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Critical Review

Principles, Advances, and Perspectives of Anaerobic Digestion of Lipids

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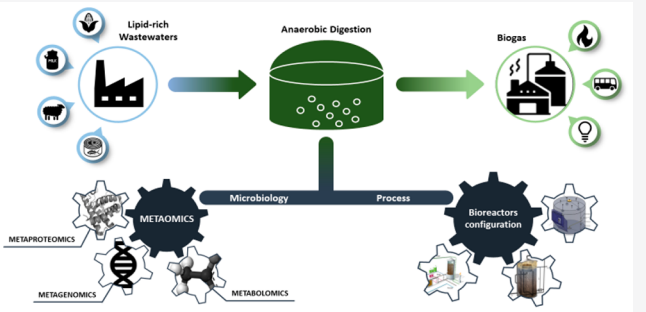
ABSTRACT: Several problems associated with the presence of lipids in wastewater treatment plants are usually overcome by removing them ahead of the biological treatment. However, because of their high energy content, waste lipids are interesting yet challenging pollutants in anaerobic wastewater treatment and codigestion processes. The maximal amount of waste lipids that can be sustainably accommodated, and effectively converted to methane in anaerobic reactors, is limited by several problems including adsorption, sludge flotation, washout, and inhibition. These difficulties can be circumvented by appropriate feeding, mixing, and solids separation strategies, provided by suitable reactor technology and operation. In recent years, membrane bioreactors and flotation-based bioreactors have been developed to treat lipid-rich wastewater. In parallel, the increasing knowledge on the diversity of complex microbial communities in anaerobic sludge, and on interspecies microbial interactions, contributed to extend the knowledge and to understand more precisely the limits and constraints influencing the anaerobic biodegradation of lipids in anaerobic reactors. This critical review discusses the most important principles underpinning the degradation process and recent key discoveries and outlines the current knowledge coupling fundamental and applied aspects. A critical assessment of knowledge gaps in the field is also presented by integrating sectorial perspectives of academic researchers and of prominent developers of anaerobic technology.



KEYWORDS: *FOG, LCFA, microbiology, bioreactor configuration, codigestion*

1. INTRODUCTION

Anaerobic digestion (AD) contributes to several sustainable development goals by combining energy and resource recovery from organic wastes and wastewaters with pollution control. The generation of a gaseous renewable energy source, the recycling of nutrients, and the low surplus sludge production, aligned with the increasing knowledge on microbiology and ecophysiology, has promoted the development of AD technologies as a sustainable treatment solution for a diverse range of wastes and wastewaters, with a significant number of worldwide full-scale implementations.[1,2] Considering the main components of organic matter in wastes/wastewaters, lipids present a high COD/TOC (chemical oxygen demand/total organic carbon) ratio and are, theoretically, ideal substrates for methane production via AD, since their degradation produces more biogas per weight of substrate, than others, i.e., 1.4 L of biogas per gram of lipids compared to 0.9 and 0.8 L g^{-1} for proteins and carbohydrates, respectively.[3]

High-rate anaerobic treatment (HRAT) technologies, mostly based on well settling granular sludge, have been established for the treatment of biodegradable industrial wastewaters, such as those from food and drink processing and pulp and paper among others, primarily applied directly on industry' sites.[1] However, when dealing with lipid-rich wastewaters, this HRAT technology is inappropriate, because the lipids and long chain fatty acids (LCFA) strongly adsorb to the sludge, leading to sludge flotation and washout and potential microbial inhibition.[4−7] Therefore, the economically feasible utilization of lipids in HRAT, and the resulting resource recovery (i.e., biogas), has been challenging.[8,9]

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The low-rate anaerobic treatment (LRAT) of solid wastes such as agricultural residues and municipal sludge is also an established practice, applying variations on the well-known continuously stirred tank reactor (CSTR). The potential to boost biogas production in these systems through the addition of lipids (fat, oil, and grease (FOG) wastes) has been demonstrated.[3] However, similarly to the HRAT, the addition of lipids can cause problems in these codigestion processes, requiring proper feeding and mixing strategies, coupled with effective monitoring of the system performance, mandatory to avoid microbial inhibition and to enhance biogas production.[10,11]

This critical review provides a synthesis of recent advancements in the AD of lipids, both in anaerobic wastewater treatment and codigestion processes, including examples of full-scale applications. Critical aspects on the microbiology and technology, linked to efficient lipids conversion, have been identified, and support is given to the more widespread utilization of lipids from wastes and wastewaters as a sustainable resource for biogas production.

1.1. Occurrence and Composition of Waste Lipids.

Lipids are ubiquitous in nature and are found in most wastes/wastewaters. The classification of "lipids" includes an extremely diverse range of compounds, which can be divided into four main groups of those most commonly found in wastewaters: triacylglycerols including LCFAs, glycolipids, phospholipids, and cholesterol.[12] From these, the most abundant are LCFAs and triacylglycerols, commonly referred to as fats and oils.[13] LCFAs have been characterized with a myriad of different chain lengths, configurations, and degrees of (un)saturation. However, only 20 LCFAs appear widely in nature, and of these, palmitic, oleic, and linoleic acids make up ∼80% of common oils and fats.[7,13,14] Unsaturated LCFAs are components of vegetable oils, while fats are normally composed of saturated fatty acids. Generally, the lipids that are present in the wastewater from industries, that use fats or oils as raw materials, are simple esters of straight chains, even-numbered long chain fatty acids, and linear polyols (triglycerides, phospholipids), as well as their hydrolysis resulting products. Their typical fatty acids composition was reviewed by Alves et al.,[3] being palmitic (C16:0) and oleic (C18:1) acids the most abundant saturated and unsaturated fatty acids, respectively.

Several food and other processing industries have wastewater streams characterized by high FOG contents, namely, dairy, slaughterhouses, edible oil production, fish canning factories, bioethanol and diesel production, and wool scouring (Table 1). The FOG content of these wastewaters is highly variable and dependent on the production process. For example, dairy processing industry wastewaters have high concentrations of fats, along with carbohydrates and proteins, which come from milk. Since the dairy industry produces many different kinds of products, the characteristics of the wastewater vary significantly according to the specific industry and the processing methods[15−17] as can be observed in Table 1, varying from 0.3 to approximately 40 g FOG L^{-1}.[18−22] For slaughterhouse wastewater, the composition of the suspended fraction is characterized by a complex mixture of fats, proteins, and fibers and varies considerably on the type of animals slaughtered and on the production process.[23,24] Regarding the fish industry, the FOG concentration is around 1−1.5 g FOG L^{-1}.[25,26] The extraction and purification of palm oil generates different kinds of wastewaters, commonly known as palm oil mill effluent (POME), where the separator sludge and sterilizer effluent are

Table 1. Typical FOG Concentrations in Wastewater of Different Industrial Wastewaters

Industrial wastewater	FOG concentration (g L^{-1})	ref
Dairy		
Milk and cream bottling plant	0.3−0.5	18
Dairy industry overall	0.3−40	19
	1.7	21
Cheese production	0.8	20
Cheese whey production	9.4	38
Ice cream	0.88−5.12	39
Slaughterhouse		
Cattle	35.8	41
	0.2−0.3	22
	1.3	25
Sheep and goat	0.1−0.4	42
Poultry	0.2−0.7	43
	38.8	44
Food industry		
Tank cleaning company	0.1−2.2	39
Fish	1	25
	1.5	26
POME		
POME	1.4−15	27
	8.8−11.4 (in COD)	28
	2.2−27.2	29
Olive oil mill		
Olive oil mill	0.3−100	31
	17.2	32
Bioethanol		
Corn-to-ethanol thin stillage	10.8−11.8	34
	13	33
Wool scouring		
Wool scouring	0.6−55	36
	10.8	37

the two most important fractions of POME,[27] which contribute to the highly polluting characteristics of this wastewater. The literature reports values from 1.4 to 27 g FOG L^{-1} in this type of wastewater.[27−29] Olive oil mill wastewaters produced by the traditional mill and press processes have a high organic fraction made up of sugars, polyphenols, polyalcohols, proteins, and lipids,[30] with characteristic values of 0.3−100 g FOG L^{-1}.[31,32] Bioethanol production from corn generates a lipid-rich stream called thin stillage, a complex wastewater containing high concentrations of carbohydrates, proteins, lipids, glycerol, and lactic acid[33,34] with FOG values accounting from 11 to 13 g L^{-1}. According to Becker et al.,[35] the major constituents of wool scouring effluent are fats and oils, and effluent characteristics vary largely between processes and materials, with values ranging from 0.6 to 55 g FOG L^{-1}.[36,37]

Often, the lipids present in these industrial wastewaters are removed by pretreatment systems, such as screening, centrifugation, sedimentation, dissolved air flotation, flocculation, or precipitation,[45] producing a more concentrated waste stream commonly referred to as FOG waste. With this procedure, a more diluted wastewater, with significantly less FOG, is obtained that can be more easily treated in traditional anaerobic and aerobic processes. The FOG waste is still frequently disposed in landfills along with waste-activated sludge. However, it is also codigested via LRAT with sewage sludge, manure, or the organic fraction of municipal solid waste, in order to increase the biogas production. The fraction of FOG

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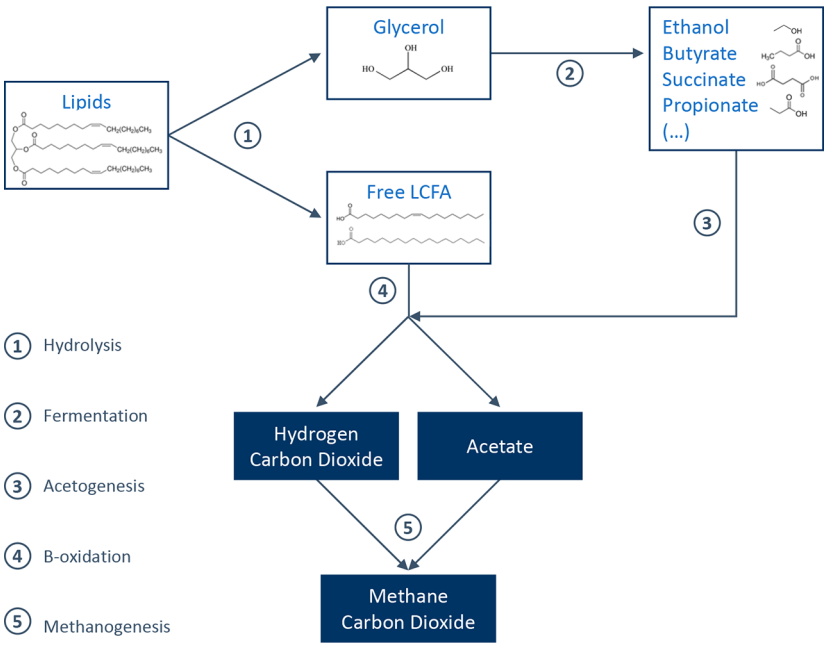

Figure 1. Pathway of anaerobic triacylglycerol biodegradation.

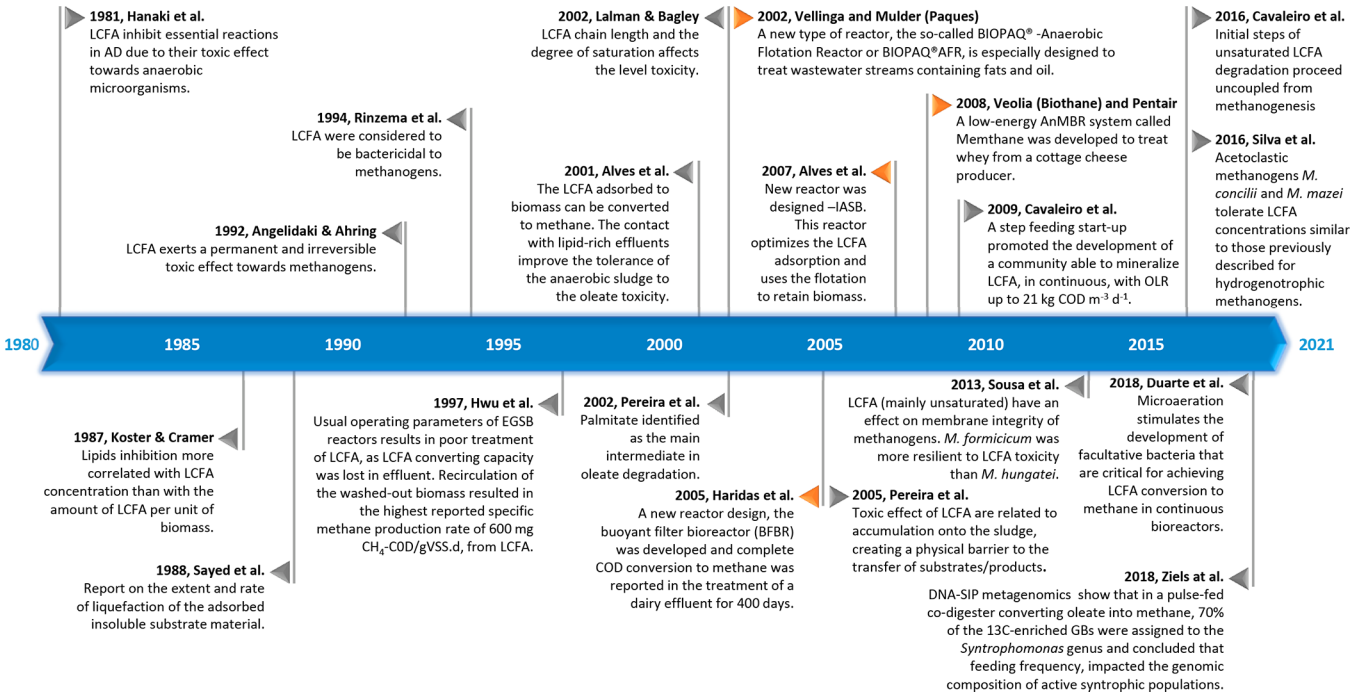

Figure 2. Timeline of key milestones in the microbiology and process research on AD of lipids. The orange markers represent the main reactors developed for AD of lipid-rich wastewaters.[50,55,60−62,64−78]

removed in the pretreatment can account for up to 85% of the total organic fraction with potential for biogas production, highlighting their importance for industry as a potential renewable energy source. This value has been reported by NVP Energy Ltd., at the Arrabawn Dairies Group's WWTP (Ireland), with a dissolved air flotation pretreatment employed, based on the wastewater generated from a milk processing industry.

Municipal wastewater streams in industrialized countries are generally characterized by a relatively low FOG content, estimated between 50 and 150 mg L^{-1}.[46] This relatively low number can be attributed to the common practice in these countries to collect FOG at the source in commercial cooking premises to prevent blockages in drains due to the solidification of fats. Interception of FOG can be achieved via grease traps, and plumbing devices at source points, before it enters the municipal wastewater systems. The produced waste stream is referred to as grease trap waste (GTW), and its composition is highly diverse, mainly dependent on the source.[47] These biosolids have started to be utilized in codigestion systems to boost the biogas yield in LRAT systems.[48,49]

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2. AD OF LIPIDS: A HISTORICAL PERSPECTIVE

During AD, triacylglycerols are hydrolyzed to glycerol and LCFA (Figure 1) in a step catalyzed by extracellular lipases produced by acidogenic bacteria.[50−52] The released LCFA are further degraded to acetate (and propionate, in the case of odd-numbered LCFA) and hydrogen via β-oxidation, which is the rate-limiting step in the degradation of lipids.[14,50,53−55] These compounds are then finally converted to methane and carbon dioxide by methanogens.[56]

Lipids hydrolysis is a surface-related process, and its rate may vary depending on the fatty acid chain length, substrate physical state (solid or liquid), and specific surface area.[90] When fat concentration is very high, hydrolysis can become the rate-limiting step in the whole anaerobic degradation process.[57] For example, in wastewater treatment systems, large insoluble droplets can be formed with concomitant low surface area for hydrolysis. However, when the lipid−water interface area is large, because of the small particle size (e.g., lipids emulsions or micelles), hydrolysis is not necessarily the rate-limiting step.[87] In this case, lipids conversion to glycerol and LCFA is regarded as a fast process, and the overall degradation of lipids is limited by LCFA degradation.[49,95−97]

Efficient methane production from FOG-containing wastewater is not easily achieved with existing conventional HRAT, mainly due to the formation of a thick layer of sludge enclosed by a whitish greasy matter on the top of the water surface.[58−60] Consequently, an important fraction of the sludge is lost by washout, and methane production decreases over time. Biogas bubbles are frequently retained in the floating hydrophobic layer, leading to foam formation, which may cause problems in the biogas line. Moreover, lipids and LCFA have been reported as toxic for the anaerobic microbial communities.[50] The identification of these problems promoted the practice that, for a long time, lipids and LCFA have been separated from the wastewater before AD, with the consequent loss of their energy potential. A significant amount of research has been performed to understand the complex phenomena of lipids biodegradation by anaerobic communities, aiming to overcome process limitations and enhance methane production from these compounds. The key milestones in the microbiology and process research on AD of lipids are summarized in Figure 2.

In 1981, pioneering work by Hanaki et al.[50] showed that lag phases preceding methane production were a consequence of LCFA accumulation and inhibition, rather than an effect of neutral lipids. In the early 1990s, LCFAs were considered to be bactericidal, exerting a permanent and irreversible toxic effect, particularly toward methanogens.[55,61] Both acetoclastic and hydrogenotrophic methanogens were reported to be inhibited in the presence of LCFA,[50] but acetoclasts were reported as more sensitive to LCFA than hydrogenotrophs.[7,50,55,62,63] In these studies, acetoclastic methanogens were unable to adapt to LCFA, after repeated exposure to toxic concentrations as well as after extended exposure to subtoxic concentrations.[55] More recently, however, Silva et al.[64] showed that pure cultures of *Methanothrix* (*Methanosaeta*) *concilii* and *Methanosarcina mazei* tolerated LCFA concentrations similar to those previously reported for hydrogenotrophic methanogens,[65] showing that these acetoclastic methanogens are more robust than considered previously, which may explain the observed prevalence of microorganisms from *Methanosarcina* and *Methanothrix* genera in anaerobic bioreactors treating LCFA-rich wastewater.

It has also been shown, however, that the nature of the lipids also influence the extent of toxicity. Unsaturated fatty acids, containing one or more double bonds (e.g., oleate, C18:1), are more toxic to microbial cells than saturated fatty acids, such as stearate (C18:0) or palmitate (C16:0),[7,66,79] and the toxicity also increases with the carbon chain length.[80]

In general, most studies have been performed within the mesophilic range (30−37 °C), but the anaerobic digestion of LCFA under thermophilic conditions (40−60 °C) has also been studied.[58,61,63,77,81] Hwu et al.[63,82] reported higher oleate conversion rates in high temperature reactors (55 °C), but oleate toxicity toward acetoclastic methanogens was also higher than at 30 °C. Since the compositions of cell membranes of thermophilic and mesophilic microorganisms are different, responses to LCFA toxicity may vary.[63] Moreover, lipids/LCFA solubilization increases with temperature, thus enhancing their bioavailability[35] and possibly their toxicity. AD of lipids at low temperatures (12−20 °C) remains seldom studied. Recently, Singh et al.[83−85] showed the potential of mesophilic sludge to produce methane from a synthetic dairy wastewater containing LCFA (33% in COD) at low temperatures (10 and 20 °C) over a 150 day bioreactor trial. Petropoulos et al.[86] assessed the lipase activity in the treatment of municipal wastewater at 4, 8, and 15 °C and concluded that, although lipases were produced at these temperatures, their activities were low and even became undetectable at 4 °C. Interestingly, these authors found that the raw wastewater presented high levels of lipase activity that was unaffected by temperature and as was shown by Keating et al.[87] no hydrolytic-based limitation was expected. Therefore, lipid-rich wastewater digestion at low temperatures should be investigated. Furthermore, comparing rates of lipid and LCFA conversion to methane across temperature ranges coupled with the identity of active microbial community members would prove valuable.

