PERGAMON
www.elsevier.com/locate/watres

PII: S0043-1354(00)00241-4

Wat. Res. Vol. 35, No. 1, pp. 255–263, 2001

Printed in Great Britain
0043-1354/00/$ - see front matter

EFFECT OF LIPIDS AND OLEIC ACID ON BIOMASS DEVELOPMENT IN ANAEROBIC FIXED-BED REACTORS. PART I: BIOFILM GROWTH AND ACTIVITY

M. M. ALVES, J. A. MOTA VIEIRA, R. M. ÁLVARES PEREIRA, M. A. PEREIRA[M] and M. MOTA*[M]

Centro de Engenharia Biológica—IBQF, Universidade do Minho, 4710-057, Braga, Portugal

(First received 31 March 1999; accepted in revised form 20 April 2000)

Abstract—Two similar anaerobic fixed-bed bioreactors which allowed the biomass to be periodically withdrawn were run in parallel. After feeding each digester with synthetic dairy wastes of different lipid content (Period I), both digesters were fed with increasing sodium oleate concentrations with skim milk as co-substrate (Period II) and oleate as the sole carbon source (Period III). In Period I, the digester fed with lipids was more efficient and exhibited lower levels of volatile fatty acids than the digester fed without lipids. The biofilm built up in the presence of lipids was thinner, but more resistant to the presence of oleate than the biofilm formed in the absence of lipids, which lost 53% of its solids after contacting with oleic acid. The specific methanogenic activity with butyrate as substrate was enhanced in the presence of lipids, but no significant effect was detected on the acetoclastic and hydrogenophilic activities, which remained similar for both digesters along the trial period. © 2000 Elsevier Science Ltd. All rights reserved

Key words—anaerobic filter, oleic acid, methanogenic activity, biofilm stability

INTRODUCTION

Lipids are one of the major components of organic matter in wastewaters and, although domestic sewage contains about 40–100 mg/l of lipids, industrial wastewaters are of greater concern when considering the potential toxicity of this type of effluents (Forster, 1992). Along with slaughterhouses and edible oil and fat refineries, dairy industries are important contributors to the total lipid emission.

In the last few years the anaerobic wastewater treatment technology was markedly improved due to the development of the upflow anaerobic sludge blanket (UASB) concept and its world-wide application—more than 900 full scale UASB reactors are currently under operation (Hulshof Pol *et al.*, 1997)—along with the more recent designs of the expanded granular sludge bed (EGSB) and the internal circulation (IC) reactors (Habets *et al.*, 1997). In such systems, biomass immobilization is achieved by self granulation, a crucial requirement that, when unsuccessful, affects the overall performance, most times irreversibly. Although there is evidence that granular sludge is more resistant to long chain fatty acid (LCFA) toxicity than suspended or flocculent sludge (Hwu *et al.*, 1996), it is also evident that granulation and/or granule stability is problematic for lipid containing wastewaters (Hawkes *et al.*, 1995; Sam-Soon *et al.*, 1991).

Problems with anaerobic treatment of lipid containing wastewater result from two phenomena: (1) adsorption of a light lipid layer around biomass particles causing biomass flotation and washout and (2) acute toxicity of LCFA against both, methanogens and acetogens, the two main trophic groups involved in LCFA degradation (Roy *et al.*, 1986; Rinzema *et al.*, 1994).

More than inhibition, flotation of granular biomass conducting to washout is the most important operational problem of the Upflow Anaerobic Sludge Blanket (UASB) Reactors. Hwu *et al.* (1998) studied the adsorption of oleic acid in relation to granular sludge flotation in a UASB reactor and concluded that granular sludge flotation occurred at concentrations far below the toxicity limit. This might suggest that complete washout of granular sludge would occur prior to inhibition. Furthermore, it is known that addition of calcium salts prevents, to some extent, inhibition problems, but has no effect upon flotation problems (Hanaki *et al.*, 1981).

In an anaerobic fixed-bed reactor, the support medium acts as a physical protective factor against

*Author to whom all correspondence should be addressed. Tel.: +351-253-604405; e-mail: mmota@deb.uminho.pt

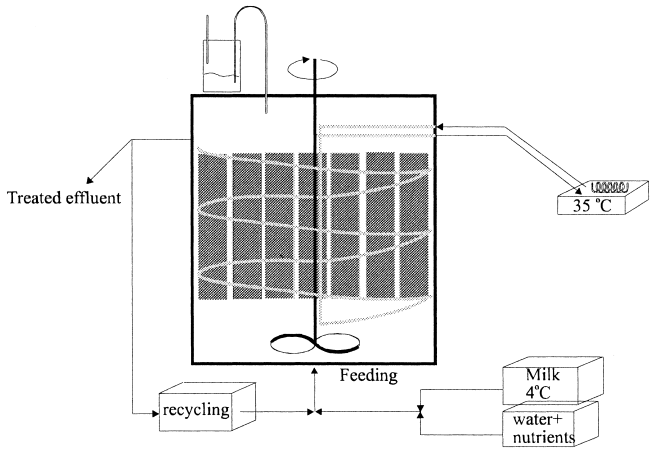

Fig. 1. Schematic representation of the fixed-bed reactor.

washout, thus being potentially attractive for biomass retention in this particular kind of wastewater. Biomass immobilization is achieved by retention in the void space of a matrix and by adhesion to its surface. Good stability and robustness can be pointed out as the main advantage of this system while channeling and clogging problems represent a major drawback. One of the most serious problems associated with the study of anaerobic filters is the difficulty of determining biomass quantity and quality as well as their evolution with time and operating conditions.

