

# Efficient Methane Production from Lipid-Rich Wastewater in High-Rate Anaerobic Treatment

Cavaleiro, A.J.<sup>a</sup>, Salvador, A.F.<sup>b</sup>, Alves, M.M.<sup>c\*</sup>

*IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal. <sup>a</sup>acavaleiro@deb.uminho.pt; <sup>b</sup>asalvador@deb.uminho.pt; <sup>c</sup>madalena.alves@deb.uminho.pt*

*\* Corresponding author: phone: +351 253 604 417; fax: +351 253 678 986*

**Abstract:** In this work, high rate anaerobic mineralization of a synthetic dairy effluent containing 50% COD as oleic acid was accomplished in two reactors operated in parallel. The anaerobic reactors were able to accommodate organic loading rates up to 21 kg COD m<sup>-3</sup> day<sup>-1</sup>, HRT of 9 hours, attaining 99% of soluble COD removal efficiency and methane yield higher than 70%. Long chain fatty acids (LCFA) accumulated inside the reactor only during the last two phases of operation and palmitic acid was the main LCFA quantified, representing 40–100% of the total LCFA detected. High specific methanogenic activity was determined at the end of the operation, in the presence of acetate (1346±87 mg COD-CH<sub>4</sub> gVS<sup>-1</sup> day<sup>-1</sup>) and H<sub>2</sub>/CO<sub>2</sub> (3582±309 mg COD-CH<sub>4</sub> gVS<sup>-1</sup> day<sup>-1</sup>). The specific activity of the anaerobic consortia present in the reactors during the operation was also determined, and a maximum value of 1170±170 mg COD-CH<sub>4</sub> gVS<sup>-1</sup> day<sup>-1</sup> was obtained. The high performance accomplished in the reactors was a consequence of the discontinuous acclimation strategy applied, that produced an anaerobic microbial community specialized in the efficient mineralization of LCFA.

**Keywords:** methane yield, sludge acclimation, sodium oleate, organic loading rate, anaerobic bioreactors

## 1. Introduction

Energy production from renewable sources is currently a priority for economical, social and environmental reasons. The organic matter present in the wastewaters is converted into biogas, a renewable energy source, during the anaerobic treatment. The energy yield of this process depends on the type of organic matter present in the wastewater and is especially high for more reduced compounds, such as long chain fatty acids (LCFA), the main products of lipids hydrolysis (Sousa, 2007). Theoretically 1.01 L of methane at standard temperature and pressure can be produced from, for instance, 1 g of oleate (unsaturated LCFA, C18:1), whereas only 0.37 L can be produced from 1 g of glucose. Therefore, wastes or wastewaters with high lipid-content represent an attractive source for methane production (Kim *et al.*, 2004).

Despite the different technologies described in the literature for the anaerobic treatment of lipid-rich effluents, lipids conversion into biogas is considered difficult and tends to decrease with the increase of the organic loading rate (OLR) applied. Consequently, reports of reactor's failure are quite frequent and the treatment of this type of wastewater is generally performed at OLR lower than  $10 \text{ kg COD m}^{-3} \text{ day}^{-1}$  (Hwu *et al.*, 1998; Jeganathan *et al.*, 2006; Kim *et al.*, 2004; Pereira *et al.*, 2002; Rinzema, 1988).

Several operational problems are described as the main causes for the difficult conversion of lipids to biogas, namely bacterial inhibition and sludge washout (Hwu, 1997; Jeganathan *et al.*, 2006; Tagawa *et al.*, 2002). These problems result mostly from LCFA accumulation onto the microbial aggregates, by mechanisms of adsorption, precipitation and entrapment (Hwu, 1997; Pereira *et al.*, 2005). Besides the potential metabolic inhibition, LCFA accumulation onto the sludge can create a physical barrier, with consequent limitations in the transport of substrates and products, namely methane (Pereira *et al.*, 2005). However, since large amounts of methane are produced when LCFA-loaded sludge is incubated in batch vials, a discontinuous operation, designed to promote LCFA accumulation during continuous feeding, and subsequent batch degradation of the biomass-associated

substrate, was proposed as a strategy for achieving efficient rates of methane production (Pereira *et al.*, 2004).

