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## EFFECTS OF LIPIDS AND OLEIC ACID ON BIOMASS DEVELOPMENT IN ANAEROBIC FIXED-BED REACTORS. PART II: OLEIC ACID TOXICITY AND BIODEGRADABILITY

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**Abstract**—Oleic acid toxicity and biodegradability were followed during long-term operation of two similar anaerobic fixed-bed units. When treating an oleate based effluent, the sludge from the bioreactor that was acclimated with lipids during the first operation period, showed a higher tolerance to oleic acid toxicity ( $IC_{50} = 137$  mg/l) compared with the sludge fed with a non-fat substrate ( $IC_{50} = 80$  mg/l). This sludge showed also the highest biodegradation capacity of oleic acid, achieving maximum methane production rates between 33 and 46  $mlCH_4(STP)/gVS.day$  and maximum percentages of methanization between 85 and 98% for the range of concentrations between 500 and 900 mg oleate/l. When oleate was the sole carbon source fed to both digesters, the biomass became encapsulated with organic matter, possibly oleate or an intermediate of its degradation, e.g. stearate that was degraded at a maximum rate of 99  $mlCH_4(STP)/gVS.day$ . This suggests the possibility of using adsorption–degradation cycles for the treatment of LCFA based effluents. Both tolerance to toxicity and biodegradability of oleic acid were improved by acclimatization with lipids or oleate below a threshold concentration. © 2000 Elsevier Science Ltd. All rights reserved

**Key words**—oleic acid toxicity, oleic acid biodegradability

### INTRODUCTION

Lipids are easily hydrolyzed to long chain fatty acids (LCFA), which are further converted to acetate and hydrogen through  $\beta$ -oxidation mechanism by the proton reducing acetogenic bacteria (Weng and Jeris, 1976). The mechanism of LCFA toxicity is related to the adsorption of the surface active acids onto the cell wall, which affects its transport and/or protective functions (Demeyer and Henderickx, 1967; Galbraith and Miller 1973). The acetate-utilizers (acetoclastic) seem to be more affected by LCFA than the  $H_2$ -utilizers (hydrogenophilic) bacteria (Hanaki *et al.*, 1981).

The existing literature suggests that LCFA exert a bactericidal effect on methanogenic bacteria and no adaptation was observed. The recovery after a lag phase usually observed in batch assays is attributed to the growth of few survivors (Rinzema *et al.*, 1994). These authors found that acetoclastic bacteria do not adapt to LCFA either upon repeated exposure to toxic concentrations, or after prolonged

exposure to non-toxic concentrations. Among other conclusions, Hanaki *et al.*, (1981) found, in batch assays, that: (1) glucose fermentation was not affected by the presence of LCFA; (2) the addition of acetate and butyrate intensified the toxic effect of LCFA; and (3) oleate was less inhibitory than a LCFA mixture. Angelidaki and Ahring (1992) suggested that the response to the addition of neutral lipids may depend upon the degree of adaptation to lipids, whereas the addition of free LCFA above a certain concentration may directly result in process failure.

So far, the question of adaptation of anaerobic sludge to lipids or LCFA has essentially been studied in batch experiments, which are very important as a complement of long-term operation data. In this work, the effect of feeding increasing concentrations of lipids on the characteristics of the sludge developed in anaerobic fixed-bed digesters was investigated by comparison with a control digester fed with a non-fat substrate. The effect of gradually shifting the feeding from the original composition, to oleate as the sole carbon source, allowed us to evaluate whether the pre-contact with lipids would increase the tolerance of acetoclastic bacteria to

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oleic acid and the biodegradation capacity of this long chain fatty acid (LCFA).

The fixed-bed reactors were specially designed to allow the biomass to be periodically withdrawn (Alves *et al.*, 1998). Sodium oleate was used as a model for LCFA because it is, in general, the most abundant of all LCFA present in wastewater (Komatsu *et al.*, 1991), has a good solubility, it is the most important LCFA produced by whole milk degradation (Hanaki *et al.*, 1981) and is one of the more toxic LCFA (Galbraith *et al.*, 1971).

