# Sequencing adsorption and degradation cycles towards the methanization of long chain fatty acids: comparison between granular and suspended sludge

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ABSTRACT

The aim of the present work was to study the maximum potential methane production in batch assays of sludge samples taken along the operation of two EGSB reactors (RI inoculated with granular sludge and RII inoculated with suspended sludge) fed with increasing oleic acid concentrations between 2 and 8 gCOD/l (HRT=1 day). After removing the residual substrate, the sludge was incubated in batch vials without any added carbon source. A maximum methane production rate of 152±21 ml$CH_{4(STP)}$/gVS.d was obtained for the RII-suspended sludge taken on day 70, when oleate at a concentration of 2 g COD/l was fed with a co-substrate (50% COD). The maximum plateau achieved in the methane production curve was 1145±307 ml$CH_{4(STP)}$/gVS, obtained for the RII-suspended sludge taken on day 162, when oleate was fed as the sole carbon source at 6 g COD/l. Methanization rate of the adsorbed substrate was enhanced under stirring conditions and was inhibited by adding oleic acid. Extraction and GC analysis confirmed that the main adsorbed substrate was palmitate, and not oleate, as previously suspected. These results evidence the advantage of sequencing adsorption and degradation cycles for the treatment of effluents with high lipid content.

KEYWORDS

EGSB; granular sludge; LCFA; oleic acid; palmitic acid

## INTRODUCTION

Lipids are one of the major components of organic matter in wastewater. Along with slaughterhouses and edible oil and fat refineries, dairy products industries are important contributors for the total lipid emission (Rinzema, 1988). Lipids are easily hydrolysed to Long Chain Fatty Acids (LCFA), which are further converted to acetate and hydrogen through β-oxidation mechanism by the proton reducing acetogenic bacteria (Weng and Jeris, 1976). LCFA are especially problematic compounds for anaerobic wastewater treatment. Tentative application of granule-based digesters to lipid-containing wastewaters revealed that, although granular sludge was more resistant to LCFA toxicity than suspended or flocculent sludge, physical stability of granules is critical for lipid containing wastewaters. This problem arises because these compounds adsorb onto the biomass and, besides the acute toxic effect, provoke granular sludge flotation, which occurs for concentrations far below the toxicity limit (Hwu *et al*., 1998). Results from batch assays suggested 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 was attributed to the growth of few survivors (Rinzema 1998). However, results from continuous experiments with a gradual replacement of a co-substrate by oleic acid ($C_{18:1}$), revealed that acetoclastic bacteria increased the tolerance to oleic acid toxicity (Alves *et al*., 2001). Moreover, a digester inoculated with an acclimated sludge exhibited higher methane yields than a digester inoculated with a non-acclimated sludge (Pereira *et al*., 2001).

In a continuous digester fed with oleate at concentrations as high as 12 gCOD/L anaerobic sludge became encapsulated by a whitish matter, which was a priory supposed to be oleic acid. When this encapsulated sludge was incubated in batch vials, after two consecutive washings for removing the residual substrate, methane was produced at a rate of 99 ml $CH_{4(STP)}$/gVSS.day, achieving a plateau of 736±20 ml

$CH_{4(STP)}$/gVSS, without any added carbon source. This methane production rate seemed to be delayed when oleic acid was added to the vials (Alves *et al*., 2001). The aim of the present work was to characterize the potential maximum methane production from the adsorbed substrate exhibited by the sludge of two EGSB reactors fed with increasing loads of oleic acid. Behaviour of granular and suspended sludge was compared. Sodium oleate was used as a LCFA model since it is, in general, the most abundant of all LCFA present in wastewater (Komatsu *et al*., 1991) as well as one of the more toxic (Galbraith *et al*., 1971).

## METHODS

### Experimental Set-up and operation mode


(a)

(b)

Figure 1. Experimental set-up: EGSB reactors (a) and batch experiments (b).