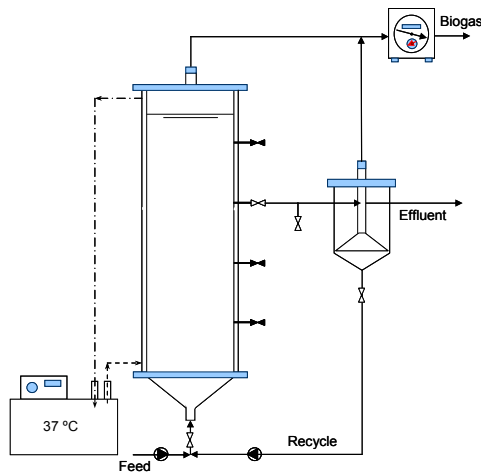
Based on this suggestion, Cavaleiro *et al.* (2007) studied the anaerobic treatment of an oleate-rich wastewater under discontinuous operation. The results obtained showed that sequencing feeding and degradation during the reactor start-up provides the ideal conditions for sludge acclimation, conducting to the development of a specialized microbial community capable of subsequent efficient methane production during continuous LCFA loadings. Therefore, this start-up strategy is a pre-requisite that should be applied when continuous treatment of lipid-rich wastewater is aimed. Clearly this kind of operation is preferable for large scale facilities, since wastewater is constantly generated and its energetic potential, in the form of biogas production, should not be wasted.

In this work, sludge previously acclimated to oleate through discontinuous operation (Cavaleiro *et al.*, 2007) was used as inoculum for the continuous high-rate treatment of an oleate-rich wastewater, in two anaerobic reactors operated in parallel. Organic loading rates were steadily increased from 5 to 31 kg COD m<sup>-3</sup> day<sup>-1</sup>, and the optimum load that allowed the maximum methane recovery was assessed.

## **2. Materials and Methods**

### **2.1. Experimental set-up**

Two anaerobic reactors were constructed in Plexiglas and were operated in parallel, at constant temperature ( $37 \pm 1$  °C). Plexiglas settlers were installed at the outlet of the reactors and the settled biomass was intermittently recycled (Figure 1). Each reactor was inoculated with 3.0 L of suspended biomass, previously acclimated to oleate through discontinuous operation (Cavaleiro *et al.*, 2007). The reactor was fed with a synthetic dairy wastewater, composed of 50 % COD-skim milk and 50 % COD-sodium oleate. This substrate was supplemented with macronutrients, micronutrients and sodium bicarbonate, as described elsewhere (Alves *et al.*, 2001).



**Figure 1.** Experimental set-up.

## 2.2. Routine analysis

Reactors' performance was monitored by measuring biogas production, influent and effluent soluble COD (centrifuged 15 min at 15000 rpm), effluent volatile fatty acids (VFA) and effluent solids. COD and solids were determined according to the Standard Methods (APHA, 1998). VFA were determined by HPLC (Jasco, Japan) using a Chrompack organic analysis column (30×6.5 mm) and a mobile phase of 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.7 mL min<sup>-1</sup>. The column was set at 60 °C and the detection was made spectrophotometrically at 210 nm. Biogas production was measured with a wet gas meter W-NK-0.5B (Shinagawa Corporation Factory, Japan) and the methane content was analyzed in a Pye Unicam GC-TCD gas chromatograph (Cambridge, England), using a Porapack Q (100-180 mesh) column. Helium was used as carrier gas (30 mL min<sup>-1</sup>) and the temperatures of the injection port, column and detector were 110, 35 and 110 °C, respectively.

