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Co-digestion of cow manure, food waste and intermittent input of fat

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ABSTRACT

Pulses of oil were added to completely mixed reactors fed with dairy cow manure and food waste, after achieving a stable performance at an organic loading rate of 4.6 ± 0.1 gCOD/($l_{reactor}$ day), an oily waste effluent from a canned fish processing industry was fed in the form of pulses. The oil concentration rose up to 9, 12, 15 and 18 $gCOD_{oil}/l_{reactor}$, after the pulse feeding in the reactor. The highest fat concentration of 18 $gCOD_{oil}/l_{reactor}$ promoted a persistent inhibition in the process of the continuous reactor, although in batch assays, the reactor content evidenced a capacity to degrade more oil and to degrade the accumulated organic matter. All the other pulses had a positive effect in the methane production. From a practical point of view, this work demonstrates that controlled intermittent inputs of oil can enhance the methane production in a co-digestion of cow manure and food waste.

1. Introduction

Anaerobic digestion is attractive for the treatment of organic wastes such as cow manure, since it produces biogas, a renewable energy source and a digestate that can be used as organic fertilizer. Because biogas plants are difficult to run with economically profitable results if the process is based only in livestock manure, co-digestion strategies are widely applied in order to enhance the methane production in agricultural biogas plants. For instance, in Denmark (Raven and Gregersen, 2007) and Germany (Weiland, 2006) the digestion of manure and organic waste is a well-established technological practice. This process consists of combining several wastes with complementary characteristics in order to improve the methane production.

In many cases the addition of co-substrates is however based on trial and error practice. Biogas plant operators know well the advantages of adding fat residues or food waste to their biogas plants. Food waste has a high potential for methane production and can be digested rapidly making it a good source of material for anaerobic co-digestion (Lay et al., 1997). According to Zhang et al. (2007), food waste collected from restaurants is a highly desirable substrate for anaerobic digesters, accomplishing 80% of the theoretical methane yield in 10 days digestion time.

Among the co-digested wastes, lipids are also one of the most used (Fernández et al., 2005). Lipids (fats, oils and greases) are one of the major organic matters found in food waste and some industrial wastewaters, such as slaughterhouses, dairy industries or fat refineries (Li et al., 2002). When compared to other organic wastes of different biochemical composition, lipids are theoretically more-interesting for biogas production, since they are reduced organic materials and have higher methane potential (Pereira et al., 2003). Inhibition related to the long chain fatty acids concentration in anaerobic digestion of lipid-rich wastes have been reported in literature, especially in reactors with a continuous feeding (Hwu et al., 1998; Rinzema et al., 1989; Pereira et al., 2003) or even in batch assays (Koster and Cramer, 1987; Lalman and Bagley, 2000, 2001, 2002; Shin et al., 2003). On the other hand, Broughton et al. (1998) stated that anaerobic digestion of sheep tallow with high lipid content was amenable to mesophilic digestion in batch. At the same time Fernández et al. (2005) reported that fats from animal and vegetable origin were almost completely degraded in high percentages in co-digestion with simulated organic fraction of municipal solid waste, confirming that anaerobic digestion of lipids is possible. Moreover, Nielsen and Ahring (2006) followed the performance of reactors fed with a mixture of pig and cattle manure with increasing pulses of 0.5 and 2.0 g_{oleate}/l and conclude that these pulses had a stimulating effect on the overall process.

The aim of this work was to study the behaviour of co-digestion of cow manure with food waste, by applying increasing concentrations of intermittent pulses of residual-oil, from a canned fish processing industry. The establishment of the optimal lipids concentration that can be added ensuring the methane enhancement, without inhibiting the process, was the specific objective of the present work.

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Table 1
Characterization of the co-substrates (results are given as means of triplicates with standard deviations).

Waste	Cow manure (g/l)	Food waste (g/kg waste)	Oily waste (g/kg waste)
Chemical oxygen demand (COD)	39 ± 8	327 ± 73	2690 ± 61
Total solids (TS)	28 ± 5	238 ± 1	971 ± 5
Volatile solids (VS)	21 ± 4	214 ± 7	972 ± 4
Total Kjeldahl nitrogen (TKN)	2 ± 1	13 ± 1	170 ± 83
Fat content	–	20 ± 8	877 ± 32

2. Methods

2.1. Substrates

Three different co-substrates were used in the anaerobic co-digestion process.