Two 10 l EGSB reactors (RI and RII) were operated in parallel with increasing oleate concentrations between 2 and 8 g COD/l. Figure 1a presents the EGSB reactors. Hydraulic retention time was set at 1 day. RI was inoculated with granular sludge and had an internal settler. RII was inoculated with suspended sludge and was equipped with an external settler (Figure 1a). The amount of adsorbed substrate and the corresponding potential maximum degradation rate were evaluated for a range of oleate loading rate between 2 and 8 kg COD/$m^3$.day. In both digesters, a significant amount of sludge accumulated in a floating layer.

### Batch experiments

For each applied oleate loading rate, samples from the bottom and top layers were collected, washed and centrifuged twice with anaerobic basal medium and incubated in batch vials of 25 ml at 37 °C, 150 rpm under strict anaerobic conditions, without any added substrate. The methane production was followed by measuring the pressure developed in each vial, using a hand held pressure transducer capable of measuring a pressure variation of two bar (0 to ± 202.6 kPa) over an output range of -200 to +200 mv (Colleran *et al*., 1992). The basal medium used in all the batch experiments was made up with demineralised 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 NaOH 8N and was prepared under strict anaerobic conditions. No calcium or trace-nutrients were added. The initial maximum methane production rate and the maximum plateau achieved were determined for each vial. Methanogenic activity, toxicity and biodegradability tests were also performed using this technique. All the batch experiments were performed in triplicate assays.

### Substrate

In the first 70 days the substrate was made of skim milk (50% COD) and oleic acid (50% COD). From the day 70 on, the carbon source was exclusively composed by oleic acid. Macro and micro-nutrients were added according to the composition described elsewhere (Alves *et al*., 2001).

### Seed Sludge

The granular sludge was obtained from an UASB treating a brewery effluent located in Oporto, Portugal. Suspended sludge was collected from a laboratorial digester treating an oleic acid synthetic effluent. Before inoculation this sludge was incubated in batch during about 15 days, in order to degrade the adsorbed substrate. 1.6 l of granular sludge (20.2 g VSS/l) and 2 l of suspended sludge (18.0 g VSS/l) were added to the digesters RI and RII, respectively. Both inocula were characterized in terms of specific methanogenic activity, oleic acid toxicity toward acetoclastic bacteria and biodegradability. Table 1 summarizes the corresponding results.

Table 1. Methanogenic activity, oleic acid toxicity and biodegradability for the granular and suspended seed sludge (±95% confidence interval).

| | | granular | suspended |
|---|---|---|---|
| Methanogenic activity in presence of: | | ($mlCH_{4(STP)}/gVSS \cdot d$) | |
| | Acetate | 327±11 | 107±6 |
| | Propionate | 160±10 | 48±14 |
| | Butyrate | (n.d.) | 52±3 |
| | Ethanol | 514±94 | 106±2 |
| | $H_2/CO_2$ | 597±16 | 487±31 |
| Oleic acid toxicity limit ($IC_{50}$) (mg/l) | | 345±26 | 133±16 |
| Biodegradability for oleate concentrations: | | ($mlCH_{4(STP)}/gVSS \cdot d$) | |
| | 100 mg/l | (n.d.) | 6±1 |
| | 300 mg/l | 14±1 | 7±2 |
| | 500 mg/l | 10±1 | 9±1 |
| | 700 mg/l | 8±1 | 8±2 |
| | 900 mg/l | 10±2 | 7±2 |

STP – Standard temperature and pressure conditions; n.d.- non-detectable

Granular sludge exhibited activities significantly higher than suspended sludge for acetoclastic, hydrogenophilic and syntrophic propionate and ethanol degrading bacteria. Only methanogenic activity with butyrate as substrate was non- detectable in this sludge whereas a value of 52 ml $CH_{4(STP)}$/gVSS.day was detected in the suspended sludge. The toxicity limit of oleic acid towards acetoclastic bacteria was higher for the granular than for the suspended sludge, which agree with the work of Hwu *et al*., (1996). The biodegradation rate was slightly, but not significantly higher in the granular than in the suspended sludge, for the range of concentrations studied.

## RESULTS AND DISCUSSION

Table 2 summarises the operating conditions and performance of RI and RII.

