ADSORPTION OF LCFA ON ANAEROBIC SLUDGE UNDER STEADY STATE AND SHOCK LOADING CONDITIONS: EFFECT ON ACETOGENIC AND METHANOGENIC ACTIVITY

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ABSTRACT

Accumulation of LCFA adsorbed onto the biomass was quantified under steady-state and shock conditions in a fixed bed reactor fed with oleic acid. The amount of adsorbed LCFA was more dependent on LCFA concentration than on LCFA loading rate and inhibited the acetogenic, acetoclastic and hydrogenophylic activity. However, even when methanogenic activity measurements indicate a severe inhibition, the anaerobic sludge was able to methanise adsorbed LCFA.

KEYWORDS

Anaerobic filter; LCFA; oleic acid; methanisation

INTRODUCTION

If compared with other organic matter, lipids are attractive for biogas production due to their high theoretical methane production. However, application of continuous anaerobic digesters to lipid-containing wastewaters is hindered by the acute toxicity of Long Chain Fatty Acids (LCFA) towards the anaerobic consortium and by the adsorption of these compounds onto the biomass, inducing sludge flotation and washout (Hanaki *et al*., 1981, Rinzema *et al*., 1994, Hwu *et al*., 1998). In spite of these problems, effective methanisation of high loads of LCFA is possible. The key is to sequence adsorption and degradation steps during the treatment process. Firstly the adsorption of LCFA should be favoured and then the methanisation of the adsorbed compounds will be efficient, provided the feed is suppressed. In a previous work, when operating continuously an EGSB reactor up to 6 kg oleate COD/m^3.day, as the sole carbon source, the methane yield was as low as 27 lCH_4/kg $COD_{removed}$, less than 10% of the theoretical value. When samples of biomass were taken from the reactor, washed and incubated in batch assays without any added carbon source, effective methanisation of the accumulated substrate was observed, achieving a maximum plateau of 1145±307 ml CH_4/g VSS (3271 mg COD-CH_4/gVSS) at a specific production rate of 85±3 ml CH_4/gVSS.day (243 mg COD-CH_4/gVSS.day) (Pereira *et al*., 2002). It was also observed that oleic acid inhibited the degradation of the adsorbed substrate which can explain the different methanisation efficiency under continuous and batch (feed suppressed) conditions. On the other hand, shear conditions were found to favour the methanisation rate of the adsorbed LCFA.

The aim of the present work was to investigate the effect of the reactor operating conditions (steady state versus organic and hydraulic shock loadings) on the accumulation of LCFA onto the anaerobic sludge and on its further degradation kinetics in batch assays.

METHODS

Experimental Set-up

A fixed bed reactor was constructed in PVC with a total volume of 86.8 liters and a diameter of 48 cm (Figure 1). 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. Each mini-bioreactor had 7.1cm 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%. This reactor configuration was described in detail elsewhere (Alves *et al*., 1998) and allowed the sampling of biomass along the operation. Operating temperature was kept constant at 35±1 °C and routine reactors performance was monitored by determining influent and effluent total and soluble Chemical Oxygen Demand (COD), influent flow rate and effluent volatile fatty acids (VFA).

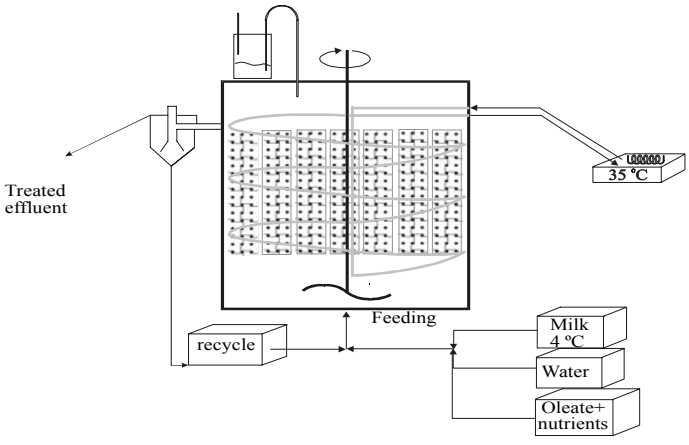

Figure 1- Experimental set-up.

