

## Minireview

# Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA)

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### Summary

**The position of high-rate anaerobic technology (HR-AnWT) in the wastewater treatment and bioenergy market can be enhanced if the range of suitable substrates is expanded. Analyzing existing technologies, applications and problems, it is clear that, until now, wastewaters with high lipids content are not effectively treated by HR-AnWT. Nevertheless, waste lipids are ideal potential substrates for biogas production, since theoretically more methane can be produced, when compared with proteins or carbohydrates. In this minireview, the classical problems of lipids methanization in anaerobic processes are discussed and new concepts to enhance lipids degradation are presented. Reactors operation, feeding strategies and prospects of technological developments for wastewater treatment are discussed. Long-chain fatty acids (LCFA) degradation is accomplished by syntrophic communities of anaerobic bacteria and methanogenic archaea. For optimal performance these syntrophic communities need to be clustered in compact aggregates, which is often difficult to achieve with wastewaters that contain fats and lipids. Driving the methane production from lipids/LCFA at industrial scale without risk of overloading and inhibition is still a challenge that has the potential for filling a gap in the existing processes and technologies for biological**

**methane production associated to waste and wastewater treatment.**

### Introduction

The range of feedstock for biogas production spans from animal waste to municipal sludge, industrial wastewater and organic fractions of municipal solid waste as well as energy crops whenever available. The wide diversity of anaerobic technologies can be grouped in two main concepts: (i) facilities aiming at industrial wastewater treatment and (ii) facilities aiming at energy production. In general, the first group comprises more sophisticated technologies that operate with low solids content, high loading rates and sludge retention time much higher than hydraulic retention time. The second group includes anaerobic digestion (AD) plants dedicated to bioenergy production, which are usually completely mixed reactors, with a simple technological design. Since anaerobic processes possess multifunctional characteristics, energy is recovered from wastewater treatment facilities whenever it is economically feasible. On the other hand, organic waste stabilization and nutrient redistribution are, besides energy production, embedded objectives of any AD plant.

From the beginning of the eighties, high-rate anaerobic wastewater treatment technology (HR-AnWT) has become a standard for a certain range of industrial wastewaters. Thousands of full-scale installations are in operation worldwide, treating mainly wastewater containing readily degradable organic pollutants such as volatile fatty acids and carbohydrates. Reliable technologies, such as the up-flow anaerobic sludge blanket (UASB) reactor and the derived designs – expanded granular sludge bed (EGSB) and internal circulation (IC) reactors – promoted the confidence in AD technology. However, HR-AnWT applications are still centred essentially in biodegradable effluents from distilleries, pulp and paper, breweries and beverage industries.

The position of HR-AnWT in the wastewater treatment and bioenergy market can be further enhanced if the range of suitable substrates is expanded. Analysing the existing technologies, applications and problems, it is

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

Component	Methanogenic reaction	Biogas (lg <sup>-1</sup> )	CH <sub>4</sub> (%)
Lipids	$C_{60}H_{90}O_6 + 24.5H_2O \rightarrow 34.75CH_4 + 15.25CO_2$	1.425	69.5
Carbohydrates	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$	0.830	50.0
Proteins	$C_{16}H_{24}O_5N_4 + 14.5H_2O \rightarrow 8.25CH_4 + 3.75CO_2 + 4NH_4^+ + 4HCO_3^-$	0.921	68.8

clear that wastewaters with high lipids content are not effectively treated yet by HR-AnWT. Nevertheless, waste lipids are ideal substrates for methane production, since theoretically their degradation produces more biogas with higher methane content, when compared with proteins or carbohydrates (Table 1).

The energy value of lipids makes them an ideal co-substrate to increase the economical feasibility of any AD plant based on co-digestion concepts. The net energy production increases significantly if a fraction of waste lipids is mixed in the feedstock. This has already been noticed by managers of AD plants, who are even willing to pay for lipids. However, without a proper feeding strategy, addition of waste lipids to an AD plant is risky, if accumulation of long-chain fatty acids (LCFA) is not prevented. Understanding anaerobic degradation of lipids has therefore an immediate economical impact on AD plants.

The existing gap in HR-AnWT for complex wastewaters with lipids and the importance of lipids as co-substrates in AD plants make this issue of global interest in the environmental technology field. In this minireview, the classical problems of lipids degradation in anaerobic processes are discussed and new concepts to avoid inhibition by lipids and to enhance degradation are presented. Reactors operation, feeding strategies and prospects of technological developments for wastewater treatment are discussed. Finally, some hints on anaerobic bacteria and microbial communities that degrade LCFA are presented.

### The role and drawbacks of LCFA in the anaerobic degradation of lipids

Lipids are LCFA bonded to glycerol, alcohols or other groups by an ester or ether linkage. Fats and oils are a subgroup of lipids that have the alcohol groups esterified with fatty acids, predominantly in the form of triglycerides (glycerol backbone with three LCFA). Fats contain saturated LCFA, and oils are normally composed of unsaturated fatty acids, which confer lower melting point.

Fats and oils are common contaminants of domestic sewage and industrial effluents from dairy industries (Perle *et al.*, 1995), slaughterhouses (Sayed *et al.*, 1988), livestock farms (Broughton *et al.*, 1998), wool scouring

facilities (Becker *et al.*, 1999) and edible oil-processing facilities (Beccari *et al.*, 1996; Becker *et al.*, 1999).

In general, hydrolysis of fats and oils to glycerol and LCFA proceeds rapidly in AD processes, resulting in the accumulation of LCFA in the wastewater (Hanaki *et al.*, 1981; Angelidaki and Ahring, 1992). Over 90% of the chemical oxygen demand (COD) present in lipids is conserved, after hydrolysis, in the LCFA (Hanaki *et al.*, 1981). The estimated biomass/substrate yield for the conversion of fat is 0.038 g VSS (g COD)<sup>-1</sup>, whereas for proteins and carbohydrates values of 0.2 and 0.35 g VSS (g COD)<sup>-1</sup>, respectively, are reported (Pavlostathis and Giraldo-Gomez, 1991).

