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REVIEW

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Behavior of lipids in biological wastewater treatment processes

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Abstract Lipids (characterized as oils, greases, fats and long-chain fatty acids) are important organic components of wastewater. Their amount, for example, in municipal wastewater is approximately 30–40% of the total chemical oxygen demand. The concern over the behavior of lipids in biological treatment systems has led to many studies, which have evaluated their removal, but still the exact behavior of lipids in these processes is not well understood. In this review, we discuss the current knowledge of how lipids/fatty acids affect both aerobic and anaerobic processes and specific methods that have been used in an attempt to enhance their removal from wastewater. Overall, the literature shows that lipids/fatty acids are readily removed by biological treatment methods, inhibitory to microbial growth as well as the cause of foaming, growth of filamentous bacteria and floc flotation.

Keywords Aerobic and anaerobic wastewater treatment · Activated sludge · Biodegradation of lipids · Fatty acids · Microbial growth · Soluble microbial products

Introduction

Lipids (characterized as oils, greases, fats and fatty acids) are one of the most important components of natural foods and many synthetic compounds and emulsions. The latter are mostly found in pharmaceutical and cosmetic industrial effluents. Further, lipids constitute one of the major types of organic matter found in municipal wastewater [70, 73], which may find their way into surface waters.

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The amount of lipid-rich wastewater increases every year due to urbanization and the development of factories. Suspended lipids can be readily removed from wastewater by physical methods. Nevertheless, chemically and/or physically stabilized lipid/water emulsions should be managed in an appropriate manner. This is necessary because lipids that pass through physicochemical treatment processes contribute to the levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) in the effluents [16, 17, 35, 45, 52]. Thus, biological treatment processes are commonly used to remove emulsified lipids from wastewater. However, the exact behavior of lipids in biological treatment systems is still not well understood. In this review, we discuss current knowledge of how lipids/fatty acids affect both aerobic and anaerobic processes, and methods that have been used in an attempt to enhance their removal from wastewater. Overall, the literature shows that lipids/fatty acids are readily removed by biological treatment methods, inhibitory to microbial growth as well as the cause of foaming, growth of filamentous bacteria and floc flotation.

Behavior of lipids in aerobic treatment processes

Aerobic treatment of lipid-rich wastewater

Aerobic treatment of lipid-rich wastewater in activated sludge system

In aerobic wastewater treatment systems, lipids are generally believed to be biodegradable and, therefore, considered as part of the organic load that is treated. However, lipids have detrimental effects on oxygen transfer. They reduce the rates at which oxygen is transferred to biofilms, thereby depriving the microorganisms of oxygen [17]. This effect results in reduced microbial activity. Young [103] carried out studies by mixing biological solids with vegetable oil and the effluent BOD characteristics correlated with the amount

of oil added. Moreover, the removal of oil by mixed microbial population was equal or better than BOD removal [103], suggesting that not only biodegradation processes occurred, but also adsorption of oil to the biomass took place. Although Hsu et al. [36] found that adsorption of lipids contributes to their removal from wastewater, Chao and Yang [17] reported that the adsorbed lipids cause a decrease in specific gravity as well as the ability of the sludge to settle, resulting in process failure. Therefore, either lipid adsorption or the resulting decrease in specific gravity of sludge influences the performance of aerobic processes such as the activated sludge process.

Hrudey [35] studied the effects of emulsified lipids on activated sludge and found them to have no inhibitory effect on substrate utilization rate over an experimental range of lipid-to-microorganisms ratio (lipid/MLSS; mixed liquor suspended solids) from 0.04 to 0.78 g lipid day^{-1} g^{-1} MLSS. Emulsified lipids did not inhibit the microbial oxygen consumption rate over an experimental range of lipid/MLSS from 0.09 to 0.5. Nevertheless, in terms of effluent wastewater quality expressed as BOD and total suspended solids, activated sludge processes overloaded with lipids are also known to show a poor performance though the lipid removal is good. Although these findings show that activated sludge can efficiently remove lipids even at high lipid loadings, Hrudey [35] also found that activated sludge process exhibited poor effluent quality as lipid/MLSS was raised beyond 0.25; no detail was given to explain this discrepancy. Hence, in accordance with Hrudey's [35] conclusions, lipids affect activated sludge microorganisms by a mechanism other than their metabolic inhibition since lipid loading did not involve any inhibition of heterotrophic bacteria in activated sludge. A study by Wakelin and Forster [101] shows that acclimatized activated sludge exhibits a higher performance than a non-acclimatized one even though the microbial growth pattern and removal of lipids and fatty acids are similar. Since activated sludge is a mixture of different microorganisms, which can be dominated by different species, their respective domination can be dictated by the type and concentration of the substrate [24, 48]. Therefore, the results reported by Hrudey and Wakelin [35, 101] suggest that the differences in the overall performances of various microbial cultures could be due to differences in enzyme systems, especially lipases [2, 72, 83, 94]. This further suggests that the use of mixed microbial cultures such as activated sludge, particularly when it has been acclimatized to lipids and fatty acids, can offer the best option for the treatment of wastewater containing these organic substrates.

