Extraction and analysis of long chain fatty acids adsorbed onto active and inactivated anaerobic sludge

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ABSTRACT

Extraction and GC analysis of the adsorbed long chain fatty acids onto active and inactivated sludge taken from two EGSB reactors fed with oleic acid as the sole carbon source at 6 kgCOD/m^3.day was determined. No gradual acclimation of sludge to this LCFA was performed. Active sludge had been overloaded with oleic acid in a previous experiment and a possible retrieval of its activity was investigated. However the results revealed that the average removal efficiency was as low as 35% and only 19% of the biogas was methane. In the inactive reactor COD removal efficiency was 14%, corresponding only to physical adsorption, because no methane was produced. Extraction and analysis of the adsorbed fatty acids revealed that in the active sludge palmitic acid was the dominant fatty acid, in average, 3 to 52 times higher than oleic acid. In the inactivated sludge the main fatty acid detected was oleic acid, 8 times higher than palmitic acid.

KEYWORDS

granular sludge, LCFA, oleic acid, palmitic acid

INTRODUCTION

The adsorption of oleic acid and other long chain fatty acids (LCFA) on the surface of anaerobic sludge is described in the literature (Hanaki *et al*, 1981, Alves *et al*., 2001). Ning *et al*. (1996), postulated that anaerobic bio-sorption is mainly a physico-chemical process, but experiments performed by Hwu *et al*. (1998) have shown that oleate became adsorbed prior to be biodegraded and that after starting the methane production, a certain amount of oleate was desorbed due to biogas release. The anaerobic degradation of oleic acid (C18:1) through β-oxidation produce a complex mixture of several by-products. For each LCFA molecule degraded, one carboxylic acid molecule containing two fewer carbons**,** one molecule of acetic acid and four electrons are produced (Jerald *et al.*, 2000). LCFA are especially problematic compounds for anaerobic wastewater treatment, because are toxic to the anaerobic consortia and provoke sludge flotation**.** In previous works (Alves *et al*, 2001) anaerobic sludge was overloaded up to 12 kg COD/m^3.d of oleic acid as the sole carbon source. When this sludge was removed from the reactor, centrifuged and washed with anaerobic buffer twice, and incubated in batch vials at 37 °C under stirring conditions (150 rpm), the adsorbed substrate produced a maximum of 736±20 ml $CH_{4(STP)}$/gVS at a rate of 99 ml $CH_{4(STP)}$/gSV.day, without any lag-phase. However, if oleate was added to this encapsulated sludge, a lag phase up to 250 hours (depending on the added oleate concentration) was observed, suggesting that the added oleate delayed the degradation of the adsorbed matter. This suggested that adsorbed LCFA was not oleic acid, but probably an intermediate of its degradation.

The aim of this work was to systematically extract and analyse the adsorbed LCFA when two digesters were continuously fed with oleic acid as the sole carbon source. One of the digesters was inoculated with active biomass and the other reactor was inoculated with inactivated biomass.

METHODS

Experimental Set-up and operation mode

Two 10 l EGSB reactors, RI and RII, were operated during 51 and 38 days, respectively with an oleate concentration of 6 g COD/L and an HRT of 1 day. Macro and micronutrients were added according to the

composition described elsewhere (Alves *et al*., 2001). RI had an internal settler and RII was equipped with an external settler. Routine reactor performance was monitored by determining influent and effluent Chemical Oxygen Demand (COD), effluent VSS, % CH_4 in the biogas and pH. In both reactors a recirculation rate of 13 l/day was applied.

Seed sludge

RI was inoculated with 1 l of suspended active sludge (43.7 gVSS/l) proceeded from a previous experiment using the same reactors, which is described in a companion paper (Pereira *et al*., 2001a). This sludge collected at the end of the referred operation had shown a certain degree of inhibition due to the overloading with oleic acid at concentrations up to 8 g COD/l. This inhibitory effect is described in the same paper. Between the end of the previous experiment and the beginning of the actual one, the biomass was kept under anaerobic conditions at 4 °C, during a total period of 5 months. At the end of this period, appearance of biomass was gelatinous, seeming to be encapsulated by a whitish matter and before inoculation it was let to degrade the adsorbed substrate in batch mode at 120 rpm and 37 °C, until no more biogas production was detected (this lasted 17 days). The choice of this particular sludge was specially made to investigate the retrieval possibility of a sludge previously inhibited by LCFA. RII was inoculated with 1 l of suspended sludge (43.7 gVSS/l) inactivated by autoclaving, as described by Hwu *et al*. (1998).

Extraction and gas chromatography (GC) analysis

Samples from the reactors were collected washed and centrifuged (4000 rpm, 10 min) twice with anaerobic basal medium. A known volume of each sample was dried at 105ºC. Dried samples were taken, and placed into separating funnels. A solution of internal standards (C7 and C15) was added to the sample, and, after acidification to pH 2, a multiple extraction with 5x1 ml of petroleum ether was applied. The ether phase was transferred to glass vials, immediately capped, and stored at –4ºC. Volatile and LCFAs concentration was determined by a gas chromatograph (CP-9001 Chrompack) equipped with a flame ionization detector (FID) and a split/splitless injector. LCFAs were separated on a FFAP-CB 25m x 0,32mm x 0,3µm column (Chrompack), using nitrogen (N_2) as carrier gas at 35KPa, 31:1 split rate. Oven temperature was 40ºC for 0,2 min, with a 5ºC/min ramp to 250ºC, and a final hold at 250ºC for 15 min.