## 2.3. Biomass sampling

A total of nine biomass samples were collected from each reactor during the experiment, as detailed in Table 1. All samples were characterized in terms of long chain fatty acids accumulation and specific methanogenic activity was determined in samples 1 and 9, in the presence of acetate and H<sub>2</sub>/CO<sub>2</sub>.

**Table 1.** Biomass sampling during the experiment

Sample n. °	Sampling time (days)	Key moment of the operation
1	0	Beginning of phase C-I
2	145	Beginning of phase C-IV
3	194	Beginning of phase C-V
4	211	During phase C-V
5	251	During phase C-V
6	301	During phase C-V
7	341	During phase C-VI
8	356	During phase C-VI
9	422	End of the operation (phase VII)

#### 2.4. Long chain fatty acids quantification

Saturated and unsaturated long chain fatty acids (LCFA) from C12 to C18, present in the liquid phase or associated to the biomass (solid phase), were extracted and quantified according to the method described by Neves *et al.* (2008). Free fatty acids present in the samples were esterified with propanol in acid medium at high temperature (100 °C) for 3.5 hours, and extracted with dichloromethane. Quantification was made in a gas chromatograph (CP-9001 Chrompack) equipped with a flame ionization detector. Fatty acids were separated on a TR-WAX (eq.CP-Sil 52 CB) 30 m x 0.32 mm x 0.25 µm capillary column, using helium (He) as carrier gas at a flow rate of 1.0 mL min<sup>-1</sup>. Initial oven temperature was 50 °C for 2 min, followed by a 10 °C min<sup>-1</sup> ramp to 225 °C and a final isothermal for 10 minutes. Detector and injector temperatures were 250 °C and 220 °C, respectively.

#### 2.5. Specific methanogenic activity

Specific methanogenic activity (SMA) was determined in batch assays with acetate (30 mM) and H<sub>2</sub>/CO<sub>2</sub> (80:20 v/v) as substrates. The basal medium used in these experiments was described elsewhere (Pereira *et al.*, 2004) and vials were prepared with 2 - 5 g VS L<sup>-1</sup>. All batch tests were performed in triplicate and were incubated at 37 °C and 150 rpm. Methane content of the biogas was measured by gas chromatography, as described in section 2.2. Methane production values were corrected for standard temperature and pressure (STP) conditions and background

production rate due to residual substrate consumption, measured in the blank controls, was subtracted.

## **2.6. Operation mode**

The OLR applied to both reactors was steadily increased from 5 to 31 kg COD m<sup>-3</sup> day<sup>-1</sup> (Table 2), by decreasing the hydraulic retention time (HRT) and keeping constant the concentration fed ( $7.4 \pm 1.6$  g COD L<sup>-1</sup>). OLR was increased only when COD removal efficiency was constant for more than 3 HRT and, simultaneously, the methane yield was higher than 60 %. R1 and R2 were operated in parallel during 337 days, but at this point a comparison between continuous and batch operation was tried. For that, the feed to R2 was stopped while keeping R1 in continuous. After 19 days in batch conditions, continuous feeding was restarted in R2 and maintained until the end of the operation (Table 2).

## **3. Results and discussion**

The performance data collected in reactors R1 and R2 during the 422 days of operation are presented in Table 2 and Figure 2. The reactors were operated in parallel from phase C-I to C-V, and the results obtained were very similar, showing that good reproducibility is possible during the anaerobic biodegradation of LCFA based effluents. Also, very high performance was observed during this period of time, with average COD removal efficiency of  $98 \pm 13$  % and methane yields higher than 72 % (Figure 2a and Table 2). A maximum methane yield of 98 % was obtained during phase C-IV in both reactors. Methane production rate increased fast and proportionally to the applied OLR, showing that there was no inhibition of the anaerobic consortia (Figure 2b). A comparison with other studies reported in the literature for lipid or LCFA-rich wastewater (Hwu *et al.*, 1998; Jeganathan *et al.*, 2006; Kim *et al.*, 2004; Pereira *et al.*, 2002; Rinzema, 1988) show that up to date these are the best results reported in terms of applied OLR and corresponding methane yields.