Aerobic treatment of lipid-rich wastewater in combined suspended and attached growth systems

To enhance biodegradation of lipids, Keenan and Sabelnikov [45] proposed the use of a combination of suspended and attached growth treatment systems using selected bacterial strains capable of degrading lipids. They found that the lipid content in the effluent wastewater could not be reduced to values below 0.3 g/l from 1.512 g/l by using a suspended growth treatment system only, whereas adding a biofilter (a solid support that could be colonized by bacteria) to the suspended growth system substantially reduced the lipid content in the wastewater effluent to 0.028 g/l. The increase in the efficiency of the system was the result of an increased concentration of bacterial cells, which was accompanied by increased microbial activity, growth and maintenance of microbial populations that were associated with attached growth systems [98]. However, the treatment system reported by Keenan and Sabelnikov [45] sporadically failed, and the content of lipids in the effluent wastewater increased to 0.386 g/l. Although the authors attributed the sporadic failures to the failure of the pH adjustment system, the complete explanation for such failures was unknown.

Biodegradation of lipids

Comparison of biodegradability of different lipids

Erhan and Kleiman [25] investigated the biodegradability of lipids and fatty acids, including soybean oil, meadow foam oil, fatty acids and oleic acid under aerobic conditions using strains of *Penicillium verucosum, Mucor racenosus* and *Enterobacter aerogens*. They reported that biodegradation of these substrates occurs relatively fast when their concentration in the medium is low, i.e., 2% in relation to water with dissolved microelements. As shown in Table 1, although the biodegradation rates increased with retention time, those for erucic estolides and meadow foam oil and its fatty acids were lower than that of either oleic acid or soybean oil. These results show that biodegradability of lipids is limited by the characteristics of their fatty acids. Loehr and Roth [55] reported that the biodegradability of long-chain fatty acids increases with their decreasing carbon chain lengths and increasing degree of unsaturation of carbon chains. This is expected since factors influencing biodegradation of organic compounds include: (1) molecular structure of the compound; (2) solubility of the compound in the aqueous medium containing the microorganisms; and (3) environmental factors, such as the effects of pH, temperature, nutrients, electron acceptor and presence or absence of oxygen [1].

Novak and Klaus [64] determined the substrate utilization rates of fatty acids (myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid) by activated sludge microorganisms. The substrate concentration of fatty acids (as sodium salts) ranged up to 300 mg/l. They found that the maximum utilization rates of 16 and 18 carbon saturated fatty acids were lower than those of unsaturated fatty acids with the same chain length (Table 2). The substrate utilization

Table 1 Biodegradation rates of soybean and meadow foam oils, oleic acid and erucic acid estolides [25]

Substrates	Biodegradation rates (%)		
	Day 3	Day 5	Day 10
Oleic acid	97	98	99
Soybean oil	87	97	99
Meadow foam oil fatty acids	78	86	97
Meadow foam oil	75	81	88
Erucic acid estolides	30	43	57

rate of myristic acid was similar to that of unsaturated fatty acids. The low substrate utilization rates of long-chain fatty acids are expected because such fatty acids are also reported to pass through biological wastewater treatment systems and are found in treated wastewater effluents [21, 70]. Peil and Gaudy [66] determined substrate utilization rates of various substrates including sugars and amino acids under similar experimental conditions as reported by Novak and Klaus [64]. Comparison of the results of these authors (Table 2) shows that unsaturated and saturated fatty acids were, respectively, degraded at rates ten and hundred times slower than the substrates studied by Peil and Gaudy [66]. This suggests that lipids are less responsive to degradation by microorganisms than other biodegradable organic substrates such as sugars and amino acids.

Moreover, the biodegradation of all saturated long-chain fatty acids (C_{12} and above) is known to be identical. They are degraded by sequential removal of two-carbon atoms via the β-oxidation pathway, resulting in release of a fatty acid shorter by two carbons and acetyl-CoA, which is then subsequently oxidized to carbon dioxide by the tricarboxylic acid cycle [57, 72]. Therefore, the differences in degradation rates of long-chain fatty acids [64] may be attributed to their solubilities. Myristic acid is more soluble than either palmitic or stearic acid [91].