The perceived toxicity of LCFA is also influenced by the structure of the reactor's inoculum; i.e., granular sludge is generally more resistant to LCFA than suspended or flocculent sludge.[54] The higher perceived toxicity observed for flocculent sludge was ascribed to its higher surface area and therefore to a higher adsorption capacity. In the granules, their three-dimensional structure offers higher protection to the methanogens, generally located in the inner layers. However, lipids also have a negative effect on granulation and maintenance of granular sludge integrity, which is critical for most HRAT reactor types.[58,63,88,89]

LCFA adsorption was initially reported as the main cause of cell damage and toxicity by limiting cell membrane transport and decreasing its protective function.[52,79,90] Even so, Koster and Cramer[62] suggested that microbial inhibition was more correlated with the fatty acids concentration than with the amount of LCFA per unit of biomass, and Hwu et al.[91] proposed that adsorption is an essential step preceding LCFA degradation. Further developments showed that microbial inhibition caused by LCFA is not permanent[59,92] and that biomass adaptation to LCFA can occur,[92] which was striking and opened new perspectives for AD of lipids (Figure 2).

Considering the various observations made by the different authors, we may postulate on two different mechanisms for LCFA interference on the microbes, previously perceived as "toxicity", in which we can differentiate between bactericidal toxicity and temporary inhibition. Bactericidal toxicity would then refer to the impact of the long hydrophobic alkyl chain on the archaeal membrane, leading to membrane leakages, lysis, and

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Table 2. Gibbs Free Energy Changes for Some of Acetogenic and Methanogenic Reactions Presumably Involved in Conversion of Fatty Acids[a]

Reaction	$\Delta G^{0\prime}$ (kJ reaction^{-1})	$\Delta G'$ (kJ reaction^{-1})
Hydrogenation		
$oleate^- + H_2 \rightarrow stearate^-$ (1)	−79	−50
One β-oxidation cycle		
$stearate^- + 2H_2O \rightarrow palmitate^- + acetate^- + 2H_2 + H^+$ (2)	+51	−23
Hydrogenation + one β-oxidation cycle		
$oleate^- + 2H_2O \rightarrow palmitate^- + acetate^- + H_2 + H^+$ (3)	−28	−73
Complete β-oxidation		
$oleate^- + 16H_2O \rightarrow 9acetate^- + 15H_2 + 8H^+$ (4)	+326	−190
Methanogenic reactions		
$acetate^- + H_2O \rightarrow HCO_3^- + CH_4$ (5)	−31	–
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ (6)	−136	–

[a]Adapted from Sousa et al. and Cavaleiro et al.[68,107]). $\Delta G^{0\prime}$: Gibbs free energy change at standard conditions (solute concentrations of 1 mol L^{-1}, gas partial pressure of 10^5 Pa, 25 °C), and pH 7. $\Delta G'$: Gibbs free energy change at nonstandard conditions (1 mmol L^{-1} for reagent LCFA, products stoichiometric accumulation, H_2 depletion to 1 Pa partial pressure, 25 °C, and pH 7).

decay of cells. This irreversible loss of the methanogenic activity of the biomass can then only be restored by growth of new cells as suggested by Rinzema et al.[55] This concept of permanent inhibition or bactericidal toxicity was questioned by Pereira et al.,[60] who observed that the accumulation of LCFAs on the methanogenic biomass prevented mass transfer from the liquid broth to the microbial cells, but cells integrity and viability was maintained. These authors showed that biomass-associated LCFA up to 5 kg kg^{-1} (expressed in COD per mass unit of volatile solids (VS)) could be degraded to methane in batch, eliminating the mass transfer limitations and restoring the methanogenic activity. Therefore, the physical inhibitions related to mass transfer limitations, imposed by the LCFA layer adsorbed onto the sludge, were proposed as the main causes for the transient inhibitory effects observed during AD of lipids.[60] By concluding that a temporary inhibition could be overcome by incubating the LCFA-loaded biomass in batch mode, thus promoting the degradation of the accumulated substrate to methane,[59,60,78] a strategy based on reactor operation in cycles of adsorption followed by degradation was proposed as a first suggestion for the treatment of wastewater with high LCFA content.[93] Later, Cavaleiro et al.[67] demonstrated that by sequencing continuous feeding phases and batch reaction phases in the start-up of an anaerobic reactor, a microbial community able to efficiently mineralize LCFA was established. In a subsequent continuous operation with high organic loading rates (OLR), up to 21 kg m^{-3} day^{-1} (expressed in COD, 50% of the COD being LCFA), stable COD to methane conversion of 80% was observed. More recently, it has been shown that long-term sludge acclimatization to lipids or LCFA-rich wastewater and limitation of excessive LCFA accumulation are beneficial for the efficient degradation of LCFA to methane.[94−96] Ideally, specific biomass-associated substrate should be kept below 1 kg kg^{-1} (expressed in COD per mass unit of VS of inoculum),[59] although well-adapted sludge could still have a good performance with approximately three times this value.[94]

In codigestion processes, microbial adaptation has been recently shown to be important for the degradation of FOG. Ziels et al.[97] highlighted the enrichment of syntrophic LCFA-degrading bacteria during the codigestion of FOG with municipal wastewater sludge. Similar observations were reported in other studies of FOG codigestion.[98−100] Moreover, Ziels et al.[101] showed that, during the anaerobic digestion of cattle manure, oleate pulse feeding (every 48 h) resulted in a higher conversion rate and functional stability than when oleate pulses were performed every 6 h. Syntrophic LCFA-degrading bacteria were significantly enriched in both codigesters relative to the control (without oleate), being more abundant in the codigester that was 48 h pulse fed. In the same line of research, Kougias et al.[102] showed that during the digestion of cattle manure a thermophilic inoculum previously exposed to LCFA was capable of degrading oleate pulses more efficiently than a non-acclimatized inoculum due to the specialization of the microbial consortium. More detailed information on the microbiology and metabolic pathways involved in the AD of lipids is presented in Section 3.

Over the years, several other strategies have been studied to overcome LCFA/lipids toxicity, namely, bioaugmentation with LCFA-degrading bacteria,[94,103] emulsification of LCFAs,[104] addition of adsorbents like bentonite,[81] and LCFA precipitation with calcium salts[50] (Section 5). Different bioreactor designs have also been tested to overcome the problems of sludge flotation and washout, which are detailed in Section 4.

3. METABOLIC PATHWAYS AND MICROBIOLOGY

To achieve an efficient anaerobic digestion of lipids, comparable with easier degradable substrates, the kinetics, metabolic pathways, and microorganisms involved need to be fully understood. Targeted "omics" approaches and improved analytical methodologies have recently offered new insights into complex microbial communities in natural and engineered environments and contributed to the understanding of microbial diversity, function, and interactions in lipid degrading communities. However, despite these recent advances, many aspects remain poorly understood, including the initial steps of unsaturated LCFA degradation and the interactions among the microorganisms involved, as for example, the role of anaerobic facultative microorganisms.

3.1. Metabolic Pathways: β-Oxidation of LCFA. Long chain fatty acids are degraded via β-oxidation. Fatty acids are actively transported inside bacterial cells[105] and activated to acyl-CoA thioesters by acyl-CoA synthetase. After this step, the fatty acyl-CoA undergoes β-oxidation. This oxidation pathway

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Environ. Sci. Technol. 2022, 56, 4749−4775

acts in a cyclic way, with each cycle resulting in the shortening of the input acyl-CoA by two carbon atoms, thus producing acetyl-CoA and hydrogen.[106] More detail on the biochemical features of LCFA biodegradation can be found in Sousa et al.[107]

Due to thermodynamic constraints, acetogenic reactions are only energetically feasible when the hydrogen concentration is kept low (Table 2),[108,109] which is generally accomplished through syntrophic cooperation of acetogenic bacteria and hydrogenotrophic methanogens[108] (reactions 4, 5 ,and 6, Table 2). This obligate relationship is essential to achieve complete LCFA conversion to methane. Alternatively to hydrogen interspecies electron transfer, direct interspecies electron transfer (DIET) may also occur. However, many claims for DIET and electrotrophy have only been suggested without adequate experimental validation.[110] Although the reactions efficiencies may be different for both situations, the overall Gibbs free energy change is the same.[111]

Unsaturated LCFA may directly undergo β-oxidation[7,112] or may need a preliminary hydrogenation step before entering the β-oxidation pathway.[14,53] However, stearate (C18:0) formation from oleate (C18:1) (reaction 1 in Table 2) was only occasionally observed, and significant accumulation of palmitate (C16:0) has been frequently reported when continuous anaerobic bioreactors are fed with oleate-rich wastewaters.[41,60,67,71,113] From a thermodynamic point of view, hydrogenation of unsaturated LCFA is favorable at standard temperature and pressure conditions, as shown by the negative Gibbs free energy change, i.e., $\Delta G^{0'} = -79$ kJ mol^{-1} (Figure S1, Table 2). However, one β-oxidation cycle is not favorable ($\Delta G^{0'} = +51$ kJ mol^{-1}, Figure S1, Table 2), requiring syntrophic cooperation with hydrogenotrophic microorganisms, which scavenge hydrogen, maintaining low hydrogen partial pressure (P_{H2}). Considering nonstandard conditions (1 mmol L^{-1} for reagent LCFA, products stoichiometric accumulation, at 298 K and pH 7), these reactions only became favorable for P_{H2} lower than 10^{-3} atm (101.3 Pa) (Figure S2a).

Nevertheless, one β-oxidation cycle can be thermodynamically feasible if it occurs after the chain saturation step, and the combination of these two reactions yielding a $\Delta G^{0'}$ is -28 kJ mol^{-1} (Figure S1, Table 2). For the nonstandard conditions previously defined, the Gibbs free energy change of oleate (C18:1) to palmitate (C16:0) conversion, for example, is still negative at high hydrogen partial pressure ($P_{H2} > 1$ atm) (Figure S2b). In contrast with this high P_{H2} value, complete palmitate degradation to acetate can only occur when $P_{H2} < 10^{-3}$ atm (Figure S2b), thus pointing to the possibility of palmitate accumulation in the medium. This analysis is further reinforced by the smaller window of opportunity of palmitate to acetate conversion (pink shadowed area in Figure S3), relative to oleate to palmitate degradation (gray shaded area in Figure S3). These windows define the conditions at which the LCFA degradation processes and the methanogenic conversions (acetoclastic and hydrogenotrophic) are energetically favorable.[114] Acetate concentration is not an important limitation for oleate to palmitate conversion (Figure S3) nor for palmitate conversion to acetate, which remains feasible at high acetate concentration, even at 30 g L^{-1} (0.5 mol L^{-1}) of acetate, for example. In summary, this analysis suggests that oleate to palmitate conversion may be predominant in oleate-fed anaerobic bioreactors and that palmitate degradation to acetate will only occur when the hydrogen partial pressure is low or the electrons are channelled via DIET.

In fact, Cavaleiro et al.[68] showed that in bioreactors in which methanogenesis was inhibited, the degradation of different unsaturated LCFA (namely, C18:2, C18:1, and C16:1) lead to the accumulation of two carbons shorter saturated LCFA. From these observations, it could be hypothesized that hydrogenation of unsaturated LCFA (e.g., oleate) is followed by one β-oxidation cycle (reactions 2 and 3, Table 2), after which two carbons shorter saturated LCFA (e.g., palmitate in oleate-fed bioreactors) would be expelled from the bacterial cells. However, this hypothesis is unlikely, as no immediate energy gain is derived from this uptake and excretion process. One alternative hypothesis is that the chain saturation step and first β-oxidation cycle might occur membrane bound, possibly outside the cell, in which the reducing equivalents generated from β-oxidation are used to reduce the double carbon bond of the unsaturated chain, producing palmitate. This, however, has never been shown and remains speculative, since the LCFA molecule needs to be activated prior to β-oxidation. Therefore, the reason why palmitate accumulates in oleate-based wastewater treatment still represents a knowledge gap. Moreover, it is still not clear whether conversion of oleate to palmitate (involving the two possible steps of hydrogenation and β-oxidation) is performed by only one or by more than one microorganism.[107] The accumulated palmitate can be further degraded by different or by the same microorganisms that performed the oleate bioconversion, since bacteria that degrade unsaturated fatty acids are also able to degrade saturated fatty acids, whereas the opposite generally does not occur.[115] However, in anaerobic reactors with mixed communities, oleate consumption is generally fast, while palmitate degradation is slow, which underpins the hypothesis that two different metabolic routes may be involved in the complete oleate degradation to methane. Therefore, the build-up of palmitate during oleate biodegradation must be deeply studied, since it is directly linked with potential solutions to increase the conversion rate of full-scale lipids AD systems. Palmitate was also the main LCFA identified in floating fat balls that were formed during the treatment of high lipid concentrations. These aggregates were mainly composed by calcium LCFA salts and were essentially unavailable to microbes.[116,117]

3.2. Microbiology of Lipids and LCFA Anaerobic Degradation. In the absence of external electron acceptors (other than CO_2), LCFA biodegradation is associated with a syntrophic cooperation between LCFA-consuming bacteria and methanogens, with the latter consuming the acetate and hydrogen formed by the bacteria and producing methane (reactions 4, 5, and 6, Table 2). None of these groups can degrade LCFA alone, so this obligate relationship between syntrophic bacteria and methanogens (especially hydrogenotrophic methanogens) is essential to achieve LCFA degradation to methane. In the absence of a hydrogen scavenger, hydrogen partial pressure increases, and LCFA conversion becomes thermodynamically unfeasible (reaction 4, Table 2).[118,119]

Syntrophomonas sapovorans was the first LCFA-degrading syntrophic bacterium isolated in coculture with *Methanospirillum hungatei*.[120] To date, 12 syntrophic strains able to convert C4, and longer fatty acids have been described.[107,121] Five of these microorganisms can grow with unsaturated LCFA,[107,121] and only one, *Thermosyntropha lipolytica*, is also able to hydrolyze lipids.[122] In general, the syntrophic LCFA degraders are also able to degrade short chain fatty acids (SCFA), while the opposite is not true. Information on syntrophic species degrading propionate and butyrate (the most important

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Environ. Sci. Technol. 2022, 56, 4749−4775

SCFA) are compiled by refs 123−125. Besides methanogens, other microorganisms can act as syntrophic partners for LCFA-degrading bacteria, e.g., hydrogen- and acetate-consuming sulfate reducing bacteria (SRB).[126] For example, Salvador et al.[127] reported a novel syntrophic relationship between an oleate-degrading bacterium, closely related to *Syntrophomonas zehnderi* and hydrogenotrophic sulfonate-reducing *Desulfovibrio*. Recently, a new study suggested that the anaerobic degradation of LCFAs could be enhanced by the presence of other electron acceptors such as iron. Cavaleiro et al.[128] investigated the effect of different substoichiometric amounts of Fe(III) on the anaerobic degradation of oleate in suspended and granular sludge. In that study, faster LCFA biodegradation was observed by suspended sludge in the presence of iron, but no noticeable effect of iron was observed with granular sludge. Regarding the microbial community composition, the results obtained suggest the occurrence of a novel microbial interaction in LCFA oxidation, involving microorganisms of the *Syntrophomonas, Geobacter*, and *Methanobacterium* genera.[128]

The microbiome of the anaerobic LCFA-degrading communities was initially studied using traditional molecular techniques (e.g., cloning and sequencing), targeting the 16S rRNA gene, focusing on phylogenetic and taxonomic characterizations.[115,129−131] In general, the relative abundance of fatty acid-degrading syntrophic bacteria in high-rate methanogenic bioreactors is low (between 0.2% and 3%), despite their importance in lipids/LCFA degradation.[107,124] Besides the genus *Syntrophomonas* from the phylum *Firmicutes* (known as a syntrophic fatty acid degrading bacteria), also *Clostridium* species were detected in several studies.[68,132−135] Members of phyla *Bacteroidetes*, *Synergistetes*, *Spirochaetes*, and *Proteobacteria* were also detected, even though their direct involvement in LCFA degradation was never demonstrated (Table 3).[130,132,136,137] These microbial groups were also detected by Nakasaki et al.,[96] which examined microbial community changes during the degradation of oil, LCFA, and glycerol. Their results showed that *Leptospirales*, *Thermobaculaceae*, *Synergistaceae*, and *Syntrophaceae* were the most abundant bacteria in both oil and LCFA experiments.[95]

In the last decades, the development of new methodologies for the detection and identification of uncultivated microorganisms has contributed to increase the knowledge about microbial diversity, their functions, and interactions in complex communities. In Table 3, microorganisms found in lipid/LCFA-rich environments are shown. Nevertheless, the role of most of these microorganisms in LCFA conversion is unknown.

While it is clear that acclimatization of the microbial community to LCFAs or lipids, both in wastewater and codigestion processes, benefits degradation and decreases inhibition through the development of specialized microbial communities, as previously described,[67,97,101] it is now important for future studies to further link microbial identification to function. Metagenomics, metatranscriptomics, metaproteomics, and metametabolomics are different approaches to address that challenge.[136] Treu et al.[138] studied the metatranscriptome of an anaerobic microbial community during LCFA exposure. Besides confirming the importance of *Syntrophomonas* species in fatty acids degradation, the authors also noted the upregulation of genes involved in "peptidoglycan biosynthesis" and in "lipopolysaccharides biosynthesis" by bacteria belonging to order *Clostridiales* to *Rykenellaceae* families and to *Halothermothrix* and *Anaerobaculum* genera. This may indicate that, by modifying their cell walls and the compositions of the lipopolysaccharides, the bacteria promote a protective mechanism to counteract the toxic/inhibitory effect of LCFA.[138] Kougias et al.[102] studied the microbial community dynamics during an inhibitory shock load induced by single pulses of oleate, using high-throughput shotgun sequencing (metagenomics). They showed that only the microorganisms associated with LCFA degradation could encode proteins related to "chemotaxis" and "flagellar assembly", which allow these microbes to move toward LCFA. Recently, Ziels et al.[70] used DNA-SIP metagenomics and showed that in a pulse-fed codigester converting oleate into methane, 70% of the 13C-enriched genome bins were assigned to the *Syntrophomonas* genus and concluded that feeding frequency impacted the genomic composition of active syntrophic populations.

