

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

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## Abstract

Pulses of fat were added to completely mixed reactors fed with dairy cow manure (CM) and food waste (FW). After achieving a stable performance at an organic loading rate (OLR) of  $4.6 \pm 0.1$  gCOD/(L<sub>reactor</sub>.day), an oily effluent (OE) from a canned fish processing industry was fed in the form of pulses, raising the lipids concentration up to 9, 12, 15 and 18 gCOD<sub>fat</sub>/L<sub>reactor</sub>. The highest fat concentration of 18gCOD<sub>fat</sub>/L<sub>reactor</sub> promoted a reversible inhibition in the methane production. 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 fat can enhance the methane production in the co-digestion of CM and FW.

## Keywords

Anaerobic digestion; biogas; cow manure; food waste; lipids; oily effluent.

## INTRODUCTION

In many commercial digestion plants the biogas yield from animal manure is often too low to make production economically viable without subsidies and/or selling prices substantially above the market rate (Raven and Gregersen, 2007). Biogas plants are difficult to run with economically profitable results, if the process is based only in livestock manure. Hence, an urgent need to enhance the methane production from biogas farm plants in order to make the process more economic is mandatory and therefore, strategies for improving the yield of biogas shall be considered.

Anaerobic co-digestion can be one of the main advantages of the anaerobic technology. This process consists of combining several wastes with complementary and balanced characteristics in order to improve the methane production. Food waste (FW) has a high potential for methane production and can be digested rapidly making it a good source of material for anaerobic co-digestion. According to Zhang et al. (2007), FW collected from restaurants is a highly desirable substrate for anaerobic digesters, accomplishing 80% of the theoretical methane yield in 10 days.

Among the co-digested wastes, lipids are also one of the most used (Fernández et al., 2005). When compared to other organic wastes of different biochemical composition, lipids are attractive for biogas production. This is due to the fact that they are reduced organic materials and have high theoretical methane potential (Pereira et al., 2003). The aim of this study was to promote the enhancement of methane production from the co-digestion of cow manure (CM) with FW using increasing intermittent pulses of residual fat from a canned fish processing industry. The increasing pulses of fat were done in order to set a concentration until which lipids can be added as an enhancement and to ascertain the concentration that hinders methane production.

## MATERIALS AND METHODS

### Substrates

Three different co-substrates were used in the anaerobic co-digestion process. (i) CM, collected in a dairy farm in the suburbs of Braga (Portugal) and stored in a refrigerator (4 °C) until use to minimize the decomposition of substrate; (ii) FW, 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. FW was ground 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 effluent (OE) collected from a canned fish processing industry. The characteristics of each substrate are presented in Table 1.

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

Waste #	CM (g/L)	FW (g/kgwaste)	OE (g/kgwaste)
Chemical Oxygen Demand (COD)	39±8	327±73	2690 ± 61
Total Solids (TS)	28±5	238±1	-
Volatile Solids (VS)	21±4	214±7	-
Total Kjeldahl Nitrogen (TKN)	2 ±1	13±1	170±83
Fat content	-	20±8	998±1

### Start-up and Operation

Four 5L mesophilic continuously stirred tank reactors (CSTR) with hydraulic retention time (HRT) of 15 days were fed with CM and FW. The digesters were inoculated with the effluent from a mesophilic lab-scale anaerobic digester fed with CM and FW. The ratio CM/FW in the feed was 1, expressed as TS. The OLR in the four reactors was  $4.6 \pm 0.1$  gCOD/(L<sub>reactor</sub>·day) with a TS/VS content in the feed of 5.2%/4.5% (w/v). Biogas was analysed for flow rate and methane content.

After a stable operation of the four reactors for 148 days, the intermittent feeding of fat was initiated. It should be noted that all co-substrates used in this work were real wastes and this can be responsible for the variations encountered along the tests. Reactor 1 (R1) was used as control and so no OE was added. In reactors R2, R3 and R4, pulses of OE, were applied, according to Table 2. After the 7<sup>th</sup> pulse (day 204) methane production in R4 decreased drastically and so no more OE was added to this reactor.