Effect of types of microbial species on biodegradation of lipids

Even though lipids are biogenic compounds, their different structures and solubilities imply that it may be difficult to induce the enzymes required for their biodegradation. Keenan and Sabelnikov [45] studied the biodegradation of corn, olive, sunflower and waste oils (obtained from a restaurant) by a variety of bacterial strains (*Acinetobacter* sp., *Rhodococcus* sp. and *Caseobacter* sp. that were isolated from different environments based on their ability to grow on vegetable and waste oils) and by commercial bacterial preparations specifically designed for lipid degradation. They found that for all bacterial strains and preparations only corn oil and waste oils supported microbial growth more efficiently than either olive or sunflower oil. Moreover, the *Caseobacter* strain and one commercial preparation could not grow on olive oil at all (Table 3). Wakelin and Forster [101] reported similar results (Table 3) that *Acinetobacter* strain was the most efficient to grow on lipids among other bacterial species tested, including *Rhodococcus rubra, Nocardia amarae*, and *Microthrix parvicella*. However, even *Acinetobacter* sp. could not reduce the content of lipids in wastewater to values lower than 0.1 g/l. From the initial lipid content of 1.5 g/l, the lowest values achieved using *Acinetobacter* sp. were 0.305 g/l for corn oil and 0.267 g/l for waste oil. Interestingly, Keenan and Sabelnikov [45] also reported that microbial growth was better on unrefined than on refined oils. In an attempt to explain this discrepancy, Keenan and Sabelnikov [45] speculated that the refined oil samples used might have contained preservatives or other compounds that limited or prevented microbial growth because good growth was observed for all the strains in experiments using unrefined sunflower. A possible explanation is that there was better growth of microorganisms on unrefined oil than on refined oil because the former contained nutrients which were removed while making refined oil.

Bioaugmentation as a method for enhancing biodegradation of lipids

The current practice to improve the biodegradation of organic compounds is bioaugmentation, which is the addition of microorganisms (indigenous or genetically modified) or enzyme preparations for bioremediation or treatment of wastewater. Bioaugmentation improves several aspects in wastewater treatment processes, such as improved degradation of organic matter, either

Table 2 Kinetic constants for long-chain fatty acids and various substrates

Source [64]		Source [66]			
Fatty acid substrates	Kinetic constants (k, h^{-1})	Sugars and other substrates	Kinetic constants (k, h^{-1})	Sugars and other substrates	Kinetic constants (k, h^{-1})
Myristic	0.0341	Glucose	0.49	Phenylalanine	0.33
Palmitic	0.0071	Lactose	0.53	Cysteine	0.16
Stearic	0.0052	Sucrose	0.55	Acetic acid	0.36
Myristoleic	0.0420	Sorbitol	0.60	Propionic acid	0.38
Palmitoleic	0.0453	Alanine	0.33	Sewage	0.49
Oleic	0.0440	Glutamic acid	0.78		
Linoleic	0.0341	Serine	0.43		
Linolenic	0.030	Hisidine	0.50		

Table 3 Microbial growth on vegetable oils

Bacteria[a]	Biomass yield (g/l) on vegetable oils[b]						
	Corn	Olive	Sunflower	Linseed	Coconut	Rapeseed	Waste oil
Source [45]							
Acinetobacter sp.	2.80	1.48	1.42	nr	nr	nr	2.49
Rhodococcus sp.	1.68	0.13	0	nr	nr	nr	0.97
Caseobacter sp.	1.17	0	0.25	nr	nr	nr	3.10
S700C	1.88	0.66	1.32	nr	nr	nr	2.75
S9004C	3.36	0	0.71	nr	nr	nr	0.92
Source [101]							
Acinetobacter sp.	2.81	2.48	nr	2.55	3.25	3.50	2.06
Rhodococcus sp.	1.10	1.61	nr	0.52	1.09	1.12	0.38
Microthrix parvicella	0.40	0.51	nr	0.32	0.25	0.44	2.40
Nocardia amarae	0.52	0.80	nr	1.08	1.31	1.50	0.52

[a] *S700C and S900C* biopreparations, [b] *Waste oil* waste oils from restaurants (unspecified), *nr* not reported

through the activity of the added microbial strains or after the transfer of degradative plasmids to activated sludge microorganisms [69]. Concerning biodegradation of lipids in wastewater, two bioaugmentation approaches are in use [45]: the use of enzyme preparations (primarily lipases) and the use of viable microorganisms.

The use of enzyme preparations is not attractive because it is only used for hydrolysis of lipids, for example fats and oils to fatty acids and glycerol [28, 29, 65]. The fatty acids liberated can form colloidal particles that aggregate and precipitate from solutions during changes in environmental conditions in the treatment system (e.g., changes in pH, temperature, salt concentration, etc.), causing clogging and process failure [17]. Therefore, this approach provides only a partial solution of the problem. Process stability may also depend on the state of the added enzyme preparations. If added in solution, enzymes would be lost from the system. It is also difficult to recover them from reactor effluents at the end of the catalytic process which is an even more expensive exercise. In contrast, immobilized enzymes would be retained within the system and most likely would have improved stability in relation to environmental conditions [28, 29]. Despite these advantages, the use of immobilized enzymes in wastewater treatment has been limited by several factors, mainly the high cost of the enzymes connected with immobilization procedures (e.g., enzyme isolation and purification, bioreactor operational stability, and bioreactor regeneration).

