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Discontinuous operation promotes efficient continuous anaerobic treatment of effluents with high lipid content

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Abstract: A mixture of skim milk and sodium oleate was fed to an upflow sludge bed reactor operated in cycles. Each cycle had a feeding phase under continuous operation and a reaction phase in batch. Five cycles were performed with organic loading rates applied during feeding phases varying between 4.4 and 8 kg $COD.m^{-3}.d^{-1}$ and a constant hydraulic retention time of 1.6 days. In the first two cycles, 70% of the methane-COD was produced in the reaction batch phase, whereas from the third to the fifth cycles, biogas production in the reaction phase was less than 3% of total production. Overall methane yields increased steadily, from 0.67 to 0.91 kg $COD\text{-}CH_4.kg\ COD\ removed^{-1}$. LCFA accumulated into the sludge in the first two cycles, being palmitate and stearate the dominant intermediates quantified. In the subsequent cycles no LCFA were detected in the solid or liquid phases. The specific methanogenic activity in the presence of acetate and H_2/CO_2 increased significantly along the operation, particularly between time zero and the end of the third cycle. These results show that a discontinuous operation promoted the development of an active anaerobic community able to efficiently convert a continuous organic load of 8.2 kg $COD.m^{-3}.d^{-1}$, from which 50% was oleate.
Keywords: Anaerobic treatment; dairy wastewater; discontinuous operation; long-chain fatty acids; oleic acid

INTRODUCTION

High rate anaerobic reactors for the treatment of complex lipid-rich wastewaters represent a market niche that still needs to be filled and opens place for research and development. In general, despite the high chemical oxygen demand (COD) removal efficiencies reported in bioreactors treating this type of wastewaters, lipids/long chain fatty acids (LCFA) conversion to biogas is not always complete and decreases sharply with the increase in the organic loading applied (Hwu, 1997; Petruy and Lettinga, 1997; Pereira *et al.*, 2002; Haridas *et al.*, 2005; Jeganathan *et al.*, 2006). Substrate accumulation during long-term operation at high LCFA-loading rates causes limitations in the transport of substrate to the biomass, with a consequent decrease on the removal efficiency and methane production (Pereira *et al.*, 2005). However, in specific conditions, LCFA accumulated onto the sludge can be efficiently mineralised (Alves *et al.*, 2001, Pereira *et al.*, 2002) and, therefore, efficient treatment of LCFA-rich wastewaters is possible and should be related to the correct equilibrium between LCFA adsorption and degradation. A discontinuous operation, in which a first step of LCFA adsorption is followed by batch degradation of the biomass-associated substrate, was suggested for the treatment of this type of wastewaters (Pereira *et al.*, 2004). However, in real wastewater treatment facilities, operation in continuous is preferable, to assure a continuous biogas production.

The aim of this work was to study the anaerobic degradation of an oleate-rich wastewater in a reactor operated in cycles, involving a feeding phase in continuous and a reaction phase in batch.

METHODS

Experimental set-up

The reactor was constructed in Plexiglas (20 L working volume) and was operated at constant temperature (37 ± 1 ºC). After day 62 the effluent entered a Plexiglas settler and the settled biomass was subsequently recycled. The reactor was inoculated with 4.2 L of suspended biomass from a local municipal sludge anaerobic digester (33 g $VS.L^{-1}$). This biomass was considered to be acclimated to fat, since in the municipal wastewater treatment plant the fat removed in the beginning of the process was periodically introduced in the anaerobic digester. 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 $NaHCO_3$, as described elsewhere (Alves *et al.*, 2001).

Routine analysis

Routine performance was monitored by measuring influent and effluent soluble (centrifuged 10 min 15000 rpm) chemical oxygen demand (COD), effluent volatile fatty acids (VFA) and effluent total and volatile solids (TS and VS). During the feeding phases, samples from the effluent were collected immediately after the settler. In the reaction phases samples were collected in the same point, representing the effluent that would leave the reactor if it would be discharged at that time. In this way, the values obtained for soluble COD and VFA in all these samples were considered to be comparable. COD and solids were determined according to Standard Methods (1998). COD removal efficiency for a cycle was calculated as the ratio between the total mass of COD removed from the liquid medium and the total mass of COD fed in the feeding phase. VFA were determined by HPLC (Jasco, Japan) using a Chrompack column (30×6.5 mm) and a mobile phase of 5 mM H_2SO_4 at 0.7 $mL.min^{-1}$. The column was set at 60 °C and the detection was made spectrophotometrically at 210 nm. Biogas production was measured with a liquid displacement gas counter and the methane content of the biogas produced during all the experiment was in the range of 63-67 %.