#### MATERIALS AND METHODS

For details on the experimental set-up, substrate, biomass separation and quantification, and analytical methods, see Part I.

##### *Sludge characterization*

Methanogenic toxicity and biodegradability tests were performed using a pressure transducer technique (Colleran *et al.*, 1992; Colleran and Pistilli, 1994). The test involves the monitoring of the pressure increase developed in sealed vials fed with non-gaseous substrates or pressure decrease in vials previously pressurised with gaseous substrates (H<sub>2</sub>/CO<sub>2</sub>:80:20). Strict anaerobic conditions were maintained. The hand-held pressure transducer used was developed at University College Galway, Ireland, and was capable of measuring a pressure variation of two bar (0–± 202.6 kPa) over a range of –200–+ 200 mv. The sensing element is connected to a digital panel module and the device is powered by a 9.0 V DC transformer.

The basal medium used in all the batch experiments was made up with demineralized water, and consisted of cysteine-HCL (0.5 g/l) and sodium bicarbonate (3 g/l) and sodium bicarbonate (3 g/l), the pH was adjusted to 7.0–7.2 with a few drops of NaOH 8N and was prepared under strict anaerobic conditions. No calcium or trace-nutrients were added.

Toxicity batch experiments were performed by adding 30 mM acetate and increasing oleate concentrations (100, 300, 500, 700 and 900 mg/l) to the sludge in batch vials. Working volume was 12.5 ml and total volume 25 ml. Fifty percent inhibition concentration (IC<sub>50</sub>) was defined as the oleate concentration that caused 50% relative activity loss. Toxicity batch experiments were performed in triplicate assays.

Biodegradability tests were performed by adding increasing oleate concentrations (100, 300, 500, 700 and 900 mg/l) to the sludge in batch vials. The maximum methane production rate (MMPR), the percentage of methanization achieved (PM) and the lag phases were determined. Background methane production due to residual substrate was discounted in the values of MMPR and PM. Biodegradability tests were performed in duplicate assays. The specific values of degradation rate and percentage of methanization were obtained by dividing the methane production rates by the VS content of each vial determined at the end of the experiment.

##### *Operation mode*

The operation mode was described in Part I. During the start-up both reactors (RI and RII) were fed with skim milk. After this period, the feeding to reactor RI was gradually shifted to whole milk, while in reactor RII skim milk feeding proceeded for 246 days (see Part I, Table 1). After this Period (I), both reactors were fed with the same substrate. Initially, increasing oleate concentrations with skim milk as co-substrate was fed (Period II) and, in

Period III, oleate was the sole carbon source fed to both digesters.

#### RESULTS AND DISCUSSION

Performance of both digesters, biomass growth and methanogenic activity were the subject of the previous paper (see Part I).

##### *Toxicity of sodium oleate towards acetoclastic bacteria*

The 50% inhibition concentration of sodium oleate (IC<sub>50</sub>) was previously determined for the acetoclastic and the hydrogenophilic bacteria present in the inoculum. The values obtained for IC<sub>50</sub> were 50 mg/l for acetoclastic bacteria and 200 mg/l for the hydrogenophilic bacteria, which confirms the higher susceptibility of acetoclastic bacteria to oleic acid, relative to hydrogenophilic bacteria, as reported by Hanaki *et al.* (1981). These authors concluded that inhibition of acetogens and acetoclastic bacteria induced a prolonged lag phase in batch assays whereas inhibition of hydrogenophilic bacteria merely caused a decrease in the hydrogen conversion rate. In addition to these considerations, the choice of acetoclastic bacteria for toxicity studies was also justified by their important metabolic role (Gujer and Zehnder, 1983).

During Period I, four toxicity tests for the sludge of each digester were performed on days 90, 132, 162 and 212 (see Part I). An example (for sludges taken on the 162nd day) of the prolonged monitoring of methane production in batch assays, fed with constant acetate concentration and increasing oleate concentrations from 100 to 900 mg/l is represented in Fig. 1.