The use of viable microorganisms is more attractive because they hydrolyze the lipids as well as biodegrade them further to carbon dioxide and water [57, 72, 101]. Lipids in this case are used as substrates for microbial growth, resulting in an increase in the concentration of microorganisms in the treatment system. As shown above, the use of appropriate microbial strains can significantly improve the removal of lipids from wastewater. In other studies, Pieper and Reineke [69] showed that pre-adaptation of microbial strains to new environments and use of recombinant strains can enhance the biodegradation of biogenic or xenobiotic organic compounds. In this context, microorganisms growing in the presence of long-chain fatty acids (linoleic, arachidonic and docosahexaenoic acids) and acetic acid have been reported to show many inducible responses, including appearance of stress proteins, which implies complex regulation of gene expression [81], and changes in physiological and morphological functions [41, 43, 44, 88]. Thus, microorganisms have a greater tolerance to changes in environmental conditions than enzyme preparations. For these reasons, addition of selected microorganisms to wastewater treatment systems is more advantageous than the use of enzymes.

Biodegradation of lipids under thermophilic conditions

Biological degradation of lipids is largely limited by their unfavorable physicochemical properties, for example, they are insoluble in water [71]. Lipids are, therefore, not readily available for microbial uptake and degradation. Biological treatment of lipid-rich wastewater under thermophilic conditions (i.e., above 60°C) is expected to be more advantageous than, for example, under mesophilic conditions [49]. This is because both the diffusion coefficients and the solubility of lipids in aqueous media increase significantly with rising temperature. Under thermophilic conditions, lipids become more accessible to microorganisms and their lipolytic enzymes.

To show this advantage, Becker and his co-workers [12] studied the aerobic thermophilic degradation of olive oil using a pure culture of bacterial strain, *Bacillus thermoleovorans* IHI-91, in a continuously operated stirred-tank reactor. They observed a lipid removal of more than 90% of the initial lipid concentration of 2 g/l at a residence time of 2 h. In subsequent experiments, Becker et al. [12] treated wool-scouring wastewater, with 15–20 g/l of lipids, under aerobic conditions at 65°C using *B. thermoleovorans* IHI-91 as an inoculum. The lipid removal was only 20–25% at a residence time of 10 h. Although these results show that aerobic thermophilic treatment of lipid-rich wastewater using *B. thermoleovorans* IHI-91 is possible, it is also clear that lipids present in industrial wastewater and common lipids such as olive oil respond

differently to microbial degradation. According to Becker et al. [12], the low degradation rates of lipids in industrial wastewater are due to the negative effects of salinity and toxic materials on the biological treatment process.

Saponification as a method for enhancing biodegradation of lipids

Another method to increase the solubility of lipids in wastewater is their saponification (chemical reaction in which lipids, i.e. triacylglycerols, are hydrolyzed into glycerol and fatty acids, then neutralized to form soaps with higher solubility). Saponification of lipids improves the biodegradation of lipids in wastewater and the reaction yield depends upon many factors such as temperature and the type of hydroxide (potassium or sodium hydroxide) used for saponification [46, 52]. Lefebvre et al. [52] used potassium hydroxide for saponification because potassium salts of fatty acids are more soluble than the analogous sodium salts. To obtain model wastewater, the products of the saponification reaction were diluted with water until the desired lipid concentrations (0–4 g/l) were obtained. The model wastewater was treated under aerobic conditions using activated sludge as an inoculum. They found that microbial growth on saponified lipid substrates followed an exponential growth pattern. For initial lipid and biomass concentrations of 3.5 g/l and 1.8 g MLSS/l, respectively, degradation of lipids began as soon as the saponified lipids were mixed with activated sludge and continued until the lipid concentration was 0.9 g/l (after 2–3 h). No further degradation was observed even after extending the retention time to 10 h. To explain this low degradation rate, Lefebvre et al. [52] showed that foaming, which occurred at the end of the culture (10 h), caused the low degradation activity and, consequently, the low lipid removal efficiency (74%). Foaming limited the degradation of lipids by activated sludge despite the beneficial effect of saponification, which improved solubility and the subsequent bioavailability of lipids.