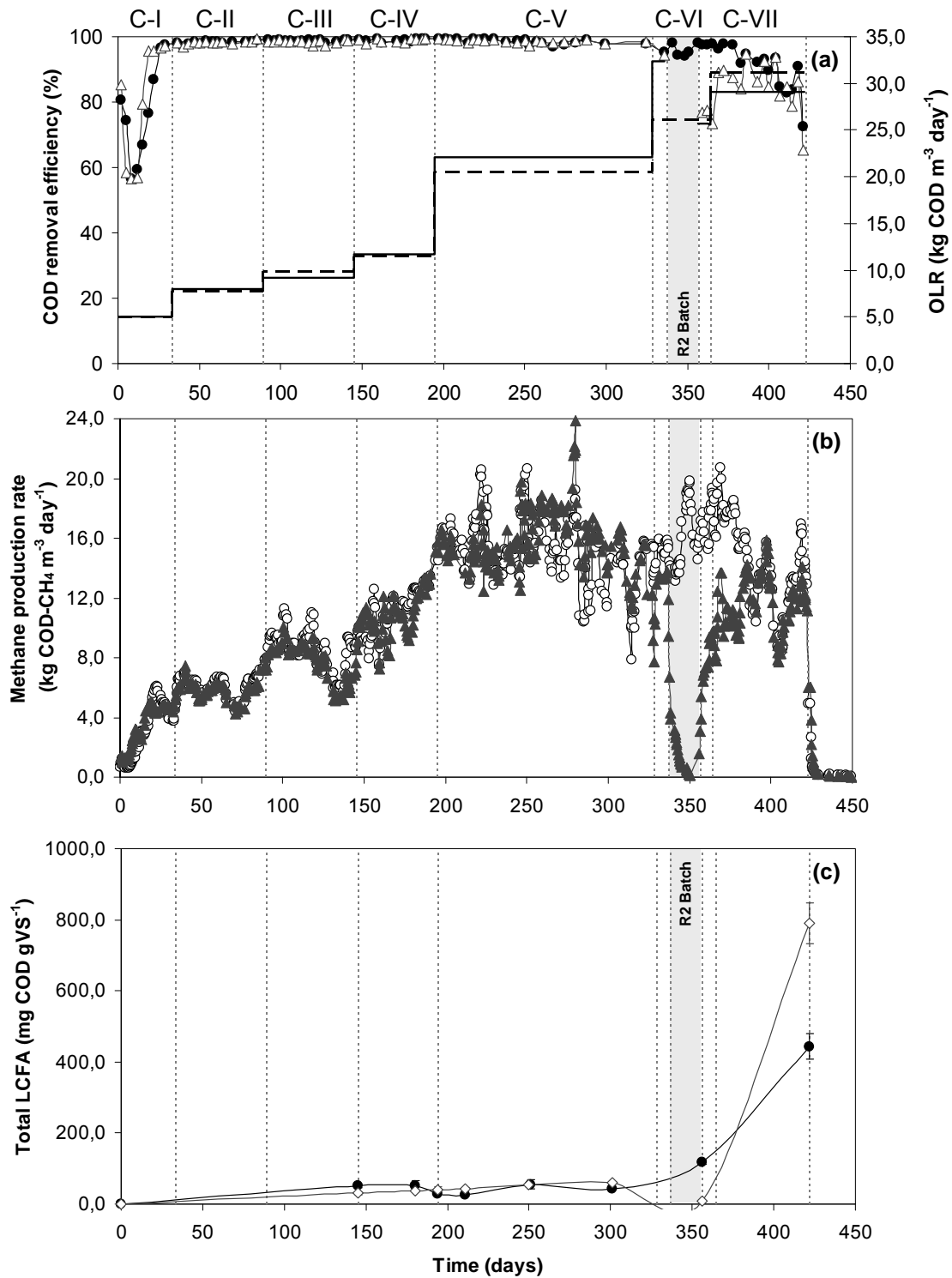
**Table 2.** Operating conditions and performance data