Typically, the sludge from RI showed a lag phase of variable length prior to methane production for concentrations higher than 300 mg/l of added oleate. After the lag-phase, acetate and oleate were virtually degraded at once. However, acetate and oleate degradation by sludge from RII followed a diauxic behavior and no lag phase was observed over the range of concentrations under study. The constant methane concentration obtained in the first stage could be correlated with the constant initial added acetate concentration. Furthermore, the second state methane production was proportional to the added oleate concentration. This behaviour was typical during Period I, but the differences between the two sludges became more pronounced towards the end of this operation period. Although no effect was evident in the specific acetoclastic activity which was enhanced for both sludges during this period (see Part I), it was clear that the pre-contact with lipids delayed the acetate degradation, when in the presence of sodium oleate.

Figure 2 represents the time course of the 50% inhibition concentration of sodium oleate towards acetoclastic bacteria (IC<sub>50</sub>) during the trial

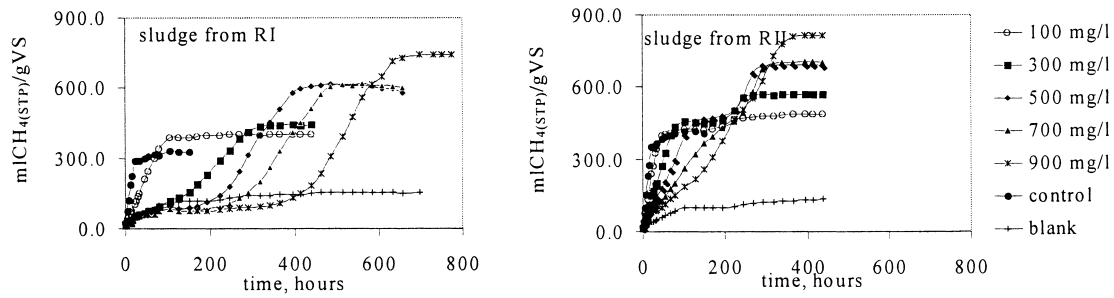


Fig. 1. Long-term course of methane production during the toxicity batch experiment (example for sludges taken on the 162nd day).

period. During Period I, the tolerance to oleate increased for both sludges, but particularly for sludge RII. However, when the feeding was changed to a blend of skim milk and sodium oleate (Period II) the sludge RII lost its resistance whereas sludge from RI achieved a maximum of tolerance to oleate during this period.

In other words, it seems that the pre-contact with lipids affected the type of tolerance developed by the sludge, the pre-exposition to lipids seeming to be beneficial to the development of resistance to oleic acid in the treatment of an effluent containing this LCFA. It is important to note that during Period II, oleate concentration in the feed was gradually increased and biomass characterization was made on day 315 when the feed consisted of 2.4 g oleate/l added to skim milk (total COD = 12 g/l). With such a high concentration of LCFA, a negative effect on acetoclastic bacteria was foreseeable. In fact the specific activity was strongly reduced during this period, but the increasing tolerance of the RI sludge may be considered as a kind of adaptation.

To estimate the threshold resistance of sludges RI

and RII, the feed to both digesters was then changed to oleate as a sole carbon source. The oleate concentration increased to 4.15 g/l (total COD = 12 g/l) and the co-substrate was eliminated. The potential specific acetoclastic activity followed the same decreasing trend observed during Period II (see Part I), and the resistance to sodium oleate decreased for both sludges. Therefore, the addition of oleate above a threshold concentration affected the acetoclastic activity as well as the tolerance of these bacteria to oleate.

Adaptation of methanogenic bacteria to LCFA is discussed in the literature, but most of the previous works report on results from batch assays (Hanaki *et al.*, 1981; Koster and Cramer, 1987; Angelidaki and Ahring, 1992) and the general conclusion is that no adaptation of methanogenic bacteria to LCFA occurs. Concepts of bactericidal effect and cytolysis are frequently associated with toxicity of LCFA (Rinzema *et al.*, 1994; Hwu and Lettinga, 1997). A time scale, compatible with the longer operating times of real digesters, should be used to assess the behaviour of anaerobic systems. In the present paper the concept of acclimatization is