At neutral pH, LCFA are ionized and so it is appropriate to refer to them according to their carboxylate form: for instance, oleate and palmitate instead of oleic and palmitic acids. The concentration of lipids and LCFA in domestic and industrial wastewater is quite diverse. Wool scouring and olive mill processes can generate effluents with lipids concentrations in the range of 5–25 g l<sup>-1</sup> (Beccari *et al.*, 1998; Becker *et al.*, 1999). Lower values were detected in a sunflower oil mill wastewater, with LCFA concentrations ranging from 0.2 to 1.3 g l<sup>-1</sup> (Saatci *et al.*, 2003). Total lipids in a dairy wastewater were reported to vary from 0.9 to 2.0 g l<sup>-1</sup> (Kim *et al.*, 2004a). In domestic sewage, lipids represent generally 20–25% of the total organic matter, with concentrations ranging from 40 to 100 mg l<sup>-1</sup> (Quémeneur and Marty, 1994). Slaughterhouses can produce effluents with a total fat matter between 0.35 and 0.52 g l<sup>-1</sup> (Sayed *et al.*, 1988).

Table 2 presents the LCFA composition of lipid-containing raw materials and wastewaters. It is very likely that the major constituents in raw materials are also present in the wastewaters generated during their processing. It is clear that palmitic acid and oleic acid are the most abundant saturated and unsaturated LCFA respectively.

Research on the application of anaerobic technology to treat wastewaters containing lipids/LCFA has been emerging in the past 25 years. As discussed below, two main problems specifically related to the treatment of these effluents were identified and characterized: (i) sludge flotation and biomass washout due to the adsorption of lipids/LCFA onto the biomass, and (ii) inhibition of acetogenic bacteria and methanogenic archaea by LCFA.

**Table 2.** LCFA commonly found in raw materials and wastewaters (showed as % of total LCFA) (adapted from Hwu, 1997).

Raw materials/wastewaters	LCFA common name (structure <sup>a</sup> )						
	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)
Palm oil (1)		1.4	42.9	0.7	4.8	39.0	10.0
Olive oil (1)			14.3	1.4	2.4	71.4	5.5
Soybean oil (1)		1.0	11.0		4.8	21.9	49.0
Cotton seed oil (1)		1.4	25.7	1.0	2.9	15.2	51.9
Cocoa butter (1)			26.7	0.5	32.9	33.8	4.3
Whole milk (2)	7.0	6.0	21.0	2.0	6.0	39.0	13.0
Chicken fat (1)		1.4	21.0	6.7	4.3	42.4	20.0
Beef tallow (1)	1.0	2.6	28.1	3.8	20.0	37.6	2.9
Domestic sewage (3)		2.2	16.4	0.9	8.1	30.5	29.2
Dairy wastewater (4)			27.0		7.0	37.0	13.0

a. Cn:d, where n is the number of carbon atoms and d the number of double bonds.

(1) Taylor (1965); (2) Hanaki and colleagues (1981); (3) Quémeneur and Marty (1994); (4) Kim and colleagues (2004a).

### LCFA adsorption onto the sludge – sludge flotation and sludge washout

Sludge flotation and sludge washout due to the adsorption of fatty matter onto the biomass are widely reported in the literature. Samson and colleagues (1985) referred the failure of an industrial scale UASB reactor treating milk fat, due to sludge flotation. Hawkes and colleagues (1995) observed poor biomass retention in four different reactors treating ice-cream wastewater with high fat content. Rinzema and colleagues (1989; 1993) tested the treatability of LCFA-containing wastewaters in UASB reactors. When the reactors were overloaded a severe washout caused by flotation was observed. Sam-Soon and colleagues (1991) used a UASB reactor to study oleic acid degradation and reported that the original inoculated granules suffered from disintegration and encapsulation by a gelatinous and whitish mass.

LCFA have an amphiphilic structure; they are composed of a hydrophobic aliphatic tail and a hydrophilic carboxylic head. From a thermodynamic viewpoint disintegration of granules can be expected because at neutral pH LCFA act as surfactants, lowering the surface tension. According to Thaveesri and colleagues (1995) the adhesion of hydrophilic cells appeared to be enhanced at a low liquid surface tension, while the adhesion of hydrophobic cells was favoured at a high surface tension. Acetogens were characterized as mostly hydrophobic and therefore the low-surface-tension environments imposed by LCFA may cause a sloughing-off from granular sludge and the selective washout of these microorganisms (Daffonchio *et al.*, 1995). Hwu *et al.* (1997a) concluded that typical operating parameters of EGSB reactors (up-flow velocity  $> 4 \text{ m h}^{-1}$ , hydraulic retention time  $< 10 \text{ h}$ ) resulted in a poor treatment of LCFA containing wastewaters. The recirculation of the washed out biomass was beneficial to enhance the overall performance (Hwu *et al.*, 1997b).

Hwu and colleagues (1998a) showed that the specific LCFA organic load necessary to induce complete sludge flotation [ $0.203 \text{ g COD (g VSS)}^{-1} \text{ day}^{-1}$ ] corresponded to a LCFA concentration of  $263 \text{ mg LCFA l}^{-1}$ , which was far below the minimum inhibitory concentration ( $401 \text{ mg LCFA l}^{-1}$ ) of methanogenesis. This suggested that deterioration of the UASB process by LCFA adsorption and consequent sludge washout are likely to occur prior to inhibition of the methanogenic archaea by the LCFA.

Jeganathan and colleagues (2006), using UASB reactors to treat a complex oily wastewater from a food industry, reported that although approximately 75% of COD was degraded to methane at an organic loading rate (OLR) of about  $2.5 \text{ g COD l}^{-1} \text{ day}^{-1}$ , the system performance declined sharply at higher loading rates. An increase in loading to  $5 \text{ g COD l}^{-1} \text{ day}^{-1}$  caused fat, oil and grease (FOG) accumulation in the sludge and increased foam production. This reduced the degradation to 40–50%. These authors also reported that accumulation of FOG in the biomass was the critical parameter governing the high-rate anaerobic reactor performance and further suggested the need for periodic reseedling of anaerobic reactor systems treating oily wastes, since the loss of sludge in the bed, due to washout, increased the FOG accumulation onto the biomass and consequent reactor failure.