**Table 2.** Concentration of fat (gCOD<sub>fat</sub>/L<sub>reactor</sub>) after the pulse feeding.

Pulse #	day	R1	R2	R3	R4
1 <sup>st</sup> , 2 <sup>nd</sup> , 3 <sup>rd</sup> , 4 <sup>th</sup> , 5 <sup>th</sup> , 6 <sup>th</sup>	148, 168, 176, 183, 190, 197	0	9	12	15
7 <sup>th</sup>	204	0	12	15	18
8 <sup>th</sup> , 9 <sup>th</sup> , 10 <sup>th</sup>	211, 218, 225	0	12	15	0

### Analytical Methods

The routine analysis (COD, pH, TS, VS and TKN) was performed according to Standard Methods (1989). Methane content in the biogas was measured by gas chromatography (GC) using a Porapak 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.

Volatile fatty acids (VFA) (acetate, propionate, iso-butyrate and n-butyrate) were determined by high-performance liquid chromatography using a Chrompack column (300x6.5 mm) and a mobile phase of sulphuric acid 5mM 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, dried and weighed.

### Biodegradability Tests

Two distinct biodegradability assays were performed with biomass collected from R1 and R4 in day 203 (end of 6<sup>th</sup> pulse) and in day 224 (9<sup>th</sup> pulse). In the first type of biodegradability tests the samples collected from the reactors were incubated in 125 mL batch vials at 37 °C, 150 rpm under strict anaerobic conditions, without any added substrate. The methane production was regularly measured by sampling the headspace and by analysing the methane content in the GC. The maximum methane yield was calculated per kgVS at the end of the experiment, in order to correct the losses after the mineralization of the CM, FW and lipids associated with the solid matrix. The maximum methane production rate (MMPR) was determined using the values of the initial slope of the methane production curve within the first 14 days. The second type of biodegradability tests included the addition of 4.8 gCOD/L of OE to the same biomass samples. These assays were assessed as previously described. All batch experiments were performed in triplicate.

### Specific methanogenic activity test (SMA)

The SMA of the biomass from the four reactors was accessed in day 203 (end of 6<sup>th</sup> pulse) and in day 224 (9<sup>th</sup> pulse), in the presence of acetate and H<sub>2</sub>/CO<sub>2</sub>. Blank controls were used for acetate (no added substrate) and for the gaseous substrate (pressurized with N<sub>2</sub>/CO<sub>2</sub>-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 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).

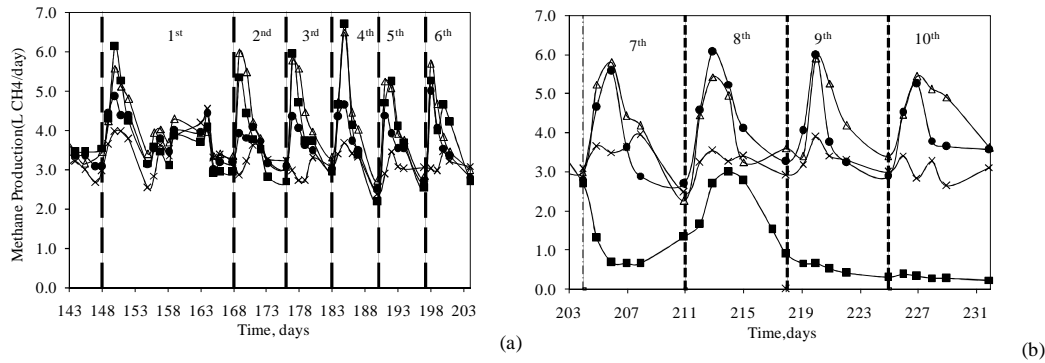
## RESULTS AND DISCUSSION

### Reactors Performance

Before the trial, all the four reactors had achieved a stable performance in the methane production (3.2± 0.2 LCH<sub>4</sub>/day), effluent VFA concentrations (≤0.5 gCOD/L), and the pH was stable between 7.7-7.9. The TS/VS (% w/v) ratio in the four reactors was 4.7/5.9 in R1 as well as in R2, and 4.9/6.0, 4.8/5.9 in R3 and R4, respectively.