When studying the hypothesis that different microorganisms may be involved in the accumulation and further degradation of palmitate in oleate-fed bioreactors, Cavaleiro et al.[68] concluded that the initial steps of unsaturated LCFA degradation can happen independently from methanogenic activity. Because facultative anaerobic bacteria became abundant, these authors suggested that these bacteria might have a role in these biochemical reactions, thus opening new possibilities besides the classical syntrophic degradation pathway[68] (Figure 2). To further investigate the role of facultative anaerobic bacteria, Duarte et al.[69] studied oleate conversion in continuous bioreactors, one operated with microaeration (−250 mV) and other under strict anaerobic conditions (−350 mV). That difference in the oxidation−reduction potential (ORP) was correlated to a higher abundance of facultative anaerobic bacteria, particularly *Pseudomonas* spp. Interestingly, microaeration also promoted the transformation of oleate to palmitate, avoiding the long-term methanogenic inhibition observed in the strict anaerobic control experiment, possibly because palmitate is less toxic to methanogens than oleate (Figure 2). In fact, the theoretical ORP value of oleate to palmitate reaction is −270 mV (calculated at standard temperature and pressure conditions, using $\Delta G^{0'}$ from Table 2, and according to Thauer et al.[155]). This value is close to the ORP measured in the microaerophilic reactor (−250 mV), where the oleate to palmitate reaction was favored. However, ORP in bioreactors (under nonstandard conditions) will vary with the soluble concentration of compounds, which for LCFA is generally difficult to determine with accuracy. Moreover, the presence of other soluble species, such as sulfur compounds or oxygen, will also influence the ORP conditions. In anaerobic bioreactors treating oleate-based wastewater, the presence of facultative anaerobic bacteria was also shown to be important because they accelerate oleate conversion to methane by protecting strict anaerobes from oxygen toxicity and also by acting as alternative hydrogen/formate and acetate scavengers for LCFA-degrading anaerobes.[156] From an applied point of view this is very important, since at industrial scale, the feeding tanks/pipelines are not kept under strict anaerobic conditions, and small amounts of oxygen can be introduced to the system. The potential role of facultative bacteria in the conversion of unsaturated to saturated LCFA is still to be disclosed, and further studies are needed to better understand the interactions between facultative anaerobic bacteria and other microorganisms within methanogenic communities in continuous bioreactors. The addition of vestigial levels of oxygen and the fine regulation of redox potential are new perspectives to investigate in this field.[69]

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. Phylogenetic Composition of LCFA/Lipids/FOG Degrading Microbial Communities in Enrichment Cultures or in Bioreactors

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
Thermophilic oleate degrading enrichment cultures	ARDRA, sequencing	*Firmicutes* (*Clostridia*) *Synergistetes* (*Sinergistia*)	*Methanobacterium thermoautotrophicum* (added to enrichment culture)	139
Bioreactors with granular and suspended sludge fed with oleate	PCR-DGGE, sequencing, FISH	*Firmicutes* (*Syntrophomonas* and others) *Proteobacteria* (*Pseudomonas* and others) *Spirochaetales*	*Methanobacterium* sp. *Methanobacterium formicium* *Methanosaeta concilii*	71
Bioreactors with granular sludge fed with increasing loads of oleic acid	PCR-DGGE, cloning, sequencing	*Pseudomonas* *Desulfovibrio*	*Methanobacterium* sp. *Methanobacterium formicium* *Methanosaeta concilii*	140
Stearate degrading enrichment cultures	Culture dependent, RFLP, FISH, sequencing	*Deltaproteobacteria* (*Syntrophus gentianae*) *Bacteroidetes* (*Cytophaga* sp. BHI60-95B)	*Methanocalculus taiwanensis* *Methanosaeta concilii*	141
Synthetic LCFA wastewater containing oleate and palmitate (chemostat cultivation)	Real-time PCR, FISH, DGGE, cloning, sequencing	*Firmicutes* (*Syntrophomonadaceae* and others) *Proteobacteria* *Bacteroidetes* *Spirochaetes*	*Methanosarcina* *Methanosaeta* *Methanospirillum*	137
Batch degradation of oleate or palmitate accumulated during continuous feeding in bioreactors	DGGE, real-time PCR, cloning, sequencing, FISH	*Firmicutes* (*Clostridiaceae*, *Syntrophomonadaceae*, uncultured) *Proteobacteria* *Bacteroidetes*	*Methanobacterium aarhusense* Methanobacterium formicicum *Methanosaeta concilii* *Methanosarcina mazei*	131
Oleate or palmitate enrichment cultures	Culture dependent, PCR-DGGE, cloning, sequencing	*Syntrophomonas*—in both enrichments *Bacteroidetes/Chlorobi* group (*Chlorobium*)—in oleate enrichment *Proteobacteria* (*Desulfovibrio*)—in oleate enrichment *Proteobacteria* (*Syntrophobacter*, *Halothiobacillus*)—in palmitate enrichment	Archaeal community not studied	115
Thermophilic or mesophilic palmitate, stearate, oleate, or linoleate enrichment cultures	RNA-SIP, cloning, FISH, RFLP, culture dependent	*Firmicutes* (*Syntrophomonas*, *Syntrophothermus*, and others) *Proteobacteria* (*Deltaproteobacteria*)	Archaeal community not studied but *Methanosaeta* detected by microscopic observation	142
Incubations with palmitate under mesophilic or thermophilic conditions	RNA-SIP, RFLP, sequencing	*Bacteroidetes* *Firmicutes* (*Clostridium*, *Syntrophomonas*, *Syntrophothermus*, *Tepidanaerobacter*, *Desulfotomaculum*, *Coprothermobacter*) *Deltaproteobacteria* (*Syntrophaceae*, *Geobacteraceae*) *Synergistetes*, *Deferribacteres*, *Bacteroidetes/Chlorobi*, *Thermotogae*, *Acidobacteria*, *Spirochaetes*, and others	Archaeal community not studied	132
Thermophilic bioreactor fed with manure with successive pulses of a LCFA mixture (oleate, stearate, palmitate)	PCR-DGGE, sequencing	*Firmicutes* (*Clostridium*, *Syntrophomonadaceae*) *Synergistetes*	*Methanosarcina*	143
Thermophilic codigestion of organic fraction of municipal solid wastes with FOG wastes	PCR-DGGE, cloning, sequencing	*Firmicutes* (*Clostridiales*, *Thermoanaerobacterales*) *Bacteroidetes* Methanothermobacter wolfeii *Thermotogales* *Synergistetes* *Thermotogae*	*Methanobacterium*, *Methanoculleus* *Methanosarcina*	144
Codigestion of dairy and poultry wastes	Cloning, sequencing	Bacterial community not studied	*Methanocorpusculum* sp. *Methanosardna barkeri* *Methanosaeta concilii*	145

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. continued

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
			Methanoculleus palmolei	
			Methanomethylovorans sp.	
Biodegradability batch tests of fresh pig/cattle slaughterhouse waste mixtures	PCR-DGGE, sequencing	*Firmicutes* (*Thermodesulfobiaceae, Syntrophomonadaceae*)	*Methanosaeta concilii*	133
		Synergistetes (*Anaerobaculum* sp.)	*Methanosarcina siciliae*	
		Bacteroidetes (*Porphyromonadaceae*)		
		Chloroflexi (*Anaerolineaceae*)		
Thermophilic bioreactor fed with manure with continuous addition of oleate	PCR-DGGE, sequencing	*Firmicutes* (*Clostidiaceae, Bacillaceae, Syntrophomonas*)	*Methanococcus*	129
		Bacteroidetes	*Methanosarcina*	
		Proteobacteria (*Pseudomonas*)	*Methanobacterium*	
		Thermotogae	*Methanosaeta*	
Oleate-rich wastewater treated in bioreactor based on a sequence of step feeding and reaction cycles	PCR-DGGE, sequencing	Bacterial community was not studied	*Methanobacterium*	146
			Methanosaeta	
Low-temperature (10 °C) anaerobic digestion of dilute dairy wastewater in an EGSB bioreactor	Real-time PCR, PCR-DGGE	*Firmicutes*	*Methanocorpusculum*	147
		Proteobacteria	*Methanospirillum hungatei*	
		Spirochaetes	*Methanosaeta concilii*	
		Bacteroidetes		
		Trichococcus		
		Proteobacteria		
CSTR codigesting fish waste and cow manure	Pyrosequencing	*Firmicutes* (*Clostridium, Syntrophomonas*, and others)	*Methanobrevibacter*	134
		Proteobacteria	*Methanoculleus*	
		Actinobacteria	*Methanosarcina*	
		Synergistetes	*Methanosaeta*	
			Tenericutes	
			Cloacimonetes (*Candidatus Cloacimonas acidaminovorans*)	
Batch-fed methanogenic bioreactors degrading oleic acid	Quantitative PCR, PCR, sequencing	*Clostridiales* (*Syntrophomonas*)	*Methanomicrobiales*	148
		Anaerolineales (*Levilinea*)	*Methanosaetaceae*	
		Synergistales (*Synesgistes*)		
		Enterobacteriales (*Escherichia, Shigella*)		
Biogas reactors disturbed with pulses of lipids	Sequencing	*Megamonas*	*Methanoculleus*	135
		Flectobacillus	*Methanocorpusculum*	
		Clostridium	*Methanocella*	
		Syntrophomonas sapovorans		
Sequencing batch reactors treating dairy wastewater and cattle manure	PCR-DGGE, sequencing	*Bacteroidales*	*Methanospirillum*	149
		Syntrophomonas	*Methanosarcinales*	
		Thermovirga	*Methanobacteriales*	
Bioreactors continuously operated with Palmitoleate (C16:1) and oleate (C18:1)	16S rRNA gene pyrosequencing	*Levilinea*	*Methanosaeta*	68
		Clostridium	*Methanospirillum*	
		Syntrophomonas	*Methanobacterium*	
		Spirochaeta	*Methanolinea*	
		Longilinea		
		Bellilinea		
		Thermanaerovibrio		

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. continued

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
		Thermanaerothrix		
		Anaerolinea		
		Syntrophobacter		
		Pseudomonas		
		Delftia		
		Curtobacterium		
		Rheinheimera		
		Petrotoga		
		Candidatus Odyssella		
Bioreactors subjected to inhibitory shock load induced by single pulses of unsaturated LCFA	Illumina HiSeq sequencing	*Clostridia* (*Syntrophomonas, Desulfotomaculum, Syntrophothermus*)	*Methanosarcina*	102
		Gammaproteobacteria	*Methanoculleus*	
Reactors codigesting three agro-industrial wastes underwent abrupt and gradual changes of LCFAs concentrations	PCR-DGGE, FISH, Illumina MiSeq sequencing	*Clostridiales*	*Methanosarcina*-related methanogens	150
		Syntrophomonadaceae (*Syntrophomonas*)		
		Synergistetes		
		Anaerobaculaceae		
		Tissierellaceae		
		Peptococcaceae		
Comparison of bioreactors fed with cattle manure after oleate addition to feeding	RNA Illumina MiSeq sequencing, shotgun reads	*Alcaligenaceae* sp.	*Methanoculleus* sp.	138
		Eubacteriaceae sp.	*Methanosarcina* sp.	
		Rikenellaceae sp.	*Methanothermobacter* sp.	
		Clostridiales sp.		
		Porphyromonadaceae sp.		
		Halothermothrix		
		Anaerobaculum		
Anaerobic codigestion of fats, oils, and grease with municipal sludge	Quantitative PCR, rRNA Illumina MiSeq sequencing	*Syntrophomonas*	*Methanosaeta*	97
		Petrimonas	*Methanospirillum*	
		Mahella		
		Levilinea		
		Sedimentibacter		
		Ornithobacterium		
		Others		
Digesters exposed to subsequent OLR increase with FOG and glycerol	Pyrosequencing phospholipids, ether-linked isoprenoids analysis	*Firmicutes* (*Cloacibacillus*)	Archaeal community not studied	151
		Sulfur-reducing bacteria (SRB)		
		Bacteroidetes		
Bioreactors subjected to inhibitory shock load induced by single pulses of unsaturated LCFA	Illumina HiSeq sequencing	*Clostridia* (*Syntrophomonas, Desulfotomaculum, Syntrophothermus*)	*Methanosarcina*	102
		Gammaproteobacteria	*Methanoculleus*	
Reactors codigesting three agro-industrial wastes underwent abrupt and gradual changes of LCFAs concentrations	PCR-DGGE, FISH, Illumina MiSeq sequencing	*Clostridiales*	*Methanosarcina*-related methanogens	150
		Syntrophomonadaceae (*Syntrophomonas*)		
		Synergistetes		
		Anaerobaculaceae		
		Tissierellaceae		

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. continued

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
		Peptococcaceae		
Comparison of bioreactors fed with cattle manure after oleate addition to feeding	RNA Illumina MiSeq sequencing, shotgun reads	*Alcaligenaceae* sp.	*Methanoculleus* sp.	138
		Eubacteriaceae sp.	*Methanosarcina* sp.	
		Rikenellaceae sp.	*Methanothermobacter* sp.	
		Clostridiales sp.		
		Porphyromonadaceae sp.		
		Halothermothrix		
		Anaerobaculum		
Anaerobic codigestion of fats, oils, and grease with municipal sludge	Quantitative PCR, rRNA Illumina MiSeq sequencing	*Syntrophomonas*	*Methanosaeta*	97
		Petrimonas	*Methanospirillum*	
		Mahella		
		Levilinea		
		Sedimentibacter		
		Ornithobacterium and others		
Sequential bench-scale respirometry experiments (thermophilic) with FOG (30%−60%) and food waste	rRNA sequencing	unclassified *Clostridiales*	*Methanoculleus*	100
		unclassified *Thermotogales*	*Methanosarcina*	
		Anaerobaculum		
		Syntrophomonas		
		Coprothermobacter		
		Lactobacillus		
		Tepidimicrobium		
		Syntrophothermus		
		Tepidanaerobacter		
Anaerobic codigesters treating manure and oleate (continuous-fed and pulse-fed at 35 °C)	16S rRNA gene amplicon sequencing of DNA-SIP samples	*Syntrophomonas*	*Methanosaeta*	70
		Thermovirga	*Methabacterium*	
		Aminivibrio		
		Candidatus Cloacamonas, Anaerofustis		
		Syntrophothermus		
		Ruminococcaceae		
		Firmicutes		
		Candidatus Parcubacteria		
		unclassified *Planctomycetes, Spirochaetae, Synergistes, Actinobacteria,* and *Bacteriodetes*		
Reactors treating oleate-based effluent under different redox conditions	16S rRNA gene Illumina MiSeq sequencing	*Stenotrophomonas*	*Methanosaeta*	69
		Delftia	*Methabacterium*	
		Leptothrix		
		Comamonas		
		Pseudomonas		
		Acinetobacteria		
		Azoarcus		
		Aeromonas		
		Microvirgula		
		Ochrobactrum		

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. continued

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
		Aquamicrobium		
EGSB for the treatment of mixed LCFA-containing synthetic dairy wastewater at 20 °C	16S rRNA amplicon sequencing	*Bacteroidia* *Clostridia* *Synergistia*	*Methanomicrobia* (*Methanobacterium*) *Methanobacteria* (*Methanosaeta*)	83
Lab-scale mesophilic (37 °C) and thermophilic (54 °C) continuous stirred tank reactors fed with cheese whey	High-throughput 16S rRNA gene amplicon sequencing	*Clostridiales* (*Clostridiales* sp. M2 and M3) *Aminobacterium colombiense* M1 *Blautia producta* sp. M20 *Erysipelatoclostridium ramosum* M6 *Pelotomaculum schinkii* M44 *Methanothermobacter* sp. T22 *Bacteroidaceae* sp. M42 *Syntrophomonas sapovorans* M45 *Verrucomicrobia* *Clostridia* sp. T3, *Clostridium* sp. T7 *Defluviitoga tunisiensis* T1 *Anaerobaculum* sp. T2 *Clostridium thermopalmarium* T13 *Sporanaerobacter acetigenes* T24 *Alkalispirillum* sp. T21 *Sinibacillus* sp. T30 *Syntrophomonas bryantii* T17 *Tepidimicrobium xylanilyticum* T12 *Bacteroidetes* sp. T36 *Clostridium ultunense* T6	*Methanoculleus palmolei* M8 *Methanomassiliicoccaceae sp. M46 and M48* *Bacteroidetes* sp. M9 *Methanimicrococcus* sp. M41 *Methanobacterium formicicum* T20	152
Batch reactors treating cooking oil, LCFA, and glycerol	16S rRNA gene Ion Torrent PGM sequencing platform	*Enterobacteriaceae* (*Enterobacter*, *Raoultella*, *Citrobater*, *Klebsiella*) *Shewanellaceae* *Clostridiaceae* *Ruminococcaceae* *Porphyromonadaceae* *Bacteroidaceae* *Spirochaetes* *Spirochaetaceae*	*Methanocorpusculum* *Methanobrevibacter*	153
Anaerobic sequencing batch reactor treating synthetic lipid-rich wastewater, comprised of glucose, acetic acid, lactic acid, and soybean oil	16S rRNA gene Illumina MiSeq sequencing	*Synergistales* *Anaerolineales* *Actinomycetales* *Nitrospirales*	*Methanobacteriales* *Methanosarcinales*	95
Fed-batch anaerobic digestion of synthetic wastewater containing oil, glycerol, or LCFAs	16S rRNA gene Illumina MiSeq sequencing	*Rikenellaceae* *Thermobaculaceae* *Anaerolineaceae* *Anaerolineaceae* *Clostridium* *Desulfovibrio*	*Methanobacterium* *Methanosaeta*	96

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 3. continued

Substrate and culture conditions	Techniques applied	Bacterial community	Archaeal community	ref
Batch codigestors treating anaerobic digestion sludge and FOG	16S rRNA gene	*Desulfovibrio aminophilus*		154
		Syntrophaceae		
		Syntrophobacter		
		Leptospirales		
		Treponema		
		Dethiosulfovibrionaceae		
		Synergistaceae		
		Kosmotoga		
		Firmicutes (*Syntrophomonas*)	*Methanobacteriales*	
	Illumina MiSeq sequencing	*Bacteroidetes* (*Fermentimonas*)	*Methanomicrobiales*	
		Proteobacteria	*Methanosarcinales* (*Methanosaeta*, *Methanosarcina*)	
		Synergistetes		
		Methanomassiliicoccales		

The microbial communities developed during the codigestion of lipids also have been the focus of recent studies. Hao et al.[157] reported that in the codigestion of waste-activated sludge and FOG an important increment of methane production was observed, probably due to the abundance of *Geobacter* species, indicating the role of direct interspecies electron transfer in FOG and activated sludge codigestion. In another study, Salama et al.[158] assessed the effect of calcium on FOG degradation. The addition of calcium promoted an increase in methane production and a shift in the microbial community, increasing the growth of bacteria from the *Clostridium*, *Syntrophomonas*, and *Sedimentibacter* genera. The genus *Methanosaeta* increased after the addition of 0.5% calcium, which is one of the factors responsible for high methane production, avoiding the inhibitory growth and toxic effects of high concentrations of FOG. In the study of Usman et al.,[154] *Syntrophomonas* and *Fermentimonas* were abundant. *Methanosaeta* were dominant in the beginning, owing to the increased presence of LCFA, but afterward were replaced by *Methanosarcina* genus, likely because of the increase in acetate concentration due to the LCFA conversion. Kurade et al.[98] compared acclimatized (fed batch over 160 days, 10 batch cycles) to nonacclimatized sludge and showed an increased LCFA degradation efficiency in the former of up to 64%, albeit LCFA degradation was still not complete within 30 days, and 56% oleate remained unconverted in the acclimatized reactor. Amha et al.[100] thoroughly evaluated the microbial community under thermophilic conditions treating a waste with up to 60% FOG. These authors highlighted that syntrophic bacteria were enriched and promoted the successful codigestion process with FOG. Moreover, their approach of jointly utilizing sequencing technology with qPCR analysis (and quantification) on specific groups (e.g., methanogens, syntrophic bacteria) was shown to be robust and beneficial for future studies in the field.

4. BIOREACTOR CONFIGURATIONS IN HIGH-RATE WASTEWATER TREATMENT

Since the 1970s, the field of anaerobic digestion has been commercially active treating waste/wastewaters from various industries with different bioreactor configurations. To ensure the uptake of AD by industry, the costs need to be competitive, both capital and operational per m^3 of waste treated. This can be achieved if the rate of degradation is increased, along with the biogas yield per m^3, especially in respect to wastewater treatment.