### Inhibition of anaerobic microbial communities by LCFA

Although derived essentially from the interpretation of batch experiments, the effect of LCFA on the methanogenic and acetogenic microorganisms is documented (Hanaki *et al.*, 1981; Koster and Cramer, 1987; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994; Lalman and

Bagley, 2000; 2001; 2002). Both acetoclastic and hydrogenotrophic methanogens are affected by LCFA, although acetoclastic methanogens are apparently more affected by the presence of these compounds (Hanaki *et al.*, 1981; Hwu and Lettinga, 1997; Alves *et al.*, 2001; Lalman and Bagley, 2001). Inhibitory effects of unsaturated LCFA are reported to be more severe than those of saturated LCFA (Lalman and Bagley, 2002).

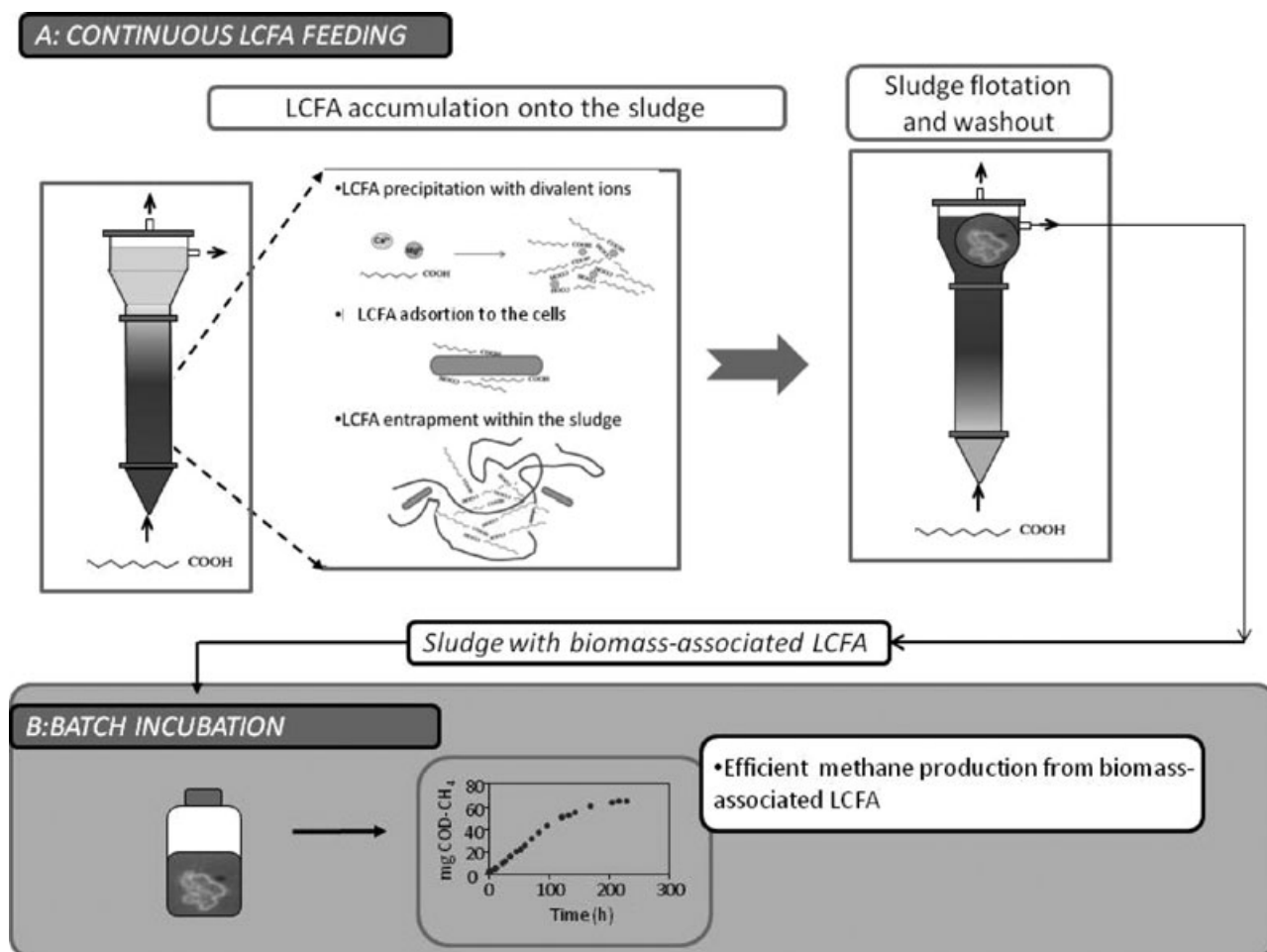
In the early 1980s, Hanaki and colleagues (1981) performed several batch experiments where they found that glucose fermentation was not affected by the presence of LCFA, the addition of acetate and butyrate intensified the toxic effect of LCFA, and oleate was less inhibitory than a LCFA mixture. Angelidaki and Ahring (1992) suggested that the response to the addition of neutral lipids may depend on the degree of biomass adaptation, whereas the addition of free LCFA above a certain concentration may directly result in process failure, due to a permanent toxic effect of these compounds towards acetogenic bacteria and methanogenic archaea. Rinzema and colleagues (1994) validated this concept and found that LCFA exerted a bactericidal effect on methanogenic archaea. This conclusion was based on the observation that acetoclastic methanogens did not adapt to LCFA neither upon repeated exposure to toxic concentrations, nor after prolonged exposure to non-toxic concentrations. The recovery after a lag phase usually observed in batch assays was ascribed to growth of a few survivors. For many years it was believed that high-rate treatment of lipid-rich effluents was not possible. Hwu (1997) tried to enhance the anaerobic treatment of wastewater containing oleic acid and found a higher susceptibility of suspended sludge than granular sludge to LCFA toxicity. This observation in batch assays, though interesting, was of little practical relevance, since granular sludge was not structurally stable when LCFA were present.