The effect of OE pulses in the methane production is presented in Figure 1 (a) for the first six pulses and Figure 1 (b) for the last four pulses. Comparing the peak values achieved in each pulse, the increase was 42 (± 15), 82 (± 12) and 80 (± 9) % in R2, R3 and R4, respectively, having as reference the value on the same day in R1, in the first six pulses. The methane enhancement in R2 and R3 expected due to the pulse of OE was 37% and 82%, respectively, in the first six inputs, which are in accordance with the obtained values. However, comparing the obtained values of R4 with R3 the expected increase was 25% and no methane enhancement was detected.

From the 7<sup>th</sup> to the 10<sup>th</sup> pulse the same behaviour was observed, the increase was 70 (±15) and 69 (±18) in R2 and R3, respectively. These results suggest that the threshold to enhance methane production is 12 gCOD<sub>fat</sub>/L<sub>reactor</sub> using intermittent inputs of the OE. In the 7<sup>th</sup> pulse (day 204) the methane production in R4 decreased to values of 0.68 LCH<sub>4</sub>/day (less than 82% of the value obtained in the same day for R1) on account of that no more pulses of OE 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. The methane decay was detected when the concentration increased up to 18 gCOD<sub>fat</sub>/L<sub>reactor</sub>. The methane production response to a given concentration of lipids input was very similar independently of former inputs to the reactor until a pulse of 18 gCOD<sub>fat</sub>/L<sub>reactor</sub>.



**Figure 1.** Methane production (LCH<sub>4</sub>/day) in the first six pulses (a) [1<sup>st</sup> to the 6<sup>th</sup> pulses in gCOD<sub>fat</sub>/L<sub>reactor</sub> were 0, 9, 12 and 15 in R1, R2, R3 and in R4, respectively] and in the last four pulses (b) [7<sup>th</sup> pulse was in gCOD<sub>fat</sub>/L<sub>reactor</sub> 0, 12, 15 and 18 in R1, R2, R3 and in R4, respectively; 8<sup>th</sup> to 10<sup>th</sup> pulse were in gCOD<sub>fat</sub>/L<sub>reactor</sub> 0, 12, 15 and 0 in R1, R2, R3 and in R4, respectively] (-x- R1, -●- R2, -△- R3 and -■- R4)

Table 3 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 OE inputs did not influence TS or VS removal in the reactors.