In the present work the effect of feeding increasing concentrations of lipids on the characteristics of the sludge developed in an anaerobic fixed-bed digester was investigated by comparison with a control digester fed with a non-fat substrate. The effect of gradually shifting the substrate in both digesters from the original composition to oleate as the sole carbon source, allowed evaluation of the effect of the pre-contact with lipids on the biomass developed in the presence of this LCFA.

Sodium oleate was used as a model for long chain fatty acid (LCFA) because it is, in general, the most abundant of all LCFA present in wastewater (Komatsu *et al.*, 1991), has a good solubility and it is the most important LCFA produced by whole milk degradation (Hanaki *et al.*, 1981).

MATERIALS AND METHODS

Experimental set-up

The bioreactor, designed to allow the periodical withdrawal of biomass, was described in detail elsewhere (Alves *et al.*, 1988) and is schematically presented in Fig. 1. Two identical units were run in parallel. Each reactor was constructed in PVC with a total volume of 86.8 l and a diameter of 48 cm. The support medium was equally divided among 27 parallel mini-bioreactors arranged in the central section, which aimed to simulate the behaviour of the support matrix in an anaerobic filter (Fig. 2). A perforated plate sustained the mini-bioreactors and a stirrer was used to homogenize the feed inlet.

Each mini-bioreactor had 7.1 cm internal diameter, a total volume of 989 cm^3 and accommodated 89 pieces of support material. The support medium consisted of PVC Raschig rings of 21 mm in size, with a specific surface area of 230 m^2/m^3 and a porosity of 92.5%. A constant recycle of 47 l/day was applied to increase the mixing and the superficial upflow velocity, which was set at 0.43 m/day.

The two bioreactors were opened at six different operation times and three of the 27 mini-bioreactors were randomly selected and replaced by new similar mini-bioreactors, which were not accounted for in the next selection.

Operating temperature was kept constant at $35 \pm 1°C$. The seed sludge was obtained from a municipal sludge digester and both digesters were inoculated with equal amounts of seed sludge (11 l with 25.7 g of volatile solids (VS) per litre). Routine reactor performance was monitored by determining influent and effluent total and soluble chemical oxygen demand (COD), influent flow rate and effluent volatile fatty acids (VFA).

Substrate

Initially the substrate consisted of whole milk (reactor RI) and skim milk (reactor RII) diluted with tap water, and supplemented with macro and micronutrients which had the following composition: *Macronutrients*—$MgSO_4.7H_2O$: 30.2 g/l; KH_2PO_4: 28.3 g/l; KCl: 45 g/l. 0.6 ml of this solution was added per gram of COD fed. *Micronutrients*—$FeCl_2.6H_2O$: 2 g/l; H_3BO_3: 0.05 g/l; $ZnCl_2$: 0.05 g/l; $CuCl_2.2H_2O$: 0.038 g/l; $MnCl_2.4H_2O$: 0.5 g/l; $(NH_4)_6Mo_7O_{24}.4H_2O$: 0.05 g/l; $AlCl_3.6H_2O$: 0.09 g/l; $CoCl_2.6H_2O$: 2 g/l; $NiCl_2.6H_2O$: 0.092 g/l; $Na_2SeO_3.5H_2O$: 0.164 g/l; EDTA: 1 g/l; Resazurin: 0.2 g/l; HCl 37%: 1 ml/l. The composition of this solution was based on the work of Zehnder *et al.* (1980). Micronutrients were supplemented to the influent feed by addition of 1 ml per litre. To give suitable alkalinity 5 g $NaHCO_3$/l

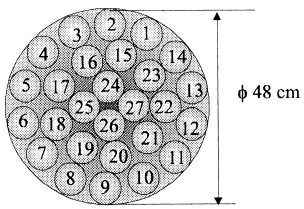

Fig. 2. Layout of the support section.

were added. In the two last operation periods both reactors were fed, initially with a mixture of skim milk and sodium oleate followed by feeding oleate as the sole carbon source. Micro and macronutrients were always added.

Analytical methods

Routine analysis. COD, volatile and total solids (VS and TS) were determined by standard methods (APHA, AWWA, WPCF, 1989). VFA were determined by HPLC (Jasco, Japan) using a Chrompack column (6.5 × 30 mm); the mobile phase was sulphuric acid (0.01 N) at a flow rate of 0.7 ml/min. The column temperature was set at 40°C and the detection was made spectrophotometrically at a wavelength of 210 nm. Methane content of biogas produced in batch experiments was measured by a Pye Unicam GCD gas chromatograph (Cambridge, England), using a Chrompack column Haysep Q (80–100 mesh). N_2 was used as carrier gas (30 ml/min) and the temperatures of injection port, column and flame ionisation detector were 120, 40 and 130°C, respectively.