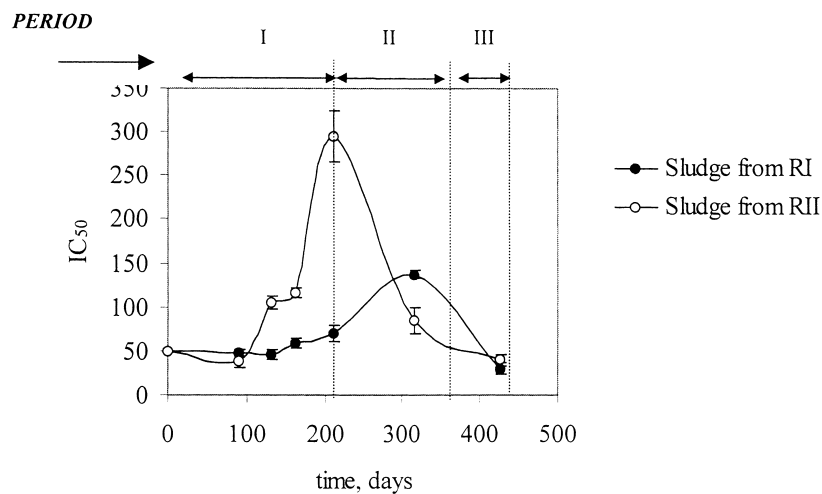


Fig. 2. Fifty percent inhibition concentration ( $IC_{50}$ ) of sodium oleate to acetoclastic bacteria. Bars represent 95% confidence intervals.

Table 1. Summary of reported works on oleic acid toxicity towards acetoclastic bacteria

Reference	Sludge origin	Type of sludge	Temperature (°C)	Calcium/magnesium	IC <sub>50</sub> (mg/l)
Koster and Cramer, 1987	UASB/potato processing	Granular	30	35 mg/l	1322
Hwu <i>et al.</i> , 1996	UASB/potato processing	Granular	40	2.7 mg/l	690
Hwu <i>et al.</i> , 1996	EGSB/milk fat	Suspended	40	2.7 mg/l	79
Hwu <i>et al.</i> , 1996	EGSB/milk fat	Granular	40	2.7 mg/l	1015
Hanaki <i>et al.</i> , 1983	Domestic sewage treatment plant	Suspended	37	no	10
This work	AF <sup>a</sup> /whole milk	Suspended	37	no	48–70
This work	AF/skim milk	Suspended	37	no	38–295
This work	AF/oleate + skim milk	Suspended	37	no	85–137
This work	AF/oleate	Suspended	37	no	30–40

<sup>a</sup>Anaerobic filter.

understood as an increasing resistance to oleic acid toxicity during a long term operation and it was proven that a gradual increase in tolerance of acetoclastic bacteria could be induced by the contact with lipids.

The obtained values for the toxicity limit (IC<sub>50</sub>) of sodium oleate towards acetoclastic bacteria agree well with those reported in the literature. Table 1 summarizes some works reporting on oleic acid toxicity towards acetoclastic bacteria. Several factors are referred to in the literature as affecting the toxicity of LCFA.

Koster and Cramer (1987) found a higher value for IC<sub>50</sub> than Hwu *et al.* (1996) for the same kind of sludge taken from the same digester, which was attributed to the higher level of calcium and to the lower temperature. Hwu *et al.* (1996) found a clear correlation between the IC<sub>50</sub> value and the specific surface area of the sludge, being the flocculent sludge much more susceptible to oleate toxicity than the granular sludge. A value of IC<sub>50</sub> 13 times higher for granular than for suspended sludge was obtained by these authors. The lower value of IC<sub>50</sub> presented in Table 1 refers to the work of Hanaki *et al.* (1983) with digested sludge from a domestic

sewage treatment plant without added calcium. When comparing values of IC<sub>50</sub>, all the parameters, temperature, the presence of calcium and magnesium cations and the sludge characteristics, must be taken into account. The obtained values for IC<sub>50</sub> in the present work ranged from 30 to 295 mg/l and were obtained for suspended sludge at 37°C without added calcium or magnesium.

#### Oleate biodegradability

Along the trial period the sludge from RI was characterized in terms of oleic acid biodegradation capacity. Table 2 summarizes the results obtained for the maximum methane production rate, the percentage of methanization and the lag phases that preceded the initial methane production.