Operation mode

During the steady state operation, which lasted 426 days, the seed sludge consisted of 11 litres of sludge (25.7 g of volatile solids (VS) per liter) and was obtained from a municipal sludge digester. Table 1 presents the operating

conditions prevailing at the moment of biomass withdrawal. The reactor was opened at six different operation times and 3 of the 27 mini-bioreactors were randomly selected and replaced by new similar mini-bioreactors, which were not accounted for in the next selection.

Table 1- Operating conditions prevailing at the moment of biomass characterization (adapted from Alves *et al*., 2001).

Period	Time (days)	Influent COD (mg/l)	HRT, (days)	Type of substrate	Organic loading (Kg COD/m^3.day)
	90	3000	0.9	skim milk (100% COD)	3.33
	132	6000	1.4	skim milk (100% COD)	4.29
I	162	9000	1.4	skim milk (100% COD)	6.43
	212	12000	1.4	skim milk (100% COD)	8.57
II	315	12000	1.4	skim milk (50% COD) + sodium oleate (50% COD)	8.57
III	426	12000	1.4	sodium oleate (100% COD)	8.57

The same reactor was used to perform two shock loading experiments (one organic and one hydraulic). The reactor was inoculated with 15 L of seed sludge containing 10 g of volatile suspended solids (VSS) per liter that was obtained from a local municipal sludge anaerobic digester. Table 2 summarises the operating conditions applied. During the start-up (Period I) the applied organic loading rate was gradually raised up to 6 Kg COD/m^3.day.

Table 2- Operating conditions during the trial period (adapted from Cavaleiro *et al*., 2001).

Period	Time (days)	Influent COD	HRT, (days)	Type of substrate	Organic loading	Remarks
	0-16	2000	4	skim milk	0.5	
	16-34	2000	2	skim milk	1	
I	34-54	4000	2	skim milk	2	
	54-83	4000	1	skim milk	4	
	83-102	4000	1	skim milk (50% COD) +sodium oleate (50% COD)	4	Introduction of oleate
	102-140	4000	0.667	skim milk (50% COD) +sodium oleate (50% COD)	6	Pre-shock conditions
II	140-144	20000	0.667	skim milk (50% COD) +sodium oleate (50% COD)	30	Organic shock
	144-230	4000	0.667	skim milk (50% COD) +sodium oleate (50% COD)	6	Pre-shock conditions
III	230-234	4000	0.133	skim milk (50% COD) +sodium oleate (50% COD)	30	Hydraulic shock
	234-286	4000	0.667	skim milk (50% COD) +sodium oleate (50% COD)	6	Pre-shock conditions

The reactor was initially fed with skim milk followed by a mixture of skim milk (50% COD) and sodium oleate (50% COD). This proportion remained constant during all subsequent operation periods. After the start-up, the applied organic loading rate was increased 5-fold (30 Kg COD/m3.day), by increasing the substrate concentration (simulation of an organic shock – Period II) or by decreasing the hydraulic retention time, HRT (simulation of a hydraulic shock – Period III). Both shocks lasted 4 days after what there was a return to pre-shock conditions (Table 2). At different moments along the shocks, the reactor was opened and 2 of the mini-bioreactors, randomly selected, were removed and replaced by new similar ones, which were not accounted for in the next selection. The substrate was diluted with tap water, and supplemented with macro and micronutrients as described elsewhere (Alves *et al*., 2001). To give suitable alkalinity, 5 g NaHCO3 were added per litre of feed.

Biomass characterisation:

All the sludge samples collected were 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 plateaux achieved were determined for each vial. The same biomass samples were also characterised in terms of specific methanogenic activity in the presence of acetate, propionate, butyrate and H_2/CO_2, using the above referred pressure transducer technique. All the batch experiments were performed in triplicate assays.

RESULTS AND DISCUSSION

Figure 2 represents the accumulation of adsorbed substrate onto the biomass under steady state (Figure 2 a) and shock loading conditions (Figure 2 b). During the steady state operation the adsorbed substrate remained constant around 400 mg COD-CH_4/gVSS, except in the period III (oleate sole carbon source) when a significant increase on the adsorbed substrate was found, attaining a maximum value of 1874 ± 51 mgCOD-CH_4/gVSS. Under shock conditions a steadily increase of adsorption was observed during the shock time followed by a return to the pre-shock values along the post-shock recovery time. This increase was significantly higher in the organic than in the hydraulic shock, achieving maximum values of 927±52 and 525±11 mg COD-CH_4/gVSS, respectively. This clearly shows that accumulation of adsorbed LCFA depends more on LCFA concentration than on LCFA loading rate. Accumulated LCFA is the result from the balance between the adsorption and degradation phenomena occurring simultaneously in the reactor. In the hydraulic shock, besides the much lower bulk oleate concentration that reduces the driving force of adsorption, the higher shear stress could have enhanced the biogas release inducing a more efficient degradation. This effect was also observed in a previous work (Pereira *et al*., 2001).