Biomass characterization. Biomass was characterized in terms of: distribution between adhered and entrapped fractions, and specific methanogenic activity with acetate, H_2/CO_2, propionate and butyrate as individual substrates

Biomass Separation And Quantification

In both experiments, the entrapped biomass was considered to be the fraction which was unattached to the support after being freely dispersed in a distilled water bath under circular movements with alternate sense for 1 min. N_2/CO_2 (80:20), was continuously flushed to keep the anaerobic environment. After successive cycles of washing with anaerobic buffer and centrifugation at 6000 rpm during 10 min, this sludge was re-suspended in an anaerobic buffer and the total volume and volatile solids (VS) content were determined. Activity tests were performed with this fraction of biomass. Adhered biomass was removed from the support by using a 0.1 N NaOH solution continuously stirred at 100 rpm during 12 h at 37°C followed by sonication, according to the procedure described by Donlon (1992). Total volume and its volatile solids content were measured.

Activity Measurements

Methanogenic activity tests were performed using the pressure transducer technique (Colleran *et al.*, 1992; Coates *et al.*, 1996). 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_2/CO_2). The non gaseous substrates were acetate, propionate and butyrate. Strict anaerobic conditions were maintained. The hand-held pressure transducer was capable of measuring a pressure increase or decrease of two bar (0– ± 202.6 kPa) over a range of −200– + 200 mV, with a minimum detectable variation of 0.005 bar. A sensing element consisting of a 2.5 mm square silicon chip with integral sensing diaphragm is connected to a digital panel meter module and the device is powered by a 7.5 V DC transformer. The basal medium used in the batch experiments, made up with distilled water, was composed of cysteine-HCl (0.5 g/l) and sodium bicarbonate (3 g/l), the pH was adjusted to 7.0–7.2 with few drops of NaOH 8 N and was prepared under strict anaerobic conditions. No calcium or trace-nutrients were added. The same technique was used to characterize the methanogenic activity of the inoculum. All methanogenic activity tests were performed in triplicate assays. The methanogenic activity values were corrected to the standard temperature and pressure conditions (STP), being expressed as $mlCH_{4(STP)}/gSV.day$.

Operation mode

During the start-up both reactors 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 was fed during 246 days. After this Period (I), both reactors were fed with the same substrate. Initially, a mixture of skim milk with sodium oleate was used, with increasing oleate concentrations and constant loading rate (Period II) and in Period III, oleate was the sole carbon source fed to both digesters. Table 1 represents the type of substrate and the total and oleate influent COD applied in RI and RII.

During Period I, the influent COD was increased from 3000 to 12,000 mg/l using whole milk and skim milk as substrate in RI and RII, respectively. The equivalent oleate COD fed to reactor RI until the 212th day, was calculated on the basis of 44% (%COD) of lipids in the whole milk producing 39% of oleic acid (Hanaki *et al.*, 1981). After the 212th and 246th days for reactors RI and RII respectively, the feeding to both reactors was similar, the total COD was kept constant at 12,000 mg/l, but the sodium oleate content was gradually increased from 1734 to 8670 mg COD/l. In the last operation period, oleate at a concentration of 4150 mg/l (12,000 mg COD/l) was the sole carbon source fed to both reactors.

RESULTS AND DISCUSSION

Performance

Figure 3 represents the applied organic loading rate, the removal efficiency (soluble COD) and the effluent VFA in RI and RII.

The applied organic loading rate was gradually increased to 8.6 Kg $COD/m^3.day$, by increasing the total COD fed to each reactor [Fig. 3(a) and Table 2]. From the 162nd day on, the OLR was kept constant (influent COD = 12,000 mg/l; HRT = 1.4 days), but the substrate composition was changed, according to Table 1. For reactor RI, the removal efficiency was always higher than 90%, but, for reactor RII, it achieved a minimum value as low as 70 % on day 114, after which the organic loading rate was reduced by increasing the Hydraulic Retention Time (HRT) from 0.92 to 1.4 days [Fig. 3(a)]. The effluent volatile fatty acids suffered a sudden increase during the same period, but returned to lower levels after the increase in HRT.

It was observed that, on average, the VFA levels of RII (without lipids) were far above (2–3 times) those of RI (with lipids). This fact is evidenced in Fig. 4 where the average pseudo steady-state effluent VFA concentrations are presented for RI and RII during Period I.

During Period II the effluent VFA levels decreased continuously with the increase in oleic acid concentration and remained stable during Period III (Fig. 5). The similarity between the pseudo-steady state average effluent VFA concentrations for RI and RII during these periods, agrees with the fact that both reactors were running under equal operating conditions.