Reactor	Phase	Time (days)	HRT (days)	OLR applied (kg COD m <sup>-3</sup> day <sup>-1</sup> )	COD removal efficiency (%)	Methane yield (%) <sup>(a)</sup>
<b>R1</b>	C-I	0 - 33	1.50 ± 0.10	5.0 ± 0.4	77.6 ± 18.8	81.5
	C-II	33 - 89	0.97 ± 0.02	7.8 ± 1.0	96.0 ± 15.1	79.5
	C-III	89 - 145	0.72 ± 0.01	9.8 ± 2.2	98.0 ± 31.6	86.0
	C-IV	145 - 194	0.49 ± 0.03	11.5 ± 2.2	99.6 ± 27.5	98.4
	C-V	194 - 328	0.37 ± 0.01	20.6 ± 4.0	99.1 ± 27.6	71.5
	C-VI	328 - 364	0.30 ± 0.00	26.1 ± 4.2	96.4 ± 22.5	60.9
	C-VII	364 - 422	0.26 ± 0.00	31.2 ± 7.9	91.3 ± 34.5	57.0
<b>R2</b>	C-I	0 - 33	1.50 ± 0.10	5.0 ± 0.9	82.4 ± 31.0	82.4
	C-II	33 - 89	0.98 ± 0.03	8.0 ± 1.0	96.0 ± 16.3	73.9
	C-III	89 - 145	0.73 ± 0.01	9.2 ± 2.0	97.6 ± 30.4	83.2
	C-IV	145 - 194	0.49 ± 0.03	11.7 ± 2.9	98.9 ± 35.9	98.0
	C-V	194 - 328	0.37 ± 0.01	22.0 ± 4.0	98.7 ± 25.3	73.3
	C-VI	328 - 337	0.29 ± 0.00	32.4	94.3	42.2 <sup>(b)</sup>
		337 - 356	- [Batch]	0.0 [Batch]	0.8	8.4 <sup>(b)</sup>
	356 - 364	0.30 ± 0.00	25.7 ± 0.6	76.3 ± 2.2	34.2	
	C-VII	364 - 422	0.28 ± 0.02	29.1 ± 7.2	85.8 ± 33.0	52.9

<sup>(a)</sup> Standard deviation lower than 10%. <sup>(b)</sup> Calculated considering the total amount of COD removed during the continuous feeding and in the batch phase (t = 328 – 356 days).

When the OLR was increased to 26-30 kg COD m<sup>-3</sup> day<sup>-1</sup> (phases C-VI and C-VII) reactors' performance clearly declined. A steady decrease of the COD removal efficiency was observed, reaching minimum values of 73 % and 65 % in R1 and R2, respectively (Table 2), and the methane yields decreased until 57 % in R1 and 53 % in R2. Methane production rate decreased and became more unstable, suggesting that the applied OLR near 20 kg COD m<sup>-3</sup> day<sup>-1</sup> should not be exceeded. VFA and LCFA accumulation was also observed in the last two phases of operation (Figure 2c and Figure 3).