For several biodegradable industrial wastewaters, HRAT has enabled high rates of degradation and biogas yields. The superior performance of these systems is based on the retention of slow-growing microorganisms inside the bioreactor, requiring a successful decoupling of solids retention time (SRT) and hydraulic retention time (HRT). The three most common mechanisms to achieve this are physical separation (e.g., by settling and/or filtration), attachment to fixed or nonfixed inert supports, and autoimmobilization or granulation.[1,159] Among these mechanisms, microbial granulation dominated the implementation of anaerobic technology in the last decades, following the development of the upflow anaerobic sludge blanket (UASB), the expanded granular sludge bed (EGSB), and the internal circulation (IC) reactors.[1] However, the improved performances of these HRAT designs did not translate across to the treatment of lipid-rich wastewaters. In these systems, the COD removal efficiencies are generally high, but

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Table 4. Operational Parameters of First-Generation Reactors Treating Lipid-Rich Wastewaters[a]

Type of reactor	Type of wastewater	Scale	Volume	OLR (in COD) ($g\ L^{-1}\ d^{-1}$)	HRT (days)	T (°C)	Influent (in COD) ($g\ L^{-1}$)	FOG ($g\ L^{-1}$)	Methane yield or Methane production	COD removal (%)	Trial duration (months)	ref
ACP	Bakery industry	Full scale	1860 m^3	3	7.8	35	23.7	5.8	ND	97	4	160
	Ice cream	Pilot	5.4 m^3	1.09	5.51	35	4.9	0.8	0.39 (L $CH_4\ g^{-1}$ CODr)[b]	81.8	9	88
AF	Slaughterhouse	Lab	2 L	0.88−11.21	7.1−0.5	37	5.2−11.4	0.2−0.7	0.05−1.10 (L $CH_4\ L^{-1}\ d^{-1}$)	28−82	13.6	24
	Dairy Industry	Full scale	12 m^3	2−4.7	7.00−1.85	35−37	10.5	1.8	30.94 (m^3 $CH_4\ d^{-1}$)	67−93	20.84	20
	Ice cream	Pilot	5 m^3	6.38	0.93	35	4.9	0.8	0.36 (L $CH_4\ g^{-1}$ CODr)[b]	81.8	15 (period of stability)	88
UASB	Slaughterhouse	Lab	10 L	2.2−5.9	5	35	10.7−29.4	3.9−16.4	0.42−0.15 (L $CH_4\ g^{-1}$ CODr)[b]	90	5.5	41
			15 L	1.2−8.9	2.5−1.25	35	3−11.1	1.1−4.9	0.55−0.18 (L $CH_4\ g^{-1}$ CODr)[b]	80−88	8.4	41
		Lab	33.5 L	2.5−19.5	0.38−0.07	30	1.5−2.2	0.05−0.10	0.24−1.87 (L $CH_4\ L^{-1}\ d^{-1}$)	53−67	2.3	45
			33.5 L	3−12	0.42−0.21	20	1.5−2.2	0.05−0.10	0.22−1.12 (L $CH_4\ L^{-1}\ d^{-1}$)	40−62	4.7	45
		Lab	2 L	1−6.5	6.5−1.2	37	5.2−11.4	0.2−0.7	0.22−1.34 (L $CH_4\ L^{-1}\ d^{-1}$)	59−91	13.7	24
	Ice cream	Pilot	5 m^3	2.19	1.62	35	4.9	0.8	0.19 (L $CH_4\ g^{-1}COD_{in}$)[c]	49	8.5 (period of stability)	88
UASB+AF	Dairy (UHT and cheese production)	Lab	10 L	0.98−15.7	1	30	10.9−20.5	0.2	ND	76−95	7.2	163
	Slaughterhouse	Lab	2 + 10 L	1.2−4.5	2.5−1.25	35	2.8−5.6	0.5−1.6	0.52−0.09 (L $CH_4\ g^{-1}$ CODr)[b]	80	5.5	41
EGSB	Slaughterhouse	Lab	2.7 L	2.1−15.8	0.79−0.22	35	1.4−4.2	0.05−0.28	ND	47−91	10	23
	POME	Lab	20.5 L	10	3	35	32.5	11	ND	93	4.3	164
	Oleic acid	Lab	1 L	2.7−1.18	1	37	3.9	ND	0.16−0.28 (L $CH_4\ L^{-1}\ d^{-1}$)	65−93	2.5	60
	Palmitic acid	Lab	1 L	2.7−1.14	1	37	3.7	ND	0.13−0.25 (L $CH_4\ L^{-1}\ d^{-1}$)	62−93	2.5	60

[a]OLR, organic loading rate (expressed in COD); ND, not determined. [b]Expressed relatively to COD removed. [c]Expressed relatively to the COD added.

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Environ. Sci. Technol. 2022, 56, 4749−4775

Table 5. Operational Parameters of Second-Generation Reactors Treating Lipid-Rich Wastewaters[a]

Type of reactor	Type of wastewater	Scale	Volume	OLR (in COD) ($g\ L^{-1}\ d^{-1}$)	HRT (d)	SRT (d)	T (°C)	Influent (in COD) ($g\ L^{-1}$)	Influent FOG ($g\ L^{-1}$)	Methane yield or Methane production	COD removal (%)	Trial duration (months)	ref
AFR	Ice cream	Full scale	511 m^3	2−6	3	90	38	4.5−25.6	2.2−12.8	0.33 (L CH_4 g CODr)[b]	90	8.1	39
	Food cleaning stream	Full scale	430 m^3	0.1−4.6	5	90	38	2.3−29.8	0.1−2.2	ND	98	10	39
	Slaughterhouse	Full scale	9000 m^3	3.3	3	20−50	28−35	11	0.6	0.33 (L CH_4 g CODr)[b]	94	12	Personal communication (Paques)
AnMBR	Corn to ethanol thin stillage	Lab	10 L	8.3	10	20	35	63.6−80.8	10.8−11.8	0.26 (L CH_4 g CODr)[b]	99	3	34
			10 L	7.8	10	30	35	63.6−80.8	10.8−11.8	0.28 (L CH_4 g CODr)[b]	99	3	34
			10 L	6.1	10	50	35	63.6−80.8	10.8−11.8	0.29 (L CH_4 g CODr)[b]	98	3	34
	Snacks factory	Pilot	760 L	1−2	4	25	30−36	8.6−14.8	0.1−0.4	0.5 (g COD-CH_4 $L^{-1}\ d^{-1}$)	91−75	0.8	171
			760 L	2−16	2.8	95	30−36	11.6−98.0	2.7−36	2.75 (g COD-CH_4 $L^{-1}\ d^{-1}$)	81−99	3.2	171
	Slaughterhouse	Pilot	50 L	4.34−15.8	3.33−1.25	ND	37	7.1−20.4	0.2−0.3	0.31−0.13 (L CH_4 g^{-1} CODr)[b]	97−60	2.37	22
	Dairy	Lab	10 L	2.3−4.7	2.2	20	35	2.6−17.6	1.7	0.31 (L CH_4 g^{-1} CODr)[b]	99	6.5	21
			10 L	2.3−4.7	2.2	30	35	2.6−17.6	1.7	0.32 (L CH_4 g^{-1} CODr)[b]	99	6.6	21
IASB	Slaughterhouse	Pilot	1.2 m^3	0.5−16	1.5−1.4	ND	30−35	0.001−44	6.7	ND	80−85	12	174

[a]OLR, organic loading rate (expressed in COD); ND, not determined. [b]Expressed relatively to the COD removed.

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Environ. Sci. Technol. 2022, 56, 4749−4775

the substrate conversion to methane tends to be incomplete,[3] mainly due to lipids/LCFA adsorption onto the sludge.

4.1. High-Rate Anaerobic Technologies (HRAT) for Lipid-Rich Wastewater Treatment. Operational parameters of diverse first-generation reactors treating lipid-rich wastewater are summarized in Table 4. The anaerobic contact process (ACP) is one of the original developments of HRAT and is constituted by a continuous stirred anaerobic digester and an external clarifier, where the anaerobic sludge is settled and returned back to the reactor.[160] In this type of system, the successful operation relies on the operation of the clarifier, and problems with sludge settleability can be partially addressed through the degasification of the reactor effluent, where the biogas is released from the sludge often allowing it to settle again. Sludge separation through flotation and not through settling is an alternative way.

The anaerobic filter (AF), upflow or downflow, is another type of HRAT, in which the reactor has support media (e.g., PVC or ceramic rings) for biomass attachment. AFs have relatively simple constructions, since there are no moving parts; however, a large reactor volume is required. Moreover, AF generally suffers from severe clogging issues due to suspended solids entrapment and biomass growth in the filter, resulting in the occurrence of channeling and short circuiting. Moreover, a high concentration of lipids in the wastewater will aggravate the clogging process,[161,162] and lipids may act as a soap, decreasing the biomass adhesion to the support.[92]

In the UASB reactor, developed by Lettinga et al.,[165] formation of highly settleable sludge aggregates (granules) takes place, combined with gas separation and sludge settling.[1] However, several reports describe difficulties when applying granular sludge reactors to lipid containing wastewaters. The granules are structurally unstable when lipids or LCFA adsorb to their surface, suffering breakage, loss of density, and thus process inhibition. Sayed et al.[45] studied the UASB reactor performance in the treatment of a slaughterhouse wastewater containing 50% of insoluble suspended COD and 5% of grease in the total solids. The process could not handle OLRs exceeding 3.5 g L^{-1} d^{-1} (in COD) at an HRT of 8 h (Table 4). At the same time, there was a deterioration of the COD removal of the system under high loading conditions. Further to this, other studies found that the operation of UASB or other granular systems is limited by components, such as milk fat and proteins, presenting low rates of anaerobic degradation and microbial inhibition problems.[88,166,167] Hawkes et al.[88] reported the performance of a pilot-scale UASB reactor treating ice cream wastewater at an OLR of 2.19 g L^{-1} d^{-1} (in COD), giving a poor performance with less than 50% COD removal efficiency (Table 4). Jeganathan et al.[41] studied the treatment of a complex oily wastewater from a slaughterhouse in two different UASB reactors and verified that, at an OLR of 3 g L^{-1} d^{-1} (in COD), FOG and COD removal efficiencies were higher than 80% (Table 4). However, the reactors performances deteriorated sharply at higher loading rates, and the presence of FOG caused a severe sludge flotation resulting in process failure. Fat, protein, and cellulose components of the POME wastewater were also reported to have an adverse impact on UASB reactors performances and caused deterioration of microbial activity and biomass washout.[168]

In the EGSB reactor design, problems have also been noted when treating lipid-rich wastewaters. In the study of Núñez and Martínez,[23] an EGSB was used for the treatment of slaughterhouse wastewater obtaining a COD removal efficiency of 65%−80%, applying an OLR (in COD) up to 15 g L^{-1} d^{-1} with a fat influent concentration of 0.15 g L^{-1}. In this study, 85% of the fats present in the wastewater were removed, and no accumulation of fats on the sludge was observed. Zhang et al.[164] treated POME wastewater in a laboratory-scale EGSB reactor at OLR (in COD) from 1.45 to 17.5 g L^{-1} d^{-1} and an HRT of 2−3 days, obtaining 90%−95% of COD removal efficiency. In this study, scum formation and sludge flotation were reported due to the presence of FOG in the raw POME and its adsorption to the granules. Pereira et al.[60] studied LCFA inhibition in a lab-scale EGSB treating oleate at an OLR (in COD) of 8 g L^{-1} d^{-1}, with a COD removal efficiency around 80% and a biogas containing 55% methane.

From these studies, it becomes clear that these HRAT reactors do not successfully deal with the commonly reported problems related to lipid-rich wastewater, namely, the loss of granular structure or unsuccessful granulation, sludge flotation, and washout. Therefore, different solutions were evaluated to overcome these problems. For example, the two-phase reactor concept[22,169] was applied to improve process stability and efficiency due to physical separation of the rate-limiting methanogenic phase. However, considering that saturated LCFA biodegradation requires syntrophic cooperation with methanogens, phase separation may not be advantageous. Inverse fluidized reactors were also used for the treatment of a dairy wastewater by Arnaiz et al.[170] with good COD removal efficiencies, but methane yields were not reported. Haridas et al.[72] developed a new reactor design, the buoyant filter bioreactor (BFBR), for the treatment of fat-rich wastewater. In this system, buoyant polystyrene beads form a granular filter bed that allows the decoupling of the SRT from the HRT. An almost complete COD conversion to methane was reported during the treatment of a dairy effluent for 400 days. When the OLR was increased, scum accumulation was observed, followed by further solubilization and degradation to methane.

4.2. Second Generation Reactors for AD of Lipids. In the last decades, novel reactor designs based on alternative sludge retention strategies have been developed up to technology readiness levels (TRL) of 8−9, which are able to deal with the main problems associated to AD of lipids. The core developments include sludge flotation as a strategy to prevent the washout of biomass.

Nowadays, there are several commercially available bioreactors suitable to treat lipid-rich wastewaters: Evoqua's ADI-BVF, Paques B.V.'s anaerobic flotation reactor (AFR), trading as BIOPAQAFR,BIOPAQ AFR, and both Biothane-Veolia's Memthane (anaerobic membrane bioreactor, AnMBR) and recently Sparthane (anaerobic sequencing batch reactor, AnSBR). All these bioreactors use flocculent sludge. In Table 5, a summary of reported operational conditions of second-generation reactors treating lipid-rich wastewater is presented.

The ADI-BVF system provides low-rate treatment for complex wastewaters, operating at lower volumetric loading rates and higher HRT than HRAT. The large volume of the reactor, the low-rate operation mode, and the sludge recycle system avoids biomass washout and guarantees very long SRT. The tank has a simple design and operation; however, due to its size, it represents a large capital investment.

The BIOPAQ AFR reactor by Paques B.V.[74] is especially designed to treat wastewater streams containing fats and oil, for example, from the dairy, poultry, and food industries. It utilizes the flotation properties of the FOG−sludge mixtures, assisting it with white-water microbubbles, derived from a small part of the

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system's produced biogas that is compressed and solubilized in the feedwater to be released in the lower part of the internally mounted anaerobic floatation unit. The effluent is withdrawn from the suspended solids free zone below the flotation layer. The flotation unit, integrated with the reactor system, retains the sludge up to concentrations of 15−30 kg per m^3 of reactor volume.[39] Therefore, it saves the biomass and the substrate solids from washout and also increases the biological activity through increasing contact with the substrate and allowing the sludge to degrade absorbed lipids. Ultimately, through an engineered robust retention system for the sludge, the BIOPAQ AFR reactor overcomes one of the common bottlenecks related to sludge washout during the anaerobic treatment of lipid-rich wastewater. A recent improvement is based on pressurizing the effluent flow of the bioreactor (including the biomass), instead of the effluent of the flotation unit only, which enhances the efficiency of the flotation process. Furthermore, less pressure is required for biomass floatation, and increased solids loading rates on the flotation unit can be applied, resulting in an even more compact system. In pilot and full-scale treatments of the complex wastewater, it was observed that during the first half-day after a nonfeeding period (e.g., a weekend) filamentous sludge developed. However, within a day after restarting feeding, the sludge becomes more compact again (Paques personal communication[40]). While this issue is easily addressed, the rapid change in biomass morphology is unclear. Since the phenomenon of developing filamentous biomass after a restart seems a generic observation in other type of plants as well (Paques personal communication[40]), the microbiological knowledge regarding floc formation and composition, its thickening, and exopolysaccharides formation should be further explored. In the AFR system itself, this filamentous biomass is retained, as sludge floatation is very efficient by directly pressurizing the biomass as explained above.

Full-scale studies performed with this reactor design showed that extremely high concentrations of fats could disturb the system, but the inhibition was reversible.[39] Therefore, managing the waste streams (for example, the high concentrated FOG streams, like ice cream, in a small buffer tank and the low to medium concentrated stream in a large buffer tank) is necessary. Both streams can be pumped in the reactor in a controlled way, avoiding extreme peaks of fat.[39] Despite the possible requirement for separate buffers, the reactor has a strong buffering capacity against spike loading of lipids. It is hypothesized that this buffering capacity is due to the adsorption of the lipids to the sludge and the degradation of the excess lipids at a later time. The reactor has a high COD removal efficiency of 90%−95%, applying an HRT of 1−8 days, dependent on substrate and volumes (Table 5). It has the ability to treat wastewater with COD concentrations of 5−70 g L^{-1}, with a maximum of 50% of the COD being lipids.[39] Microbiologically, the flocculent biomass has proven ideal for this reactor system, with high methanogenic activities recorded, despite the complex substrates treated. The AFR system is applied for full-scale treatment of various fat or oil containing wastewaters as dairy wasters, meat processing wastewater, tank cleaning wastewater, and fish processing wastewater. The system is very robust, and the sludge is well retained, even if there is an upset in load. The sludge has, in the case of a higher fat concentration in the reactor, a tendency to float which is an advantage in this system as it is designed to retain by means of flotation. Therefore the system shows high flexibility for changes in loading rates and types of waste.[39]

Other commercially available technologies to treat lipid-rich wastewaters include the anaerobic membrane system offered by Biothane-Veolia B.V., the Memthane. Saddoud and Sayadi[22] who studied the application of an AnMBR for the treatment of slaughterhouse wastewater, with an operational OLR (in COD) from 4.34 to 15.8 g L^{-1} d^{-1}, achieved a COD removal efficiency up to 94%. Dereli et al.[34] studied the performance of a lab scale AnMBR treating lipid-rich corn to ethanol thin stillage at different SRTs, achieving removal efficiencies up to 99% with an OLR (in COD) up to 8 g L^{-1} d^{-1}. These results were obtained applying SRTs of 20 and 30 days, where LCFA precipitations with cations or adsorption onto biomass of LCFA were the dominant mechanism for LCFA removal. Results showed that high amounts of COD originating from lipids accumulated as very large LCFA precipitates (denominated fat balls) at short SRTs, meaning that COD bioconversion was, in fact, less. Ramos et al.[171] studied the performance of a pilot AnMBR treating lipid-rich wastewater from a snacks factory, where satisfactory results were obtained with an OLR below 2 g L^{-1} d^{-1} (in COD) with acclimated sludge, without inhibitory effects. Szabo-Corbacho et al.[21] studied the performance of an AnMBR treating synthetic dairy wastewater, at two different SRTs (20 and 40 days), with a working OLR of 4.7 g L^{-1} d^{-1} (in COD), obtaining efficiencies of more than 99% organic matter removal and a very low LCFA accumulation inside the system. Biothane commissioned nine full-scale AnMBRs (Memthane systems), using tubular inside-out polymeric membranes in cross-flow skids.[1] Other companies, for example, Kubota,[172] are implementing submerged AnMBRs in which the membranes are mounted inside the bioreactor or in a separate membrane tank. While membrane-based bioreactors offer a solution for lipid-rich wastewaters, their economic viability due to high operation costs related to membrane filtration proves difficult for standard treatment of wastewater, unless there is downstream water reuse, where high effluent quality is demanded, and other membrane systems (i.e., reverse osmosis) are in operation. Therefore, further novel systems have been developed and tested at pilot scale. Sparthane, a sequencing batch reactor (AnSBR) also by Biothane, takes another approach to address the problem of lipid degradation through a patented batch sequence of a stirred reactor, batch degassing tank, and semicontinuous settling tank.[173] Similar in setup to the ACP, it can, however, accept high-loading rates from 8 to 10 g L^{-1} d^{-1} (of total COD), under mesophilic conditions. Preacidification is core to the process, ensuring a balanced liquid matrix of compounds that are degradable by the flocculent microbial community, avoiding denaturation of proteins and temporary inhibition of lipid degradation. Stringent monitoring positively influences the separation and clarification steps, limiting the growth of filamentous bacteria, to ensure sludge settleability and thus easy clarification, solving previously documented issues with the contact reactor process.[1] The batch sequencing is further supported by the microbial findings of Cavaleiro et al.[67] and Ziels et al.,[101] suggesting that this approach increased the ability to rapidly degrade lipids. Ziels et al.[101] further supported this work with quantification of the syntrophic communities and their resulting increase from batch feedings, albeit in a codigestion system. Overall, as the system is expanded with full-scale reference sites presently, it offers a full degradation of complex lipid-rich wastewaters in a strategic yet operationally candid manner.

Another technology especially designed for the treatment of wastewater with high lipids contents, not yet commercially

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Environ. Sci. Technol. 2022, 56, 4749−4775

available, is the Inverted Anaerobic Sludge Bed (IASB) reactor.[3,9,73] Similar to the BIOPAQ AFR, the IASB reactor uses the sludge flotation properties, resulting from lipids/LCFA adsorption, to retain the sludge and the LCFA in the system. Adsorption is promoted by mixing the feed with the recycled sludge, and this mixture is fed from the top. The recycle line and a gas lift effect assist in the internal mixture of the reactor content. Sludge separation is performed at the bottom. A pilot-scale IASB reactor (1.2 m^3) was operated for the treatment of a slaughterhouse wastewater, at an OLR (in COD) from 0.5 to 16 g L^{-1} d^{-1}, with 63% as fat.[174] COD removal efficiencies higher than 80% were achieved, and excessive LCFA accumulation was prevented, showing its capacity for the treatment of complex wastewater with high quality fluctuations.