From the above results, it is understood that the presence of lipids and oleic acids in the feed

Table 1. Type of substrate, total and oleate influent COD applied in RI and RII

Period	Time (days)	RI			RII		
		Type of substrate	Total COD (mg/l)	Oleate COD (mg/l)	Type of substrate	Total COD (mg/l)	Oleate COD (mg/l)
I	0–71	Skim milk	3000	0	Skim milk	3000	0
	71–76	blend	3000	491		3000	0
	76–90	Whole milk	3000	534		3000	0
	90–132		6000	962		6000	0
	132–162		9000	1562		9000	0
	162–212		12,000	1968		12,000	0
II	212–246	Skim milk + oleic acid	12,000	1734	Skim milk	12,000	0
	246–272		12,000	1734	Skim milk + oleic acid	12,000	1734
	272–294		12,000	3468		12,000	3468
	294–333		12,000	6936		12,000	6936
	333–363		12,000	8670		12,000	8670
III	363–426	Oleic acid	12,000	12,000	Oleic acid	12,000	12,000

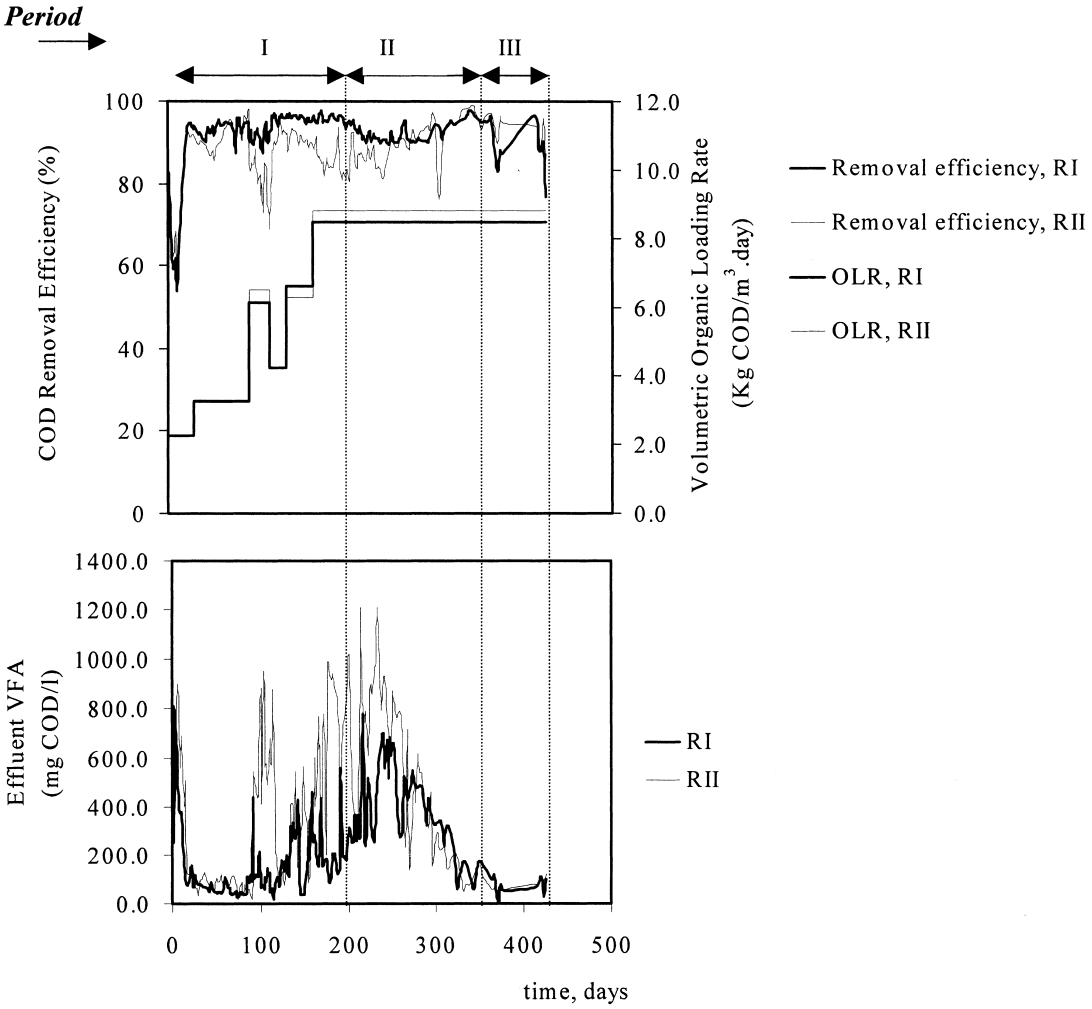

Fig. 3. Operating conditions and performance of RI and RII. Soluble COD removal efficiency (a) and effluent volatile fatty acids (b).