(i) Cow manure, collected in a dairy farm in the suburbs of Braga (Portugal) and stored at 4 °C, until use to minimize the decomposition of substrate; (ii) food waste, which was a composite sample (one week based) from the waste produced in the restaurant of the University of Minho, located in "Campus de Gualtar", Braga, Portugal. Food waste was crushed to 1–3 mm particle size and stored at 4 °C during 5 days, until the end of the collecting process. Then it was mixed and stored at –18 °C; (iii) fat, was an oily waste effluent collected from a canned fish processing industry. The characteristics of each substrate are presented in Table 1.

2.2. Start-up and operation

Four 5 l mesophilic (37 °C) continuously stirred tank reactors with hydraulic retention time of 15 days were fed with cow manure and food waste. The digesters were inoculated with the effluent from a stable laboratory mesophilic anaerobic digester fed with cow manure and food waste. The ratio cow manure/food waste in the feed was 1 expressed as total solids (TS), meant for equal amount of both co-substrates expressed as TS. The organic loading rate in the four reactors was 4.6 ± 0.1 gCOD/($l_{reactor}$ day) with a TS/VS content in the feed of 5.2%/4.5% (w/v).

After a stable operation of the four reactors for 148 days, the intermittent feeding of fat was initiated. It should be noted that variations encountered within the test results can be justified by the heterogeneity of the real wastes used as co-substrates in this work.

Reactor 1 (R1) was used as control and so no oily waste was added. In reactors R2, R3 and R4, pulses of oil, were applied, according to Table 2. After the 7th pulse (day 204) methane production in R4 decreased drastically and so no more oily waste was added to this reactor.

Table 2
Punctual loading concentrations of fat ($gCOD_{oil}/l_{reactor}$).

Day (pulse)	R1 ($gCOD_{oil}/l_{reactor}$)	R2 ($gCOD_{oil}/l_{reactor}$)	R3 ($gCOD_{oil}/l_{reactor}$)	R4 ($gCOD_{oil}/l_{reactor}$)
148 (1st)	0	9	12	15
168 (2nd)	0	9	12	15
176 (3rd)	0	9	12	15
183 (4th)	0	9	12	15
190 (5th)	0	9	12	15
197 (6th)	0	9	12	15
204 (7th)	0	12	15	18
211 (8th)	0	12	15	0
218 (9th)	0	12	15	0
225 (10th)	0	12	15	0

2.3. Analytical methods

The routine analysis (COD, pH, TS, VS and TKN) was performed according to standard methods (APHA et al., 1989).

Methane content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 mesh) column, with He as the carrier gas at 30 ml/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110 °C, 110 °C and 35 °C, respectively. Biogas flow rate was measured by using a Ritter Milligascounter (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany).

Volatile fatty acids (VFA) (acetate, propionate, iso-butyrate and n-butyrate) were determined by high-performance liquid chromatography using a Chrompack column (300 × 6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 ml/min. The column was set at 60 °C and the detection was by spectrophotometry at 220 nm. The total fat content was extracted with diethyl ether in a Soxtec System HT2 1045 extraction unit produced by Tecator (Official Methods of Analysis 2003.05, 2007). The Soxtec System is an extraction unit in which a thimble containing the oil matrix was immersed in a boiling solvent. The oily waste was added to thimbles previous weight and placed in the apparatus. A previous weight cup containing boiling stones and 50 ml of diethyl ether was placed in the apparatus. The thimbles were lowered to the "boiling" position, and heat was applied to the plates. The temperature of the circulating heating fluid was 90 °C. The condensers were connected to a recirculation cold bath. After 40 min the thimbles were raised to the "rinsing" position for 40 min. The solvent collection knob was closed and when no additional solvent could be seen collecting in the condensers, the cups were removed and placed in the hood to eliminate last traces of solvent. The samples were weighed and dried a second time for 15 min or until a constant weight was reached.