Lalman and Bagley (2002) reported only a small inhibition of hydrogenotrophic methanogens in the presence of linoleate (C18:2), oleate (C18:1) and stearate (C18:0), individually or in mixture. The mechanism of inhibition by LCFA was related to their adsorption onto the cell surface, affecting the transport and/or protective functions of the cell (Demeyer and Henderickx, 1967; Galbraith and Miller, 1973; Rinzema, 1988). This led to the hypothesis that the inhibitory effect of LCFA is determined by the LCFA : biomass surface ratio, although in several other studies the LCFA concentration was found to determine inhibition by LCFA (Rinzema *et al.*, 1994; Kim *et al.*, 2004a). However, other authors showed that inhibition by LCFA is not permanent and that adaptation of biomass to lipids/LCFA can occur (Broughton *et al.*, 1998; Alves *et al.*, 2001; Kim *et al.*, 2004a; Pereira *et al.*, 2004).

### New concepts on LCFA inhibition and degradation

As a consequence of LCFA accumulation onto the sludge, treatment of lipids/LCFA-containing wastewaters in conventional up-flow anaerobic reactors can lead to complete sludge washout and process failure, which seems to be more due to particular problems of fluid dynamics than to microbial activity inhibition. In fact, microbial injury due to intensive (high concentration) and extended (long time) contact between anaerobic sludge and LCFA was found to be less severe than could be expected (Alves *et al.*, 2001; Pereira *et al.*, 2002a; 2004; 2005). When performing a routine assessment of the specific methanogenic activity of sludge collected from a continuous reactor fed with oleic acid, a surprisingly high methane production was observed in the blank vials, where no external substrate was added (Alves *et al.*, 2001). The observed methane production resulted from the degradation of substrate that accumulated onto the sludge during the reactor's operation, contradicting the accepted theories of permanent LCFA toxicity and inhibition. This finding led to the development of new concepts and encouraged the study of the microbiology of LCFA degradation in anaerobic bioreactors. The scheme presented in Fig. 1 illustrates the sequence of continuous LCFA feeding, LCFA accumulation and consequent sludge flotation and washout. Subsequent batch incubation of the sludge taken from the reactor, containing biomass-associated LCFA, results in methane production. The proposed mechanisms of LCFA accumulation onto the biomass are adsorption, entrapment within the flocks structure and precipitation with divalent ions (Pereira *et al.*, 2005). Fig. 2 shows a microbial aggregate collected from a UASB reactor fed with oleic acid. The whitish matter embedding the cells consisted, in that case, of more than 80% palmitic acid (Pereira *et al.*, 2005).

The difference in properties of granular and suspended sludge in terms of their capacity to accumulate and to degrade the accumulated (or biomass associated) LCFA was studied by Pereira and colleagues (2002a). Two EGSB reactors were fed with oleic acid-based synthetic effluents. One EGSB was inoculated with suspended sludge and the other one with granular sludge. Sludge samples were collected at different operating times and incubated in batch vials, where the methane production from the degradation of the accumulated LCFA was monitored. Suspended sludge turned out to be more efficient than granular sludge to accumulate and to degrade LCFA. A maximum specific LCFA accumulation of  $3271 \pm 877$  mg COD-LCFA (g VSS)<sup>-1</sup>, and a maximum methane production rate of  $434 \pm 60$  mg COD-CH<sub>4</sub> (g VSS)<sup>-1</sup> day<sup>-1</sup> was exhibited by the suspended sludge, which was about 1.3 and 3 times, respectively, the maximum values exhibited by the granular sludge

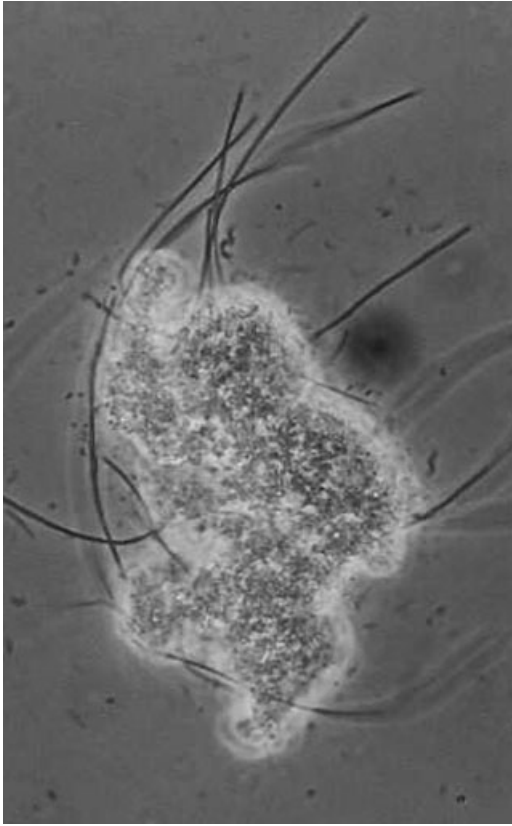


**Fig. 1.** Schematic representation of the phenomena of LCFA accumulation onto the sludge during the continuous operation of a reactor fed with LCFA, sludge flotation, sludge washout and methane production in batch vials from the degradation of the biomass-associated LCFA (adapted from Sousa *et al.*, 2009).

(Pereira *et al.*, 2002a). Additionally, for the suspended sludge, the methane production rate in batch was enhanced by stirring and was inhibited by the addition of oleic acid. Extraction and GC analysis revealed that the main adsorbed substrate was palmitate, and not oleate. Apparently, the conversion of oleate to palmitate occurred easily in the reactor, but further degradation of palmitate did not occur.

Pursuing the investigation, the specific methanogenic activity (SMA) of sludge samples, containing biomass-associated LCFA content between 1000 and 5000 mg COD-LCFA (g VSS)<sup>-1</sup>, was determined before and after the conversion to methane of that embedded LCFA (Pereira *et al.*, 2004). Acetate and H<sub>2</sub>/CO<sub>2</sub> were used as individual substrates for SMA experiments. In general, the loaded sludge had no quantifiable activity except with H<sub>2</sub> as substrate (Table 3). After the degradation of the biomass-associated LCFA, a significant increase on the SMA was observed for the selected substrates.