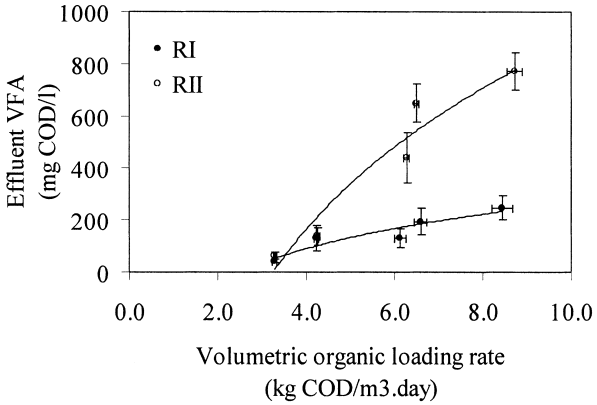

Fig. 4. Effluent average pseudo steady-state VFA concentrations vs applied volumetric organic loading rate during Period I. Bars represent 95% confidence intervals.

decreased the effluent VFA levels. The possibility of an inhibition of acetogenic bacteria involved in β-oxidation by lipids, would imply an accumulation of non-degraded substrate affecting the overall performance of RI, which was not observed [Fig. 3(a)]. In fact from that figure, during Period I, RI, which was fed with lipids was even more efficient than RII (fed without lipids).

The results confirmed that lipids and sodium oleate were retained in the reactor and effectively removed from the substrate. As the methane content of the biogas was not measured, it was not definitely proven that biodegradation occurred. Sayed *et al.* (1987) found a discrepancy between methane production and COD removal efficiency, which was attributed to adsorption of LCFA onto the biomass. Rinzema (1988) verified the accumulation of LCFA around the biomass, affecting the transport of substrates and products. This fact was also reported by Hanaki *et al.* (1981) who showed that LCFA adsorbed to the biomass in 24 h.

On the other hand, it is known that the presence of calcium reduces the inhibitory effect of LCFA, by lowering their soluble (available) concentration through the production of a calcium–LCFA precipitate. The "free" oleate concentration depends on oleate and calcium concentration in the feed and on the solubility product of the calcium oleate salt (Roy *et al.*, 1985). The effect of magnesium salts should be similar. Rinzema (1988) observed the accumulation of a LCFA salt precipitate in a UASB. By adding Ca^{2+} at stoichiometric concentration to a digester fed with lauric acid, this author found that inhibition started at 1500 mg lauric acid/l instead of 100 mg lauric acid/l without calcium. Hanaki *et al.* (1981) found also that the addition of $CaCl_2$ reduced the lag-phase observed before methane production in batch assays.

In the present work, if the lipids had been precipitated with the Ca^{2+} and Mg^{2+} cations, the effective applied organic loading rate in RI (with lipids) would be lower than that applied in RII (without lipids), justifying the lower VFA levels and higher removal efficiency. Considering the contribution of

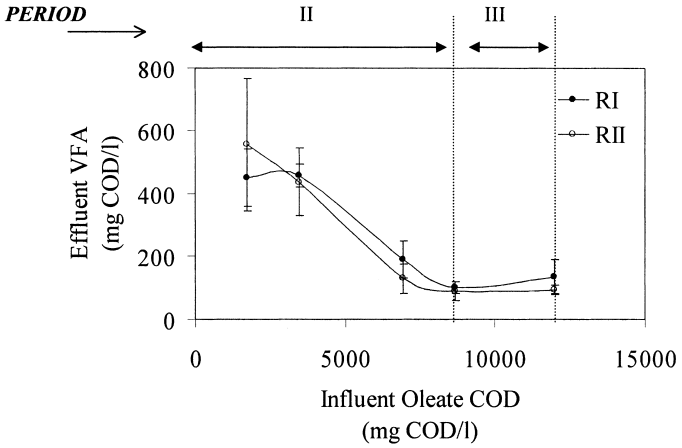

Fig. 5. Effluent average pseudo steady-state VFA concentrations vs influent oleate COD during Periods II and III. Bars represent 95% confidence intervals.

Table 2. Molar ratio oleate/($Ca^{2+} + Mg^{2+}$) fed along the trial period ± 95% confidence intervals

		Molar ratio oleate/($Ca^{2+} + Mg^{2+}$)	
Period	Time, days	RI	RII
I	0–29	–	–
	29–71	–	–
	71–76	0.50 ± 0.01	–
	76–90	0.54 ± 0.01	–
	90–114	0.82 ± 0.01	–
	114–132	0.71 ± 0.05	–
	132–162	1.10 ± 0.01	–
	162–212	1.25 ± 0.01	–
II	212–272	1.04 ± 0.01	1.04 ± 0.01
	272–294	2.10 ± 0.01	2.09 ± 0.01
	294–333	4.27 ± 0.04	4.26 ± 0.03
	333–363	5.46 ± 0.02	5.49 ± 0.02
III	363–426	7.79 ± 0.08	7.73 ± 0.04

tap water, milk and macronutrients to the calcium and magnesium content, the molar ratio oleate/($Ca^{2+} + Mg^{2+}$) was determined along all the trial period (Table 2). Values exceeding the stoichiometric value 2 were observed during Period II and a maximum of 7.7 mole of oleate/mole ($Ca^{2+} + Mg^{2+}$) was achieved in Period III. The exceeding oleate concentration that was not precipitated with calcium and magnesium salts could be adsorbed onto the biomass or biodegraded, but this experiment was not conclusive about what mechanism was responsible for the oleate elimination.

Biomass characterisation

At six different times, summarized in Table 3, the biomass was characterized in terms of: (1) distribution between adhered and entrapped fractions; (2) methanogenic activity of different trophic groups.