**Table 3.** TS and VS reduction (%) (Results are given as means of triplicates with standard deviations).

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

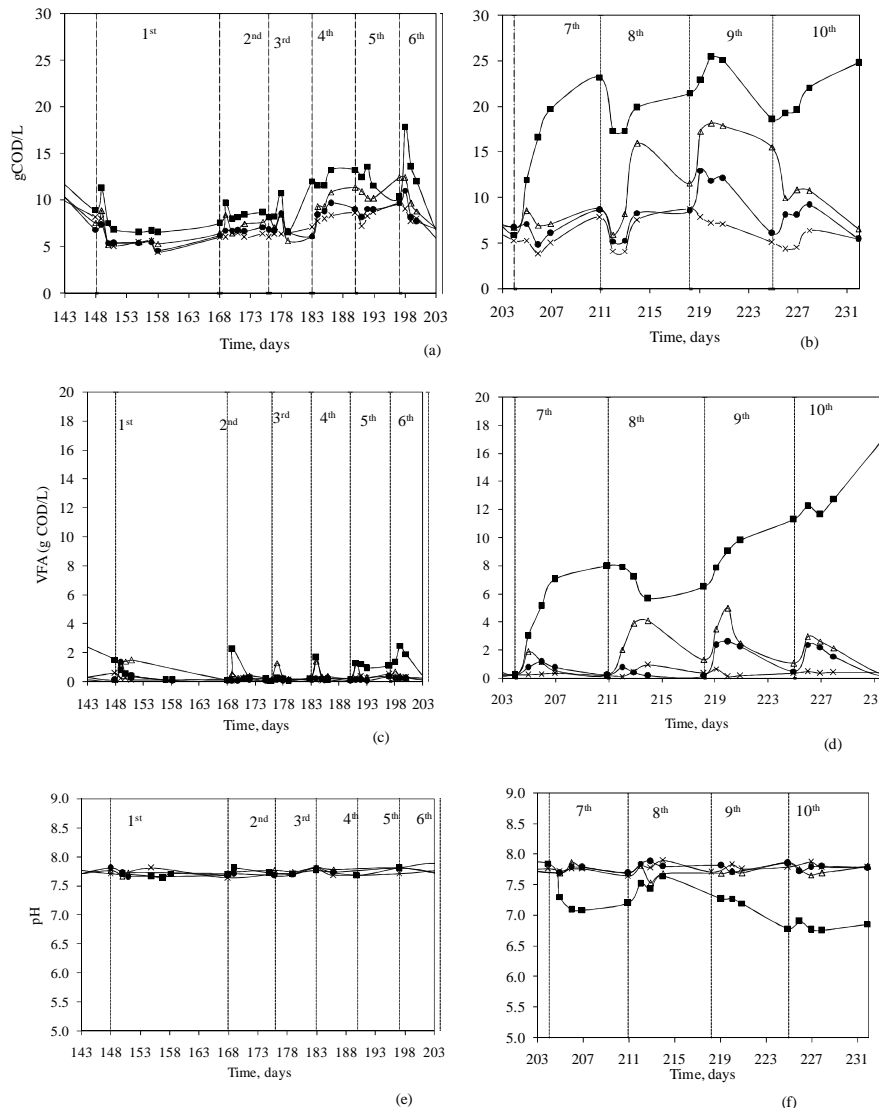
The COD profile in the reactors effluent is depicted in Figure 2 (a) for the first six inputs and Figure 2 (b) for the last four inputs. In the first six inputs, the effluent COD is very similar for all the four reactors, only R4 after the 6<sup>th</sup> input presents a slightly higher value when compared to the other reactors. The soluble COD presents a peak (17.9 gCOD/L) in R4, decreasing to values similar to the other reactors. This peak value was twice the value obtained in R1 on the same day. Likewise to methane production, the soluble COD response to a given concentration of lipids was very similar independently of former inputs to the reactor until a pulse of 18 gCOD<sub>fat</sub>/L<sub>reactor</sub>.

When the concentration of lipids applied was 9 gCOD<sub>fat</sub>/L<sub>reactor</sub> the value attained for the soluble COD was very similar to R1. In the pulses of 12 and 15 gCOD<sub>fat</sub>/L<sub>reactor</sub> the maximum value of soluble COD attained was 13 and 18 gCOD/L in the all trial. 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 OE, indicating that the system presented a difficulty in degrading the accumulated soluble COD.

Analyzing the VFA dynamics in the reactors (Figures 2 (c) and (d)) the maximum value of VFA attained with the inputs (with the exception of the higher concentration of lipids applied in R4) was determined on the day of higher methane production. After the 7<sup>th</sup> input the VFA levels in R4 increased significantly attaining values of 8, 11, 17 gCOD/L at the end of the 7<sup>th</sup>, 9<sup>th</sup> and 10<sup>th</sup> inputs respectively.

After the day 204, the pH values in R4 decreased (Figure 2 (e) and (f)), although the measured values were always higher than 6.5, this parameter did not recover until the end of the experiment