2.4. Methane potential assays

Two distinct methane potential assays were performed with biomass collected from R1 and R4 in day 203 (end of 6th pulse) and in day 224 (9th pulse). In the first type of methane potential assays, the samples collected from the reactors were incubated in 125 ml batch vials at 37 °C, with a stirring speed of 150 rpm under strict anaerobic conditions, without any added substrate. The methane production was regularly measured by gas chromatography. The maximum methane yield was calculated per kg VS added in each vial. The maximum methane production rate (MMPR) was determined using the values of the initial slope of the methane production curve.

The second type of methane potential tests included the addition of 4.8 g COD/l of oily waste to the same biomass samples. These assays were assessed as previously described. All batch experiments were performed in triplicate.

2.5. Specific methanogenic activity test (SMA)

The SMA of the biomass from the four reactors was assessed in day 203 (end of 6th pulse) and in day 224 (9th pulse), in the pres-

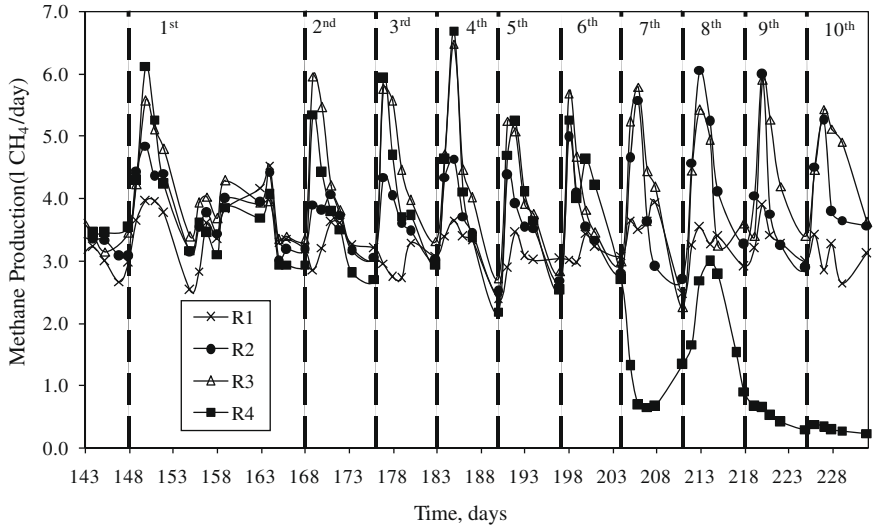

Fig. 1. Time course of methane production (l CH_4/day). (1st to the 6th pulse were 0, 9, 12 and 15 $gCOD_{oil}/l_{reactor}$ in R1, R2, R3 and R4, respectively; 7th pulse was 0, 12, 15 and 18 $gCOD_{oil}/l_{reactor}$ in R1, R2, R3 and R4, respectively; 8th to 10th pulse were 0, 12, 15 and 0 $gCOD_{oil}/l_{reactor}$ in R1, R2, R3 and R4, respectively).

ence of 30 mM of acetate and pressurized with H_2/CO_2 (80/20 (v/v)) at 1 bar. Blank controls were used for acetate (no added substrate) and for the gaseous substrate (pressurized with N_2/CO_2-80/20 (v/v) at 1 bar). Strict anaerobic conditions were maintained.

SMA values were determined by dividing the initial linear slope of the methane production curve by the VS content of each vial measured at the end of the experiment. The volume of methane produced was corrected to the standard temperature and pressure conditions (STP – 1 atm and 273 K).

3. Results and discussion

3.1. Reactors performance

Before the trial all the four reactors were run for 148 days and had achieved a stable performance in terms of methane production (3.2 ± 0.2 l CH_4/day) and VFA concentrations (⩽0.5 gCOD/l), the pH was stable and between 7.7 and 7.9. The percentage of solids reduction in the four reactors was 47, 48, 50 and 48 for TS and 60, 59, 60 and 59 for VS in R1, R2, R3 and R4, respectively.

