EFFECT OF LIPIDS ON BIOMASS DEVELOPMENT IN ANAEROBIC FIXED-BED REACTORS TREATING A SYNTHETIC DAIRY WASTE

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ABSTRACT

The aim of this work was to follow the evolution in quantity and quality of the biomass developed during the operation of two anaerobic fixed bed reactors treating a synthetic dairy waste with different lipid contents. The feasibility of a special reactor design that allowed the biomass withdrawal with minimum operation disturbances was tested. The HRT was maintained at 1.5 days and the influent concentration was gradually increased from 3 to 12 g COD/l. Initially, one reactor was loaded with skim milk and the other one with whole milk, with equal organic loading rates (OLR). The effect of lipids was evaluated in terms of reactor performance, total, adhered and entrapped biomass and evolution of biomass quality determined in batch assays by: (i) measuring of potential specific methanogenic activity against direct (acetate, H_2/CO_2) and indirect (propionate and butyrate) substrates; (ii) measuring of the resistance of acetoclastic bacteria to the presence of sodium oleate. The lipids reduced the adhered fraction of biomass. The methanogenic activity against butyrate was enhanced in the presence of lipids, but no significant effect was detected on the other measured activities. The biomass taken from the reactor fed with lipids was more susceptible to the presence of sodium oleate, but, over the operation period, this susceptibility was reduced.

INTRODUCTION

Lipids are, in general, one of the major components of organic matter in wastewater and dairy products industries are important contributors for the total lipids emission (Rinzema, 1988). The conversion of lipids to long chain fatty acids (LCFA) and glycerol is not rate limiting (Hanaki *et al.*, 1981), but LCFA are known to be inhibitors at very low concentrations and may cause severe damage in anaerobic treatment systems.

Two main phenomena are usually associated with problems caused by anaerobic treatment of lipid containing wastewater: (i) the adsorption of a light lipid layer around biomass particles that affects its settling characteristics, increasing the washout probability; (ii) the strong inhibitory effect of LCFA, that affects cell membrane functions which is more important to acetoclastic than to hydrogenophilic methanogens (Hanaki *et al.*, 1981). These facts might influence the performance of high rate anaerobic treatment systems in different ways. Rinzema (1988) found that, more than inhibition, flotation of granular biomass conducting to washout, was the most important operational problem of the Upflow Anaerobic Sludge Blanket (UASB) Reactors. Addition of calcium salts prevented, to some extent, inhibition problems, but not flotation problems. Hwu *et al.*, (1996) compared the toxicity of LCFA to sludge from different origins and concluded that granular sludge was more resistant to LCFA inhibition than suspended and flocculent sludge.

In an Anaerobic Fixed Bed Reactor the support medium acts as a physical protective factor against washout, providing a potential attractive way for biomass retention for this particular type of wastewater. Several advantages and some disadvantages are usually appointed to the anaerobic filters (Young, 1991). Certainly one of the most serious problems associated with the study and knowledge of anaerobic filters is the difficulty of determining biomass quantity and quality as well as its evolution with time and operating conditions.

The aim of this work was to study the biomass development in two anaerobic fixed bed reactors treating synthetic dairy wastes with different lipid contents. The reactors were specially designed to allow the biomass to be periodically withdrawn. 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 is the most important LCFA produced by whole milk degradation (Hanaki *et al.*, 1981). The batch assays were performed using a pressure transducer technique (Colleran *et al.*, 1992).

MATERIALS AND METHODS

Experimental set-up

Each reactor was constructed in PVC with a total volume of 86.8 litres and a diameter of 48 cm. The support medium was equally divided among 27 parallel minireactors arranged in the central section of the reactors, located at 10 cm from the bottom. A perforated plate was used to sustain the minireactors and a stirrer was used to distribute the feed. Fig. 1 represents the reactor scheme. The support medium consisted of PVC raschig rings of 21 mm in size, with a specific surface area of 230 m2/m3 and a porosity of 92.5%.