This result demonstrated that the inhibition by LCFA is reversible in the range of LCFA content between 1000 and 5000 mg COD-LCFA (g VSS)<sup>-1</sup>, contradicting the previously reported bactericidal or permanent toxic effects of LCFA (Rinzema, 1988; Angelidaki and Ahring, 1992). This opened new scenarios for the anaerobic treatment of wastewater with high lipid content. The low SMA measured before the depletion of the biomass-associated LCFA may result from a strong effect of transport (diffusion) limitations imposed by the LCFA layer surrounding the cells, which could hamper the access of the added substrates, as well as the subsequent biogas release. This is reinforced by the fact that with H<sub>2</sub>, the smallest molecule used as electron donor, methane production was directly observed, suggesting a fast transport of this small compound through the LCFA layer. Transport limitations phenomena may also be responsible for the observed lag phases that previously have been ascribed to mechanisms of cell wall damage and bactericidal



**Fig. 2.** Example of a microbial aggregate collected from a lab-scale UASB reactor fed with oleic acid.

effects. However, although the hypothesis of transport limitations seems to be reasonable, a reversible metabolic inhibitory effect would result in similar observations. Probably both phenomena are involved, their relative importance being dependent on the specific LCFA content of the sludge (Pereira *et al.*, 2004).

The evidence of physical inhibition due to transport limitation effects was further clarified by Pereira and colleagues (2005). The important conclusion was reinforced; the inhibitory effect on the methanogenic activity, after the contact of the biomass with LCFA, is reversible.

From this ensemble of new results it became clear that LCFA-rich wastewater treatment can be feasible.

Sequencing phases of continuous feeding and batch degradation of the accumulated substrate was then postulated as a possible practical solution for the treatment of this kind of effluents.

### New reactor concept for HR-AnWT of complex wastewater with high lipid content

Since the success of conventional anaerobic treatment systems is based on optimization of biomass sedimentation, flotation leads to washout and subsequent process disruption. Table 4 gives an overview of studies in which LCFA-containing wastewater was treated in anaerobic bioreactor studies. Problems of sludge flotation or unsuccessful granulation were ascribed to treatment failure of industrial and pilot scale UASB reactors treating lipids/LCFA-containing wastewater (Samson *et al.*, 1985; Rinzema, 1988; Sam-Soon *et al.*, 1991; Hawkes *et al.*, 1995; Hwu, 1997). Reasonable solutions to overcome flotation problems were searched: sieve drums, biomass recirculation or partial phase separation were tentatively applied for that purpose (Rinzema, 1988; Hamdi *et al.*, 1992; Hwu, 1997; Beccari *et al.*, 1998). To prevent washout induced by LCFA adsorption, a sequential process with a feeding and a reaction phase was suggested as the preferred technology for anaerobic LCFA removal from wastewater (Pereira *et al.*, 2005). It was further postulated that the specific contact area between bacteria and LCFA should be maximized to minimize mass transfer limitations. The sequential process was applied at lab scale by Cavaleiro and colleagues (2009) and the results obtained showed that this operating mode was the best for sludge acclimation. After the third cycle a specialized anaerobic microbial community was developed, able to efficiently convert the LCFA to methane. Continuous treatment was then applied, achieving volumetric loading rates up to 21 kg COD m<sup>-3</sup> day<sup>-1</sup> with 72% conversion efficiency to methane. From the current problems encountered at industrial scale with LCFA and the research results from Pereira and colleagues (2002a,b,c; 2004; 2005) and Cavaleiro and colleagues (2009), two main principles may be postulated for the design of a reactor capable of high-rate anaerobic treatment of

**Table 3.** Specific methanogenic activities exhibited by three different sludges, before and after the conversion to methane of the biomass-associated LCFA (adapted from Pereira *et al.*, 2004).

Sludge-specific LCFA content [mg COD-LCFA (g VSS) <sup>-1</sup> ]	Specific methanogenic activity [mg COD-CH <sub>4</sub> (g VSS) <sup>-1</sup> day <sup>-1</sup> ]			
	Acetate		H <sub>2</sub> /CO <sub>2</sub>	
	Before	After	Before	After
1221 ± 144	143 ± 29	326 ± 13	1462 ± 94	1670 ± 81
2838 ± 63	0	579 ± 4	1218 ± 1	2817 ± 146
4571 ± 257	0	533 ± 95	401 ± 21	2709 ± 38

**Table 4.** Treatment of wastewater containing lipids and LCFA in different anaerobic reactors (adapted from Sousa, 2007).