The effect of oil pulses in the methane production is presented in Fig. 1 and Table 3 summarizes the obtained peaks of methane production after the pulse feeding, in terms of percentage of increase, relative to the control R1 in the same day. A comparison with the expected values is also provided. In the first six pulses, the expected methane enhancement in R2 and R3 was attained. On the other hand, when compared to R1, the 15 $gCOD_{oil}/l_{reactor}$ pulse should enhance 135% the methane production in R4. The expected peaks values ought to be 5.25 l CH_4 higher than R1, nonetheless this was not observed. From the 7th to the 10th pulse the obtained methane peaks were somewhat lower than expected, although the same behaviour was observed. In R4, when the oil pulse concentration increased up to 18 $gCOD_{oil}/l_{reactor}$ (7th pulse-day 204), the methane production decreased to values of 0.68 l CH_4/day, about 18% of the value obtained in R1. On account of that, no more pulses of oil were added to this reactor. From day 212 to 215, a slight increase in the methane production was observed, although it did not achieve the values obtained in R1, which was being fed in the same conditions. It can therefore be concluded that in R4 a long term inhibition from lipids was observed. The oil added in the 12 $gCOD_{oil}/l_{reactor}$ pulse was 86% converted into methane, whereas in the 15 $gCOD_{oil}/l_{reactor}$ pulse only 55% of methane recovery was observed. Therefore, it is suggested that the threshold to enhance methane production, using intermittent inputs of the oily waste in a co-digestion process of manure and food waste, was 12 $gCOD_{oil}/l_{reactor,}$ in order to avoid long term accumulation of lipids.

Table 4 presents the overall average TS and VS percentage of reduction in the four reactors during the experiment, considering samples collected twice a week during all the trial. In general oily waste inputs did not influence TS or VS removal in the reactors.

The effluent soluble COD, total VFA, individual VFA (acetate, propionate, i-butyrate and n-butyrate) and pH are depicted in Fig. 2 for all four reactors.

In the first six pulses, the effluent soluble COD is very similar for all the four reactors, only R4 after the 6th pulse presents a slightly higher value, achieving a peak of 17.9 gCOD/l decreasing, afterwards, to values similar to the other reactors. This peak value was twice the value obtained in R1 at the same day. When the concentration of lipids applied was 9 $gCOD_{oil}/l_{reactor}$, the value attained for the soluble COD was very similar to the control reactor R1. In the pulses of 12 and 15 $gCOD_{oil}/l_{reactor}$ the maximum value of soluble COD attained was 13 and 18 gCOD/l. An increase in the soluble COD was detected in R4, matching the methane production decline. The values of soluble COD in this reactor did not decrease until the end of the experiment even with no more addition of oil, confirming the persistent inhibition of the system.

Table 3
Peak of methane production after the pulse feeding, in terms of percentage of increase relative to the control R1 in the same day. (Expected theoretical increase percentage). (Results are given as means with standard deviations).

Pulse #	Day #	R1	R2	R3	R4
1st to 6th	148, 168, 176, 183, 190, 197	–	42 ± 15(41)	82 ± 12(88)	80 ± 9(135)
7th to 10th	204, 211, 218, 225	–	70 ± 15(88)	69 ± 18(135)	(182)

Table 4
TS and VS reduction (%).

Reduction (%)	R1	R2	R3	R4
TS	46.7 ± 6.7	45.8 ± 5.6	43.7 ± 6.2	45.1 ± 5.4
VS	53.5 ± 6.1	52.1 ± 5.0	49.1 ± 5.7	51.0 ± 4.8