Specific methanogenic activity and toxicity tests

Biomass samples, collected at the beginning of the operation, at the end of the third and fifth cycles, were characterized in terms of specific methanogenic activity (SMA) with acetate (30 mM) and H_2/CO_2 (80:20 V/V) as substrates. The influence of sodium oleate concentrations between 300 and 900 $mg.L^{-1}$ on the acetoclastic activity was determined for the inoculum. The basal medium used in the batch experiments was described elsewhere (Pereira *et al.*, 2002). All batch tests were performed in triplicate, at 37 ºC, 150 rpm. Methane production values were corrected for standard temperature and pressure conditions (STP) and background production rate due to residual substrate, measured in the blank controls, was subtracted. The methane content of the biogas was measured by a Pye Unicam GC-TCD gas chromatograph (Cambridge, England), using a Chrompack column Haysep Q (80-100 mesh). Helium was used as carrier gas (30 $mL.min^{-1}$) and the temperature of the injection port, column and detector was 110, 35 and 110 ºC, respectively.

Quantification of the biomass-associated long-chain fatty acids

Biomass-associated long-chain fatty acids (LCFA) were extracted and quantified according to the method described by Neves *et al.* (2007), in samples collected at the beginning of each phase during the experiment. Free LCFA present in the samples, either in the solid or in the liquid phases, were esterified with propanol in acid medium at high temperature (100 ºC) for 3.5 hours, and extracted with dichloromethane. Quantification was made in a gas chromatograph (CP-9001 Chrompack) equipped

with a flame ionization detector. LCFA were separated on a eq.CP-Sil 52 CB 30 m x 0.32 mm x 0.25 μm column (Teknokroma, Tr-wax), using helium (He) as carrier gas at 1.0 $mL.min^{-1}$. Oven temperature was 50ºC for 2 min, with a 10ºC.min^{-1} ramp to 225ºC and with a final isothermal for 10 minutes. Detector and injector temperatures were 250 ºC and 220 ºC, respectively.

RESULTS AND DISCUSSION

The influence of oleate concentration on the acetoclastic activity of the inoculum (considered to be acclimated to fat) was determined in batch assays (Figure 1). No significant differences were observed in the initial slope of the methane production curves for all the oleate concentrations added and there were no lag phases in the range of concentrations under study.

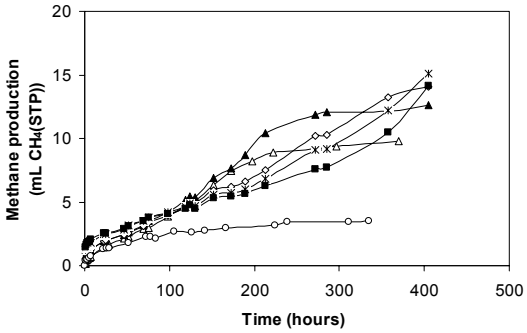

Figure 1. Methane production curves obtained during the toxicity batch test performed with the inoculum, when oleate was added at concentrations of 0 (only acetate added) (Δ), 300 (▲), 500 (◇), 700 (∗) and 900 (■) $mg.L^{-1}$. Blanks assays (no acetate or oleate added) are also presented (o).

The reactor was operated in cycles, each one with two phases: feeding (in which the reactor was continuously fed with the wastewater to be treated) and reaction (in which the feeding was stopped, i.e., batch conditions). Five cycles were performed over 213 days, with different combinations of organic loading rate and feeding phase time, but with a constant hydraulic retention time (HRT) of 1.6 days. Performance data collected over time were used to define the feeding time in each cycle. After a reaction phase, feeding was restarted (beginning of a new feeding phase/cycle) when biogas production due to the degradation of the substrate accumulated inside the reactor stopped. Operation mode and performance data are presented in Table 1.