In general, it was shown that there was an increase in the methane production rate and in the percentage of methanization for all concentrations of oleate until Period II, when oleate was fed with a co-substrate. This sludge exhibited the highest capacity of oleate biodegradation evidenced by the highest methane production rates, the highest percentages of methanization and the lowest lag phases. The maximum value of methane production

Table 2. Results from the biodegradability batch experiments<sup>a</sup>

Period	Time, days		Oleate concentration in the batch vial (mg/l)				
			100	300	500	700	900
I	90	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	3 ± 2	8 ± 2	3 ± 2	7 ± 2	13 ± 4
		PM (%)	77 ± 21	72 ± 6	58 ± 7	61 ± 7	61 ± 9
		Lag phases (hours)	0	265	240	273	343
I	132	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	0	0	0	18 ± 4	28 ± 5
		PM (%)	0	9 ± 15	22 ± 7	46 ± 5	36 ± 11
		Lag phases (hours)	0	255	260	273	294
I	162	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	3 ± 2	0	10 ± 2	33 ± 6	43 ± 2
		PM (%)	0	45 ± 8	66 ± 9	62 ± 3	54 ± 4
		Lag phases (hours)	0	274	200	279	323
I	212	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	9 ± 3	0	3 ± 3	18 ± 1	14 ± 1
		PM (%)	139 ± 52	6 ± 5	32 ± 12	77 ± 1	65 ± 5
		Lag phases (hours)	0	267	230	218	267
II	315	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	15 ± 6	12 ± 3	33 ± 1	43 ± 3	46 ± 1
		PM (%)	63 ± 18	87 ± 16	98 ± 6	86 ± 10	90 ± 2
		Lag phases (hours)	0	72	98	87	99
III	426	MMPR (mlCH <sub>4</sub> (STP)/gVS.day)	7 ± 1	11 ± 10	0	0	19 ± 13
		PM (%)	0	35 ± 13	57 ± 27	80 ± 11	91 ± 7
		Lag phases (hours)	0	90	130	145	251

<sup>a</sup>Maximum methane production rate (MMPR) ± 95% confidence interval, percentage of methanization (PM) ± 95% confidence interval and LAG phases.

Table 3. Maximum plateaux achieved and methane production rate in "blank-vials" batch experiments along the trial period

Period	Time, days	Maximum plateaux (mlCH <sub>4</sub> (STP)/gSV)		Methane production rate (mlCH <sub>4</sub> (STP)/gSV.day)	
		RI	RII	RI	RII
I	90	116 ± 7	171 ± 9	21 ± 2	32 ± 4
	132	154 ± 32	163 ± 17	22 ± 5	25 ± 3
	162	157 ± 4	141 ± 19	21 ± 2	20 ± 3
	212	133 ± 4	133 ± 7	17 ± 1	21 ± 8
II	315	59 ± 4	79 ± 5	18 ± 1	24 ± 2
III	426	736 ± 20	656 ± 18	99 ± 13	84 ± 8

rate obtained for this sludge, 46 mlCH<sub>4</sub>(STP)/gSV.day (= 131 mg COD-CH<sub>4</sub>/gVS.day) is similar to that obtained by Hwu *et al.* (1997), who found 129 mg COD-CH<sub>4</sub>/gVSS.day in batch assays with the washed out biomass from a EGSB reactor, but under thermophilic conditions. The sludge collected at the end of the experiment, when oleate was the sole carbon source fed to the reactor, exhibited percentages of methanization higher than 80% for the concentrations of 700 and 900 mg/l, but methane production rates decreased significantly and lag phases increased.

#### Methane production due to background substrate

The low methane production rates obtained in the biodegradability batch experiments for the sludges samples from RI at the end of the operation resulted, in part, from the extremely high residual methane production due to background substrate. In fact, when performing the blank vials where no substrate was added, an extremely high background methane production was detected, when compared with the corresponding production obtained with the other sludges. Table 3 represents the maximum plateaux in terms of methane production achieved for each characterized sludge, and the corresponding methane production rates.