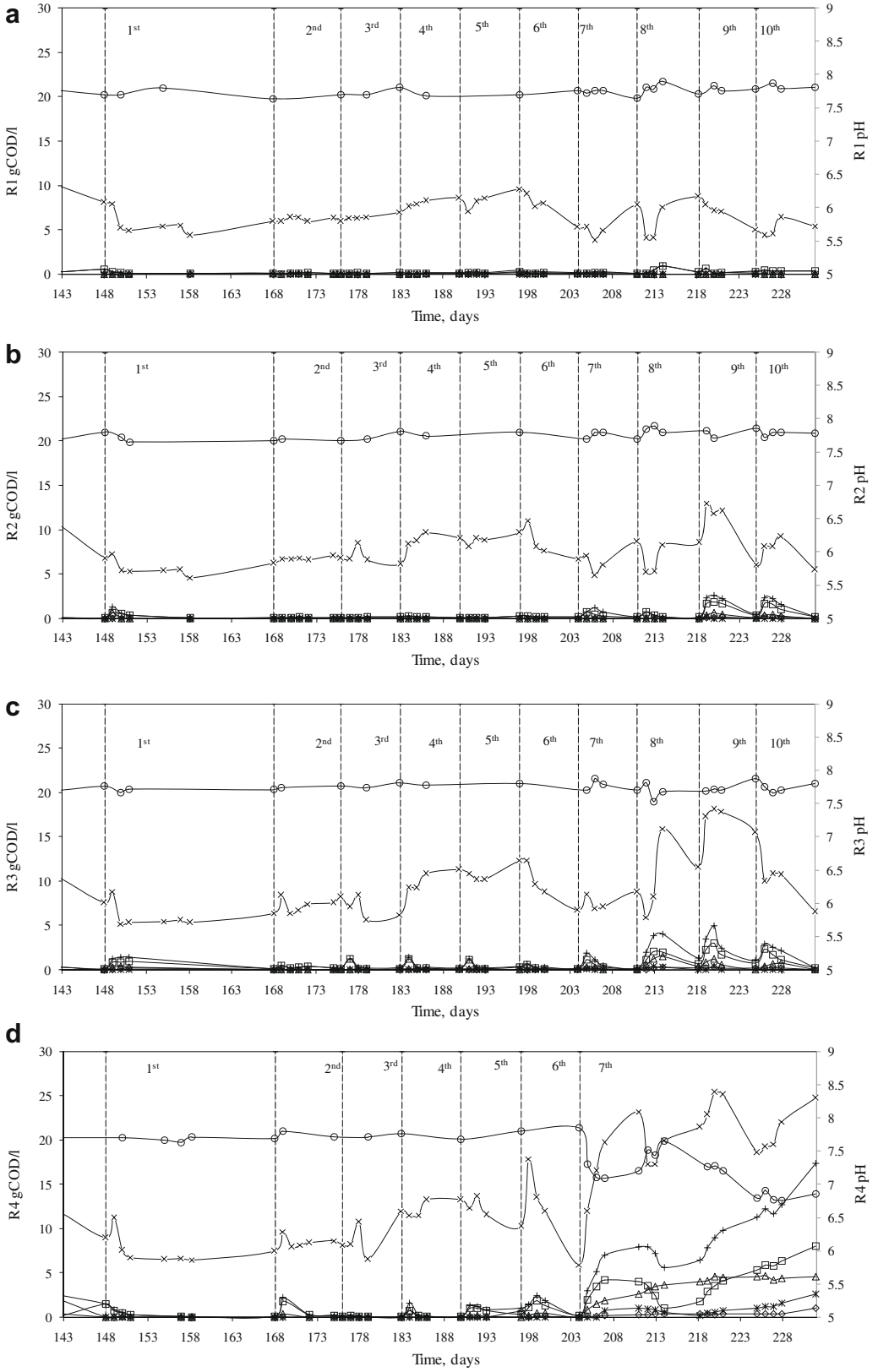

Fig. 2. Effluent soluble COD (-×-), total VFA (-+-), acetic acid (-□-), propionic acid (-△-), iso-butyric acid (-◇-), n-butyric acid (-∗-) and pH (-○-) in R1 (a), R2 (b), R3 (c) and R4 (d). (1st to the 6th pulse were 0, 9, 12 and 15 $gCOD_{oil}/l_{reactor}$ in R1, R2, R3 and R4, respectively; 7th pulse was 0, 12, 15 and 18 $gCOD_{oil}/l_{reactor}$ in R1, R2, R3 and R4, respectively; 8th to 10th pulse were 0, 12 and 15 $gCOD_{oil}/l_{reactor}$ in R1, R2, and R3, respectively).

VFA dynamics followed the general trend presented by the soluble COD. After the 7th pulse, the VFA levels in R4 increased significantly, attaining values of 8, 11, 17 gCOD/l on days 210, 224 and 232, respectively. In this case, the VFA composition in acetate was 51% on day 210 and 47% on days 224 and 232.