Type of wastewater	Type of reactor	Temperature (°C)	HRT (days)	OLR (g COD l <sup>-1</sup> day <sup>-1</sup> )	Specific OLR [g COD (g VSS) <sup>-1</sup> day <sup>-1</sup> ]	COD removal (%)	CH <sub>4</sub> yield [(g COD) <sup>-1</sup> ]	Reference
LCFA mixture	EGSB	30	0.25–0.13	4–8	0.20–0.41	44–69	ND	Hwu <i>et al.</i> (1998b)
LCFA mixture	EGSB	55	0.25–0.13	4–8	0.23–0.47	66–73	ND	Hwu <i>et al.</i> (1998a)
LCFA mixture	UASB	35	0.7–1.2	3.2–9.4	0.09–0.25	82–93	ND	Kim <i>et al.</i> (2004b)
LCFA mixture <sup>a</sup>	CSTR + UASB	35	2.9	0.2–2.7	ND	60–95	ND	Pereira <i>et al.</i> (2002b)
Oleate (+milk) <sup>b</sup>	EGSB	37	1	4–8	1.39–2.78	69–97	0.03–0.28	Pereira <i>et al.</i> (2005)
Oleate (+milk) <sup>b</sup>	AF	37	3.3–0.64	0.7–12.5	0.09–1.67	80–95	0.09–0.36	Miranda <i>et al.</i> (2006)
Oleate	EGSB	37	1.18	3.3	0.85	75–86	0.03	
Palmitate	EGSB	37	1.14	3.2	0.83	90–95	0.03	
Saccharose + oleate	UASB	35	1	4.2–6.3	0.25–0.38	76–90	ND	
Saccharose + oleate	DAEB	35	1	4.2–6.3	NA	77–93	ND	
Saccharose + stearate	UASB	35	1	4.2–6.4	0.25–0.38	77–93	ND	
Saccharose + stearate	DAEB	35	1	4.2–6.4	NA	76–90	ND	Gavala <i>et al.</i> (1999)
Dairy wastewater	UASB	35	6–40	2.0–4.5	ND	79–99	ND	Arnaiz <i>et al.</i> (2003)
Dairy wastewater	IFB	35	63.6–3	0.5–10	ND	75–98	ND	
Dairy wastewater	ITB	35	66.6–3	0.5–12	ND	75–98	ND	
Dairy wastewater	BFBR	–	0.3–0.5	10 (up to)	ND	85–90	Approximately 0.37	Haridas <i>et al.</i> (2005)
Ice-cream factory wastewater	AF	35	0.9	6.4	ND	67	0.36	Hawkes <i>et al.</i> (1995)
	Contact process	35	5.5	1.1	ND	82	0.39	
	Fluidized bed reactor	35	1.5	4.2	ND	56	0.37	
	UASB	35	1.6	2.2	ND	49	0.19	
Food-processing wastewater	Multi-stage UASB	55	0.14	50	2.29	60–70	ND	Tagawa <i>et al.</i> (2002)
Food-processing wastewater	UASB	35	5	2.7–5.2	0.19–0.37	94–98	0.24–0.32	Jeganathan <i>et al.</i> (2006)
Food-processing wastewater	UASB	35	2.5–1.25	1.3–8.0	0.07–0.42	84–89	0.24–0.48	
	(PBR+) UASB	35	2.5–1.25	1.3–4.2	0.06–0.18	86–90	0.18–0.42	
Slaughterhouse wastewater	EGSB	35	0.2	15 (up to)	ND	67	ND	Núñez and Martínez (1999)
Slaughterhouse wastewater	ASBR	25	2.9	31.3 <sup>c</sup>	ND	94	ND	Masse <i>et al.</i> (2002)
Slaughterhouse wastewater	UASB	33	0.3–0.1	13–30	ND	60–93	0.20–0.28	Torkian <i>et al.</i> (2003)
Slaughterhouse wastewater	Draw-and-fill reactor	35	20	0.20	–	48	0.30 <sup>d</sup>	Fountoulakis <i>et al.</i> (2008)
Slaughterhouse + olive mill wastewater (1:1)		35	20	2.1	–	85	0.17 <sup>d</sup>	
Olive mill wastewater		35	20	4	–	75	0.11 <sup>d</sup>	
Palm oil mill wastewater	MABR	–	3–10	1.6–5.3	ND	87–95	0.32–0.42	Faisal and Umno (2001)
Palm oil mill wastewater	UASFF	38	3–1.5	1.8–23.2	0.18–1.23	89–97	0.31–0.35	Najafpour <i>et al.</i> (2006)
Sunflower oil factory wastewater	UASB	37	2–2.8	1.6–7.8	ND	87	0.16–0.35	Saatci <i>et al.</i> (2003)

a. Glucose was used as a co-substrate during reactor operation.

b. Skim milk was used as co-substrate during the start-up period.

c. Calculated with basis on the 1 h time feeding of the reactor (subsequent reaction and settling phases lasted for 69 h).

d. ICH<sub>4</sub> (g COD added)<sup>-1</sup>.

HRT, hydraulic retention time; OLR, organic loading rate; COD, chemical oxygen demand; UASB, up-flow anaerobic sludge bed reactor; CSTR, continuously stirred tank reactor; EGSB, expanded granular sludge bed reactor; DAEB, down-flow anaerobic expanded bed reactor; IFB, inverse fluidized bed reactor; ITB, inverse fluidized bed reactor; BFBR, buoyant filter bioreactor; AF, anaerobic filter; PBR, packed bed reactor (containing immobilized lipase beads); ASBR, anaerobic sequencing batch reactor; UASFF, up-flow anaerobic sludge-fixed film reactor; MABR, modified anaerobic baffled bioreactor; ND, not determined; NA, not applicable; –, information not available.

**Table 5.** Summary of kinetics data on anaerobic LCFA degradation.

Substrate	KS kg COD m <sup>-3</sup>	Y COD/COD <sup>a</sup>	μ <sub>max</sub> day <sup>-1</sup>	Reference
Oleate/manure/oil	0.058	0.05	0.55	Angelidaki <i>et al.</i> (1999)
Stearate	0.295	0.055	0.1	Novak and Carlson (1970)
Palmitate	0.41	0.054	0.11	Novak and Carlson (1970)
Myristate	1.23	0.053	0.08	Novak and Carlson (1970)
Oleate	9.21	0.054	0.44	Novak and Carlson (1970)
Linoleate	5.19	0.055	0.55	Novak and Carlson (1970)
Slaughterhouse (stearate)	0.1	0.021	7.7	Salminen <i>et al.</i> (2000)
Slaughterhouse (palmitate)	0.1	0.004	0.89	Salminen <i>et al.</i> (2000)
Slaughterhouse	0.102	–	–	Masse <i>et al.</i> (2002)
Oleate + skim milk	–	0.11–0.20	0.15–0.25	Alves (1998)
LCFA oxidation	0.105–3.18	0.06–0.16	0.085–0.55	Pavlostathis and Giraldo-Gomez (1991)

KS, half-saturation constant; Y, biomass/substrate yield; μ<sub>max</sub>, maximum specific growth rate.

a. produced biomass COD/consumed substrate COD.

LCFA-containing wastewater. These constitute the base of a new reactor concept, designated as Inverted Anaerobic Sludge Bed (IASB) reactor (Alves *et al.*, 2007): (i) maximize the contact area between biomass and LCFA to optimize LCFA adsorption, and (ii) use flotation as the primary biomass retention technique. These two principles imply that conventional primary biomass retention techniques such as granulation or biomass fixation cannot be applied. However, a settling step is still needed, because sludge settles well again after effective LCFA conversion. This settled sludge can subsequently be intimately contacted with LCFA-containing wastewater to maximize adsorption. Thus, a sludge recycle loop should be present over the reactor. This loop could further provide the mild shear stress needed to maximize the sludge surface area. Additionally, it provides the means to control mixing intensity inside the reactor and limit possible mass transfer limitations even further. A pilot scale proof of concept is running presently in a slaughterhouse located in the Nord of Portugal.