The high values of background methane production obtained for both sludges at the end of the operation indicate that adsorbed organic mat-

ter, possibly oleate or an intermediate of its degradation is still available for degradation. During Period II, when oleate was fed with a co-substrate, no significant background methane production was detected, and was reduced by comparison with the values of Period I (Table 3). It was also observed that the highest background methane production rate was obtained for the highest concentration of background substrate. Furthermore, the obtained values are significantly higher (two-fold) than the maximum values obtained from the oleate biodegradability tests (Table 2). Figure 3 shows the cumulative methane production in the blank vials performed with the sludge RI and RII collected at the end of the experiment, in comparison with the sludge collected on day 315.

When performing the biodegradability tests with the sludge collected at the end of the operation, a lag phase was observed prior to the initial methane production, for concentrations higher than 300 mg/l (Table 2, last line). This indicates that the degradation of the adsorbed organic matter was delayed by the added oleic acid, suggesting that this LCFA exerted a toxic effect on the degradation of the adsorbed matter. A possible explanation is that the adsorbed matter was a less toxic intermediate of oleic acid degradation. According to the degradation pathways of oleic acid described by Weng and Jeris (1976), the first step in oleate degradation

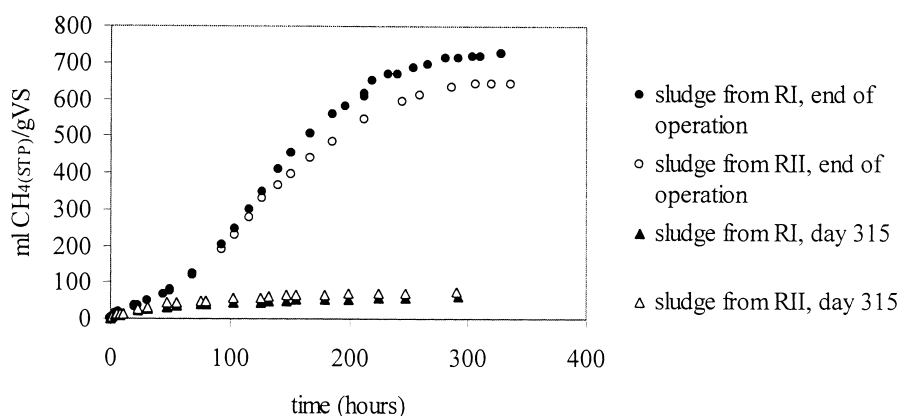


Fig. 3. Methane production in batch experiments without any added substrate for sludges RI and RII taken on day 315 and at the end of the operation.

is the reduction of oleate to stearate, which is a saturated LCFA, less toxic than oleic acid. If stearate was adsorbed, instead of oleate, the toxic effect of the added oleate would be better understood.

LCFA adsorption was referred to in several works (Hanaki *et al.*, 1981). Hwu *et al.* (1998) studied the biosorption of oleic acid in relation to the flotation in UASB reactors. Oleate adsorption was found to be highly concentration-dependent in batch experiments and induced the inhibition of methanogenic bacteria by increasing the lag-phases before initial methane production, whereas in UASB reactors sludge flotation was related to LCFA/sludge loading rates. This author also verified that, after the initial adsorption, a certain desorption occurred which was biologically mediated, probably induced by biogas production. Hanaki *et al.* (1981) concluded that oleate adsorbed to be biomass within 24 h. However, in none of these works was it proven that the adsorbed product was effectively oleic acid.