The results reported by Lefebvre et al. [52] are in accordance with the results reported by Kellel et al. [46], who observed that the length of hydraulic retention time of aerobic degradation of lipids could be reduced by saponification. According to Hsu et al. [36], both hydrolysis of triacylglycerols and lipidic phase solubilization and emulsification by a saponification step could be responsible for the increase in biodegradation of lipids. Hence, unsaponified lipids limit bacterial growth, whereas saponified lipids allow exponential growth of bacteria.

Foaming and bulking in biological wastewater treatment systems

Lipids as the cause of foaming and bulking

The benefit of the saponification step in aerobic biological treatment of lipid-rich wastewater is limited by foaming. The presence of lipids in wastewater is related to occurrences of troublesome foam [58]. Aeration enhances foaming because it enhances both saponification and emulsification of lipids. Foaming in aerated cultures could be due to pH variations, for example long-chain fatty acid salts are relatively unstable at pH below 7 [52], which could affect the lipid solubility and emulsification. Lefebvre et al. [52] suggested that such foaming problems could be reduced by discontinuous aeration. But the effect of aeration is not the only cause of foaming in biological wastewater treatment systems; many other factors also cause foaming. In fact, prolonged periods of absence of oxygen favor the growth of filamentous bacteria that cause foaming and bulking [22, 42]. Pernelle et al. [68] have reported that substrate overload induces the growth of filamentous bacteria (*Nostocoida limicola, Haliscomenobacter hydrossis*, and *Thiothrix nivea*). This is so because substrate overloads cause a sudden increase in BOD and a fall in dissolved oxygen concentration, which consequently induces growth of filamentous bacteria. Moreover, Galbraith and Miller [27] showed that fatty acid adsorption onto the surface of bacteria was promoted by low pH, resulting in formation of foam. Hence, foam also builds up from lipid/floc aggregates. Therefore, the growth of filamentous bacteria is induced by oxygen shortage and many other factors including biological ones [22].

A detailed study to characterize the substrate uptake by filamentous bacteria was conducted by Andreasen and Nielsen [3, 4]. They studied the ability of *Microthrix parvicella*, which is one of the filamentous bacteria responsible for bulking and foaming in many activated sludge treatment plants [5, 87], to take up various organic compounds under aerobic, anaerobic and anoxic conditions. They used the following substrates: (1) simple organic substrates: acetic acid, propionic acid, butyric acid, glucose, ethanol, glycerine, leucine; and (2) complex organic substrates: sodium dodecyl sulfate, octadecanol, palmitic acid, oleic acid and trioleic acid. They found that none of the simple organic substrates were taken up by *M. parvicella*. For complex organic substrates, only oleic, palmitic and trioleic acids were taken up by *M. parvicella*. Neither sodium dodecyl sulfate nor octadecanol could support the growth of *M. parvicella*. Slijkhuis [85] also reported similar results that *M. parvicella* could not grow on these simple organic substrates. However, in the presence of Tween 80, addition of acetate, butyrate and medium-chain fatty acids (C_8–C_{11}) enhanced the growth of *M. parvicella*. This is in accordance with the results of Maloy et al. [57], who reported that long-chain fatty acids (C_{12}–C_{18}) activate the transport system for medium-chain fatty acids in *Escherichia coli*, indicating that medium-chain fatty acids support microbial growth only in the presence of long-chain fatty acids.

Other researchers [23, 42] have shown that anoxic and anaerobic conditions applied in nutrient removal activated sludge plants stimulate the growth of filamentous bacteria. Anoxic conditions are applied in

connection with nitrogen removal, where organic substrates are metabolized with nitrate as an electron acceptor (denitrification). Anaerobic conditions are applied to enhance the biological removal of phosphorus. This process is based on the capability of certain phosphorus accumulating organisms (PAO) to store large amounts of polyphosphate as an energy source under aerobic or anoxic conditions. Subsequently, under anaerobic conditions, PAO use the stored energy to take up organic substrates [99]. Therefore, the results reported by Andreasen and Nielsen [3, 4] show that the presence of lipids (triacylglycerols and long-chain fatty acids) in wastewater promotes the growth of filamentous bacteria under aerobic, anaerobic or anoxic conditions. In accordance with Andreasen and Nielsen's [4] conclusion, a better control strategy against filamentous bacteria should include a better understanding of the behavior and effect of lipids on activated sludge.