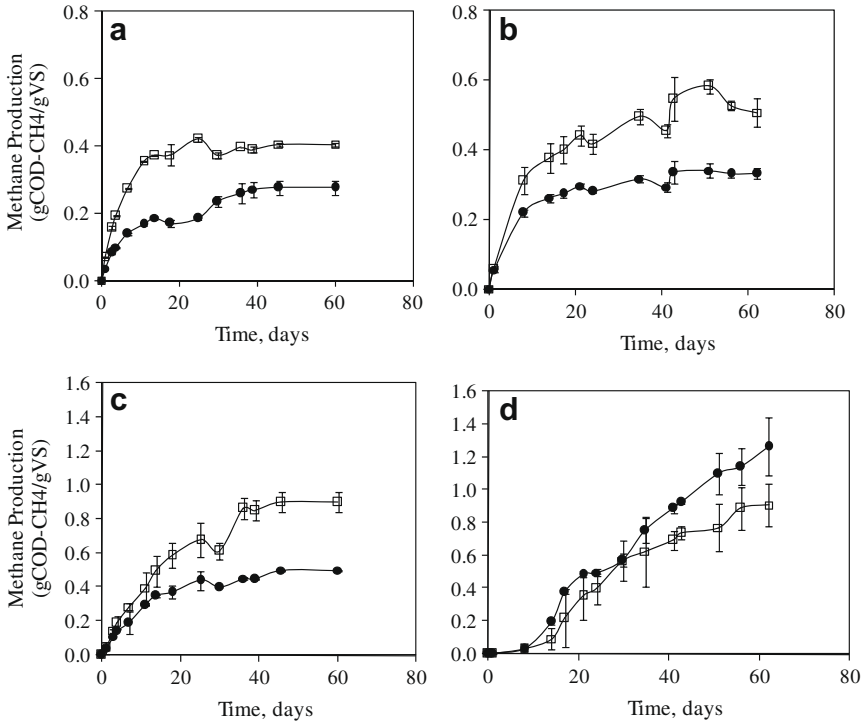

Fig. 3. Methane production (gCOD-CH_4/gVS) from the methane potential tests in R1 day 203 (a), R1 day 224 (b), R4 day 203 (c) and R4 day 224 (d) (-●- biomass collected from the reactor, -□- biomass with additionally 4.8 gCOD/l of oily waste). The error bars represent the standard deviation.

After the day 204, the pH values in R4 decreased (Fig. 2d), although the measured values were always higher than 6.5, this parameter did not recover until the end of the experiment, similarly to the soluble COD and VFA contents.

3.2. Methane potential assays

The time course of the cumulative methane production in the methane potential tests is depicted in Fig. 3 for the controls and for the tests with 4.8 gCOD/l of oily waste. Biomass collected from R1 and R4 on days 203 and 224 was used in the assays. The control tests without addition of oily waste, accounted for the methane production due to the residual substrate. All the tests were performed with the reactor effluent, without any pre-treatment of degassing or washing.

The experiment with the biomass collected from R4 in day 224 was done to determine if the inhibition observed in the methane production in the reactor was permanent or reversible (Fig. 3d).

From Fig. 3d it is clear that, in batch conditions, after a lag-phase of approximately 10 days, the consortium collected from R4 on day 224 (when methane production was already inhibited) started to mineralise the residual substrate, likely including lipids or long chain fatty acids adsorbed or entrapped onto the biomass and fibres, as was previously suggested by Pereira et al. (2005).

The information presented in Table 5 evidences that the presence of oil enhanced the maximum methane production rate (MMPR) in all assays, with the exception of the sample collected from R4 on day 224. Therefore, the presence of oily waste increased the rate of methane production in the co-digestion of cow manure and food waste, except when the solid matrix was severely loaded with lipids as was the case of R4.

The response of R1 due to the addition of 4.8 $gCOD_{oil}$/l was similar in the days 203 and 224 with an average increase of 0.13 gCOD-CH_4/gVS. In the biomass collected from R4 on day 203, the addition of 4.8 $gCOD_{oil}$/l, equivalent to 0.38 $gCOD_{oil}$/gVS, promoted an increase of 0.4 gCOD-CH_4/gVS, suggesting a complete mineralisation of the added oily waste, whereas in R1, only 34% of the expected methane production was observed. Therefore, the anaerobic community that was previous submitted to the oily pulses performed a better conversion of the fat added to methane.