Biomass distribution. The characterized biomass represents only the biomass accumulated in the support matrix and is always referred as "supported biomass" (Table 4). Accumulation of biomass in the bottom section of the bioreactor, below the perforated plate (Fig. 1), was not taken into account.

The presence of lipids (Period I) reduced the concentration of adhered biomass. Consequently RII evidenced a biofilm thicker than the one found for RI. Moreover, the difference of adhered biomass in RII (without lipids) in comparison with RI (with lipids) increased with the applied organic and lipid loading rate. For the highest lipid concentration of Period I, RII showed an adhered biomass 3-fold higher than RI's.

Although no value of adhered biomass was obtained for RI during Period II, this difference was clearly smaller in Period III, where only 23% more adhered biomass was observed in RII than in RI.

It was also observed that in RI the biofilm (expressed in gVS/m^2) was slightly growing through all the trial period. The effect of changing the feed from whole milk to oleate in this reactor induced the formation of an even thicker biofilm. However, in RII, the introduction of oleate in the substrate altered markedly the previously formed biofilm with more than 50% of the adhered biomass being effectively removed from the support. It may be concluded that the biofilm formed in contact with lipids was more resistant to the presence of oleate than the biofilm formed in the absence of lipids.

The behaviour of the entrapped biomass was inverse. During Period I, RI exhibited concentrations of volatile solids in the void space between 75 and 196% more than RII. These differences were reduced, as expected, during Periods II and III. Except for Period II, total supported biomass was significantly higher in RI than in RII. The distribution of biomass in both digesters is evidenced in Fig. 6, which represents the ratio adhered/total supported biomass against the operation time.

In RII it was observed that the adhered fraction of biomass increased up to 40% during Period I. In RI, which received equivalent loads of whole milk, the adhered fraction of biomass was very low, decreasing from 11.8% to 6.6% during the same period. After the introduction of oleate in the feed, a sharp decrease from 40 to 14% in the adhered fraction of biomass was observed in RII, but not in RI, where it remained nearly constant (Fig. 6).

Values of volatile solids (VS), which account for all the organic matter, should be carefully analyzed, because the increase in the volatile solids after

Table 3. Operating conditions prevailing at the moment of biomass characterization

				Type of substrate	
Period	Time (days)	Influent COD (mg/l)	HRT (days)	RI	RII
I	90	3000	0.9	Whole milk	Skim milk
	132	6000	1.4	Whole milk	Skim milk
	162	9000	1.4	Whole milk	Skim milk
	212	12,000	1.4	Whole milk	Skim milk
II	315	12,000	1.4	Skim milk + 2400 mg/l sodium oleate	Skim milk + 2400 mg/l sodium oleate
III	426	12,000	1.4	Sodium oleate (4150 mg/l)	Sodium oleate (4150 mg/l)

Table 4. Distribution between adhered and entrapped biomass ±95% confidence intervals

Period	Time (days)	Adhered biomass (gVS/m^2)		Entrapped biomass (gVS/l void)		Total supported biomass (gVS/l reactor)	
		RI	RII	RI	RII	RI	RII
I	90	0.9 ± 0.1	0.5 ± 0.1	1.7 ± 0.1	0.6 ± 0.1	1.2 ± 0.1	0.4 ± 0.1
	132	1.0 ± 0.2	1.4 ± 0.2	2.6 ± 0.1	1.0 ± 0.1	1.6 ± 0.1	0.8 ± 0.1
	162	1.1 ± 0.1	2.8 ± 0.3	3. 9 ± 0.1	2.2 ± 0.1	2.6 ± 0.1	1.8 ± 0.1
	212	1.4 ± 0.1	5.5 ± 0.3	4.8 ± 0.2	2.1 ± 0.1	3.2 ± 0.1	2.2 ± 0.1
II	315	–[a]	14.0 ± 1.6	7.8 ± 0.1	4.7 ± 0.1	4.9 ± 0.1[b]	5.1 ± 0.3
III	426	5.4 ± 0.1	6.6 ± 0.2	15.4 ± 0.7	10.3 ± 0.4	10.3 ± 0.4	7.4 ± 0.3

[a]Not determined.
[b]Only entrapped biomass.

Period I, may represent biomass and adsorbed organic matter.

Methanogenic activity. The specific methanogenic activities with acetate and H_2/CO_2 and with two indirect methanogenic substrates (propionate and butyrate) were determined for the entrapped biomass taken at the previously defined times (Table 3, Fig. 7). On day 90, only acetoclastic and hydrogenophilic activities were measured. Due to practical limitations, the acetoclastic activity was not determined on the 132nd day and in the last operation period the acetoclastic was the unique activity measured.

The acetoclastic activity was similar for the sludge of both reactors through all the trial period. Moreover an enrichment of this activity was observed during Period I being this fact particularly relevant for the sludge exposed to increasing lipid concentrations (Fig. 7). However, when the feeding was changed to a blend of skim milk and oleate (Period II) and subsequently to oleate only (Period III), the acetoclastic activity showed a clear decreasing trend for both digesters which were, at that time, under identical operating conditions.