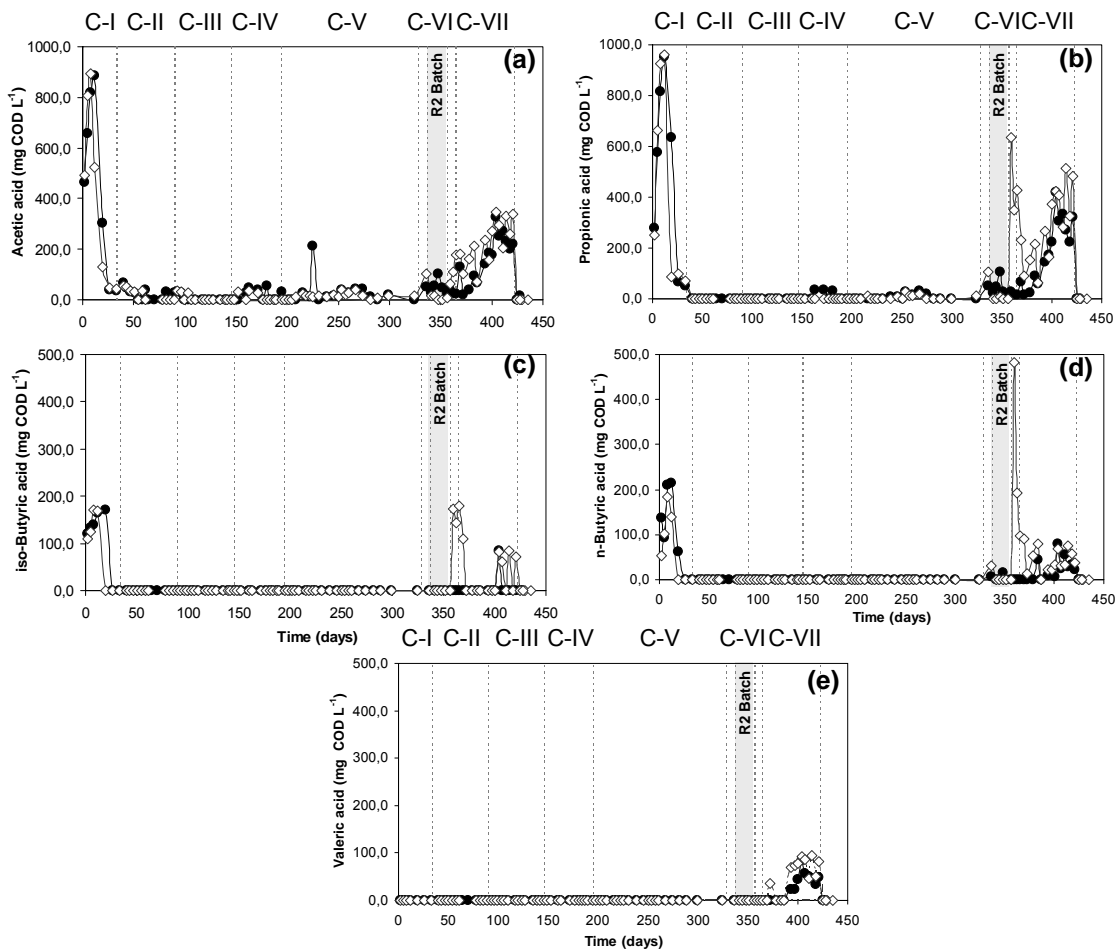
After the first 30 - 40 days, VFA levels stabilized below 50 mg COD L<sup>-1</sup> in terms of individual acids, and only increased again during phase C-VI and C-VII, although never exceeding 1000 mg COD-total VFA L<sup>-1</sup> (Figure 3).



**Figure 2.** (a) OLR applied during the experiment in R1 (----) and R2 (—); COD removal efficiency in R1 (●) and R2 (Δ). (b) Methane production rate in R1 (○) and R2 (▲). (c) Total LCFA quantified during the experiment in R1 (●) and R2 (◇).



During all the operation, acetic and propionic acids were the main VFA quantified (Figure 3a and b) and valeric acid was only detected during phase C-VII (Figure 3e), possibly as a result of cell lysis (Grobicki and Stuckey, 1991). During the batch period in R2, acetic and propionic acid levels became insignificant (lower than 25 mg COD L<sup>-1</sup>), but higher values were transiently detected in R2 after the restart of the continuous feeding, comparatively to R1.