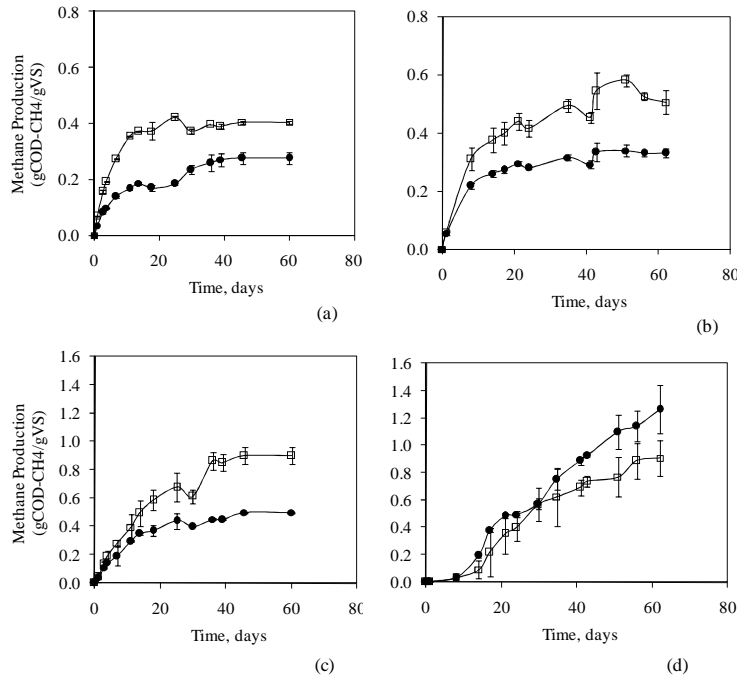
similarly to the soluble COD and VFA contents.



**Figure 2.** Effluent COD (gCOD/L), VFA (gCOD/L), pH profile in the first six pulses (a, c, e) [1<sup>st</sup> to the 6<sup>th</sup> pulses in  $\text{gCOD}_{\text{fat}}/\text{L}_{\text{reactor}}$  were 0, 9, 12 and 15 in R1, R2, R3 and in R4, respectively] and in the last four inputs (b, d, f) [7<sup>th</sup> pulse was in  $\text{gCOD}_{\text{fat}}/\text{L}_{\text{reactor}}$  0, 12, 15 and 18 in R1, R2, R3 and in R4, respectively; 8<sup>th</sup> to 10<sup>th</sup> pulse were in  $\text{gCOD}_{\text{fat}}/\text{L}_{\text{reactor}}$  0, 12, 15 and 0 in R1, R2, R3 and in R4, respectively] (-x- R1, -●- R2, -△- R3 and -■- R4).

### Biodegradability Tests

The time course of the cumulative methane production in the biodegradability tests is depicted in Figure 3 for the controls and for the tests with 4.8g COD/L of OE. Biomass collected from R1 and R4 on days 203 and 224 was used in the assays. From the results of the biomass collected from R4 on day 203 and the same biomass with the addition of OE (Figure 3 (c)) it is reasonable to presume that the anaerobic consortium could cope with the increase in the lipids concentration in order to augment the pulse to 18g  $\text{COD}_{\text{fat}}/\text{L}_{\text{reactor}}$ . Nonetheless, the reactors performance was not in accordance with the results from the batch biodegradability assays. 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 (Figure 3 (d)).



**Figure 3.** Methane production (gCOD-CH<sub>4</sub>/gVS) from the biodegradability tests in R1 day 203 (a), R1 day 224 (b), R4 day 203 (c) and R4 day 224 (d) (-●- biomass collected from the reactor - control, -□- biomass with additionally 4.8gCOD/L of OE). The error bars represent the standard deviation.

From Figure 3 (d) 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 mineralize the long chain fatty acids (LCFA) adsorbed/accumulated onto the biomass.

From Table 4 it can be observed that the biomass collected from R4 on day 203 could mineralize as well the input of 4.8g COD/L of OE, improving the methane production from 0.5 to 0.9 gCOD-CH<sub>4</sub>/gVS.

Table 4. MMPR (gCOD-CH<sub>4</sub>/gVS.day) and maximum methane yield (gCOD-CH<sub>4</sub>/gVS) obtained in biodegradability assays (results are given as means of triplicates with standard deviations).

Day	Biomass	MMPR (gCOD-CH <sub>4</sub> /gVS.day)	Maximum methane yield (gCOD-CH <sub>4</sub> /gVS)
203	R1	0.0126±0.001	0.28±0.02
	R1+ 4.8g COD/L OE	0.0260±0.001	0.40±0.01
	R4	0.0245±0.001	0.50±0.01
	R4+ 4.8g COD/L OE	0.0345±0.007	0.90±0.06
224	R1	0.0185±0.001	0.37±0.01
	R1+ 4.8g COD/L OE	0.0273±0.003	0.50±0.04
	R4	0.0132±0.001	1.26±0.13*
	R4+ 4.8g COD/L OE	0.0064±0.043	0.91±0.18*

\* Maximum methane production after 62 days in batch conditions, not the maximum that could be achieved.