The commercial need for the treatment of complex lipid-rich wastewaters has driven the field toward market ready systems and technologies, as listed and detailed above. The possibility of directly treating lipid-rich wastewater anaerobically has been accomplished, and the high return in biogas coupled with the savings in pretreatments for FOG separation contributes to counterbalance any extra operating and capital expenses. With the implementation of these second-generation AD reactors, the main issues of the first-generation are solved, i.e., LCFA inhibition, sludge washout, low removal efficiency, allowing to treat high OLR (up to 16 g L^{-1} d^{-1} in COD) with high removal efficiencies, and keeping a more stable reactor performance.

5. ALTERNATIVE STRATEGIES FOR IMPROVING AD OF LIPIDS

Besides the development of novel reactor configurations, other strategies have been studied to improve AD of LCFA/lipids. For example, addition of calcium ions[175] or inert materials, for example, activated carbon, bentonite, or other clays,[81] was tested, considering that these materials can reduce LCFA/lipids bioavailability through mechanisms of precipitation or adsorptions, thus decreasing their potential toxicity. These strategies intended to reduce LCFA bioavailability and thus decrease their toxicity. The mitigation of LCFA inhibition by the addition of cations and natural adsorbents has been recently reviewed by Elsamadony et al.[176] For example, recently, Salama et al.[158] tested the application of calcium (0.1−1%) in order to overcome the inhibition caused by 2% of FOG in bioreactors. The addition of 0.5% calcium was best, promoting a 6-fold increase in the biomethane production and a reduction in the outlet COD from 131 to 14−64 g L^{-1}. Mixing the calcium with FOG before feeding the reactor was advantageous, since it reduced the growth-inhibitory effects of FOG at the process start up.

The use of conductive materials (e.g., ferric oxyhydroxide, magnetite, and granular activated carbon) recently has improved the methane production rate from dairy wastewaters.[177,178] Also, biomethane potential assays using oleate and granular activated carbon (GAC) (0−33 g L^{-1}) were performed by Tan et al.[179] The authors suggested that GAC addition promotes the faster consumption of both volatile fatty acid and LCFA, particularly palmitate. During oleate degradation, the presence of GAC decreased the lag phase for methane production. These authors postulate that since the electron transfer via direct interspecies electron transfer (DIET) is higher than via hydrogen, the potential shift from indirect hydrogen transfer to the DIET pathway, induced by the presence of GAC, may result in a more efficient conversion of LCFA to methane.[179] Despite of the use of conductive materials to promote DIET in processes of AD of lipids recently being studied, their application in both HRAT or LRAT systems needs to be further explored.

Additionally, the implementation of microaeration also has been shown as a promising strategy to enhance the digestion of lipids/LCFA-rich wastewaters, since it promotes oleate conversion to palmitate (which is less toxic to the microorganisms than oleate), avoiding a severe inhibition of methanogens.[69]

Biogas upgrading from anaerobic digestion of waste frying oils (WFO) was obtained in a biogas-lift bioreactor in which gas and liquid recirculations were applied. In this reactor, 1.4 times more biogas, with higher methane content (79%), was obtained when compared with the control reactor without gas recirculation (67%). This improvement resulted from the enrichment of hydrogenotrophic methanogens. Biogas recirculation thus appears as a promising strategy to enhance biomethane production from lipids.[180]

Despite of all the achievements, the basic issue of LCFA inhibition and palmitate accumulation are still not clearly understood, and their comprehension might boost process performance. This could allow true high-rate (<24 h) digestion, larger energy gains (even at low, psychrophilic, temperatures), and ultimately lead to further implementation of resource recovery from lipids in wastewater.

6. CODIGESTION OF FOG IN LOW-RATE ANAEROBIC TREATMENT OF SOLID WASTES

The use of anaerobic digestion to treat solid wastes, including sewage sludge or agricultural residues (e.g., manure), has been widely implemented as an efficient method to reduce the carbon footprint of solid waste over the past two decades.[181] Codigestion of these core, abundant wastes, with various cosubstrates, such as food waste (including household organic waste) and FOG-based waste, is regularly performed whenever possible, since this increases the overall biogas and methane yield of the AD plants.[182,183] Specifically, the addition of FOG-rich waste has been shown to be beneficial for biogas production rates. For instance, Angelidaki and Ahring[32] showed that the codigestion of FOG (oil mill effluent) with manure (50:50 and 75:25, FOG/manure in VS) allowed conversion of 85% of the lipids, and the methane production increased when the reactor feed was changed from manure alone to manure and FOG, from 1.2 to approximately 2.5 L d^{-1} (50:50, FOG/manure) and to 3.1 L d^{-1} (75:25, FOG/manure). More recently, a work from Wu et al.[184] demonstrated that the addition of grease trap waste could increase methane yield up to 68% compared to monodigestion of food waste. The scaling of the process to full-scale codigestion systems has been compiled and examined by Salama et al.,[183] detailing the potential biogas production worldwide in full-scale WWTPs conducting codigestion of FOG-rich wastes.

Despite the benefit of increased methane production potential, some problems are associated with the presence of FOG, namely, microbial inhibition (observable as a reduction in biogas production) or physical issues such as digester foaming, which is frequently reported during the codigestion of FOG wastes and has the potential to clog the gas collection and handling systems.[10] While clearly beneficial, the practice of adding FOG to both municipal and agricultural waste in anaerobic digesters is limited by the accumulation of LCFA and the potential inhibition of the overall AD process.[5,150,185] In these cases, theoretical methane production is not easily achieved. The threshold that maximizes the methane productivity, avoiding inhibition, is a critical point to be

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Environ. Sci. Technol. 2022, 56, 4749−4775

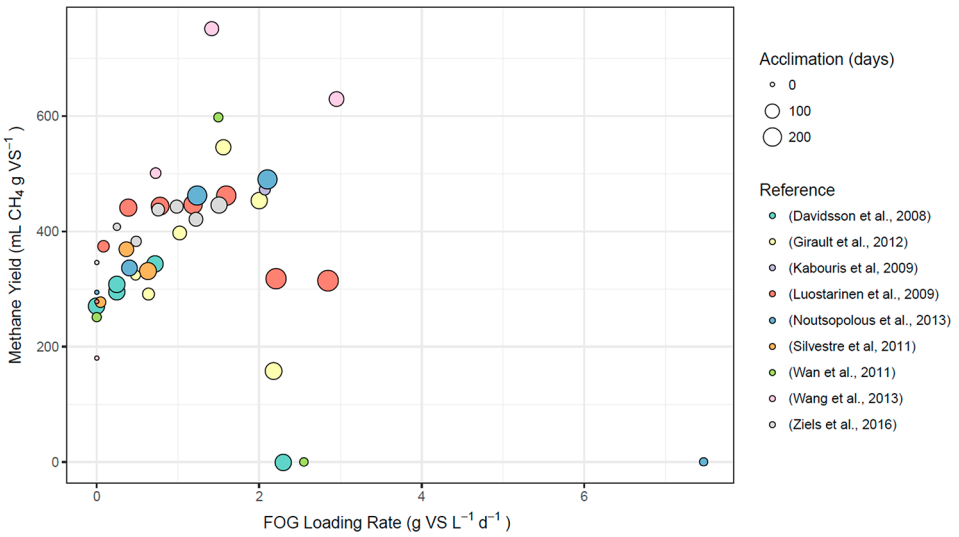

Figure 3. Summary of the methane yields obtained in several studies versus the FOG loading rate (expressed in VS) applied. References:[97,188−195]

addressed. For example, Usman et al.[154] advised to operate in codigestion processes of sludge and FOG on the concentration range of 0.1−1.5% (v/v), increasing the methane production steadily, while Neves et al.[186] showed that intermittent pulses of oily waste improved the methane production during the codigestion of food waste and cow manure (10%, v/v), up to an influent oil concentration (in COD) of 12 g L^{-1} (corresponding to an oil/manure ratio of 5%, v/v). The same codigestion system could endure recurrent pulses of oil at a COD concentration of 15 g L^{-1}, but a pulse of 18 g L^{-1} (in COD) resulted in a persistent system failure. Threshold values (expressed in COD per mass unit of total solids) of 180−220 and 120−150 g kg^{-1} were also established for total LCFA and palmitate, respectively, that should not be surpassed in order to prevent reactor failure in the codigestion of cow manure with food waste and oil.[187] In this work, C16:0 was also the major detected LCFA adsorbed/accumulated onto the solid matrix.

Methane yield obtained in several studies is summarized in Figure 3, according to the FOG loading rate applied and respective sludge acclimation time. In most of the cases, a linear tendency between the methane yield and the FOG loading rate can be observed, until a FOG loading rate (in VS) of approximately 2 g L^{-1} d^{-1}. The study of Luostarinen et al.[188] was an exception. This work showed that the methane yield remained approximately constant despite the increase of the FOG loading rate, probably due to the acclimation effect. In most studies, an increase over 2 g L^{-1} d^{-1} (in VS) promoted a decrease in the methane yield.

FOG or LCFA generally do not exceed 50% of the organic load (in COD) applied to low-rate digesters, although in the work of Ziels et al.[101] oleate reached 64% of the applied OLR. Further to the amount of FOG, the rate and type of feeding—pulse or continuous—has been outlined as key factors.[54,97,101] The feeding of reactors via continuous or pulse feeding of FOG has been tested at several locations, and results indicated that process performance improved while the microbial community was more stable when pulse feeding was applied.[54,97,101,196] This is consistent with previous research performed on solid wastes' ADs, as for example the work of De Vrieze et al.,[197] which showed that pulse feeding and/or variations in the substrate composition promoted higher functional stability of the anaerobic microbial communities during sewage sludge digestion.

While significant progress has been made in the field of solid wastes codigestion with FOG, mainly on cosubstrates ratios evaluations, and more recently linking microbial community to process changes, future investigations are still needed, specifically to identify the effects of the high variability associated with lipid-rich substrates in industrial systems and study the ability of these systems to accept high levels of lipids in a resilient manner. Such studies would add to the knowledge, robustness, and therefore confidence on the application of high levels of lipids at industrial full-scale systems, allowing a more ubiquitous use of lipids as substrate for biogas production. Besides biogas, other intermediates of AD of lipids, for example, medium chain fatty acids, are interesting target products to address in the future.

6.1. Pretreatment Strategies. Several pretreatment strategies have been tested to enhance the hydrolysis of lipid-rich wastes and improve their bioavailability and further anaerobic biodegradation. Detailed information on this topic can be found in the review of Salama et al.[183] Physical pretreatments include grinding and/or maceration, high-pressure homogenization, application of high temperature, microwaves, or ultrasounds.[183] These techniques destroy aggregated particles, decrease the particles' sizes, and disrupt the cells' structure. For example, microwave (MW) pretreatment of a mixture of thickened waste-activated sludge and FOG improved its solubilization up to 68% prior to the AD process and increased the methane yields up to 137% relative to the control.[198] Besides promoting lipids hydrolysis, the MW energy can also be used to break LCFA into shorter chain fatty acids, thus reducing LCFA inhibition. Similar results were also reported for the application of MW-enhanced advanced oxidation treatment of FOG.[199,200] Regarding ultrasonication, contradictory results have been presented by different authors. For example, Moisan[201] reported enhanced solubilization of LCFA, and consequent methane production, by applying ultrasonication on FOG, but Li et al.[202] reported that this pretreatment did not improve methane production in the codigestion of waste-activated sludge and FOG. Even more, longer lag phases were recorded in the codigestion of the pretreated samples. These negative effects of the pretreatment

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Environ. Sci. Technol. 2022, 56, 4749−4775

may be associated with the release of LCFA during the hydrolysis of lipids that may inhibit the microbial communities. Similar effects were also reported by Cirne et al.[203] and Cavaleiro et al.,[204] highlighting that even when the hydrolysis of FOG is improved by the pretreatment complete conversion of the substrates to methane can still be controlled by LCFA accumulation/biodegradation.

Chemical pretreatments change the molecular structure of the substrate, generally through the addition of acids or bases.[205−207] Thermochemical (saponification)[202,206,208,209] or enzymatic hydrolysis[57,210−212] have also been tested, as well as bioaugmentation with lipase-producing microorganisms.[203,213] Enzymatic hydrolysis of FOG was accelerated by ultrasonication.[214] Several patents and commercial products using microorganisms and/or enzyme pools for the biological treatment of FOG are available.[215] Commercial lipases are usually of microbial origin, namely, from bacteria, yeasts, and filamentous fungi. The use of viable microorganisms is more attractive than the addition of enzyme preparations, due to the high cost of the enzymes.[216,217]

In general, the costs of the pretreatments should be balanced by the benefits and must be in line with the funding available for each treatment system. In several situations, despite the potential benefits of the pretreatments, the net energy values are negative, mostly due to the high electrical energy consumption necessary to perform the pretreatments.[183,198] Therefore, further developments are still needed to turn these processes economically feasible.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

AD of lipids is a complex process that proceeds close to the thermodynamic minimum of life, being highly dependent on specific and complex microbial interactions. Significant progress has been made in the past two decades regarding fundamental knowledge in microbiology, biochemical pathways, and new reactor configurations, which have been translated into the market. The main challenges of the field have been tackled, allowing researchers to overcome the classical problems of microbial inhibition and sludge flotation and washout at higher loads. Therefore, AD of lipids is now a mature technology, which offers excellent opportunities for successful lipid valorization over long-term operation of stable full-scale systems.

Nevertheless, some issues are still challenging and constrain a wider implementation of AD of lipids:

(i) The equilibrium between LCFA accumulation and biodegradation to methane is still not mastered. Extremely high concentrations of fat lead to LCFA accumulation that hinder the bioconversion. Therefore, managing waste streams is currently necessary. This is a main critical point observed in pilot/full-scale operation of AD systems that may be tackled through the development of novel strategies that accelerate LCFA biodegradation and further conversion to methane (e.g., microaeration or addition of conductive materials).

(ii) The effect of lipids/LCFA on the structure and integrity of sludge is only poorly perceived yet, which most likely have a direct impact on process performance. In-depth studies on flocs formation, spatial organization within microbial aggregates, and exopolysaccharides formation are essential.

(iii) The reasons for success or failure of the biological processes are still to be unveiled, and this also applies to AD of lipids in codigestion processes. Additionally, it would be important to define early warning parameters to prevent reactor failure.

These issues call for further research, development, and innovation, targeting high-rate methane production from lipids and promoting AD of lipids as a hub in the bioenergy market. The production of medium chain fatty acids and/or other valuable compounds also represents an interesting alternative to biogas, which is highly relevant in the quest for a carbon-neutral world.

New strategies such as microaeration or addition of conductive materials are promising to boost methane production from lipids. Regarding microaeration in AD of lipids, fine-tuning the redox potential conditions can promote the partial detoxification of LCFA, likely triggering a more active methanogenic community thriving on lipids. Yet, the mechanisms involved and the interactions between facultative anaerobes and methanogens are new research topics in the field that still require additional studies, for example, by using pure cultures or synthetic microbial consortia. Concerning the application of conductive materials, those may act upon interspecies electron transfer or/and methanogenic activity which, otherwise, will rate limit the process. A deeper comprehension of the pathways and functional regulations in the mixed microbial communities performing AD of lipids in the presence of conductive materials is essential for an effective management of this approach.

Coupling the current methods used in the field with multiomics and advanced visualization, isotope probing, and detailed reactor data will increase the knowledge of AD of lipids. However, it is worth noting that reference genomic databases for the field need to be expanded, as only limited data are currently available. Thus, further holistic metagenome and metatranscriptome studies need to be performed.

The above mentioned research directions, together with novel strategies to improve the efficiency and interaction of the microorganism involved in the degradation of lipids, as well as the close collaboration between industry and academia, will most likely bring the AD of lipids to a higher maturity level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c08722.

Diagram with possible intermediates during unsaturated LCFA catabolism and Gibbs free energy changes of each metabolic step. Effect of hydrogen partial pressure on Gibbs free energy change of some partial reactions possibly involved in unsaturated LCFA degradation. Hydrogen and acetate as thermodynamic constraints on methanogenic LCFA degradation. (PDF)

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Author Contributions

The cofirst authors are BCH, MSD and MAS. The manuscript was written through contributions of all authors. The corresponding author made the final revision, and integrated comments from all coauthors. All authors have given approval to the final version of the manuscript.

Notes

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■ REFERENCES

(1) van Lier, J. B.; van der Zee, F. P.; Frijters, C. T. M. J.; Ersahin, M. E. Celebrating 40 Years Anaerobic Sludge Bed Reactors for Industrial Wastewater Treatment. *Rev. Environ. Sci. Biotechnol.* **2015**, *14* (4), 681−702.

(2) van Lier, J. B.; Mahmoud, N.; Zeeman, G. Anaerobic Wastewater Treatment. In *Biological Wastewater Treatment: Principles, Modeling and Design*, 2nd ed.; Chen, G., Ekama, G. A., van Loosdrecht, M. C. M., Brdjanovic, D., Eds.; IWA Publishing: London, 2020; pp 701−756. DOI: 10.2166/9781789060362_0701.

(3) Alves, M. M.; Pereira, M. A.; Sousa, D. Z.; Cavaleiro, A. J.; Picavet, M.; Smidt, H.; Stams, A. J. M. Waste Lipids to Energy: How to Optimize Methane Production from Long-Chain Fatty Acids (LCFA). *Microb. Biotechnol.* **2009**, *2* (5), 538−550.

(4) Rodríguez-Méndez, R.; Le Bihan, Y.; Béline, F.; Lessard, P. Long Chain Fatty Acids (LCFA) Evolution for Inhibition Forecasting during Anaerobic Treatment of Lipid-Rich Wastes: Case of Milk-Fed Veal Slaughterhouse Waste. *Waste Manag.* **2017**, *67*, 51−58.

(5) Chen, Y.; Cheng, J. J.; Creamer, K. S. Inhibition of Anaerobic Digestion Process: A Review. *Bioresour. Technol.* **2008**, *99* (10), 4044−4064.

(6) Palatsi, J.; Laureni, M.; Andrés, M. V.; Flotats, X.; Nielsen, H. B.; Angelidaki, I. Strategies for Recovering Inhibition Caused by Long Chain Fatty Acids on Anaerobic Thermophilic Biogas Reactors. *Bioresour. Technol.* **2009**, *100* (20), 4588−4596.

(7) Lalman, J. A.; Bagley, D. M. Anaerobic Degradation and Methanogenic Inhibitory Effects of Oleic and Stearic Acids. *Water Res.* **2001**, *35* (12), 2975−2983.

(8) Dereli, R. K.; Heffernan, B.; Grelot, A.; Van Der Zee, F. P.; Van Lier, J. B. Influence of High Lipid Containing Wastewater on Filtration Performance and Fouling in AnMBRs Operated at Different Solids Retention Times. *Sep. Purif. Technol.* **2015**, *139*, 43−52.

(9) Cavaleiro, A. J.; Picavet, M. A.; Sousa, D. Z.; Stams, A. J. M.; Pereira, M. A.; Alves, M. M. Anaerobic Digestion of Lipid-Rich Waste. In *Hydrocarbon and Lipid Microbiology Protocols: Pollution Mitigation and Waste Treatment Applications*; Springer Protocols Handbooks book series; McGenity, T. J., Timmis, K. N., Nogales, B., Eds.; Springer, 2015; pp 221−236. DOI: 10.1007/8623_2015_136.

(10) Long, J. H.; Aziz, T. N.; Reyes, F. L. D. L. D. L.; Ducoste, J. J. Anaerobic Co-Digestion of Fat, Oil, and Grease (FOG): A Review of Gas Production and Process Limitations. *Process Saf. Environ. Prot.* **2012**, *90* (3), 231−245.