Behavior of lipids in anaerobic treatment processes

Treatment of lipid-rich wastewater in upflow anaerobic sludge bed reactors

The treatment of lipid-rich wastewater is still a challenge. In addition to aerobic wastewater treatment systems, anaerobic systems are also widely used for treatment of lipid-rich wastewater [11, 26, 60, 78, 79]. Most importantly, high-rate anaerobic treatment systems have been developed. Among these systems, the upflow anaerobic sludge bed (UASB) reactor is the most widely used in the treatment of domestic and industrial wastewater due to its low-cost and adequate treatment efficiency, which is connected with its ability to retain high biomass concentrations despite the upflow velocity of the wastewater and the production of biogas, and to accommodate low concentrations of oxygen without negatively affecting the integrity or metabolic activity of the granular biomass [31, 53, 82, 89]. In UASB reactors, the biomass is retained as granules, formed by the natural self-immobilization of the bacteria. Although UASB reactors have been well characterized, and their usefulness for treatment of municipal and industrial wastes well documented [31, 100], their treatment failures have also been reported when treating lipid-rich wastewater [75]. As shown in Table 4, both low and high COD removals are experienced during the treatment of wastewater containing long-chain fatty acids in UASB reactors. COD removal efficiency of 97% is the highest reported for the anaerobic treatment of wastewater containing long-chain fatty acids [39]. The failures are attributed mainly to two problems: (1) the occurrence of flotation of sludge granules and fatty matter even at very low loadings [38, 75], and (2) inhibitory effects of long-chain fatty acids on anaerobic microorganisms [37, 47, 51, 76].

Adsorption and biodegradation of long-chain fatty acids in UASB reactors

A detailed study to characterize the adsorption and biodegradation of a long-chain fatty acid (oleic acid, $C_{18:1}$) and the subsequent effect of sludge flotation in UASB reactors was conducted by Hwu et al. [38]. The concentration of oleic acid ranged from 300–2,000 mg/l at retention times of 0 to 40 h. During the first day of the experiment, they found that sludge granules removed 40–70% of oleic acid from the wastewater, while only less than 1% of methane (in relation to oleic acid removed) was produced. Similarly Sam-Soon et al. [80] reported a 65% COD removal efficiency of oleic acid in a UASB reactor, but the methane production was not found to be equivalent to the COD removal. Such a discrepancy between COD removal and methane production rate was encountered because of adsorption of long-chain fatty acid onto sludge. Since oleic acid was adsorbed before its degradation, this indicates that the primary mechanism for COD removal of long-chain fatty acids in UASB reactors is biosorption rather than biodegradation.

Moreover, Hwu et al. [38] observed that oleic acid adsorption was significantly concentration dependent (i.e., the amount of oleic acid adsorbed increased with the increase in the initial oleic acid concentration added). They observed that the concentration of residual oleic acid increased after the first adsorption (where the residual oleic acid concentration decreased). According to Hwu et al. [38], the increase in the concentration of residual oleic acid was due to its desorption from the sludge granules. Furthermore, Hwu et al. [38] showed that desorption was accompanied by a significant increase in methane production at all oleic acid concentrations tested. Following desorption, the concentration of residual oleic acid decreased again, and the production of methane increased. This indicated that the adsorbed oleic acid was gradually degraded. However, complete degradation of oleic acid occurred only at the lowest concentrations studied (150 and 300 mg/l).

The phenomenon of desorption was earlier reported by Tsezos and Bell [95]. They reported that biodegradation of organic molecules is accompanied by their desorption from microbial biomass. The study by Hwu et al. [38] also showed that desorption did not occur when dead biomass was used. Therefore, the observed phenomenon of desorption of long-chain fatty acid from sludge granules is a biologically mediated process, which needs further investigations.

Adsorption of lipids as the cause of sludge flotation in UASB reactors

In studies to show the relationship between sludge loading rate (COD/g VSS day) and sludge flotation in UASB reactors treating a mixture of long-chain fatty acids (35% palmitic, 15% stearic and 50% oleic acid)

Table 4 Treatment of wastewater containing lipids and long-chain fatty acids in UASB reactors

Type of wastewater	Temp (°C)	Hydraulic retention time (d)	Organic load (g COD/l.d)	COD removal (%)	Source, Ref
Ice-cream	Ambient temp	1.6	2.2	50	[34]
Olive oil	35	0.2	1.03	76	[13]
Olive oil	35	16	8000	89	[13]
Olive mill effluent	55	15	3.5	90	[14]
Oleate	30	0.6	4.2	65	[80]
Oleate	55	1	8	97	[39]
Edible oil	37	26	7.8	87	[78]
Sewage	20	1	3.8	85	[77]
Sewage	35	28	2.3	76	[77]
Dairy effluent	35	0.33	5	60	[61]
Dairy effluent	35	4.5	2.04	67	[90]

[38] and lauric acid [74], the researchers reported that the level of flotation was directly proportional to the loading rates. Moreover, the time required for complete flotation to occur was shorter at higher loading rates. Their results suggest that adsorption of fatty matter on sludge particles subsequently causes sludge flotation and treatment failure. Additionally, the results reported by Hwu et al. [38] show that at about 0.200 g COD/g VSS day, the corresponding long-chain fatty acids concentration (ca. 260 mg/l) was far below the minimum inhibition concentration (401 mg/l) of long-chain fatty acids to methanogenesis [6, 37]. Therefore, under practical conditions, complete bed washout is likely to be encountered before inhibition of methanogenic bacteria. As a result, Hwu et al. [38] concluded that sludge flotation is caused by adsorption and depends on the loading rates of long-chain fatty acids. Therefore, the deterioration of the UASB treatment process is mainly due to sludge flotation rather than to the inhibition of the methanogenic bacteria by adsorbed long-chain fatty acids.