Table 2. Operating conditions and performance of RI and RII (±95% confidence intervals).

| **PERIOD** | **Time (d)** | **HRT (±0.01) (d)** | **Influent COD (g/l)** | **Influent oleate COD (g/l)** | **COD Removal efficiency (%)** | | **Effluent VSS (g/l)** | | **Methane production** $lCH_4/d$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | RI | RII | RI | RII | RI | RII |
| I | 0-70 | 1.01 | 3.8 (±0.3) | 1.9 (±0.2) | 96.5 (±0.6) | 85.8 (±3.2) | 0.38 (±0.07) | 0.65 (±0.04) | 10.6 (±1.0) | 7.9 (±1.2) |
| II | 70-119 | 1.01 | 3.8 (±0.3) | 3.8 (±0.3) | 83.4 (±4.8) | 74.4 (±5.5) | 0.85 (±0.22) | 0.72 (±0.15) | 2.3 (±0.5) | 2.6 (±0.5) |
| III | 119-162 | 1.01 | 6.2 (±0.7) | 6.2 (±0.7) | 74.2 (±3.8) | 74.6 (±2.9) | 1.96 (±0.43) | 1.57 (±0.17) | 1.6 (±0.2) | 2.0 (±0.8) |
| IV | 162-219 | 1.01 | 8.2 (±0.5) | 8.2 (±0.5) | 68.8 (±3.4) | 69.4 (±5.5) | 2.71 (±0.57) | 2.50 (±0.58) | 1.5 (±0.2) | 2.2 (±0.6) |

During the first 70 days skim milk was introduced as a co-substrate, representing 50% of the total COD fed. For the two first operating conditions, RI exhibited higher removal efficiencies and higher methane production than RII, but in the two last operation periods, higher methane productions and lower VSS levels were obtained in RI. From the day 70 on, oleate was the sole carbon source fed to both digesters, and the methane production decreased to 20 to 30% of the initial value. Along the trial period the methane yield decreased from 289 and 242 l$CH_4$/kg $COD_{removed}$ to 27 and 39 l $CH_4$/kg $COD_{removed}$ in RI and RII, respectively. Figure 2 represents the results from the batch experiments for the sludge taken from RI and RII in the bottom and top layers at the end of operating periods I, II, III, and IV. Table 3 summarises the obtained results.