Table 5
MMPR (gCOD-CH_4/gVS day) and maximum methane yield (gCOD-CH_4/gVS) obtained in biodegradability assays (results are given as means of triplicates with SD).

Day	Biomass	MMPR (gCOD-CH_4/gVS day)	Maximum methane yield (gCOD-CH_4/gVS)
203	R1	0.020 ± 0.001	0.28 ± 0.02
	R1 + 4.8 gCOD/l oil	0.039 ± 0.001	0.40 ± 0.01
	R4	0.037 ± 0.001	0.50 ± 0.01
	R4 + 4.8 gCOD/l oil	0.051 ± 0.007	0.90 ± 0.06
224	R1	0.026 ± 0.001	0.37 ± 0.01
	R1 + 4.8 gCOD/l oil	0.038 ± 0.003	0.50 ± 0.04
	R4	<0.010	nd
	R4 + 4.8 gCOD/l oil	<0.010	nd

nd – not determined.

3.3. SMA tests

On days 203 and 224, respectively, the end of 6th and 9th pulses SMA tests were performed with acetate and H_2/CO_2 as individual substrates (Fig. 4). Samples collected from all the oily waste concentration pulses applied were assessed. The samples are from the solid matrix (not as homogenous as a liquid matrix) explaining the differences between the triplicates and the significant standard deviations obtained.

For the pulses of 12 and 15 $gCOD_{oil}/l_{reactor}$, two different reactors were analysed and the behaviour was similar, irrespective of the past different history of each reactor.

From Fig. 4 it is feasible to realize that the result of the SMA in acetate presents an enhancement for the pulse concentration of 12 $gCOD_{oil}/l_{reactor}$. Above this value, the SMA value in the presence of acetate decreases and for the pulse concentration of 18 $gCOD_{oil}$/

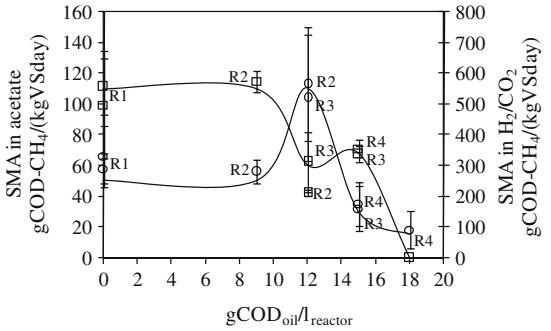

Fig. 4. SMA measured in the presence of acetate (-○-) and H_2/CO_2 (-□-) after addition of different concentrations of oily waste pulses. The error bars represent the standard deviation. [0 $gCOD_{oil}/l_{reactor}$ samples collected on days 203 and 224 in R1, which was devoid from oil pulses; 9 $gCOD_{oil}/l_{reactor}$ samples collected on day 203 in R2; 12 $gCOD_{oil}/l_{reactor}$ samples collected on days 203 and 224 in R3 and R2, respectively; 15 $gCOD_{oil}/l_{reactor}$ samples collected on days 203 and 224 in R4 and R3, respectively; 18 $gCOD_{oil}/l_{reactor}$ samples collected on day 224 in R4].

$l_{reactor}$ attained the lowest value. Possibly, the drop in methane production in R4 is due to the fact that lipids adsorbed, accumulated or entrapped onto the biomass promote a physical/chemical barrier delaying the transfer of substrates and products, as previously described by Pereira et al. (2005).

4. Conclusions

Co-digestion processes of manure and food waste can be improved by addition of oily wastes. The threshold input of oily waste to enhance the methane production in the co-digestion of cow manure and food waste was 12 $gCOD_{oil}/l_{reactor}$, considering the mixture of lipids present in the oily waste added. This corresponds to a continuous feeding of 100/10 ($V_{manure}/V_{food\ waste}$) with intermittent oil pulses of 5% (V_{oil}/V_{manure}). A pulse feeding of 18 $gCOD_{oil}/l_{reactor}$ induced a persistent inhibition of the process, detected by the decrease in pH to a minimum of 6.5 and an increase in effluent soluble COD and VFA.

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