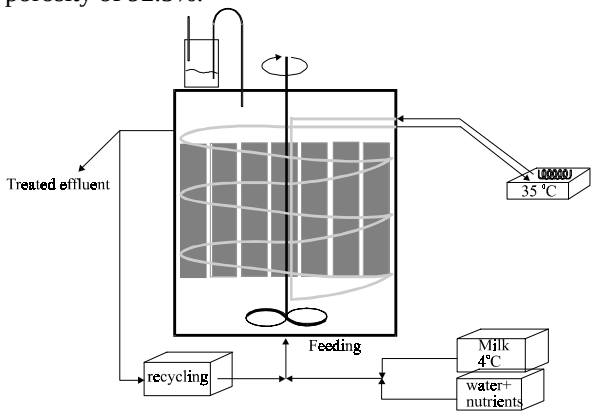

Fig. 1 - Schematic representation of the fixed bed reactors

The reactor temperature was kept constant at 35±1 °C. The seed sludge was obtained from a municipal sludge digester. Both reactors were inoculated with equal amounts of seed sludge (11 litres with 25.7 gVSS/L). Routine reactor performance was monitored by determining influent and effluent Chemical Oxygen Demand (COD), influent flow rate and effluent volatile fatty acids (VFA). Periodically each reactor was opened and three of the 27 minireactors were randomly selected and replaced by three other similar minireactors without biomass.

Biomass separation and quantification

Entrapped biomass was considered to be the fraction which was unattached to the support when it was put in a distilled water bath. N_2/CO_2 (80:20), was continuously flushed in order to keep the anaerobic environment. After centrifugation at 6000 rpm during 10 min, this fraction was ressuspended in an anaerobic buffer and total volume and its volatile solids (VS) content were determined. Activity and toxicity testes were performed with this fraction of biomass. Adhered biomass was removed from the support using a 0.1 N NaOH solution stirred in an orbital shaker at 100 rpm, followed by sonication (Donlon, 1992). Total volume and its volatile solids content were measured.

Substrate

Initially the substrate consisted of whole milk (reactor A) and skim milk (reactor B) diluted with tap water, 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. 5 g/l of CaCO3 were added. 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: 1g/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 of feed. In the last operation period both reactors were fed with skim milk and sodium oleate with micro and macronutrients.

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 column Chrompack (cat nº28350); the mobile phase was sulphuric acid (0.01N) 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 wave length of 210 nm. Methane content of biogas was measured by a Pye Unicam GCD gas chromatograph (Cambridge, England) using a column Chrompack 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.

Activity measurements. Methanogenic activity and toxicity tests were performed using a pressure transducer technique (Colleran et al., 1992). 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 (H2/CO2). Strict anaerobic conditions were maintained. The hand held pressure transducer used was developed at University College Galway, Ireland and was capable of measuring a pressure increase or decrease of two bar (0 to ± 202.6 kPa) over a range of -200 to +200 mv. The sensing element is connected to a digital panel module and the device is powered by a 9.0 V DC transformer. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Performance

During the start-up both reactors were fed with skim milk. After this period, the feeding to reactor A was gradually shifted to whole milk, while in reactor B the skim milk was fed during 246 days (Table I).

Table I - Type of substrate fed to reactors A and B

Reactor with lipids (A)		Reactor without lipids (B)	
Time (days)	Substrate	Time (days)	Substrate
0-29	skim milk	0-246	skim milk
29-71 71-76 76-212	skim milk blend whole milk		
212-315	skim milk +sodium oleate	246-315	skim milk +sodium oleate

As can be seen in Fig. 1 (a) and (b) the applied organic loading rate was increased gradually to 8.6 Kg COD/m3.day (day 162), by increasing the total COD fed to each reactor. After the 162th day the OLR was kept constant, but the substrate composition was changed. Fig. 1 (b) represents the total COD fed and the equivalent COD of the oleate fed to both reactors. It can be seen that until the 246th day, no oleate was added to reactor B and the equivalent oleate COD fed to reactor A (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).