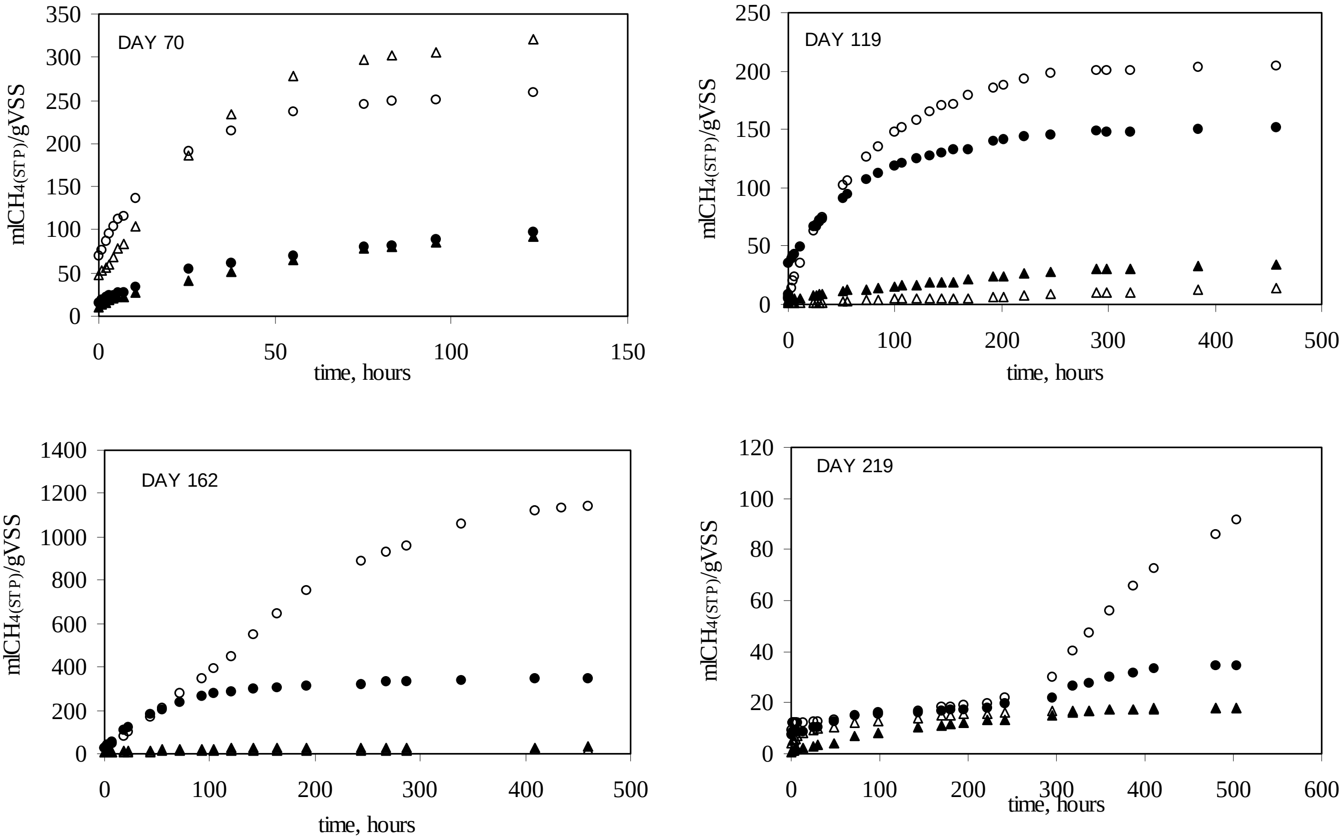


Figure 2. Results from the batch experiments. (•) RI top, (o) RII top, (▲) RI bottom, (△) RII bottom.

On day 162, when oleate was fed at 6 kg COD/$m^3$.d, the highest plateau in the methane production curve was obtained for the RII-top sludge (1145±307 ml $CH_{4(STP)}$/gVSS) which was more than three fold the obtained in the RI-top sludge. For the bottom sludges, no significant differences were obtained (Table 3).

Table 3. Maximum Plateaux (ml $CH_{4(STP)}$/gVSS) and methane production rate (ml $CH_{4(STP)}$/gVSS.day) obtained in the batch experiments (± 95% confidence intervals)

| | | RI top | RII top | RI bottom | RII bottom |
|---|---|---|---|---|---|
| DAY 70 | Maximum "plateau" | 97±13 | 260±28 | 92±6 | 321±42 |
| | Methane production rate | 40±4 | 152±21 | 35±6 | 133±17 |
| DAY 119 | Maximum "plateau" | 152±6 | 204±41 | 34±12 | 14±2 |
| | Methane production rate | 30±3 | 50±7 | 4±1 | 1±1 |
| DAY 162 | Maximum "plateau" | 349±38 | 1145±307 | 34±2 | 19±3 |
| | Methane production rate | 70±2 | 85±3 | 5±1 | 3±1 |
| DAY 219 | Maximum "plateau" | 35±4 | 111±24 | 18±1 | 18±3 |
| | Methane production rate | 4±1 | 7±1 | 3±1 | 2±1 |

For the oleate organic load of 8 kg COD/$m^3$.d (Period IV), a clear inhibition of the adsorbed substrate degradation was observed. A lag-phase of 300 hours preceded the initial methane production and a clear decrease on the methane production rates and plateaux were observed. When comparing the methane production rates in continuous operation and in batch mode for the oleate loading rate of 6 kg COD/m3.d, in RII, it is concluded that more methane would be produced per day if the feed was suppressed, provided that optimal conditions were assured. In fact, considering the amount of VSS present in the RII top layer (30 g), 2.6 l CH4 would be produced per day, whereas in continuous mode only 2 l CH4 were produced (Table 3). This suggests that adsorbed matter can be degraded efficiently, if no oleate is present. This was confirmed in batch assays (Figure 3a). When oleate was added to a sample of washed-encapsulated sludge methane production rate was significantly lower than the exhibited by the sludge without any added substrate. A decrease from 41 ml $CH_{4(STP)/}$gVSS.day to 8.1 ml $CH_{4(STP)/}$gVSS.day, was detected when 100 mg/l oleic acid were added.