RESULTS AND DISCUSSION

Figure 1 a and b represents the time course of the COD removal efficiency of RI and RII, as well the methane content of the RI biogas and the pH in the feed, RI and RII.

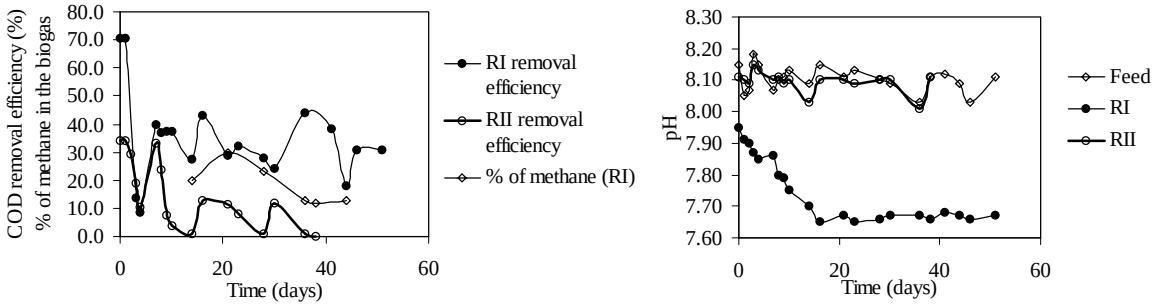

Figure 1 – Time course of COD removal efficiency, %methane in the biogas (a) and pH (b) in RI and RII.

Although removal efficiency and % of methane were very low along the trial period, a clear difference between RI and RII was observed either in terms of removal efficiency (average value of 35% in RI and 14% in RII) as well as in terms of methane content (19% and 0% in RI and RII, respectively). Also the different pH revealed that at least some biological activity was detected in RI, but not in RII. These results clearly demonstrate that in these conditions, the sludge previously inhibited by oleic acid had no capacity to recover its previous activity (Pereira *et al*., 2001a). However, it should be noted that RI and RII were operated at an extremely high load of oleate as the sole carbon source (6 kgCOD/m^3.day) without any start-up strategy for adaptation. In fact, previous experiments evidenced that if a gradual adaptation with a co-

substrate is performed, a similar sludge, previously inhibited by oleic acid, was more resistant to oleic acid toxicity and allowed higher methane yields when incubated in continuous reactors. (Pereira *et al*., 2001b).

On day 28, samples from feed and from sludge from RI and RII were taken for extraction and GC analysis. Results are summarized on Table 1. Result obtained for the feed confirmed within an error of 10% the obtained value from the COD analysis. In the active reactor the main LCFA adsorbed was palmitate exhibiting a concentration 2.8 times than the exhibited by oleic acid. In the inactivated sludge, however, oleic acid adorbed and a concentration 8 times than palmitic acid was detected. Analysis to active-sludge, repeated on days 30 and 37, revealed the same pattern with palmitic acid to be 3.6 and 52 times the concentration detected for oleic acid, respectively. The increasing accumulation of the ratio palmitic/oleic acid along the operation can be due to an inhibition of palmitate degradation by the continuously fed oleic acid, as reported elsewhere (Pereira *et al.,* 2001a). From the present results it is evident that transformation of oleic to palmitic acid is a fast and non-limiting step in oleic acid degradation. Accumulation of palmitic acid adsorbed onto the sludge suggests that its further degradation is a difficult step at least under continuous operation.

Table 1 – Analysis of long chain fatty acids on the feed and on sludge samples from RI and RII, on day 28.

Long chain fatty acids	mg /mg dry weight		
	Feed	RI	RII
Hexanoic acid	0.0110	Nd	Nd
Octanoic acid	0.0133	0.0104	0.0033
Decanoic acid	0.0002	Nd	Nd
Lauric acid	0.0003	Nd	Nd
Miristic acid	0.0118	0.0129	0.0109
Palmitic acid	0.0042	0.2541	0.0346
Estearic acid	0.0069	0.0401	0.0164
Oleic acid	0.3414	0.0901	0.2771

CONCLUSIONS

Extraction and GC analysis of the adsorbed fatty acids onto active and inactivated sludge taken from two EGSB reactors fed with oleic acid as the sole carbon source at 6 kgCOD/m^3.day was determined. In the active reactor removal efficiency was in average as low as 35% and only 19% of the biogas was methane. In the inactive reactor COD removal efficiency was 14%, corresponding only to physical adsorption, because no methane was produced. Extraction and analysis of the adsorbed fatty acids revealed that in the active sludge palmitic acid was the dominant fatty acid, in average, 3 to 52 times higher than oleic acid. In the inactivated sludge the main fatty acid detected was oleic acid and exhibited a concentration 8 times higher than palmitic acid. Transformation of oleic to palmitic acid is a fast and non-limiting step in anaerobic oleic acid degradation. Accumulation of palmitic acid adsorbed onto the sludge suggests that its further degradation is a difficult step.

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