#### CONCLUSIONS

1. When treating an oleate based effluent, the sludge that had been pre-exposed to lipids showed higher tolerance to oleic acid toxicity than the sludge that had been fed with a non-fat substrate. The contact with lipids induced the development of resistance to oleate toxicity.
2. The sludge that had been acclimatized with lipids showed an increasing biodegradation capacity of oleic acid along the trial period. Maximum methane production rates between 33 and 46 mlCH<sub>4</sub>(STP)/gVS.day and maximum percentages of methanization between 85 and 98% were obtained for the range of concentrations between 500 and 900 mg/l. The long term contact with lipids, or oleate below a threshold concentration, induced adaptation to biodegradation of oleic acid.
3. When oleate was the sole carbon source fed to both digesters, an exceptionally high and fast background methane production was obtained from the "blank-vials" batch experiments where no substrate was added. The methane production rate from this adsorbed matter (99 and 84 mlCH<sub>4</sub>(STP)/gVS.day, for RI and RII, respectively) was two-fold higher than the maximum oleate degradation rate measured in the biodegradability tests.
4. When oleate was added to the encapsulated sludge, a lag phase of variable duration (depending on the added oleate concentration) was observed, suggesting that the added oleate delayed the degradation of the adsorbed matter.
5. The hypothesis that oleate was reduced to stearate prior to adsorption was considered, but needs experimental validation.
6. The delay imposed in the methane production, by the addition of oleic acid to the encapsulated sludge, limits the application of continuous high rate anaerobic digesters to LCFA based effluents. However, the high degradation rate of the adsorbed organic matter, suggests the possibility of using adsorption-degradation cycles for the treatment of effluents with high lipid content.

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#### REFERENCES

- Alves M. M., Pereira M. A., Bellouti M., Álvares Pereira M. R., Mota Vieira J. A., Novais J. M. and Mota M. (1998) A new method to study interactions between biomass and packing material in anaerobic filters. *Biotechnology Techniques* **12**(4), 277–283.
- Angelidaki I. and Ahring B. K. (1992) Effects of free long-chain fatty acids on thermophilic-anaerobic digestion. *Applied Microbiology and Biotechnology* **37**, 808–812.
- Colleran E. and Pistilli A. (1994) Activity test system for determining the toxicity of xenobiotic chemicals to the methanogenic process. *Annals in Microbiology and Enzymology* **44**, 1–20.
- Colleran E., Concannon F., Goldem T., Geoghegan F., Crumlish B., Killilea E., Henry M. and Coates J. (1992) Use of methanogenic activity tests to characterize anaerobic sludges, screen for anaerobic biodegradability and determine toxicity thresholds against individual anaerobic trophic groups and species. *Water Science and Technology* **25**(7), 31–40.
- Demeyer D. I. and Henderickx H. K. (1967) The effects of C18 unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochemistry and Biophysics Acta* **137**, 484–497.
- Galbraith H. and Miller T. B. (1973) Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. *Journal of Applied Bacteriology* **36**, 647–658.
- Galbraith H., Miller T. B., Paton A. M. and Thomson J. K. (1971) Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *Journal of Applied Bacteriology* **34**, 803–813.
- Gujer W. and Zehnder A. J. B. (1983) Conversion processes in anaerobic digestion. *Water Science and Technology* **15**(8/9), 127–167.
- Hanaki K., Ishikawa T. and Matsuo T. (1983) Inhibitory and stimulative effects of oleate on methanogenesis from acetate in anaerobic digestion. *Technological Report Tohoku University* **48**, 123–135.
- Hanaki K., Matsuo T. and Nagase M. (1981) Mechanisms of inhibition caused by long chain fatty acids in anaerobic digestion process. *Biotechnology and Bioengineering* **23**, 1591–1610.
- Hwu C.-S. and Lettinga G. (1997) Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme and Microbial Technology* **21**, 297–301.
- Hwu C.-S., Donlon B. and Lettinga G. (1996) Comparative toxicity of long-chain fatty acid to anaerobic

- sludges from various origins. *Water Science and Technology* **34**(5/6), 351–358.
- Hwu C.-S., Molenaar G., Garthoff J., van Lier J. B. and Lettinga G. (1997) Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics. *Biotechnology Letters* **19**(5), 447–451.
- Hwu C.-S., Tseng S.-K., Yuan C.-Y., Kulik Z. and Lettinga G. (1998) Biosorption of long-chain fatty acids in UASB treatment process. *Water Research* **32**(5), 1571–1579.
- Komatsu T., Hanaki K. and Matsuo T. (1991) Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Water Science and Technology* **23**(7/9), 1189–1200.
- Koster I. W. and Cramer A. (1987) Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Applied and Environmental Microbiology* **53**, 403–409.
- Rinzema A., Boone M., van Knippenberg K. and Lettinga G. (1994) Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research* **66**(1), 40–49.
- Weng C.-N. and Jeris J. S. (1976) Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Research* **10**, 9–18.