(11) Wallace, T.; Gibbons, D.; O'Dwyer, M.; Curran, T. P. International Evolution of Fat, Oil and Grease (FOG) Waste Management - A Review. *J. Environ. Manage.* **2017**, *187*, 424−435.

(12) Abraham, W. R. Biosynthetic Oils, Fats, Terpenes, Sterols, Waxes: Analytical Methods, Diversity, Characteristics. In *Handbook of Hydrocarbon and Lipid Microbiology*; Timmis, K. N., Ed.; Springer: Berlin, Heidelberg, 2010; pp 79−95. DOI: 10.1007/978-3-540-77587-4.
(13) Gunstone, F. D.; Harwood, J. L. Occurence and Characterisation of Oils and Fats. In *The Lipid Handbook*; Gunstone, F. D., Harwood, J. L., Dijkstra, A. J., Eds.; CRC Press - Taylor & Francis Group, 2007; pp 37−143.
(14) Novak, J. T.; Carlson, D. a. Kinetics of Anaerobic Long Chain Fatty Acid Degradation. *Water Pollut. Control Fed.* **1970**, *42* (11), 1932−1943.
(15) Demirel, B.; Yenigun, O.; Onay, T. T. Anaerobic Treatment of Dairy Wastewaters: A Review. *Process Biochem.* **2005**, *40* (8), 2583−2595.
(16) Karadag, D.; Koroglu, O. E.; Ozkaya, B.; Cakmakci, M. A Review on Anaerobic Biofilm Reactors for the Treatment of Dairy Industry Wastewater. *Process Biochem.* **2015**, *50*, 262−271.
(17) Traversi, D.; Bonetta, S.; Degan, R.; Villa, S.; Porfido, A.; Bellero, M.; Carraro, E.; Gilli, G. Environmental Advances Due to the Integration of Food Industries and Anaerobic Digestion for Biogas Production: Perspectives of the Italian Milk and Dairy Product Sector. *Bioenergy Res.* **2013**, *6* (3), 851−863.
(18) Ince, O. Performance of a Two-Phase Anaerobic Digestion System When Treating Dairy Wastewater. *Water Res.* **1998**, *32* (9), 2707−2713.
(19) Arbeli, Z.; Brenner, A.; Abeliovich, A. Treatment of High-Strength Dairy Wastewater in an Anaerobic Deep Reservoir: Analysis of the Methanogenic Fermentation Pathway and the Rate-Limiting Step. *Water Res.* **2006**, *40* (19), 3653−3659.
(20) Omil, F.; Garrido, J. M.; Arrojo, B.; Méndez, R. Anaerobic Filter Reactor Performance for the Treatment of Complex Dairy Wastewater at Industrial Scale. *Water Res.* **2003**, *37* (17), 4099−4108.
(21) Szabo-Corbacho, M. A.; Pacheco-Ruiz, S.; Míguez, D.; Hooijmans, C. M.; García, H. A.; Brdjanovic, D.; van Lier, J. B. Impact of Solids Retention Time on the Biological Performance of an AnMBR Treating Lipid-Rich Synthetic Dairy Wastewater. *Environ. Technol.* **2021**, *42*, 597−608.
(22) Saddoud, A.; Sayadi, S. Application of Acidogenic Fixed-Bed Reactor Prior to Anaerobic Membrane Bioreactor for Sustainable Slaughterhouse Wastewater Treatment. *J. Hazard. Mater.* **2007**, *149* (3), 700−706.
(23) Núñez, L.; Martínez, B. Anaerobic Treatment of Slaughterhouse Wastewater in an Expanded Granular Sludge Bed (EGSB) Reactor. *Water Sci. Technol.* **1999**, *40* (8), 99−106.
(24) Ruiz, I.; Veiga, M. C.; De Santiago, P.; Blázquez, R. Treatment of Slaughterhouse Wastewater in a UASB Reactor and an Anaerobic Filter. *Bioresour. Technol.* **1997**, *60* (3), 251−258.
(25) Maya-Altamira, L.; Baun, A.; Angelidaki, I.; Schmidt, J. E. Influence of Wastewater Characteristics on Methane Potential in Food-Processing Industry Wastewaters. *Water Res.* **2008**, *42* (8−9), 2195−2203.
(26) Achour, M.; Khelifi, O.; Bouazizi, I.; Hamdi, M. Design of an Integrated Bioprocess for the Treatment of Tuna Processing Liquid Effluents. *Process Biochem.* **2000**, *35* (9), 1013−1017.
(27) Igwe, J. C.; Onyegbado, C. C. A Review of Palm Oil Mill Effluent (POME) Water Treatment. *Glob. J. Environ. Res.* **2007**, *1* (2), 54−62.
(28) Ismail, I.; Hassan, M. A.; Abdul Rahman, N. A.; Soon, C. S. Thermophilic Biohydrogen Production from Palm Oil Mill Effluent (POME) Using Suspended Mixed Culture. *Biomass and Bioenergy* **2010**, *34* (1), 42−47.
(29) Poh, P. E.; Yong, W. J.; Chong, M. F. Palm Oil Mill Effluent (POME) Characteristic in High Crop Season and the Applicability of High-Rate Anaerobic Bioreactors for the Treatment of Pome. *Ind. Eng. Chem. Res.* **2010**, *49* (22), 11732−11740.
(30) Boari, G.; Brunetti, A.; Passino, R.; Rozzi, A. Anaerobic Digestion of Olive Oil Mill Wastewaters. *Agric. Wastes* **1984**, *10* (3), 161−175.
(31) Azbar, N.; Bayram, A.; Filibeli, A.; Muezzinoglu, A.; Sengul, F.; Ozer, A. A Review of Waste Management Options in Olive Oil Production. *Crit. Rev. Environ. Sci. Technol.* **2004**, *34* (3), 209−247.
(32) Angelidaki, I.; Ahring, B. K. Codigestion of Olive Oil Mill Wastewaters with Manure, Household Waste or Sewage Sludge. *Biodegradation* **1997**, *8* (4), 221−226.
(33) Kim, Y.; Mosier, N. S.; Hendrickson, R.; Ezeji, T.; Blaschek, H.; Dien, B.; Cotta, M.; Dale, B.; Ladisch, M. R. Composition of Corn Dry-Grind Ethanol by-Products: DDGS, Wet Cake, and Thin Stillage. *Bioresour. Technol.* **2008**, *99*, 5165−5176.
(34) Dereli, R. K.; van der Zee, F. P.; Heffernan, B.; Grelot, A.; van Lier, J. B. J. B. Effect of Sludge Retention Time on the Biological Performance of Anaerobic Membrane Bioreactors Treating Corn-to-Ethanol Thin Stillage with High Lipid Content. *Water Res.* **2014**, *49*, 453−464.
(35) Becker, P.; Köster, D.; Popov, M. N.; Markossian, S.; Antranikian, G.; Märkl, H. The Biodegradation of Olive Oil and the Treatment of Lipid-Rich Wool Scouring Wastewater under Aerobic Thermophilic Conditions. *Water Res.* **1999**, *33* (3), 653−660.
(36) Bisschops, I.; Spanjers, H. Literature Review on Textile Wastewater Characterisation. *Environ. Technol.* **2003**, *24* (11), 1399−1411.
(37) Lapsirikul, W.; Ho, G.; Cord-Ruwisch, R. Mechanisms in Anaerobic Bioflocculation of Wool Scouring Effluent. *Water Res.* **1994**, *28* (8), 1749−1754.
(38) Saddoud, A.; HassaÑ, I.; Sayadi, S. Anaerobic membrane reactor with phase separation for the treatment of cheese whey. *Bioresource Technol.* **2007**, *98*, 2102−2108.
(39) Frijters, C. T. M. J.; Jorna, T.; Hesselink, G.; Kruit, J.; van Schaick, D.; van der Arend, R. Experiences with Anaerobic Treatment of Fat-Containing Food Waste Liquids: Two Full Scale Studies with a Novel Anaerobic Flotation Reactor. *Water Sci. Technol.* **2014**, *69* (7), 1386−1394.
(40) Paques, personal communication, 2021.
(41) Jeganathan, J.; Nakhla, G.; Bassi, A. Long-Term Performance of High-Rate Anaerobic Reactors for the Treatment of Oily Wastewater. *Environ. Sci. Technol.* **2006**, *40* (20), 6466−6472.
(42) Manjunath, N. T.; Mehrotra, I.; Mathur, R. P. Treatment of Wastewater from Slaughterhouse by DAF-UASB System. *Water Res.* **2000**, *34* (6), 1930−1936.
(43) Del Nery, V.; de Nardi, I. R.; Damianovic, M. H. R. Z.; Pozzi, E.; Amorim, A. K. B.; Zaiat, M. Long-Term Operating Performance of a Poultry Slaughterhouse Wastewater Treatment Plant. *Resour. Conserv. Recycl.* **2007**, *50* (1), 102−114.
(44) Nakhla, G.; Al-Sabawi, M.; Bassi, A.; Liu, V. Anaerobic Treatability of High Oil and Grease Rendering Wastewater. *J. Hazard. Mater.* **2003**, *102* (2−3), 243−255.
(45) Sayed, S.; van Campen, L.; Lettinga, G. Anaerobic Treatment of Slaughterhouse Waste Using a Granular Sludge UASB Reactor. *Biol. Wastes* **1987**, *21*, 11−28.
(46) Pastore, C.; Pagano, M.; Lopez, A.; Mininni, G.; Mascolo, G. Fat, Oil and Grease Waste from Municipal Wastewater: Characterization, Activation and Sustainable Conversion into Biofuel. *Water Sci. Technol.* **2015**, *71*, 1151−1157.
(47) Kobayashi, T.; Kuramochi, H.; Maeda, K.; Tsuji, T.; Xu, K. Dual-Fuel Production from Restaurant Grease Trap Waste: Bio-Fuel Oil Extraction and Anaerobic Methane Production from the Post-Extracted Residue. *Bioresour. Technol.* **2014**, *169*, 134−142.
(48) Kashi, S.; Satari, B.; Lundin, M.; Horváth, I. S.; Othman, M. Application of a Mixture Design to Identify the Effects of Substrates Ratios and Interactions on Anaerobic Co-Digestion of Municipal Sludge, Grease Trap Waste, and Meat Processing Waste. *J. Environ. Chem. Eng.* **2017**, *5* (6), 6156−6164.
(49) Grosser, A. The Influence of Decreased Hydraulic Retention Time on the Performance and Stability of Co-Digestion of Sewage Sludge with Grease Trap Sludge and Organic Fraction of Municipal Waste. *J. Environ. Manage.* **2017**, *203*, 1143−1157.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775

(50) Hanaki, K.; Matsuo, T.; Nagase, M. Mechanism of Inhibition Caused by Long-Chain Fatty Acids in Anaerobic Digestion Process. *Biotechnol. Bioeng.* **1981**, *23* (7), 1591−1610.
(51) Masse, L.; Massé, D. I.; Kennedy, K. J.; Chou, S. P. Neutral Fat Hydrolysis and Long-Chain Fatty Acid Oxidation during Anaerobic Digestion of Slaughterhouse Wastewater. *Biotechnol. Bioeng.* **2002**, *79* (1), 43−52.
(52) Heukelekian, A. H.; Mueller, P. Transformation of Some Lipids in Anaerobic Sludge Digestion. *Sewage Ind. Waste.* **1958**, *30* (9), 1108−1120.
(53) Weng, C.; Jeris, J. S. Biochemical Mechanisms in the Methane Fermentation of Glutamic and Oleic Acids. *Water Res.* **1976**, *10* (1), 9−18.
(54) Hwu, C.-S.; Donlon, B.; Lettinga, G. Comparative Toxicity of Long-Chain Fatty Acid to Anaerobic Sludges from Various Origins. *Water Sci. Technol.* **1996**, *34* (5-6), 351−358.
(55) Rinzema, A.; Boone, M.; van Knippenberg, K.; Lettinga, G. Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion. *Water Environ. Res.* **1994**, *66* (1), 40−49.
(56) Bryant, M. P. Microbial Methane Production - Theoretical Aspects. *J. Anim. Sci.* **1979**, *48* (1), 193−201.
(57) Masse, L.; Masse, D.I.; Kennedy, K.J. Effect of Hydrolysis Pretreatment on Fat Degradation during Anaerobic Digestion of Slaughterhouse Wastewater. *Process Biochem. 38* **2003**, *38* (9), 1365−1372.
(58) Hwu, C.-S.; Molenaar, G.; Garthoff, J.; Van Lier, J. B.; Lettinga, G. Thermophilic High-Rate Anaerobic Treatment of Wastewater Containing Long-Chain Fatty Acids: Impact of Reactor Hydrodynamics. *Biotechnol. Lett.* **1997**, *19* (5), 447−451.
(59) Pereira, M. A.; Sousa, D. Z.; Mota, M.; Alves, M. M. Mineralization of LCFA Associated with Anaerobic Sludge: Kinetics, Enhancement of Methanogenic Activity, and Effect of VFA. *Biotechnol. Bioeng.* **2004**, *88* (4), 502−511.
(60) Pereira, M. A.; Pires, O. C.; Mota, M.; Alves, M. M. Anaerobic Biodegradation of Oleic and Palmitic Acids: Evidence of Mass Transfer Limitations Caused by Long Chain Fatty Acid Accumulation onto the Anaerobic Sludge. *Biotechnol. Bioeng.* **2005**, *92* (1), 15−23.
(61) Angelidaki, I.; Ahring, B. K. Effects of Free Long-Chain Fatty Acids on Thermophilic Anaerobic Digestion. *Appl. Micriobiology Biotechnol.* **1992**, *37*, 808−812.
(62) Koster, I. W.; Cramer, A. Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. *Appl. Environ. Microbiol.* **1987**, *53* (2), 403−409.
(63) Hwu, C.; Lettinga, G. Acute Toxicity of Oleate to Acetate-Utilizing Methanogens in Mesophilic and Thermophilic Anaerobic Sludges. *Enzyme Microb. Technol.* **1997**, *21*, 297−301.
(64) Silva, S. A.; Salvador, A. F.; Cavaleiro, A. J.; Pereira, M. A.; Stams, A. J. M.; Alves, M. M.; Sousa, D. Z. Toxicity of Long Chain Fatty Acids towards Acetate Conversion by Methanosaeta Concilii and Methanosarcina Mazei. *Microb. Biotechnol.* **2016**, *9*, 514−518.
(65) Sousa, D. Z.; Salvador, A. F.; Ramos, J.; Guedes, A. P.; Barbosa, S.; Stams, A. J. M.; Alves, M. M.; Pereira, M. A. Activity and Viability of Methanogens in Anaerobic Digestion of Unsaturated and Saturated Long-Chain Fatty Acids. *Appl. Environ. Microbiol.* **2013**, *79* (14), 4239−4245.
(66) Lalman, J.; Bagley, D. M. Effects of C18 Long Chain Fatty Acids on Glucose, Butyrate and Hydrogen Degradation. *Water Res.* **2002**, *36* (13), 3307−3313.
(67) Cavaleiro, A. J.; Salvador, A. F.; Alves, J. I.; Alves, M. Continuous High Rate Anaerobic Treatment of Oleic Acid Based Wastewater Is Possible after a Step Feeding Start-Up. *Environ. Sci. Technol.* **2009**, *43* (8), 2931−2936.
(68) Cavaleiro, A. J.; Pereira, M. A.; Guedes, A. P.; Stams, A. J. M.; Alves, M. M.; Sousa, D. Z. Conversion of Cn-Unsaturated into Cn-2-Saturated LCFA Can Occur Uncoupled from Methanogenesis in Anaerobic Bioreactors. *Environ. Sci. Technol.* **2016**, *50* (6), 3082−3090.
(69) Duarte, M. S.; Silva, S. A.; Salvador, A. F.; Cavaleiro, A. J.; Stams, A. J. M. M.; Alves, M. M.; Pereira, M. A. Insight into the Role of Facultative Bacteria Stimulated by Microaeration in Continuous Bioreactors Converting LCFA to Methane. *Environ. Sci. Technol.* **2018**, *52* (11), 6497−6507.
(70) Ziels, R. M.; Sousa, D. Z.; Stensel, H. D.; Beck, D. A. C. DNA-SIP Based Genome-Centric Metagenomics Identifies Key Long-Chain Fatty Acid-Degrading Populations in Anaerobic Digesters with Different Feeding Frequencies. *ISME J.* **2018**, *12* (1), 112−123.
(71) Pereira, M. A.; Pires, O. C.; Mota, M.; Alves, M. M. Anaerobic Degradation of Oleic Acid by Suspended and Granular Sludge: Identification of Palmitic Acid as a Key Intermediate. *Water Sci. Technol.* **2002**, *45* (10), 139−144.
(72) Haridas, A.; Suresh, S.; Chitra, K. R.; Manilal, V. B. The Buoyant Filter Bioreactor: A High-Rate Anaerobic Reactor for Complex Wastewater - Process Dynamics with Dairy Effluent. *Water Res.* **2005**, *39* (6), 993−1004.
(73) Alves, M. M.; Picavet, M. A.; Pereira, M. A.; Sousa, D. Z.; Cavaleiro, A. J. Novel Anaerobic Reactor for the Removal of Long Chain Fatty Acids from Fat Containing Wastewater. World Patent 2007058557, 2007.
(74) Vellinga, S. H. J.; Mulder, R. Method and Device for the Anaerobic Purification of a Slurry Which Contains Organic Constituents. World Patent 02076893, 2002.
(75) Bouman, R.; Heffernan, B. *AnMBR, Anaerobic Membrane Bioreactor: From Concept to Full-Scale and Future Outlook*; Veolia Water Technologies, 2016.
(76) Sayed, S.; van der Zanden, J.; Wijffels, R.; Lettinga, G. Anaerobic Degradation of the Various Fractions of Slaughterhouse Wastewater. *Biol. Wastes* **1988**, *23* (2), 117−142.
(77) Hwu, C.-S.; Van Beek, B.; Van Lier, J. B.; Lettinga, G. Thermophilic High-Rate Anaerobic Treatment of Wastewater Containing Long-Chain Fatty Acids: Effect of Washed out Biomass Recirculation. *Biotechnol. Lett.* **1997**, *19* (5), 453−456.
(78) Alves, M. M.; Mota Vieira, J. A.; Álvares Pereira, R. M.; Pereira, M. A.; Mota, M. Effects of Lipids and Oleic Acid on Biomass Development in Anaerobic Fixed-Bed Reactors. Part II: Oleic Acid Toxicity and Biodegradability. *Water Res.* **2001**, *35* (1), 264−270.
(79) Demeyer, D. I.; Henderickx, H. K. The Effect of C18 Unsaturated Fatty Acids on Methane Production in Vitro by Mixed Rumen Bacteria. *Biochem. Biophys. Acta* **1967**, *137*, 484−497.
(80) Galbraith, H.; Miller, T. B. Physicochemical Effects of Long Chain Fatty Acids on Bacterial Cells and Their Protoplasts. *J. Appl. Bacteriol.* **1973**, *36* (4), 647−658.
(81) Angelidaki, I.; Petersen, S. P.; Ahring, B. K. Effects of Lipids on Thermophilic Anaerobic Digestion and Reduction of Lipid Inhibition upon Addition of Bentonite. *Appl. Microbiol. Biotechnol.* **1990**, *33* (4), 469−472.
(82) Hwu, C.; van Lier, J. B.; Lettinga, G. Physicochemical and Biological Performance of Expanded Granular Sludge Bed Reactors Treating Long-Chain Fatty Acids. *Process Biochem.* **1998**, *33* (1), 75−81.
(83) Singh, S.; Rinta-Kanto, J. M.; Kettunen, R.; Tolvanen, H.; Lens, P.; Collins, G.; Kokko, M.; Rintala, J. Anaerobic Treatment of LCFA-Containing Synthetic Dairy Wastewater at 20 °C: Process Performance and Microbial Community Dynamics. *Sci. Total Environ.* **2019**, *691*, 960−968.
(84) Singh, S.; Holohan, B. C.; Mills, S.; Castilla-Archilla, J.; Kokko, M.; Rintala, J.; Lens, P. N. L.; Collins, G.; O'Flaherty, V. Enhanced Methanization of Long-Chain Fatty Acid Wastewater at 20°C in the Novel Dynamic Sludge Chamber-Fixed Film Bioreactor. *Front. Energy Res.* **2020**, *8*, 1−13.
(85) Singh, S.; Rinta-Kanto, J. M.; Lens, P. N. L.; Kokko, M.; Rintala, J.; O'Flaherty, V.; Ijaz, U. Z.; Collins, G. Microbial Community Assembly and Dynamics in Granular, Fixed-Biofilm and Planktonic Microbiomes Valorizing Long-Chain Fatty Acids at 20 °C. *Bioresour. Technol.* **2022**, *343*, 126098.
(86) Petropoulos, E.; Dolfing, J.; Yu, Y.; Wade, M. J.; Bowen, E. J.; Davenport, J.; Curtis, T. P. Lipolysis of Domestic Wastewater in Anaerobic Reactors Operating at Low Temperatures. *Environ. Sci. Water Res. Technol.* **2018**, *4* (7), 1002−1013.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775