In a preliminary experiment (first cycle) an organic loading rate of 4.4 ± 0.6 kg $COD.m^{-3}.d^{-1}$ (7.2 ± 1.0 g $COD.L^{-1}$) was applied during the feeding phase, but after 17 days the reactor content was whitish and biogas production became insignificant (not shown), suggesting that substrate was mainly accumulating inside the reactor. In the following two cycles (second and third) a similar organic loading rate was applied during a similar period of time (17 days). Based on methane production rate during the third cycle, an increase in the feeding time was introduced in the fourth cycle (28 days), although applying the same organic loading rate. In the fifth cycle it was decided to increase the organic loading rate (8.2 kg $COD.m^{-3}.d^{-1}$), through an increase in the concentration fed (13.1 g $COD.L^{-1}$), maintaining a similar feeding time (22 days). Reaction phases were extended until no biogas production was detected, turning to be longer than necessary and therefore, optimization is required in further experiments.

Table 1. Operational and performance data during the experiment.

Cycle	1st		2nd		3rd		4th		5th	
Feeding (F) or Reaction (R)	**F**	**R**	**F**	**R**	**F**	**R**	**F**	**R**	**F**	**R**
Organic loading rate ± 0.6 (kg COD.m^{-3}.d^{-1})	4.4	-	4.4	-	4.4	-	4.4	-	8.2	-
Concentration ± 1.0 (g COD.L^{-1})	7.2	-	7.2	-	7.2	-	7.2	-	13.1	-
HRT±0.01 (days)	1.6	-	1.6	-	1.6	-	1.6	-	1.6	-
Recycle flow rate (L.d^{-1})	0	0	0	26.5	26.5	26.5	26.5	26.5	13.4	13.4
Phase time (days)	17	28	17	38	17	21	28	15	22	9
Influent COD (g)	1496	0	1496	0	1496	0	2464	0	3608	0
Removed COD (g)	nd	nd	1017	48	1251	3	2424	0	3499	0
COD removal efficiency (%)	nd	nd	68	3.2	84	0.2	98	0	97	0
COD converted to methane (g)	*	314	217	494	882	30	1292**	18	3138	36
Overall methane yield (g COD-CH_4 g COD removed^{-1})	nd		0.67		0.73		0.54**		0.91	

(*) Quantification of the biogas produced was not exact. A decrease in biogas production was detected along the feeding phase and an overall biogas production less than 35 L can be estimated, corresponding to less than 100 g COD converted to methane. (**) Problems with the biogas counter. Real values are higher than the ones presented. nd – not determined.

Overall COD removal efficiency increased steadily from cycle to cycle, from a minimum value of 71 % to a maximum value around 98 % in the last two cycles. In all cycles, COD removal developed mainly during the feeding phase, and the contribution of the reaction phase was always lower than 3.2 %. However, in the first two cycles, more than 70 % of the methane-COD was produced in the reaction batch phase, whereas from the third to the fifth cycles, methane production in the reaction phase was less than 3 % of total production, showing that almost all the substrate fed was degraded during the feeding phase. A steadily increase was observed in the overall methane yield, from a minimum value of 0.67 kg COD-CH_4.kg COD removed^{-1}, obtained in the second cycle, to a maximum value of 0.91 kg COD-CH_4.kg COD removed^{-1}, observed in the fifth cycle.

Figure 2(a) presents the cumulative methane production during the experiment, where the shift in the methane production from the reaction to the feeding phase is evidenced. Also the maximum plateau achieved was improved from the first to the fourth cycle. In the fifth cycle, in spite of applying a double loading rate, an average specific methane production of 240 mg COD-CH_4.gVS^{-1}.d^{-1} was obtained, considering the final VS concentration inside the reactor (32.5 g VS.L^{-1}). Soluble COD and VFA values increased during the feeding phase of the second cycle and decreased in the reaction phase (Figure 2(b)). In the third and fourth cycles, COD and VFA peaked during the feeding phases, although the maximum achieved in the fourth cycle was only 35 and 16 % of the COD and VFA peaks detected in the third cycle. In the fifth cycle there was no detection of a significant VFA and COD concentrations. In these last three cycles, when reaction phase started, the amount of soluble COD present inside the reactor was less than 300 mg COD.L^{-1} (Figure 2(b)), suggesting that in these cycles the reactor was able to operate continuously, being unnecessary the existence of a batch-reaction phase.