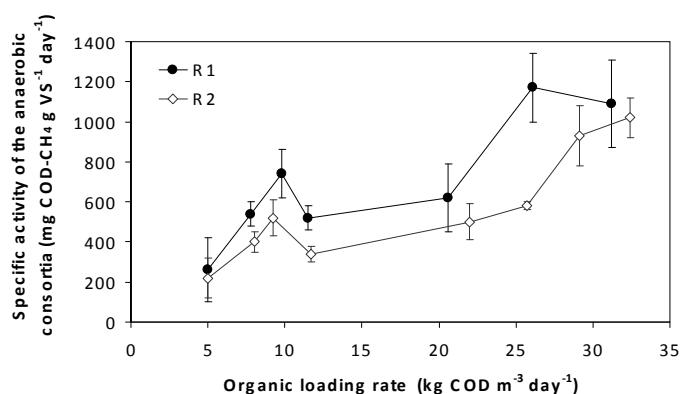


**Figure 3:** VFA quantified in R1 (●) and R2 (◇) during the experiment: (a) acetic acid, (b) propionic acid, (c) iso-butyric acid, (d) n-butyric acid and (e) valeric acid.

Total LCFA accumulation was only observed during the last two phases, reaching maximum values of  $443 \pm 36$  and  $790 \pm 58$  mg COD-LCFA g VS<sup>-1</sup> in R1 and R2, respectively (Figure 2c). A comparison with other studies (Pereira *et al.*, 2004 and

2005) show that these values are relatively low, especially when considering the high OLR applied in this study, which reflects the good biodegradation capacity of both consortia. Palmitic acid was the dominant LCFA, representing 40 – 100 % of all LCFA detected, although oleic (0 – 28 %), stearic (0 – 20 %) and myristic (3 – 20 %) acids were also present in the samples collected during the operation. Throughout the batch period imposed to R2, LCFA values became insignificant, due to the degradation of the accumulated substrate.

The SMA of the microbial communities present in the reactors R1 and R2 at the end of the operation showed that the methanogens were very active, in the presence of acetate ( $1346 \pm 87$  mg COD-CH<sub>4</sub> g VS<sup>-1</sup> day<sup>-1</sup>) and in the presence of H<sub>2</sub>/CO<sub>2</sub> ( $3582 \pm 309$  mg COD-CH<sub>4</sub> g VS<sup>-1</sup> day<sup>-1</sup>). The specific activity of the whole anaerobic consortia present inside the reactors during the experiment was also calculated, considering the methane production rate recorded along time and the VSS levels measured periodically in the reactors (Figure 4). A maximum value of  $1170 \pm 170$  mg COD-CH<sub>4</sub> g VS<sup>-1</sup> day<sup>-1</sup> was determined, when the applied OLR was higher than  $26$  kg COD m<sup>-3</sup> day<sup>-1</sup>. The maximum value reported up to date in the literature for the anaerobic treatment of oleate is  $600$  mg COD-CH<sub>4</sub> g VS<sup>-1</sup> day<sup>-1</sup> (Hwu, 1997), which is around 50 % of the obtained in the present work.



**Figure 4:** Specific activity of the anaerobic consortia present inside the reactors during the operation, as a function of the OLR applied.

The batch period applied to R2 in phase C-VI aimed the study of the influence on the subsequent reactor performance of allowing the degradation of the accumulated substrate. A comparison between the results obtained in R1 and R2 during phases C-VI and C-VII show that apparently the batch phase did not have a stimulating effect on the efficiency of LCFA conversion to methane, since the performance of both reactors became similar soon after continuous feeding was restarted in R2. This fact is probably a consequence of the sludge acclimation and of the high OLR applied.