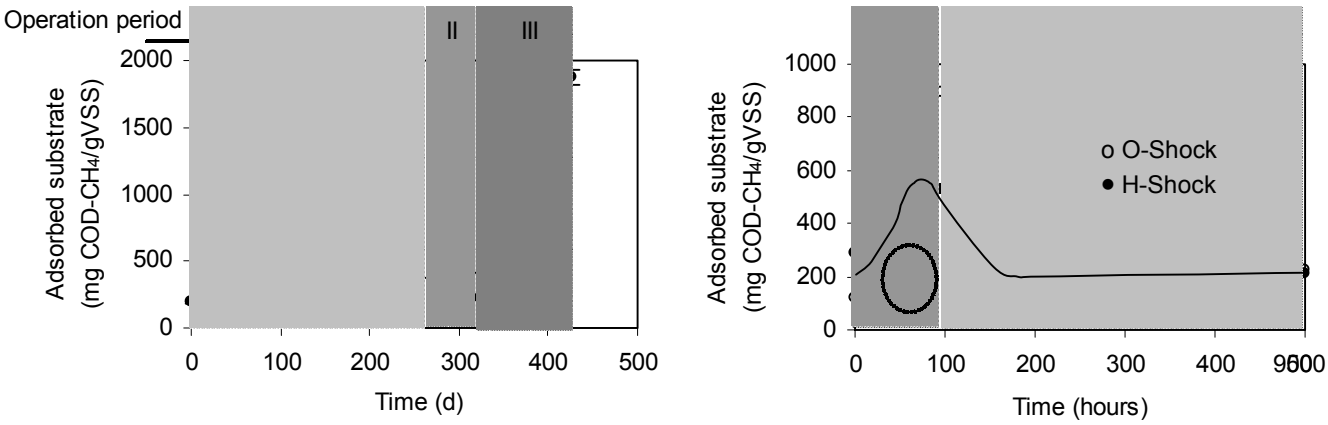

Figure 2 – Accumulation of adsorbed substrate under steady-state (a) and shock loading (b) conditions.

The occurrence of LCFA adsorption is reported in the literature (Rinzema *et al*., 1994, Hwu *et al*., 1998), but quantification and effect of this phenomenon on the acetoclastic, hydrogenophilic and acetogenic activity are not well documented. Figure 3 represents the effect of the accumulated LCFA on the specific methanogenic activity with acetate (a), propionate (b), butyrate (c) and H_2/CO_2 (d) as substrates. A clear decreasing trend between all activities and the accumulated LCFA was observed, more pronounced for the acetogenic (Figure 3 (b and c)) than for the acetoclastic and hydrogenophilic activity (Figure 3 (a and d)).