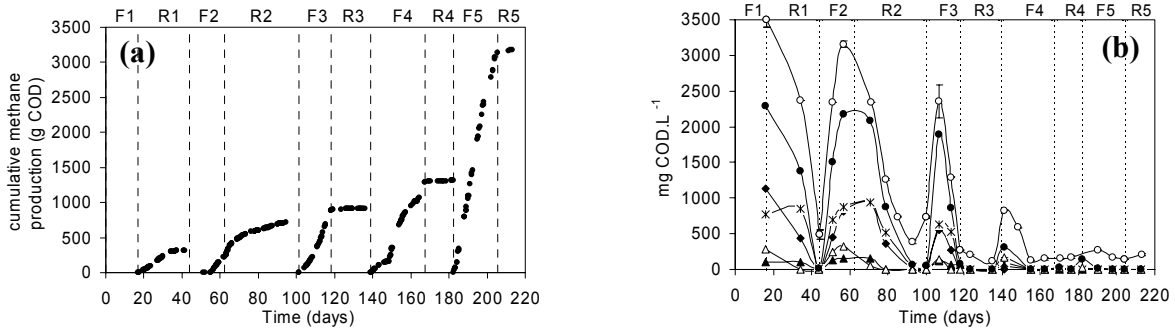

Figure 2. Time course of (a) cumulative methane production in the feed (F) and reaction (R) phases and (b) effluent concentration of soluble COD (o), total VFA (•), acetate (◆), propionate (∗), iso-butyrate (▲) and n-butyrate (Δ).

The results presented suggest that through the first two cycles the anaerobic consortium acclimated to the substrate, until an active community was obtained, able to efficiently degrade the substrate fed in continuous. Specific methanogenic activity (SMA) in the presence of acetate and H_2/CO_2 was determined in biomass samples collected in the beginning of the operation, at the end of the third and at the end of the fifth cycles (Table 2). A significant increase of both activities was observed between time zero and the end of the third cycle. These results agree well with the performance data of the reactor.

LCFA accumulation onto the sludge was observed during the first cycle and in the feeding phase of the second cycle, reaching a maximum value of 1.7 g COD-LCFA.gVS^{-1} (Figure 3). This value is significantly higher than the one suggested by Pereira *et al.* (2004) as the value that would lead to the maximal mineralization rate (1 g COD-LCFA.gVS^{-1}). Palmitate and stearate were the dominant intermediates quantified. In the reaction phase of the second cycle biodegradation of accumulated LCFA proceeded efficiently and in the third, fourth and fifth cycles almost no LCFA accumulation occurred.

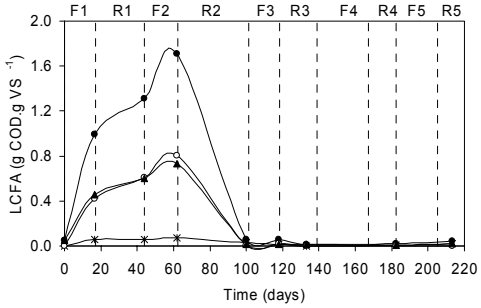

Figure 3. Time course of total LCFA (•), stearate (o), palmitate (▲) and oleate (∗).

Table 2. SMA (mL $CH_{4(STP)}.gVS^{-1}.d^{-1}$) in the presence of acetate and H_2/CO_2.

SMA in the presence of:	Inoculum	End of the 3rd cycle	End of the 5th cycle
Acetate	0	179 ± 18	246 ± 7
H_2/CO_2	56 ± 6	531 ± 10	512 ± 24

The results presented show that through the first two cycles the anaerobic consortium acclimated to the substrate, developing a microbial community able to efficiently convert the substrate fed in continuous mode to methane.

CONCLUSIONS

These results show that two cycles of a discontinuous operation, combining feeding and batch operating modes, promoted the development of an active anaerobic community able to convert a continuous organic load of 8.2 kg $COD.m^{-3}.d^{-1}$, 50% as oleate, with a methane yield of 0.91 kg $COD\text{-}CH_4$.kg COD $removed^{-1}$. Operating in discontinuous mode promotes the ideal conditions for an efficient continuous high rate anaerobic treatment of complex wastewaters with high lipid content. Sludge acclimated to discontinuous or pulsed feeding of fat is probably the best inoculum to use for that purpose. Although process optimization is required, the potential of presented results is unquestionable, considering new technological development for high rate anaerobic treatment of complex effluents with fat.

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