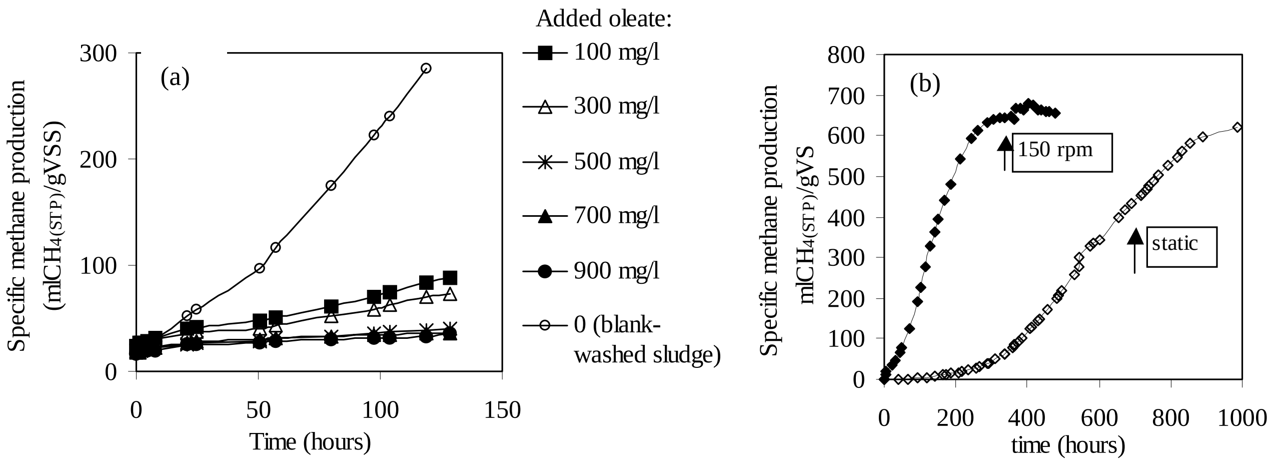


Figure 3. Specific methane production in batch assays of washed sludge: (a) effect of adding oleate; (b) effect of stirring at 150 rpm.

This aspect is of utmost importance, as it clearly indicates that for the degradation of the adsorbed matter, oleic acid should be absent, evidencing the need for sequencing cycles of adsorption and degradation towards the methanization of this type of compounds. Experiments made under static and stirring conditions led to the conclusion that methane production rate is significantly enhanced by stirring as can be observed in Figure 3b. A lag phase of about 300 hours was observed preceding the initial methane production under static conditions. As the substrate is already in intimate contact with the biomass, substrate diffusion limitations are not expected to limit the degradation rate. However, product diffusion limitation, e.g. biogas release was observed to be difficult under static condition, which may justify the different methane production patterns.

Figure 4 represents an aspect of the encapsulated sludge where a very clear whitish zone should represent the adsorbed substrate. The obtained results on the oleic acid inhibitory effect on the degradation of adsorbed substrate suggested that the adsorbed substrate is not oleic acid, but possibly an intermediate of its degradation. Extraction and GC analysis according to the method described in a companion paper (Pires *et al.*, 2001) confirmed that palmitate was the main compound detected and that only traces of oleate and stearate were present (Figure 5). This suggests that palmitate degradation is inhibited by oleic acid.

## CONCLUSIONS

Operation of two EGSB reactors (RI inoculated with granular sludge and RII inoculated with suspended sludge) fed with oleic acid as the sole carbon source revealed that methane production decreased to 20% to 30% of the value exhibited when a co-substrate was fed as 50% COD. Methane yields as low as 25 l$CH_4$/kg COD removed, were obtained. Maximum rate of methane production due to degradation of adsorbed substrate in batch vials, was 152±21 ml$CH_{4(STP)/}$gVS.d obtained for the suspended sludge taken on day 70, when oleate at a concentration of 2 g COD/l was fed with a co-substrate (50% COD). The maximum plateau achieved in the methane production curve was 1145±307 ml$CH_{4(STP)/}$gVS, obtained for the RII-suspended sludge taken on day 162, when oleate was fed as the sole carbon source at 6 g COD/l.