#### **4. Conclusions**

This work clearly demonstrates that efficient methane production is possible during continuous high rate anaerobic treatment of LCFA based effluents. The anaerobic reactors were able to accommodate organic loading rates up to 21 kg COD m<sup>-3</sup> day<sup>-1</sup>, HRT of 9 hours, attaining 99 % of soluble COD removal efficiency and methane yield higher than 70 %. The high performance accomplished in the reactors was a consequence of the discontinuous acclimation strategy applied, that produced an anaerobic microbial community specialized in the efficient mineralization of LCFA.

***Acknowledgments:** The authors thank to Jorge Cunha and Filipa Pereira for their contribution in the experimental work. The financial support from Fundação para a Ciência e Tecnologia (FCT), through the project FAT-METHANE (POCTI/CTA/46328/2002) and the PhD grant given to AJ Cavaleiro (SFRH/BD/24256/2005) is gratefully acknowledged.*

#### **References**

Alves, M.M., Mota Vieira, J.A., Álvares Pereira, R.M., Pereira, M.A. and Mota, M., 2001. Effects of lipids and oleic acid on biomass development in anaerobic fixed bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Res.* 35, 264–270.

APHA/AWWA/WEF, 1998. *Standard Methods for the Examination of Water and Wastewater*. 20<sup>th</sup> ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

- Cavaleiro, A.J., Alves, J.I. and Alves, M.M., 2007. Discontinuous operation promotes efficient continuous anaerobic treatment of effluents with high lipid content. Proceedings of the 11<sup>th</sup> IWA World Congress on Anaerobic Digestion, Brisbane, Austrália. 133.
- Grobicki, A. and Stuckey, D.C., 1991. Performance of the anaerobic baffled reactor under steady-state and shock loading conditions. *Biotechnol. Bioeng.* 37, 344-355.
- Hwu, C.-S., 1997. Enhancing anaerobic treatment of wastewaters containing oleic acid. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Hwu, C.-S., van Lier, J.B. and Lettinga, G., 1998. Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochem.* 33, 75-81.
- Jeganathan, J., Nakhla, G. and Bassi, A., 2006. Long-term performance of high-rate anaerobic reactors for the treatment of oily wastewater. *Environ. Sci. Technol.* 40, 6466-6472.
- Kim, S.H., Han, S.K. and Shin, H.S., 2004. Two-phase anaerobic treatment system for fat-containing wastewater. *J. Chem. Technol. Biotechnol.* 79, 63-71.
- Neves, L., Pereira, M.A., Mota, M. and Alves, M.M., 2008. Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids. *Bioresour. Technol.* (*in press*).
- Pereira, M.A., Mota, M. and Alves, M.M., 2002. Operation of an anaerobic filter and an EGSB reactor for the treatment of an oleic acid-based effluent: influence of inoculum quality. *Process. Biochem.* 37, 1025-1031.
- Pereira, M.A., Sousa, D.Z., Mota, M. and Alves, M.M., 2004. Mineralization of LCFA associated to anaerobic sludge: kinetics, transport limitations, enhancement of methanogenic activity and effect of VFA. *Biotechnol. Bioeng.* 88, 502-511.
- Pereira, M.A., Pires, O.C., Mota, M. and Alves, M.M., 2005. Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnol. Bioeng.* 92, 15-23.
- Rinzema, A., 1988. Anaerobic treatment of wastewater with high concentration of lipids and sulphate. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Sousa, D.Z., 2007. Ecology and physiology of anaerobic microbial communities that degrade long chain fatty acids. PhD thesis, Universidade do Minho, Braga, Portugal. <<http://repositorium.sdum.uminho.pt/handle/1822/6287>>.
- Tagawa, T., Takahashi, H., Sekiguchi, Y., Ohashi, A. and Harada, H., 2002. Pilot-plant study on anaerobic treatment of a lipid- and protein-rich food industrial wastewater by a thermophilic multi-staged UASB reactor. *Wat. Sci. Technol.* 45, 225-230.