Treatment of lipid-rich wastewater in modified UASB reactors

In general, the ability of lipids to form floating aggregates limits the biological treatment of lipid-rich wastewater. Gujer and Zehnder [32] demonstrated that low density of the floating aggregates slows the biodegradation of lipids. In order to improve lipid biodegradation in such troublesome systems, Rinzema [74] proposed rigorous mixing as a means of maintaining good contact between bacteria and lipids in the anaerobic digester. In this regard, Li et al. [54] proposed a two-stage anaerobic digestion process consisting of a mixing unit and a high solids digestion unit for treatment of lipid-rich wastewater. By using such a system, Li et al. [54] reported lipids removal efficiency of 86–93% with methane production of 60–65%. However, the degradation efficiency decreased at loading rates above 20 and 33 kg COD/m^3 day under mesophilic (35°C) and thermophilic (55°C) conditions, respectively. At higher loading rates, low degradation of lipids is expected because the higher intensity of biogas production tends to cause sludge washout from the reactor [92]. To solve this problem, Van Lier et al. [96, 97] introduced a new concept of multi-stage UASB reactor, which consists of a number of gas–solids separators. Further, Tagawa et al. [93] investigated the ability of a multi-stage UASB reactor under thermophilic conditions (55°C) to treat lipid-rich wastewater at retention times from 0 to 600 days. For a 50 kg COD/m^3.d, the reactor achieved a soluble COD removal of 90%, but the overall COD removal (based on the total effluent COD) was very unsatisfactory at only 60–70%. They attributed the poor performance of the multi-stage UASB reactor to the presence of high concentration of magnesium and calcium ions in the wastewater. The presence of long-chain fatty acids along with magnesium and calcium ions in the reactor produced extensive scum due to formation of insoluble calcium soaps, which hindered the contact between substrate and sludge microorganisms. This subsequently caused the deterioration of sludge methanogenic activity.

Lettinga [53] carried out further modifications of the UASB reactor. He found that a modified-UASB reactor system (so-called expanded granular sludge bed reactor, EGSB), which is characterized by high upflow velocities (>4 m/h) and short hydraulic retention times (<10 h), mixes the substrate and biomass well. Rinzema et al. [74] took advantage of the EGSB reactors and observed that the removal of lauric acid from wastewater significantly improved when EGSB reactors were used. They observed no flotation of granular sludge, and achieved a high volumetric loading rate of 31.4 g COD/l day

The influence of co-substrates on the biodegradation of lipids in EGSB reactors

Hwu et al. [40] studied the influence of hydrodynamics, temperature, and co-substrates (glucose and acetate) on the performance of anaerobic digestion of oleic acid in EGSB reactors. Under similar experimental conditions, as in studies by Rinzema [74] and Syutsubo et al. [92] (upflow velocity of 3.4–4 m/h), and in the absence of co-substrates, COD removal efficiencies of 66 and 73%

were attained under thermophilic (55°C) conditions at hydraulic retention times of 3 and 6 h, respectively. Whereas under mesophilic conditions, COD removal efficiencies were 44 and 69%, respectively. The highest methane production achieved was only 15% in a thermophilic reactor operated at a retention time of 6 h. Both thermophilic and mesophilic reactors failed due to severe washout of sludge granules at a retention time of 0.6 h, and non-degraded fatty matter frequently appeared in both reactors. However, when the reactors were operated at a constant retention time of 24 h, in the presence of glucose and acetate, COD removal efficiencies of 82–89% were obtained and no significant washout or flotation of sludge granules or fatty matter was observed.

Comparison of the results reported by Hwu et al. [40] with those by Rinzema et al. [74] shows that the treatment efficiency of oleic acid was much lower than that of lauric acid although both were treated in EGSB reactors and under similar conditions. To explain this difference in treatment efficiency, Hwu et al. [40] noted that this was due to different molecular sizes of the hydrophobic aliphatic chains of the two compounds; oleic acid is more hydrophobic (leading to lower biodegradation) than lauric acid. However, addition of co-substrates improved the degradation of oleic acid. Similar results were reported by Beccari et al. [10] who observed that oleic acid was not degraded in the absence of an easily biodegradable substrate such as glucose.