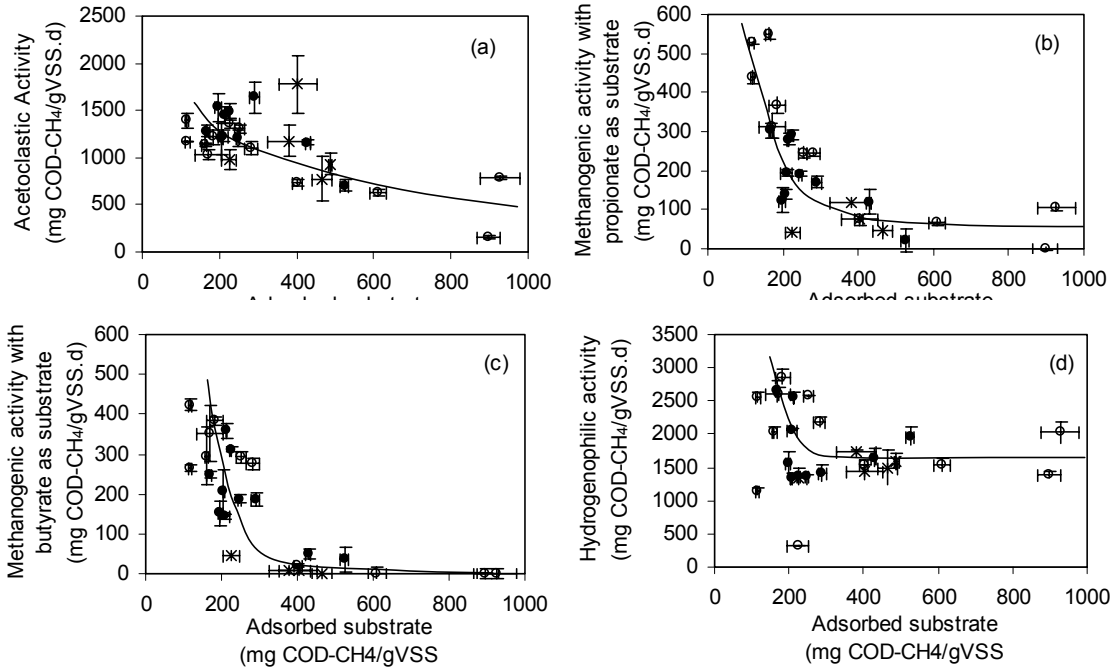

Figure 3. Effect of adsorbed LCFA on the specific methanogenic activity with acetate (a), propionate (b), butyrate (c) and H_2/CO_2(d) as substrates. Biomass samples taken under steady-state (∗), hydraulic shock(•) and organic shock (o) conditions.

Although the methanogenic activity was severely inhibited by the adsorbed LCFA it is unlikely that acetogenic and methanogenic populations were killed due to the bactericidal effect of these compounds, as suggested by Rinzema *et al* (1994), since the biomass was able to produce methane exclusively from the adsorbed substrate (Figure 5 a, b). Comparison between Figures 3 and 4 evidences that the increase in the amount of adsorbed LCFA decreased the methanisation rate from acetate, propionate, butyrate and H_2/CO_2 (added substrates), but enhanced its own degradation rate to methane. This suggests that transport of substrates form the bulk liquid to the cells was hindered by the adsorbed layer of LCFA, being this step overcome when the substrate was already in intimate contact with the cells. This result highlights the ability of anaerobic sludge to methanise LCFA even when methanogenic activity measurements indicate a severe inhibition and contradict the theory of bactericidal effect of LCFA towards acetogenic and methanogenic bacteria suggested by Rinzema *et al*, (1994).

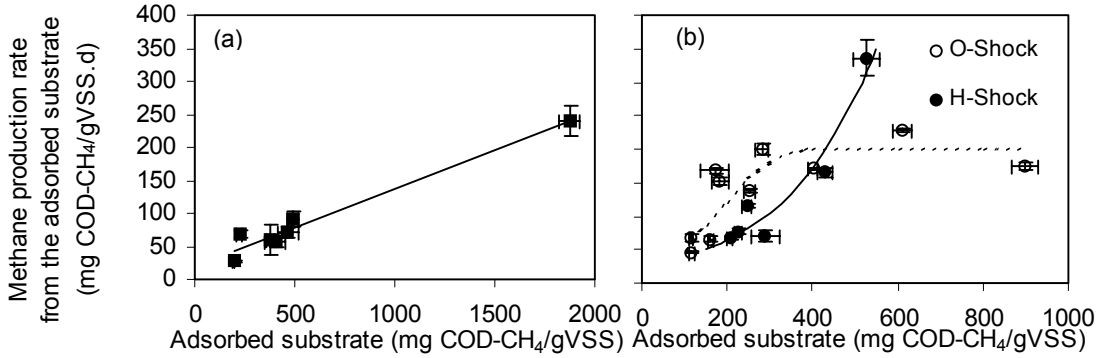

Figure 4- Kinetics of methane production in batch assays due to the degradation of adsorbed LCFA

CONCLUSIONS

Quantification of adsorbed LCFA when a fixed bed reactor was fed with oleic acid under steady-state and shock loading conditions led to the conclusion that it was more dependent on LCFA concentration than on LCFA loading rate. Acetogenic, acetoclastic and hydrogenophylic activity were severely inhibited by a layer of adsorbed LCFA. However, even when methanogenic activity measurements indicate a severe inhibition, the anaerobic sludge was able to methanise adsorbed LCFA highlighting the importance of transport limitations besides inhibitory effects.

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