The degradation of the adsorbed substrate was inhibited by adding oleic acid and was enhanced by stirring conditions. Extractions and Gas Cromatography (GC) analysis of the adsorbed substrate revealed that is was mainly composed of palmitic acid, suggesting the accumulation of this LCFA due to inhibition by oleic acid of its further degradation. When treating effluents with high lipid content, it should be advantageous to run sequencing cycles of adsorption and degradation in order to enhance the methane production. Provided that it can be retained in the reactor, suspended sludge is advantageous over granular sludge due to its higher capacity of LCFA adsorption and degradation.

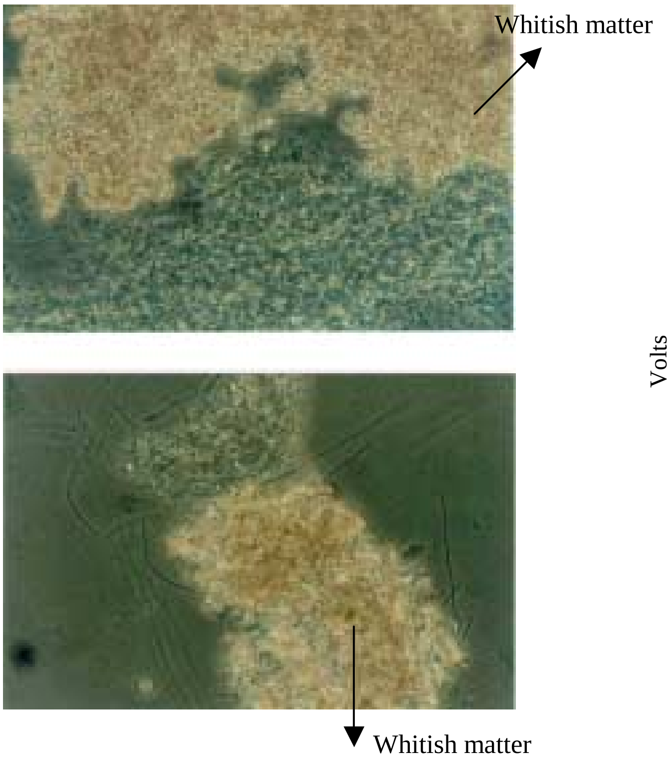


Figure 4 – Microscopic examination of encapsulated sludge.

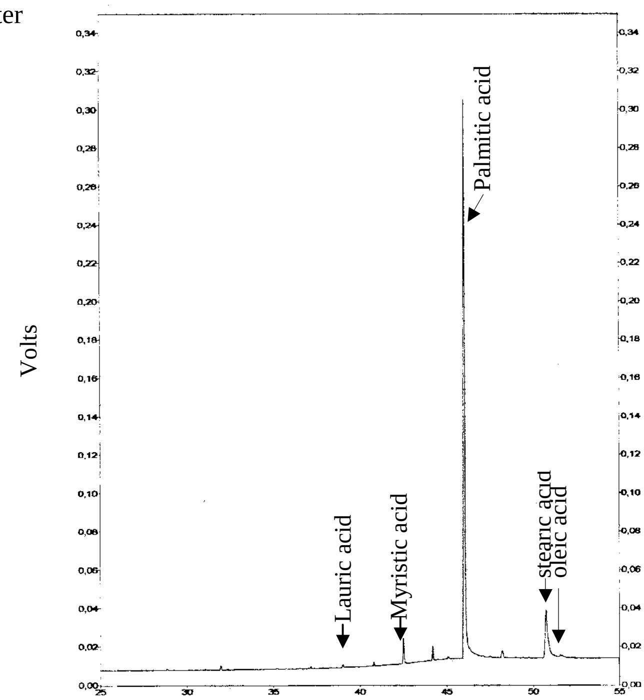


Figure 5 – example of a cromatogram obtained by GC analysis of extracted lipidic mater

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