### Kinetics of LCFA degradation

Existing information about the kinetics of LCFA degradation under anaerobic conditions is not vast (Table 5). The complex nature of LCFA–cells interaction, and the potential of biomass acclimation, make it difficult to obtain objective data about kinetics of LCFA degradation and inhibition by LCFA. In the Anaerobic Digestion Model (ADM) No. 1 inhibition under transient high LCFA concentrations is not described (Batstone *et al.*, 2002).

The specific methane production rate from LCFA has been reported by several authors. Hwu and colleagues (1997b) reported a value of 600 mg COD (g VSS)<sup>-1</sup> day<sup>-1</sup> in the washed-out biomass from an EGSB reactor fed with oleic acid. Pereira and colleagues (2004) reported kinetic values of degradation of biomass-associated LCFA; a maximum of 434 mg COD-CH<sub>4</sub> (g VSS)<sup>-1</sup> day<sup>-1</sup> was obtained for a sludge containing a specific LCFA content of

743 mg COD-LCFA (g VSS)<sup>-1</sup>, while a value of 241 mg COD-CH<sub>4</sub> (g VSS)<sup>-1</sup> day<sup>-1</sup> was obtained for a sludge containing a specific LCFA content of 3272 mg COD-LCFA (g VSS)<sup>-1</sup>. A simple Haldane substrate inhibition model was not adequate to describe the kinetics of methane production from biomass-associated LCFA. Cavaleiro and colleagues (2009) reported a maximum value of 1170 mg COD-CH<sub>4</sub> (g VSS)<sup>-1</sup> day<sup>-1</sup> in a continuous reactor fed with high loads of skim milk and oleate.

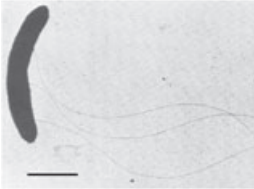
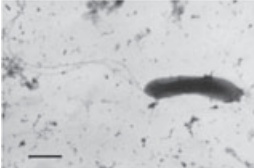

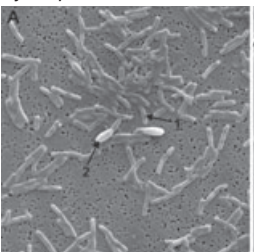
### Microbiology of anaerobic LCFA degradation

LCFA degradation in anaerobic bioreactors is accomplished by syntrophic communities of acetogenic bacteria and methanogenic archaea. LCFA are converted to acetate and hydrogen by obligate hydrogen-producing acetogens – OHPA (Schink, 1997). To date, only 14 acetogenic microorganisms have been described that degrade butyrate or higher fatty acids in syntrophy with hydrogen-consuming microorganisms (McInerney *et al.*, 2008). They all belong to the families *Syntrophomonadaceae* (McInerney, 1992; Zhao *et al.*, 1993; Wu *et al.*, 2006; Sousa *et al.*, 2007a) and *Syntrophaceae* (Jackson *et al.*, 1999), and live together with hydrogen-consuming methanogenic archaea or sulfate-reducing bacteria. However, only seven species are described that can use straight-chain LCFA with more than 12 carbon atoms: *Syntrophomonas sapovorans* (Roy *et al.*, 1986), *Syntrophomonas saponavida* (Lorowitz *et al.*, 1989), *Syntrophomonas curvata* (Zhang *et al.*, 2004), *Syntrophomonas zehnderi* (Sousa *et al.*, 2007a), *Syntrophomonas palmitatica* (Hata-moto *et al.*, 2007a), *Thermosyntropha lipolytica* (Svetlitshnyi *et al.*, 1996) and *Syntrophus aciditrophicus* (Jackson *et al.*, 1999).

Among these microorganisms only four species have the capability of utilizing mono- and/or polyunsaturated LCFA (with more than 12 carbon atoms): *S. sapovorans* (Roy *et al.*, 1986), *S. curvata* (Zhang *et al.*, 2004), *T.*



**Table 6.** Characteristics of some syntrophic LCFA-degrading bacteria (adapted from Sousa, 2007).

LCFA-degrading bacteria	Morphological characteristics	LCFA utilization range
<i>Syntrophomonas sapovorans</i> <sup>a</sup> 	Short curved rods (0.5 × 2.5 μm) Slightly motile Gram-negative Two to four flagella Non-spore forming	Degrades linear saturated fatty acids with 4–18 carbon atoms in co-culture with <i>Methanospirillum hungatei</i> . Mono- and di-unsaturated LCFA, such as oleate (C18:1) and linoleate (C18:2), are also oxidized by the co-culture
<i>Syntrophomonas curvata</i> <sup>b</sup> 	Slightly curved rods (0.5–0.7 × 2.3–4.0 μm) Motile Gram-negative One or three flagella inserted in both poles Non-spore forming	Degrades linear saturated fatty acids with 4–18 carbon atoms in co-culture with <i>M. hungatei</i> . Oleate (C18:1) is also oxidized by the co-culture
<i>Syntrophus aciditrophicus</i> <sup>c</sup> 	Rod-shaped cells (0.5–0.7 × 1.0–1.6 μm) Non-motile Gram-negative Non-spore forming	Degrades linear saturated fatty acids with more than four carbon atoms (C4:0 to C8:0, C16:0, C18:0) in co-culture with H <sub>2</sub> -utilizing <i>Desulfovibrio</i> sp. or <i>Methanospirillum hungatei</i>
<i>Syntrophomonas zehnderi</i> <sup>d</sup> 	Curved rods (approximately 0.4–0.7 × 2.0–4.0 μm) Variable response to Gram staining Slight twitching Motility Spore formation during growth on oleate in co-culture with a methanogen that utilizes hydrogen and formate	Degrades oleate, a mono-unsaturated fatty acid, and straight-chain fatty acids C4:0–C18:0 in syntrophic association with <i>Methanobacterium formicicum</i>

a. Roy and colleagues (1986).

b. Zhang and colleagues (2004).

c. Jackson and colleagues (1999).

d. Sousa and colleagues (2007a).

Bars equal 1 μm, except for picture <sup>d</sup> where the bar represents 10 μm.