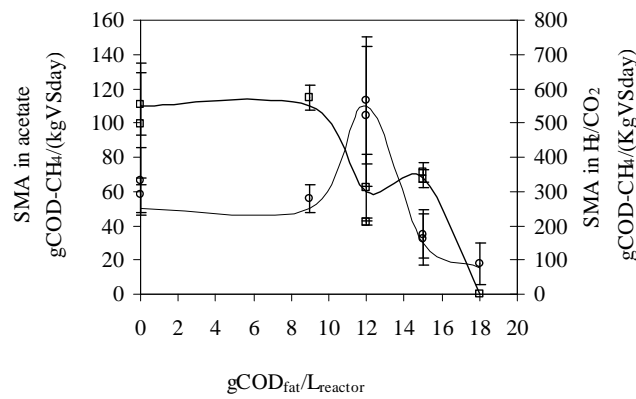
The information presented in Table 4 implies that the presence of OE enhanced the MMPR in all assays, with the exception of the sample collected from R4 on day 224. Actually, in the present work the presence of OE enhanced the MMPR of the co-digestion of CM and FW.

The biodegradability tests using R1 biomass presented analogous results (MMPR and methane

production yield) independently of the biomass collecting day. In fact, the response to the addition of OE was very similar with an increase of 0.12 and 0.13 gCOD/gVS on day 203 and 204, respectively.

### Specific methanogenic activity test

It is important to intensify the knowledge on the dynamics of some key trophic groups during the digestion process, especially when the conditions are not steady and inputs/organic shocks are applied to stable processes. Figure 4 illustrates the results of the SMA tests in the presence of acetate and H<sub>2</sub>/CO<sub>2</sub>. The assays were done on day 203 and 224, respectively the end of 6<sup>th</sup> and 9<sup>th</sup> pulses. Samples collected from all lipids concentration applied were assessed. The error bars represent the standard deviation, which in some cases are significant hence. The samples are from a solid matrix (not as homogenous as a liquid matrix) explaining the differences between the triplicates. All the results for the same applied lipid concentration input were very concordant independently of the day or reactor of biomass collection, pointing out to a similar performance.



**Figure 4.** SMA results in the presence of acetate (-o-) and in the presence of H<sub>2</sub>/CO<sub>2</sub> (-□-) in gCOD-CH<sub>4</sub>/(kgVS.day). The error bars represent the standard deviation.

From Figure 4 it is feasible to realize that the SMA in acetate presents an enhancement for a lipid concentration of 12 gCOD<sub>fat</sub>/L<sub>reactor</sub>. Above this value, the SMA value in the presence of acetate decreases and at 18 gCOD<sub>fat</sub>/L<sub>reactor</sub> attains the lowest value achieved. Possibly the drop in methane production in R4 is due to the fact that LCFA adsorbed onto the biomass promote a physical/chemical barrier delaying the transfer of substrates and products as previously described by Pereira et al. (2005).

In the presence of H<sub>2</sub>/CO<sub>2</sub>, the SMA values started to decrease for input values above 9 gCOD<sub>fat</sub>/L<sub>reactor</sub> as can be seen in Figure 4.

### CONCLUSIONS

The data suggest that the threshold to enhance methane production in the co-digestion of CM and FW is 12 gCOD<sub>fat</sub>/L<sub>reactor</sub>, considering the mixture of lipids present in the OE added. Above this value methane decay was detected attaining almost null production at 18 gCOD<sub>fat</sub>/L<sub>reactor</sub>. All the results for the same lipid concentration input are very concordant independently of day or reactor from when or where the biomass was collected, indicating a similar performance. From a practical point of view, this work demonstrates that controlled intermittent inputs of fat can improve the methane production of the co-digestion of CM and FW.

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