It was then concluded that the contact with lipids did not affect the acetoclastic activity, which was even enhanced, but the contact with oleate arose a reduction of this activity. This can be related with the suggestion of Angelidaki and Ahring (1992) 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.

The above-mentioned reasons are pointed out to explain the effluent VFA levels observed, and can explain the behaviour of acetoclastic bacteria during the trial period. The influent molar ratio oleate/$(Ca^{2+} + Mg^{2+})$ achieved the stoichiometric value of 2 only in Period II, and increased until 7.7 in Period III (Table 2). This could indicate that during Period I all the lipids were precipitated as calcium and magnesium salts and thus their effect on consortium behaviour was, *a priori*, eliminated. The effect of LCFA on the activity can be considered as a result of a combination of two independent phenomena: the inhibitory effect and the adsorption on the cell wall. Both act synergistically decreasing

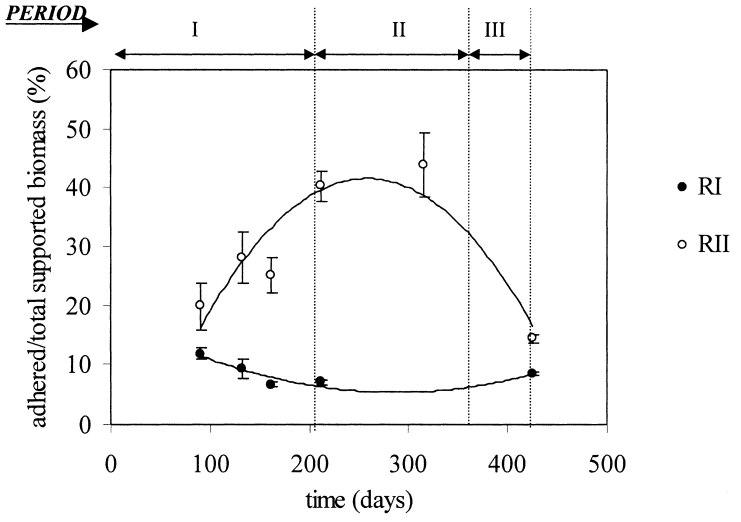

Fig. 6. Distribution of supported biomass. Bars represent 95% confidence intervals.

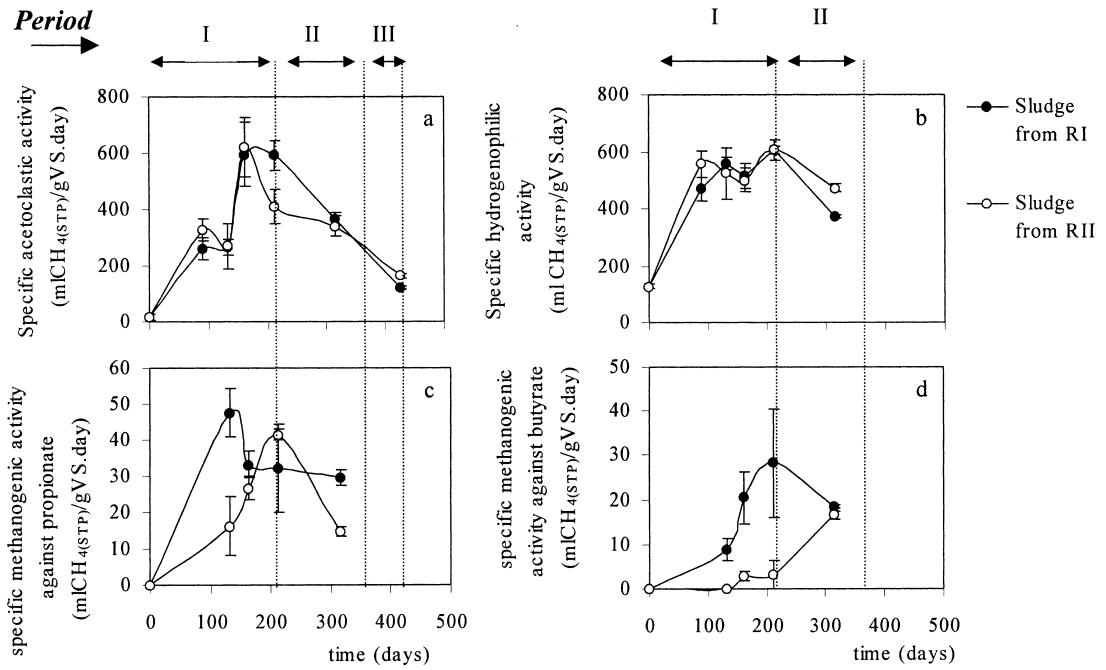

Fig. 7. Specific methanogenic activity with acetate (a), H_2/CO_2 (b), propionate (c) and butyrate (d) as substrate. Bars represent 95% confidence intervals.

the measured specific activity. The hydrogenophilic activity, which measures the activity of a specially important group that acts syntrophically with hydrogen producing acetogenic bacteria such as LCFA-degraders, was also very close between sludge from RI and RII over all trial periods. As observed for the acetoclastic bacteria, a decrease in hydrogenophilic activity was observed in Period II, but for Period III no values of this activity were determined.