(87) Keating, C.; Hughes, D.; Mahony, T.; Cysneiros, D.; Ijaz, U. Z.; Smith, C. J.; O'Flaherty, V. Cold Adaptation and Replicable Microbial Community Development during Long-Term Low-Temperature Anaerobic Digestion Treatment of Synthetic Sewage. *FEMS Microbiol. Ecol.* **2018**, *94* (7), 1−14.
(88) Hawkes, F. R.; Donnelly, T.; Anderson, G. K. Comparativem Performance of Anaerobic Digesters Operating on Ice-Cream Wastewater. *Water Rsearch* **1995**, *29* (2), 525−533.
(89) Sam-Soon, P.; Loewenthal, R.; Wentzel, M.; Marais, G. A Long-Chain Fatty Acid, Oleate, as Sole Substrate in Upflow Anaerobic Sludge Bed (UASB) Reactor Systems. *Water SA* **1991**, *17* (1), 31−36.
(90) Galbraith, H.; Miller, T. B. Effect of Metal Cations and PH on the Antibacterial Activity and Uptake of Long Chain Fatty Acids. *J. Appl. Microbiol.* **1973**, *36*, 635−646.
(91) Hwu, C.-S.; Tseng, S.-K.; Yuan, C.-Y.; Kulik, Z.; Lettinga, G. Biosorption of Long-Chain Fatty Acids in UASB Treatment Process. *Water Res.* **1998**, *32* (5), 1571−1579.
(92) Alves, M.; Mota Vieira, J.; Álvares Pereira, R.; Pereira, M.; Mota, M. Effect of Lipids and Oleic Acid on Biomass Development in Anaerobic Fixed-Bed Reactors. Part I: Biofilm Growth and Activity. *Water Res.* **2001**, *35* (1), 255−263.
(93) Pereira, M. A.; Mota, M.; Alves, M. M. Degradation of Oleic Acid in Anaerobic Filters: The Effect of Inoculum Acclimatization and Biomass Recirculation. *Water Environ. Res.* **2001**, *73* (5), 612−621.
(94) Silva, S. A.; Cavaleiro, A. J.; Pereira, M. A.; Stams, A. J. M.; Alves, M. M.; Sousa, D. Z. Long-Term Acclimation of Anaerobic Sludges for High-Rate Methanogenesis from LCFA. *Biomass and Bioenergy* **2014**, *67*, 297−303.
(95) Nakasaki, K.; Koyama, M.; Maekawa, T.; Fujita, J. Changes in the Microbial Community during the Acclimation Process of Anaerobic Digestion for Treatment of Synthetic Lipid-Rich Wastewater. *J. Biotechnol.* **2019**, *306* (July), 32−37.
(96) Nakasaki, K.; Nguyen, K. K.; Ballesteros, F. C.; Maekawa, T.; Koyama, M. Characterizing the Microbial Community Involved in Anaerobic Digestion of Lipid-Rich Wastewater to Produce Methane Gas. *Anaerobe* **2020**, *61*, 102082.
(97) Ziels, R. M.; Karlsson, A.; Beck, D. A. C. C.; Ejlertsson, J. J.; Yekta, S. S.; Bjorn, A.; Stensel, H. D.; Svensson, B. H. Microbial Community Adaptation Influences Long-Chain Fatty Acid Conversion during Anaerobic Codigestion of Fats, Oils, and Grease with Municipal Sludge. *Water Res.* **2016**, *103*, 372−382.
(98) Kurade, M. B.; Saha, S.; Salama, E. S.; Patil, S. M.; Govindwar, S. P.; Jeon, B. H. Acetoclastic Methanogenesis Led by Methanosarcina in Anaerobic Co-Digestion of Fats, Oil and Grease for Enhanced Production of Methane. *Bioresour. Technol.* **2019**, *272*, 351−359.
(99) Kurade, M. B.; Saha, S.; Kim, J. R.; Roh, H.-S.; Jeon, B.-H. Microbial Community Acclimatization for Enhancement in the Methane Productivity of Anaerobic Co-Digestion of Fats, Oil, and Grease. *Bioresour. Technol.* **2020**, *296*, 122294.
(100) Amha, Y. M.; Sinha, P.; Lagman, J.; Gregori, M.; Smith, A. L. Elucidating Microbial Community Adaptation to Anaerobic Co-Digestion of Fats, Oils, and Grease and Food Waste. *Water Res.* **2017**, *123*, 277−289.
(101) Ziels, R. M.; Beck, D. A. C.; Stensel, H. D. Long-Chain Fatty Acid Feeding Frequency in Anaerobic Codigestion Impacts Syntrophic Community Structure and Biokinetics. *Water Res.* **2017**, *117*, 218−229.
(102) Kougias, P. G.; Treu, L.; Campanaro, S.; Zhu, X.; Angelidaki, I. Dynamic Functional Characterization and Phylogenetic Changes Due to Long Chain Fatty Acids Pulses in Biogas Reactors. *Sci. Rep.* **2016**, *6*, 1−10.
(103) Cavaleiro, A. J.; Sousa, D. Z.; Alves, M. M. Methane Production from Oleate: Assessing the Bioaugmentation Potential of Syntrophomonas Zehnderi. *Water Res.* **2010**, *44* (17), 4940−4947.
(104) Eftaxias, A.; Diamantis, V.; Michailidis, C.; Stamatelatou, K.; Aivasidis, A. Comparison of Anaerobic Digesters Performance Treating Palmitic, Stearic and Oleic Acid: Determination of the LCFA Kinetic Constants Using ADM1. *Bioprocess Biosyst. Eng.* **2020**, *43* (7), 1329−1338.
(105) Mackie, R. I.; White, B. A.; Bryant, M. P. Lipid Metabolism in Anaerobic Ecosystems. *Crit. Rev. Microbiol.* **1991**, *17* (6), 449−479.
(106) DiRusso, C. C.; Black, P. N.; Weimar, J. D. Molecular Inroads into the Regulation and Metabolism of Fatty Acids, Lessons from Bacteria. *Prog. Lipid Res.* **1999**, *38* (2), 129−197.
(107) Sousa, D. Z.; Smidt, H.; Alves, M. M.; Stams, A. J. M. M. Ecophysiology of Syntrophic Communities That Degrade Saturated and Unsaturated Long-Chain Fatty Acids. *FEMS Microbiol. Ecol.* **2009**, *68* (3), 257−272.
(108) Schink, B. Energetics of Syntrophic Cooperation in Methanogenic Degradation. *Microbiol. Mol. Biol. Rev.* **1997**, *61* (2), 262−280.
(109) Stams, A. J. M.; De Bok, F. A. M.; Plugge, C. M.; Van Eekert, M. H. A.; Dolfing, J.; Schraa, G. Exocellular Electron Transfer in Anaerobic Microbial Communities. *Environ. Microbiol.* **2006**, *8* (3), 371−382.
(110) Lovley, D. R. Electrotrophy: Other Microbial Species, Iron, and Electrodes as Electron Donors for Microbial Respirations. *Bioresour. Technol.* **2022**, *345*, 126553.
(111) Wang, Z.; Wang, T.; Si, B.; Watson, J.; Zhang, Y. Accelerating Anaerobic Digestion for Methane Production: Potential Role of Direct Interspecies Electron Transfer. *Renew. Sustain. Energy Rev.* **2021**, *145*, 111069.
(112) Lalman, J. A.; Bagley, D. M. Anaerobic Degradation and Inhibitory Effects of Linoleic Acid. *Water Res.* **2000**, *34* (17), 4220−4228.
(113) Broughton, M. J.; Thiele, J. H.; Birch, E. J.; Cohen, A. Anaerobic Batch Digestion of Sheep Tallow. *Water Res.* **1998**, *32* (5), 1423−1428.
(114) Dolfing, J.; Larter, S. R.; Head, I. M. Thermodynamic Constraints on Methanogenic Crude Oil Biodegradation. *ISME J.* **2008**, *2* (4), 442−452.
(115) Sousa, D. Z.; Pereira, M. A.; Stams, A. J. M.; Alves, M. M.; Smidt, H. Microbial Communities Involved in Anaerobic Degradation of Unsaturated or Saturated Long-Chain Fatty Acids. *Appl. Environ. Microbiol.* **2007**, *73* (4), 1054−1064.
(116) Dereli, R. K.; van der Zee, F. P.; Heffernan, B.; Grelot, A.; van Lier, J. B. Effect of Sludge Retention Time on the Biological Performance of AnMB Treating Corn-to-Ethanol Thin Stillage with High Lipid Content. *Water Res.* **2014**, *49*, 453−464.
(117) Pitk, P.; Palatsi, J.; Kaparaju, P.; Fernández, B.; Vilu, R. Mesophilic Co-Digestion of Dairy Manure and Lipid Rich Solid Slaughterhouse Wastes: Process Efficiency, Limitations and Floating Granules Formation. *Bioresour. Technol.* **2014**, *166*, 168−177.
(118) Schink, B. Energetics of Syntrophic Cooperation in Methanogenic Degradation. *Microbiol. Mol. Biol. Rev.* **1997**, *61* (2), 262−280.
(119) McInerney, M. J.; Struchtemeyer, C. G.; Sieber, J.; Mouttaki, H.; Stams, A. J. M. M.; Schink, B.; Rohlin, L.; Gunsalus, R. P. Physiology, Ecology, Phylogeny, and Genomics of Microorganisms Capable of Syntrophic Metabolism. *Ann. N.Y. Acad. Sci.* **2008**, *1125*, 58−72.
(120) Roy, F.; Samain, E.; Dubourguier, H. C.; Albagnac, G. Synthrophomonas Sapovorans Sp. Nov., a New Obligately Proton Reducing Anaerobe Oxidizing Saturated and Unsaturated Long Chain Fatty Acids. *Arch. Microbiol.* **1986**, *145*, 142−147.
(121) Zhang, F.; Liu, X.; Dong, X. Thermosyntropha Tengcongensis Sp. Nov., a Thermophilic Bacterium That Degrades Long-Chain Fatty Acids Syntrophically. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 759−763.
(122) Svetlitshnyi, V.; Rainey, F.; Wiegel, J. Thermosyntropha Lipolytica Gen. Nov., Sp. Nov., a Lipolytic, Anaerobic, Alkalitolerant, Thermophilic Bacterium Utilizing Short- and Long-Chain Fatty Acids in Syntrophic Coculture with a Methanogenic Archaeum. *Int. J. Syst. Bacteriol.* **1996**, *46* (4), 1131−1137.
(123) Schink, B.; Stams, A. J. M. Syntrophism Among Prokaryotes. *In The Prokaryotes: Prokaryotic Communities and Ecophysiology* **2013**, 1−528.
(124) Stams, A. J. M.; Sousa, D. Z.; Kleerebezem, R.; Plugge, C. M. Role of Syntrophic Microbial Communities in High-Rate Methanogenic Bioreactors. *Water Sci. Technol.* **2012**, *66* (2), 352−362.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775

(125) Stams, A. J. M. M. Metabolic Interactions between Anaerobic Bacteria in Methanogenic Environments. *Antonie Van Leeuwenhoek* **1994**, *66* (1−3), 271−294.
(126) Sousa, D. Z.; Alves, J. I.; Alves, M. M.; Smidt, H.; Stams, A. J. M. M. Effect of Sulfate on Methanogenic Communities That Degrade Unsaturated and Saturated Long-Chain Fatty Acids (LCFA). *Environ. Microbiol.* **2009**, *11* (1), 68−80.
(127) Salvador, A. F.; Cavaleiro, A. J.; Paulo, A. M. S.; Silva, S. A.; Guedes, A. P.; Pereira, M. A.; Stams, A. J. M.; Sousa, D. Z.; Alves, M. M. Inhibition Studies with 2-Bromoethanesulfonate Reveal a Novel Syntrophic Relationship in Anaerobic Oleate Degradation. *Appl. Environ. Microbiol.* **2019**, *85* (2), 1−12.
(128) Cavaleiro, A. J.; Guedes, A. P.; Silva, S. A.; Arantes, A. L.; Sequeira, J. C.; Salvador, A. F.; Sousa, D. Z.; Stams, A. J. M.; Alves, M. M. Effect of Sub-Stoichiometric Fe(Iii) Amounts on Lcfa Degradation by Methanogenic Communities. *Microorganisms* **2020**, *8* (9), 1375.
(129) Baserba, M. G.; Angelidaki, I.; Karakashev, D. Effect of Continuous Oleate Addition on Microbial Communities Involved in Anaerobic Digestion Process. *Bioresour. Technol.* **2012**, *106*, 74−81.
(130) Pereira, M. A.; Roest, K.; Stams, A. J. M.; Mota, M.; Alves, M.; Akkermans, A. D. L. Molecular Monitoring of Microbial Diversity in Expanded Granular Sludge Bed (EGSB) Reactors Treating Oleic Acid. *FEMS Microbiol. Ecol.* **2002**, *41* (2), 95−103.
(131) Sousa, D. Z.; Pereira, M. A.; Smidt, H.; Stams, A. J. M.; Alves, M. M. Molecular Assessment of Complex Microbial Communities Degrading Long Chain Fatty Acids in Methanogenic Bioreactors. *FEMS Microbiol. Ecol.* **2007**, *60* (2), 252−265.
(132) Hatamoto, M.; Imachi, H.; Yashiro, Y.; Ohashi, A.; Harada, H. Diversity of Anaerobic Microorganisms Involved in Long-Chain Fatty Acid Degradation in Methanogenic Sludges as Revealed by RNA-Based Stable Isotope Probing. *Appl. Environ. Microbiol.* **2007**, *73* (13), 4119−4127.
(133) Palatsi, J.; Viñas, M.; Guivernau, M.; Fernandez, B.; Flotats, X. Anaerobic Digestion of Slaughterhouse Waste: Main Process Limitations and Microbial Community Interactions. *Bioresour. Technol.* **2011**, *102* (3), 2219−2227.
(134) Solli, L.; Håvelsrud, O. E.; Horn, S. J.; Rike, A. G. A Metagenomic Study of the Microbial Communities in Four Parallel Biogas Reactors. *Biotechnol. Biofuels* **2014**, *7* (146), 1−15.
(135) De Francisci, D.; Kougias, P. G.; Treu, L.; Campanaro, S.; Angelidaki, I. Microbial Diversity and Dynamicity of Biogas Reactors Dueto Radical Changes of Feedstock Composition. *Bioresour. Technol.* **2015**, *176*, 56−64.
(136) Salvador, A. F. F. *Functional Analysis of Syntrophic LCFA-Degrading Microbial Ecosystems*. Dissertation, Universidade do Minho, 2013.
(137) Shigematsu, T.; Tang, Y.; Mizuno, Y.; Kawaguchi, H.; Morimura, S.; Kida, K. Microbial Diversity of Mesophilic Methanogenic Consortium That Can Degrade Long-Chain Fatty Acids in Chemostat Cultivation. *J. Biosci. Bioeng.* **2006**, *102* (6), 535−544.
(138) Treu, L.; Campanaro, S.; Kougias, P. G.; Zhu, X.; Angelidaki, I. Untangling the Effect of Fatty Acid Addition at Species Level Revealed Different Transcriptional Responses of the Biogas Microbial Community Members. *Environ. Sci. Technol.* **2016**, *50* (11), 6079−6090.
(139) Menes, R. J.; Fernández, A.; Muxí, L. Physiological and Molecular Characterisation of an Anaerobic Thermophilic Oleate-Degrading Enrichment Culture. *Anaerobe* **2001**, *7*, 17−24.
(140) Pereira, M. A.; Roest, K.; Stams, A. J. M.; Akkermans, A. D. L.; Amaral, A. L.; Pons, M. N.; Ferreira, E. C.; Mota, M.; Alves, M. M. Image Analysis, Methanogenic Activity Measurements, and Molecular Biological Techniques to Monitor Granular Sludge from an EGSB Reactor Fed with Oleic Acid. *Water Sci. Technol.* **2003**, *47* (5), 181−188.
(141) Grabowski, A.; Blanchet, D.; Jeanthon, C. Characterization of Long-Chain Fatty-Acid-Degrading Syntrophic Associations from a Biodegraded Oil Reservoir. *Res. Microbiol.* **2005**, *156* (7), 814−821.
(142) Hatamoto, M.; Imachi, H.; Ohashi, A.; Harada, H. Identification and Cultivation of Anaerobic, Syntrophic Long-Chain Fatty Acid-Degrading Microbes from Mesophilic and Thermophilic Methanogenic Sludges. *Appl. Environ. Microbiol.* **2007**, *73* (4), 1332−1340.
(143) Palatsi, J.; Illa, J.; Prenafeta-Boldú, F. X.; Laureni, M.; Fernandez, B.; Angelidaki, I.; Flotats, X. Long-Chain Fatty Acids Inhibition and Adaptation Process in Anaerobic Thermophilic Digestion: Batch Tests, Microbial Community Structure and Mathematical Modelling. *Bioresour. Technol.* **2010**, *101* (7), 2243−2251.
(144) Martín-González, L.; Castro, R.; Pereira, M. A.; Alves, M. M.; Font, X.; Vicent, T. Thermophilic Co-Digestion of Organic Fraction of Municipal Solid Wastes with FOG Wastes from a Sewage Treatment Plant: Reactor Performance and Microbial Community Monitoring. *Bioresour. Technol.* **2011**, *102* (7), 4734−4741.
(145) Zhang, Y.; Zamudio Cañas, E. M.; Zhu, Z.; Linville, J. L.; Chen, S.; He, Q. Robustness of Archaeal Populations in Anaerobic Co-Digestion of Dairy and Poultry Wastes. *Bioresour. Technol.* **2011**, *102* (2), 779−785.
(146) Salvador, A. F.; Cavaleiro, A. J.; Sousa, D. Z.; Alves, M. M.; Pereira, M. A. Endurance of Methanogenic Archaea in Anaerobic Bioreactors Treating Oleate-Based Wastewater. *Appl. Microbiol. Biotechnol.* **2013**, *97* (5), 2211−2218.
(147) Bialek, K.; Cysneiros, D.; O'Flaherty, V. Low-Temperature (10°C) Anaerobic Digestion of Dilute Dairy Wastewater in an EGSB Bioreactor: Microbial Community Structure, Population Dynamics, and Kinetics of Methanogenic Populations. *Archaea* **2013**, *2013*, 1−10.
(148) Ziels, R. M.; Beck, D. A. C.; Martí, M.; Gough, H. L.; Stensel, H. D.; Svensson, B. H. Monitoring the Dynamics of Syntrophic *β*-Oxidizing Bacteria during Anaerobic Degradation of Oleic Acidby Quantitative PCR. *FEMS Microbiol. Ecol.* **2015**, *91* (4), 1−13.
(149) Toumi, J.; Miladi, B.; Farhat, A.; Nouira, S.; Hamdi, M.; Gtari, M.; Bouallagui, H. Microbial Ecology Overview during Anaerobic Codigestion of Dairy Wastewater and Cattle Manure and Use in Agriculture of Obtained Bio-Fertilisers. *Bioresour. Technol.* **2015**, *198*, 141−149.
(150) Regueiro, L.; Carballa, M.; Lema, J. M. Microbiome Response to Controlled Shifts in Ammonium and LCFA Leveis in Co-Digestion Systems. *J. Biotechnol.* **2016**, *220*, 35−44.
(151) Ferguson, R.; Coulon, F.; Villa, R. Organic Loading Rate: A Promising Microbial Management Tool in Anaerobic Digestion. *Water Res.* **2016**, *100*, 348−356.
(152) Treu, L.; Tsapekos, P.; Peprah, M.; Campanaro, S.; Giacomini, A.; Corich, V.; Kougias, P. G.; Angelidaki, I. Microbial Profiling during Anaerobic Digestion of Cheese Whey in Reactors Operated at Different Conditions. *Bioresour. Technol.* **2019**, *275*, 375−385.
(153) Nzila, A.; Razzak, S. A.; Sankara, S.; Nazal, M. K.; Al-Momani, M.; Kang, G. U.; Ibal, J. C.; Shin, J. H. Characterisation and Microbial Community Analysis of Lipid Utilising Microorganisms for Biogas Formation. *PLoS One* **2019**, *14* (11), e0224989.
(154) Usman, M.; Salama, E. S.; Arif, M.; Jeon, B. H.; Li, X. Determination of the Inhibitory Concentration Level of Fat, Oil, and Grease (FOG) towards Bacterial and Archaeal Communities in Anaerobic Digestion. *Renew. Sustain. Energy Rev.* **2020**, *131* (March), 110032.
(155) Thauer, R. K.; Jungermann, K.; Decker, K. Energy Conservation in Chemotrophic Anaerobic Bacteria. *Bacteriol. Rev.* **1977**, *41* (1), 100−180.
(156) Duarte, M. S.; Salvador, A. F.; Cavaleiro, A. J.; Stams, A. J. M.; Pereira, M. A.; Alves, M. M. Multiple and Flexible Roles of Facultative Anaerobic Bacteria in Microaerophilic Oleate Degradation. *Environ. Microbiol.* **2020**, *22* (9), 3650−3659.
(157) Hao, J.; de los Reyes, F. L.; He, X. Fat, Oil, and Grease (FOG) Deposits Yield Higher Methane than FOG in Anaerobic Co-Digestion with Waste Activated Sludge. *J. Environ. Manage.* **2020**, *268*, 110708.
(158) Salama, E. S.; Jeon, B. H.; Kurade, M. B.; Patil, S. M.; Usman, M.; Li, X.; Lim, H. Enhanced Anaerobic Co-Digestion of Fat, Oil, and Grease by Calcium Addition: Boost of Biomethane Production and Microbial Community Shift. *Bioresour. Technol.* **2020**, *296*, 122353.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775