*lipolytica* (Svetlitsnyi *et al.*, 1996) and the recently isolated *S. zehnderi* (Sousa *et al.*, 2007a) (Table 6). The latter bacterium was obtained from an anaerobic bioreactor treating an oleate-based effluent.

To date there are few studies on the microbial diversity of anaerobic communities that degrade LCFA. Microbial diversity of granular and suspended sludge was assessed during a long-term operation of two anaerobic up-flow bioreactors fed with increasing concentrations of oleate. Due to partial flotation caused by LCFA accumulation, a physical segregation of the sludge in two fractions was observed: a whitish floating fraction, at the top, and a settled fraction at the bottom (Pereira *et al.*, 2002c).

Differences in the community structure between these two fractions were assessed by comparison of denaturing gradient gel electrophoresis (DGGE) profiles. Similar-

ity indices between bottom and top sludge fractions attained values as low as 56.7% and 29.4% for the granular and suspended sludge respectively. Additionally, a shift in the community structure was observed in both sludges during the operation. Similarity indices between the original granular and suspended sludges and the respective top sludge fractions at the end of the operation were 17.3% and 15.2%.

Shigematsu and colleagues (2006) used a 16S rRNA gene approach to study the microbial communities present in a chemostat fed with a mixture of oleic and palmitic acids. Members belonging to *Syntrophomonadaceae* were detected in the chemostat, although the most predominant microorganisms belonged to the *Bacteroidetes* and *Spirochaetes* phyla. Based on this fact, the authors suggested that members of those phyla could play a role in LCFA

degradation. The diversity and dynamics of biomass in reactors that treat saturated (palmitate) and unsaturated (oleate) LCFA were studied by 16S ribosomal RNA gene-targeted molecular techniques (Sousa *et al.*, 2007b,c). These studies revealed that the bacterial community of the sludges is quite complex. Besides bacteria known to be able to degrade LCFA, bacteria were detected that are likely involved in the degradation of short-chain fatty acids, suggesting that during LCFA degradation short-chain fatty acids are formed from LCFA. Bacterial communities were dominated by members of the *Clostridiaceae* and *Syntrophomonadaceae* families (Sousa *et al.*, 2007b). However, a significant part of the retrieved bacterial 16S rRNA gene sequences (53%) were most similar to those of yet uncultured microorganisms, with the majority assigned to the phylum *Firmicutes*. Members of *Proteobacteria* and *Bacteroidetes* were also found.

A further interesting observation was the difference in the dominant population obtained with oleate and palmitate. The oleate-degrading community is able to rapidly degrade palmitate, which is obvious as palmitate is a key intermediate in oleate degradation. However, the consortium enriched with palmitate degraded oleate only poorly. This seems to reflect the above-mentioned characteristics of LCFA-degrading bacteria. All bacteria that degrade unsaturated fatty acids also degrade saturated fatty acids, but the opposite is not the case. The likely biochemical mechanism to degrade unsaturated fatty acids seems to be a coupled hydrogenation and  $\beta$ -oxidation. Besides OHPA that degrade unsaturated LCFA, bacteria exist that have the ability to hydrogenate unsaturated LCFA to saturated LCFA (Maia *et al.*, 2007; Paillard *et al.*, 2007).

Hatamoto and colleagues (2007b,c) used stable isotope probing with  $^{13}\text{C}$  palmitate as a substrate to identify the microorganisms directly involved in palmitate degradation. Members of the phyla *Bacteroidetes* and *Spirochaetes*, the family *Syntrophaceae* within the *Deltaproteobacteria*, and members of the family *Syntrophomonadaceae* and genus *Clostridium* within the *Firmicutes* were found in clone libraries from heavy rRNA fractions. These results confirm that phylogenetically diverse bacterial groups were active *in situ* in the degradation of LCFA under methanogenic conditions.

The effect of sulfate addition to methanogenic LCFA-degrading sludges was studied (Sousa *et al.*, 2009). When sludges were exposed to sulfate, sulfide was produced and methane formation decreased. Nevertheless, although many sulfate-reducing bacteria are known that can degrade LCFA, OHPA remained dominantly present in the reactors even after a long exposure to high sulfate concentrations (Sousa *et al.*, 2009). The results suggest that hydrogen consumption by methanogens is taken over by hydrogen-consuming sulfate reducers, which are known to have a higher affinity for hydrogen than methanogens.

## Conclusions and future perspectives

Lipids are suitable substrates for high-rate anaerobic wastewater treatment and are also ideal co-substrates for AD plants. Provided the appropriate technology is utilized and the right feeding strategy is followed, lipids can be effectively converted to methane by syntrophic consortia of acetogenic bacteria and methanogenic archaea. Driving the methane production from lipids/LCFA at industrial scale, without risk of inhibition, is still a challenge that has the potential for filling a gap in the existing processes and technologies for biomethane production associated to waste and wastewater treatment. A new reactor concept is proposed that provide primary biomass retention through flotation and secondary biomass retention through settling. The potential of this reactor to treat effluents with high concentrations of lipids/LCFA is being explored at pilot scale.

The types of bacteria involved in the methanogenic conversion of LCFA are known and the biochemical mechanism of LCFA degradation by  $\beta$ -oxidation is rather well understood. However, the initial steps in the anaerobic conversion of unsaturated LCFA remain unclear. Besides OHPA that degrade unsaturated LCFA, bacteria exist that have the ability to hydrogenate unsaturated LCFA to saturated LCFA. The position of the latter bacteria in LCFA degradation in bioreactors requires further study. In principle this conversion can be coupled to growth and these bacteria may compete with hydrogenotrophic methanogens for hydrogen.

LCFA require the syntrophic cooperation of OHPA and methanogens. These syntrophic communities perform optimally when they are organized in micro-colonies; at short intermicrobial distances the rate of interspecies hydrogen transfer is enhanced. Presently, it is unclear how the fatty structure of the substrate interferes with these communities. It is not clear how the micro-colonies develop in a fatty matrix and what is the effect on hydrogen transfer. As hydrogen is poorly soluble in water, it cannot be excluded that interspecies hydrogen transfer is enhanced when the matrix is LCFA rather than water.

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