There are two more possible reasons to explain the results by these authors [10, 40, 74]. First, fatty acids are not only classified by their chain lengths, but also by their degree of unsaturation. The unsaturation of the studied fatty acids (oleic and lauric) should be taken into account. In principal, both saturated and unsaturated fatty acids are degraded via β-oxidation. While the degradation of saturated fatty acids follows the classic β-oxidation pathway, the degradation of unsaturated fatty acids requires auxiliary enzymes in addition to the enzymes for degradation of saturated fatty acids, e.g., NADHPH-dependent reductase and isomerase [50, 84, 86]. These enzymes are metabolically essential in biodegradation of unsaturated fatty acids because the former acts like a mechanism for breaking down the intermediates and the latter as a detoxification mechanism [84]. Hence, the degradation of oleic acid is more metabolically demanding than that of lauric acid. This can be one of the reasons why unsaturated long-chain fatty acids cause severe inhibition of microbial activity and growth [6, 33, 51]. Second, considering that oleic acid was easily degraded in the presence of glucose, this can be explained by the co-utilization phenomenon, which enhances biodegradation by increasing the biomass of the biograders [15, 20, 30, 48, 56]. Hence, the presence of easily degradable glucose enhances the biodegradation of oleic acid due to the effect of co-utilization. Therefore, apart from the effects of the reactor hydrodynamics, the successful anaerobic digestion of lipids and fatty acids (e.g., oleic acid) seems to require the addition of an easily biodegradable co-substrate.

The influence of byproducts on the biodegradation of lipids in EGSB reactors

Pereira et al. [67] carried out studies to characterize the anaerobic degradation of long-chain fatty acids in EGSB reactors. The reactors were continuously fed with oleic acid (50% COD) and co-substrate, skimmed milk, (50% COD) as carbon sources. From day 70, the carbon source was exclusively oleic acid. Their results showed that methane production decreased to 20–30% of the value obtained when 50% COD fed was a co-substrate. Considering that adsorption occurs before biodegradation [38], the results reported by Pereira et al. [67] indicate that degradation of the adsorbed substrate was inhibited when the concentration of oleic acid was increased. Chromatographic analysis of the extracted adsorbed matter showed that the adsorbed substrate was mainly composed of palmitic acid and not oleic acid, which was added as the initial substrate. Because palmitic acid was not initially added to the medium, Pereira et al. [67] concluded that it was a by-product of the biodegradation of oleic acid. Palmitic acid accumulated and adsorbed onto the sludge in the presence of oleic acid. When oleic acid was removed from the medium, β-oxidation of the adsorbed palmitic acid occurred with methane production. Therefore, the results reported by Pereira et al. [67] show that the presence of oleic acid inhibits further β-oxidation of palmitic acid. Hence, when treating lipid-rich wastewater, it should be advantageous to run sequencing cycles of adsorption and degradation in order to enhance complete removal of lipids.

Concluding remarks

The preceding sections show the challenges posed by the presence of lipids in wastewater. Hence new approaches and methods (both biological and physicochemical) are still required to fully understand the behavior of lipids in biological wastewater treatment processes and to enhance their removal. Chang et al. [16] has proposed the use of a combination of membrane technology (i.e., ultrafiltration) and ozone treatment. Through reviewing the literature on plant design and operation, Yuan and Blackall [104] proposed that optimization of microbial community structure could serve as a new way for improving the performance of biological wastewater treatment systems. This can be achieved by use of submerged membrane bioreactors as reported by Witzig et al. [102]. In our recent study [18], in which we used lipids as the main organic pollutant and compared the influence of soluble microbial products (SMP—organic compounds produced by microorganisms as they degrade substrates) on biomass concentration and removal of soluble BOD and COD, we found that washing the

biomass was analogous to the use of submerged membrane bioreactors. In such systems, removal of soluble BOD and COD increases and biomass concentration increases steadily to a constant value. This shows that a microbial community that sufficiently consumes organic matter develops under such conditions.

Although many researchers have studied the characteristics of SMP and kinetics of their production and biodegradation [7–9, 62, 63], the exact behavior of SMP and their influence on microbial activities are not well known. In this regard, Chipasa and Mędrzycka [19] have reported that microbial production of more or less refractory organic matter depends on whether microorganisms were developed in the presence or reduced levels of SMP. The efficiency of a biological process to remove organic matter also depends on these conditions because past substrates influence future functional properties of microorganisms [19]. Since lipids/fatty acids are one of the major components of SMP found in biological wastewater effluents [8, 21, 59, 61, 90], another promising approach to enhance their biological removal from wastewater is the control of the accumulation of SMP, which is one of the possible causes of bioreactor failures and loss of biomass activity reported in the literature. Therefore, this approach is advantageous because it stabilizes not only the microbial community but also the biodegradation process [18, 19].

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