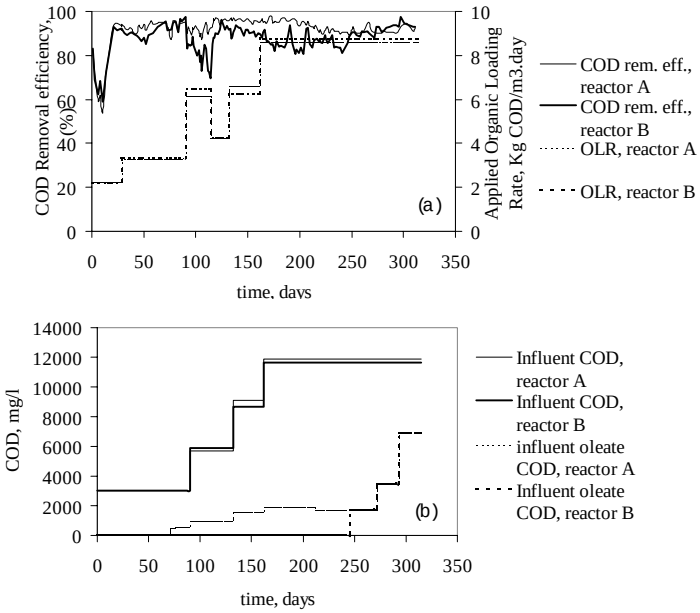

Fig. 1 - Operating conditions of reactor A and B. Removal efficiency and applied organic loading rate (a). Influent total and oleate COD (b).

After the 212 and 246th days for reactors A and B respectively, the feeding to both reactors was similar, the total COD was kept constant, but the sodium oleate content was gradually increased and at the end of the operation the sodium oleate content represented half of the total COD (Fig. 1b). The removal efficiency was always higher than 90% for reactor A and for reactor B it decreased to an average value of 76% during the period from the 90th to the 114th days, after which the organic loading rate was reduced by increasing the Hydraulic Retention Time - HRT- (Fig. 1 a). The results confirm that lipids and sodium oleate were retained in the reactor and removed from the substrate. The question is to know exactly how much was degraded, how much accumulated around the biomass or precipitated as a calcium oleate. As the methane content of the biogas was not measured, it was not proven that biodegradation occurred. Sayed *et al*. (1987) found a big difference between efficiency of methane production and efficiency of COD removal. Rinzema (1988) verified the accumulation of Long Chain Fatty Acids (LCFA) salts around the biomass, affecting transport properties of substrates and products. This fact had already been reported by Hanaki *et al*, (1982) who showed that LCFA produced by lipid degradation adhered to the biomass in 24 hours. On the other hand it is known that the presence of calcium reduces the inhibitory effect of LCFA through the production of a calcium - LCFA precipitate. However, the stoichiometric molar ratio oleate/Ca^{2+} of 2 was largely exceeded in this work; values of 2.9 and 8.6 were achieved in the two last operation periods resulting in a high supernatant concentration of sodium oleate (Roy *et al*., 1985).

Biomass characterisation

The biomass was characterised after five operation periods corresponding to the days 90, 132, 162, 212 and 315 and to the OLR of 3.2, 4.2, 6.6, 8.6 and 8.6 Kg

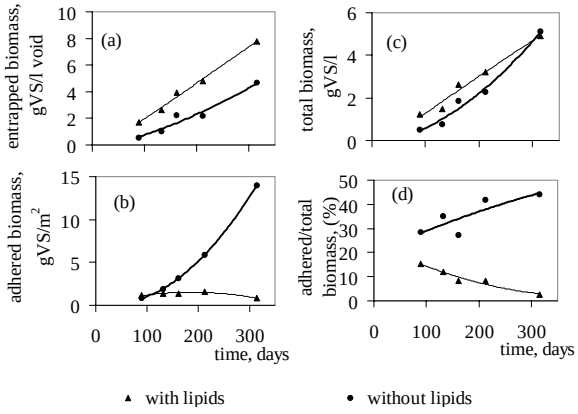

Fig. 2 - Biomass distribution. Entrapped (a), adhered (b), total (c) and % of adhered to total (d).