(159) Lettinga, G.; Field, J.; Van Lier, J.; Zeeman, G.; Hulshoff Pol, L. W. Advanced Anaerobic Wastewater Treatment in the near Future. *In Water Science and Technology* **1997**, *35*, 5−12.
(160) Shin, B. S.; Eklund, C. W.; Lensmeyer, K. V. Bakery Waste Treatment by an Anaerobic Contact Process. *Res. J. Water Pollut. Control Fed.* **1990**, *62* (7), 920−925.
(161) Rajeshwari, K.; Balakrishnan, M.; Kansal, A.; Lata, K.; Kishore, V. V. State-of-the-Art of Anaerobic Digestion Technology for Industrial Wastewater Treatment. *Renew. Sustain. Energy Rev.* **2000**, *4* (2), 135−156.
(162) Borja, R.; Banks, C. J. Anaerobic Digestion of Palm Oil Mill Effluent Using an Up-Flow Anaerobic Sludge Blanket Reactor. *Biomass and Bioenergy* **1994**, *6* (5), 381−389.
(163) Gomes, D. R. S.; Papa, L. G.; Cichello, G. C. V.; Belançon, D.; Pozzi, E. G.; Balieiro, J. C. C.; Monterrey-Quintero, E. S.; Tommaso, G. Effect of Enzymatic Pretreatment and Increasing the Organic Loading Rate of Lipid-Rich Wastewater Treated in a Hybrid UASB Reactor. *Desalination* **2011**, *279* (1−3), 96−103.
(164) Zhang, Y.; Yan, L.; Chi, L.; Long, X.; Mei, Z.; Zhang, Z. Startup and Operation of Anaerobic EGSB Reactor Treating Palm Oil Mill Effluent. *J. Environ. Sci.* **2008**, *20* (6), 658−663.
(165) Lettinga, G.; van Velsen, A. F. M.; Hobma, S. W.; de Zeeuw, W.; Klapwijk, A. Use of the Upflow Sludge Blanket (USB) Reactor Concept for Biological Wastewater Treatment, Especially for Anaerobic Treatment. *Biotechnol. Bioeng.* **1980**, *22* (4), 699−734.
(166) Perle, M.; Kimchie, S.; Shelef, G. Some Biochemical Aspects of the Anaerobic Degradation of Dairy Wastewater. *Water Res.* **1995**, *29* (6), 1549−1554.
(167) Vidal, G.; Carvalho, A.; Méndez, R.; Lema, J. M. Influence of the Content in Fats and Proteins on the Anaerobic Biodegradability of Dairy Wastewaters. *Bioresour. Technol.* **2000**, *74* (3), 231−239.
(168) Zinatizadeh, A. A. L.; Mohamed, A. R.; Mashitah, M. D.; Abdullah, A. Z.; Isa, M. H. Optimization of Pre-Treated Palm Oil Mill Effluent Digestion in an up-Flow Anaerobic Sludge Fixed Film Bioreactor: A Comparative Study. *Biochem. Eng. J.* **2007**, *35*, 226−237.
(169) Kim, S.-H.; Han, S.-K.; Shin, H.-S. Two-Phase Anaerobic Treatment System for Fat-Containing Wastewater. *J. Chem. Technol. Biotechnol.* **2004**, *79* (1), 63−71.
(170) Arnaiz, C.; Buffiere, P.; Elmaleh, S.; Lebrato, J.; Moletta, R. Anaerobic Digestion of Dairy Wastewater by Inverse Fluidization: The Inverse Fluidized Bed and the Inverse Turbulent Bed Reactors. *Environ. Technol.* **2003**, *24* (11), 1431−1443.
(171) Ramos, C.; García, A.; Diez, V. Performance of an AnMBR Pilot Plant Treating High-Strength Lipid Wastewater: Biological and Filtration Processes. *Water Res.* **2014**, *67* (2009), 203−215.
(172) Kanai, M.; Ferre, V.; Wakahara, S.; Yamamoto, T.; Moro, M. A Novel Combination of Methane Fermentation and MBR - Kubota Submerged Anaerobic Membrane Bioreactor Process. *Desalination* **2010**, *250* (3), 964−967.
(173) *SPARTHANE ™ (AnSBR): The Ultimate Anaerobic Technology for Treatment of Wastewaters High in Fat and Solids*; Veolia Water Technologies, 2020.
(174) Picavet, M.; Alves, M. A Compact High-Rate Anaerobic Reactor Configuration for the Treatment of Effluents with High Lipid Content. *AD12 World Congress on Anaerobic Digestion*, Mexico, 2010.
(175) Roy, F.; Albagnac, G.; Samain, E. Influence of Calcium Addition on Growth of Highly Purified Syntrophic Cultures Degrading Long-Chain Fatty Acids. *Appl. Environ. Microbiol.* **1985**, *49* (3), 702−705.
(176) Elsamadony, M.; Mostafa, A.; Fujii, M.; Tawfik, A.; Pant, D. Advances towards Understanding Long Chain Fatty Acids-Induced Inhibition and Overcoming Strategies for Efficient Anaerobic Digestion Process. *Water Res.* **2021**, *190*, 116732.
(177) Martins, G. M.; Salvador, A. F.; Pereira, L.; Alves, M. M. Methane Production and Conductive Materials: A Critical Review. *Environ. Sci. Technol.* **2018**, *52* (18), 10241−10253.
(178) Baek, G.; Jung, H.; Kim, J.; Lee, C. A Long-Term Study on the Effect of Magnetite Supplementation in Continuous Anaerobic Digestion of Dairy Effluent - Magnetic Separation and Recycling of Magnetite. *Bioresour. Technol.* **2017**, *241*, 830−840.
(179) Tan, L. C.; Lin, R.; Murphy, J. D.; Lens, P. N. L. Granular Activated Carbon Supplementation Enhances Anaerobic Digestion of Lipid-Rich Wastewaters. *Renew. Energy* **2021**, *171*, 958−970.
(180) Duarte, M. S.; Sinisgalli, E.; Cavaleiro, A. J.; Bertin, L.; Alves, M. M.; Pereira, M. A. Intensification of Methane Production from Waste Frying Oil in a Biogas-Lift Bioreactor. *Renew. Energy* **2021**, *168*, 1141−1148.
(181) Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M. S.; Fonoll, X.; Peces, M.; Astals, S. A Critical Review on Anaerobic Co-Digestion Achievements between 2010 and 2013. *Renew. Sustain. Energy Rev.* **2014**, *36*, 412−427.
(182) Park, K. Y.; Jang, H. M.; Park, M. R.; Lee, K.; Kim, D.; Kim, Y. M. Combination of Different Substrates to Improve Anaerobic Digestion of Sewage Sludge in a Wastewater Treatment Plant. *Int. Biodeterior. Biodegrad.* **2016**, *109*, 73−77.
(183) Salama, E. S.; Saha, S.; Kurade, M. B.; Dev, S.; Chang, S. W.; Jeon, B. H. Recent Trends in Anaerobic Co-Digestion: Fat, Oil, and Grease (FOG) for Enhanced Biomethanation. *Prog. Energy Combust. Sci.* **2019**, *70*, 22−42.
(184) Wu, L. J.; Kobayashi, T.; Kuramochi, H.; Li, Y. Y.; Xu, K. Q.; Lv, Y. High Loading Anaerobic Co-Digestion of Food Waste and Grease Trap Waste: Determination of the Limit and Lipid/Long Chain Fatty Acid Conversion. *Chem. Eng. J.* **2018**, *338*, 422−431.
(185) Regueiro, L.; Veiga, P.; Figueroa, M.; Alonso-Gutierrez, J.; Stams, A. J. M.; Lema, J. M.; Carballa, M. Relationship between Microbial Activity and Microbial Community Structure in Six Full-Scale Anaerobic Digesters. *Microbiol. Res.* **2012**, *167* (10), 581−589.
(186) Neves, L.; Oliveira, R.; Alves, M. M. Co-Digestion of Cow Manure, Food Waste and Intermittent Input of Fat. *Bioresour. Technol.* **2009**, *100* (6), 1957−1962.
(187) Neves, L.; Oliveira, R.; Alves, M. M. Fate of LCFA in the Co-Digestion of Cow Manure, Food Waste and Discontinuous Addition of Oil. *Water Res.* **2009**, *43* (20), 5142−5150.
(188) Luostarinen, S.; Luste, S.; Sillanpää, M. Increased Biogas Production at Wastewater Treatment Plants through Co-Digestion of Sewage Sludge with Grease Trap Sludge from a Meat Processing Plant. *Bioresour. Technol.* **2009**, *100* (1), 79−85.
(189) Davidsson, Å.; Lövstedt, C.; La Cour Jansen, J.; Gruvberger, C.; Aspegren, H. Co-Digestion of Grease Trap Sludge and Sewage Sludge. *Waste Manag.* **2008**, *28* (6), 986−992.
(190) Silvestre, G.; Rodríguez-Abalde, A.; Fernández, B.; Flotats, X.; Bonmatí, A. Biomass Adaptation over Anaerobic Co-Digestion of Sewage Sludge and Trapped Grease Waste. *Bioresour. Technol.* **2011**, *102* (13), 6830−6836.
(191) Wan, C.; Zhou, Q.; Fu, G.; Li, Y. Semi-Continuous Anaerobic Co-Digestion of Thickened Waste Activated Sludge and Fat, Oil and Grease. *Waste Manag.* **2011**, *31* (8), 1752−1758.
(192) Girault, R.; Bridoux, G.; Nauleau, F.; Poullain, C.; Buffet, J.; Peu, P.; Sadowski, A. G.; Béline, F. Anaerobic Co-Digestion of Waste Activated Sludge and Greasy Sludge from Flotation Process: Batch versus CSTR Experiments to Investigate Optimal Design. *Bioresour. Technol.* **2012**, *105*, 1−8.
(193) Kabouris, J. C.; Tezel, U.; Pavlostathis, S. G.; Engelmann, M.; Dulaney, J.; Gillette, R. A.; Todd, A. C. Methane Recovery from the Anaerobic Codigestion of Municipal Sludge and FOG. *Bioresour. Technol.* **2009**, *100* (15), 3701−3705.
(194) Noutsopoulos, C.; Mamais, D.; Antoniou, K.; Avramides, C.; Oikonomopoulos, P.; Fountoulakis, I. Anaerobic Co-Digestion of Grease Sludge and Sewage Sludge: The Effect of Organic Loading and Grease Sludge Content. *Bioresour. Technol.* **2013**, *131*, 452−459.
(195) Wang, L.; Aziz, T. N.; De los Reyes, F. L. Determining the Limits of Anaerobic Co-Digestion of Thickened Waste Activated Sludge with Grease Interceptor Waste. *Water Res.* **2013**, *47* (11), 3835−3844.
(196) Wu, L. J.; Kobayashi, T.; Li, Y. Y.; Xu, K. Q.; Lv, Y. Determination and Abatement of Methanogenic Inhibition from Oleic and Palmitic Acids. *Int. Biodeterior. Biodegrad.* **2017**, *123*, 10−16.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775

(197) De Vrieze, J.; Verstraete, W.; Boon, N. Repeated Pulse Feeding Induces Functional Stability in Anaerobic Digestion. *Microb. Biotechnol.* **2013**, *6* (4), 414−424.
(198) Alqaralleh, R. M.; Kennedy, K.; Delatolla, R. Microwave vs. Alkaline-Microwave Pretreatment for Enhancing Thickened Waste Activated Sludge and Fat, Oil, and Grease Solubilization, Degradation and Biogas Production. *J. Environ. Manage.* **2019**, *233*, 378−392.
(199) Srinivasan, A.; Saha, M.; Caufield, K.; Abida, O.; Liao, P. H.; Lo, K. V. Microwave-Enhanced Advanced Oxidation Treatment of Lipids and Food Wastes. *Water. Air. Soil Pollut.* **2018**, *229* (7), 227 DOI: 10.1007/s11270-018-3894-y.
(200) Ries, S.; Srinivasan, A.; Abida, O.; Caufield, K.; Liao, P. H.; Lo, K. V. Microwave Enhanced Advanced Oxidation Treatment of Fat, Oil and Grease (FOG) with Organic Co-Substrates. *Environ. Technol.* **2021**, *42* (28), 4500−4510.
(201) Moisan, M. *Ultrasonic Pretreatment for Anaerobic Digestion: A Study on Feedstock, Methane Yield, and Energy Balance*. Dissertation, University of Guelph, Ontario, Canada, 2012.
(202) Li, C.; Champagne, P.; Anderson, B. C. Effects of Ultrasonic and Thermo-Chemical Pre-Treatments on Methane Production from Fat, Oil and Grease (FOG) and Synthetic Kitchen Waste (KW) in Anaerobic Co-Digestion. *Bioresour. Technol.* **2013**, *130*, 187−197.
(203) Cirne, D. G.; Björnsson, L.; Alves, M.; Mattiasson, B. Effects of Bioaugmentation by an Anaerobic Lipolytic Bacterium on Anaerobic Digestion of Lipid-Rich Waste. *J. Chem. Technol. Biotechnol.* **2006**, *81* (11), 1745−1752.
(204) Cavaleiro, A. J.; Ferreira, T.; Pereira, F.; Tommaso, G.; Alves, M. M. Biochemical Methane Potential of Raw and Pre-Treated Meat-Processing Wastes. *Bioresour. Technol.* **2013**, *129*, 519−525.
(205) Masse, L.; Kennedy, K. J.; Chou, S. Testing of Alkaline and Enzymatic Hydrolysis Pretreatments for Fat Particles in Slaughterhouse Wastewater. *Bioresour. Technol.* **2001**, *77* (2), 145−155.
(206) Harris, P. W.; Schmidt, T.; McCabe, B. K. Evaluation of Chemical, Thermobaric and Thermochemical Pre-Treatment on Anaerobic Digestion of High-Fat Cattle Slaughterhouse Waste. *Bioresour. Technol.* **2017**, *244*, 605−610.
(207) Linyi, C.; Yujie, Q.; Buqing, C.; Chenglong, W.; Shaohong, Z.; Renglu, C.; Shaohua, Y.; Lan, Y.; Zhiju, L. Enhancing Degradation and Biogas Production during Anaerobic Digestion of Food Waste Using Alkali Pretreatment. *Environ. Res.* **2020**, *188* (June), 109743.
(208) Battimelli, A.; Carrère, H.; Delgenès, J.-P. Saponification of Fatty Slaughterhouse Wastes for Enhancing Anaerobic Biodegradability. *Bioresour. Technol.* **2009**, *100* (15), 3695−3700.
(209) Li, C.; Champagne, P.; Anderson, B. C. Enhanced Biogas Production from Anaerobic Co-Digestion of Municipal Wastewater Treatment Sludge and Fat, Oil and Grease (FOG) by a Modified Two-Stage Thermophilic Digester System with Selected Thermo-Chemical Pre-Treatment. *Renew. Energy* **2015**, *83*, 474−482.
(210) Leal, M. C. M. R.; Freire, D. M. G.; Cammarota, M. C.; Sant'Anna, G. L. Effect of Enzymatic Hydrolysis on Anaerobic Treatment of Dairy Wastewater. *Process Biochem.* **2006**, *41* (5), 1173−1178.
(211) Adulkar, T. V.; Rathod, V. K. Pre-Treatment of High Fat Content Dairy Wastewater Using Different Commercial Lipases. *Desalin. Water Treat.* **2015**, *53* (9), 2450−2455.
(212) Cirne, D. G.; Paloumet, X.; Björnsson, L.; Alves, M. M.; Mattiasson, B. Anaerobic Digestion of Lipid-Rich Waste—Effects of Lipid Concentration. *Renew. Energy* **2007**, *32* (6), 965−975.
(213) Witharana, A.; Manatunge, J.; Ratnayake, N.; Nanayakkara, C. M.; Jayaweera, M. Rapid Degradation of FOG Discharged from Food Industry Wastewater by Lipolytic Fungi as a Bioaugmentation Application. *Environ. Technol. (United Kingdom)* **2018**, *39* (16), 2062−2072.
(214) Adulkar, T. V.; Rathod, V. K. Ultrasound Assisted Enzymatic Pre-Treatment of High Fat Content Dairy Wastewater. *Ultrason. Sonochem.* **2014**, *21* (3), 1083−1089.
(215) Cammarota, M. C.; Freire, D. M. G. A Review on Hydrolytic Enzymes in the Treatment of Wastewater with High Oil and Grease Content. *Bioresour. Technol.* **2006**, *97* (17), 2195−2210.
(216) Sharma, R.; Chisti, Y.; Banerjee, U. C. Production, Purification, Characterization, and Applications of Lipases. *Biotechnol. Adv.* **2001**, *19* (8), 627−662.
(217) Hasan, F.; Shah, A. A.; Hameed, A. Industrial Applications of Microbial Lipases. *Enzyme Microb. Technol.* **2006**, *39* (2), 235−251.

https://doi.org/10.1021/acs.est.1c08722
Environ. Sci. Technol. 2022, 56, 4749−4775