Concerning the methanogenic activity against propionate and butyrate, it should be said that, since these substrates are indirect methanogenic substrates, a valid measurement of the maximum specific methanogenic activity against these acids can only be obtained when the acetoclastic and hydrogenophilic activities are not rate-limiting (Dolfing and Bloemen, 1985). In the present work this condition prevailed for all samples. A clear enhancement of methanogenic activity against butyrate was observed during Period I for the reactor RI (Fig. 7). After the introduction of sodium oleate in the feed of RII (Period II) butyrate activity was significantly increased and achieved a value close to the one measured for the biomass taken from RI. This result agrees with the suggestion by Rinzema *et al.* (1994) that a low LCFA or lipid concentration in the feed promotes the growth of acetogenic bacteria involved in β-oxidation.

Methanogenic activity against propionate in biomass taken from RII showed a maximum at the end of Period I, but comparison with biomass from RI was difficult because no particular trend was observed for this sludge, probably because propionate is only a minor intermediate of oleate degradation (Weng and Jeris, 1976).

CONCLUSIONS

1. More than 90% of COD was removed from a synthetic effluent containing 12 g COD/l of sodium oleate as a sole carbon source, even with a molar ratio oleate/$(Ca^{2+} + Mg^{2+})$ of 7.7.
2. The exposition to lipids affected the biomass distribution between the support surface and the void space of the matrix.
3. The biofilm formed in the reactor exposed to lipids (RI) was thin, but it increased in thickness when oleate was introduced in the feeding, as the sole carbon source.
4. The biofilm formed in the absence of lipids (reactor RII), although thicker than the one from RI, was reduced by more than 50% of its solids content, when oleate became the sole carbon source in the feed.
5. The butyrate activity was enhanced by the presence of lipids, but no effect was observed for the acetoclastic and the hydrogenophilic activities, which remained similar for both digesters along the trial period.

Acknowledgements—The authors gratefully acknowledge

Magda Lacerda, Nuno Cruz, Olga Torres and Luisa Pinto e Silva for their valuable participation in the laboratory work.

REFERENCES

Alves M. M., Pereira M. A., Bellouti M., Álvares Pereira M. R., Mota Vieira J. A., Novais J. M. and Mota M. (1988) 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.

APHA, AWWA and WPCF (1989) *Standard Methods for the Examination of Water and Wastewater*, 17th ed. APHA, Washington DC.

Coates J. D., Coughlan M. F. and Colleran E. (1996) Simple method for the measurement of the hydrogenotrophic methanogenic activity of anaerobic sludges. *Journal of Microbiological Methods* **26**, 237–246.

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.

Dolfing J. and Bloemen W. G. B. M. (1985) Activity measurements as a tool to characterize the microbial composition of methanogenic environments. *Journal of Microbiological Methods* **4**, 1–12.

Donlon B. (1992) Acetogenesis, bacterial adhesion and lignocellulose conversion. PhD thesis, University College, Galway, Ireland.

Forster C. F. (1992) Oils, fats and greases in wastewater treatment. *Journal of Chemical Technology and Biotechnology* **55**, 402–404.

Habets L. H. A., Engelaar A. J. H. H. and Groeneveld N. (1997) Anaerobic treatment of inuline effluent in an internal circulation reactor. *Water Science and Technology* **35**(10), 189–197.

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**, 1591–1660.

Hawkes F. R., Donnely T. and Anderson G. K. (1995) Comparative performance of anaerobic digesters operating on ice-cream wastewater. *Water Research* **29**(2), 525–533.

Hulshof Pol L., Euler H., Eitner A. and Grohganz D. (1997) GTZ sectoral project "Promotion of Anaerobic Technology for the Treatment of Municipal and Industrial Sewage and Waste". In *Proc. 8th International Conference on Anaerobic Digestion, Sendai, Japan, Vol. 2*, pp. 285–292.

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., 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.

Rinzema A. (1988) Anaerobic treatment of wastewater with high concentration of lipids or sulfate. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

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.

Roy F., Albagnac G. and Samain E. (1985) Influence of calcium addition on growth of highly purified sintrophic cultures degrading long chain fatty acids. *Applied and Environmental Microbiology* **49**, 702–705.

Roy F., Samain E., Dubourguier H. C. and Albagnac G. (1986) *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Archives of Microbiology* **145**, 142–147.

Sam-soon P., Loewenthal R. E., Wentzel M. C. and Marais GvR (1991) A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems. *Water SA* **17**(1), 31–36.

Sayed S., van Campen L. and Lettinga G. (1987) Anaerobic treatment of slaughterhouse waste using a granular sludge UASB reactor. *Biological Wastes* **21**, 11–28.

Weng C.-N. and Jeris J. S. (1976) Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Research* **10**, 9–18.

Zehnder A. J. B., Huser B. A., Brock T. D. and Wuhrmann K. (1980) Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. *Archives of Microbiology* **124**, 1–11.