COD/m^3.day with oleate. The distribution between adhered and entrapped biomass is represented in Fig. 2. As can be observed, the presence of lipids reduced the fraction of adhered biomass (Fig. 2 b and d). However entrapped biomass was higher for the reactor fed with lipids (Fig. 2 a) and no significant differences were observed between total accumulated biomass for the two reactors (Fig. 2 c).

Fig. 3 represents the evolution of methanogenic activities against direct (Fig. 3 a and b) and indirect substrates (Fig.3 c and d). The presence of lipids did not influence significantly the acetoclastic activity, which followed a similar trend for both reactors, with a maximum for the 162th day (OLR=6.6 Kg COD/m3.day). The hydrogenophilic activity was also very similar for reactors A and B and propionate activity did not followed any particular trend. However, butyrate activity was clearly enhanced in the reactor fed with lipids. Furthermore, it was significantly increased after the introduction of sodium oleate in reactor B and achieved a value close to the butyrate activity measured for the biomass from reactor A.

The resistance of acetoclastic bacteria to the presence of sodium oleate was measured in terms of

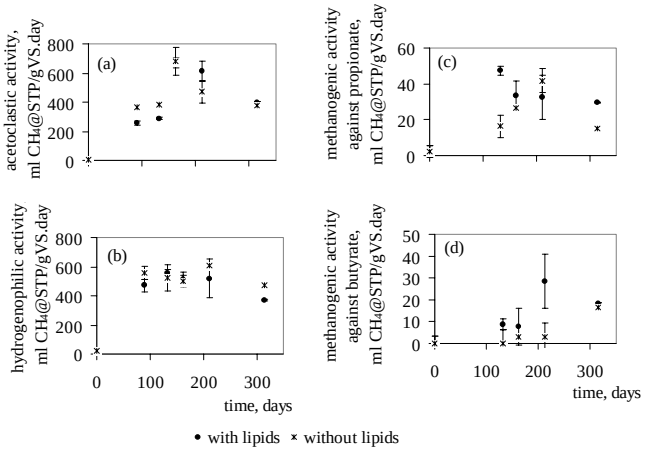

Fig. 3 - Specific methanogenic activity against acetate (a), H2/CO2 (b), propionate (c) and butyrate (d).

the fifty percent inhibition concentration of sodium oleate -IC50- to the acetoclastic bacteria (Fig. 4). In general it was verified that the previous contact with lipids or oleate rendered the biomass more susceptible to the presence of this LCFA. In general a longer lag phase was observed for biomass A than for biomass B before acetate degradation in the presence of oleate. The gradual increase in IC50 during the operation of reactor A suggests the adaptation of acetoclastic bacteria to this toxic.

CONCLUSIONS

The removal of more than 90% of COD was possible from an synthetic effluent with 6 g COD/l of sodium oleate even with molar ratio oleate/Ca^{2+} much more than 2. The presence of lipids affected the distribution of adhered and entrapped biomass without affecting the total amount of biomass accumulated. The butyrate activity was enhanced by

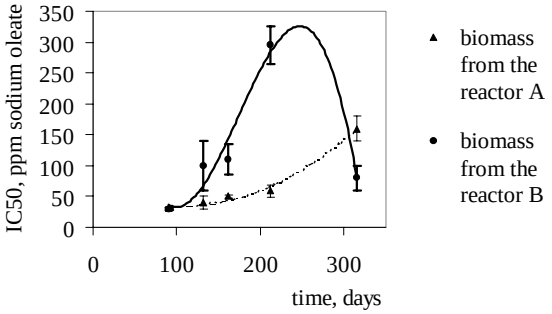

Fig. 4 - Fifty percent inhibition concentration (IC 50) of sodium oleate to acetoclastic bacteria

the presence of lipids and the contact with lipids rendered the acetoclastic bacteria more susceptible to the presence of sodium oleate. However the increase in IC50 during a long term operation of a reactor fed with lipids suggest the adaptation of acetoclastic bacteria to this toxic.

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