-scale municipal WWTP serving a metropolitan area. Total number of LCFA species and maximum concentrations were respectively 16 and 11963 mg/L for PS and 11 and 927 mg/L for SS produced prior to AD. Palmitate was the most common LCFA with the highest concentration found in both PS and SS samples reaching 40–55% of the total LCFA concentration. Palmitate showed the highest presence in summer in PS opposite to SS. Palmitate, stearate and oleate were the most common and high concentration LCFA species. Other LCFA occurred at low concentration ranges especially in the summer months. The results indicated a significant adsorption and accumulation pattern on the PS solids pointing out to both industrial intrusion of FOG and heavy mineral oils banned from discharge and dense social activities such as intense food/restaurant sector having a high usage of vegetable oils and

animal fat in a highly populated metropolitan area. LCFA content consisted mostly of saturated species meaning that slow degradation rates and accumulation in the anaerobic digesters and stabilized sludge should be expected. The concentration levels in PS reflected a seasonal change and were above inhibitory levels for any biological process where a conventional dilution rate (1/HRT) of 0.05 (+/-0.005) complied with the threshold values for mixed sludge if their augmentation in the sludge thickener (with a possible start of anaerobic hydrolysis reaction), synergistic effect and accumulation in the digester were neglected. This emphasized the importance of the dilution degree in the feeding of anaerobic digesters as high HRTs/low OLRs are needed for maximizing the dilution and biodegradation time, thus, biomethanation efficiency, as non-degraded LCFA are prone to be present in the final stabilized biosolid and affect their final disposal method. The future study needs to focus on the accumulation and/or synergistic effects of LCFA and optimization of the operational parameters for mono-digestion of PS and SS in municipal WWTPs. A secondary investigation in the biological unit of the municipal WWTPs not having primary settling units (not producing PS) would enlighten any inhibitory level of LCFA for the activities the activated sludge biomass.

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DATA AVAILABILITY STATEMENT

The author confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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