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Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animal and vegetable origin

Anna Fernández, Antoni Sánchez* and Xavier Font

Escola Universitària Politècnica del Medi Ambient

Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1

08100-Mollet del Vallès (Barcelona), Spain.

* Corresponding author: Dr. Antoni Sánchez

Escola Universitària Politècnica del Medi Ambient

Universitat Autònoma de Barcelona

Rbla Pompeu Fabra 1, 08100-Mollet del Vallès (Barcelona), Spain

Phone: 34-93-5796784

Fax: 34-93-5796785

E-mail address: asanchez@eupma.uab.es

Abstract

The potential of mesophilic anaerobic digestion for the treatment of fats of different origin through co-digestion with the organic fraction of municipal solid wastes (OFMSW) has been evaluated. Co-digestion process was conducted in a pilot plant working in semi-continuous regime in the mesophilic range (37 ºC) and the hydraulic retention time was 17 days. During the start-up period the digester was fed with increasing quantities of a simulated OFMSW (diluted dry pet food). When the designed organic loading was reached, co-digestion process was initiated. The fat used consisted of waste from the food industry (animal fat), its composition was very similar to that of the simulated OFMSW in relation to the long chain fatty acid (LCFA) profile. Fat content in the feedstock was gradually increased up to 28%, and hence the organic loading, with stable operation of the digester. Animal fat was suddenly substituted by vegetable fat maintaining the organic loading. No accumulation of LCFA or volatile fatty acids (VFA) was detected in either case. After a short adaptation period, total fat removal throughout the experiment was over 88%, whereas biogas and methane yields were very similar to those of simulated OFMSW. In conclusion, anaerobic co-digestion of OFMSW and fat wastes appears to be a suitable technology to treat such wastes, obtaining a renewable source of energy from biogas.

Keywords: Anaerobic Processes, Biogas, Fat, Long-Chain Fatty Acids, Organic Fraction of Municipal Solid Wastes, Waste Treatment.

1. Introduction

During the last few decades, anaerobic digestion of organic matter has been presented as a suitable technology used for treatment of organic wastes and production of energy from combustion of biogas [1-3]. Anaerobic digestion technology has evolved quickly and, at present, can be competitive with aerobic systems, especially for treating industrial wastewater and organic solid wastes with high organic loading [4].

In anaerobic digestion, co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics, being one of the main advantages of the anaerobic technology. There is abundant literature about the utilization of co-digestion, such as co-digestion of organic fraction of municipal solid wastes and agricultural residues [5,6], organic solid wastes and sewage sludge [7] or more specific wastes [8].

Recent works on co-digestion have been focused on the search of synergisms or antagonisms among the co-digested substrates [9]. For instance, the optimization of the carbon to nitrogen ratio when co-digesting municipal wastes and sewage sludge is pointed as beneficial to methane yield [10]. The improvement of the buffer capacity is also reported as a positive effect in the co-digestion process [11]. On the other hand, some authors have shown negative results in co-digestion processes, which are attributed to the specific characteristics of the digested wastes [12]. Additionally, the configuration of the anaerobic reactor (batch or continuous, one or two stages, mesophilic or thermophilic) has been the objective of some works [13,14]. However, there is scarce information about the response of an anaerobic reactor treating a typical waste when several co-substrates of similar

biochemical composition but with different reported levels of inhibition to the anaerobic microbial communities are co-digested. This is of special interest in the operation of industrial anaerobic digesters, where the feed co-substrates composition may be variable.

Among the co-digested wastes, one of the most commonly used are lipids. Lipids, characterized either as fats or oils and greases, are one of the major organic matters found in food wastes and some industrial wastewaters, such as those coming from slaughterhouses, dairy industries or fat refineries [15]. Lipids included in waste and wastewater consist mainly of triacylglycerides and long-chain fatty acids (LCFAs). Triacylglicerides can be hydrolyzed to LCFAs and glycerol. The profile of LCFAs found in wastes and wastewaters usually depends on the origin (animal or vegetable) of the lipids; however, LCFAs usually contain an even number of carbon atoms, typically within 14-24. LCFAs are successively degraded via the β-oxidation pathway to acetate and hydrogen, which in turn are converted to methane [16,17].

If compared with other organic matter of different biochemical composition, lipids are attractive for biogas production. This is due to the fact that they are reduced organic materials and have a high theoretical methane potential [18]. However, anaerobic treatment of organic wastes with high lipid content presents several problems. On the one hand, adsorption of lipids onto biomass can cause sludge flotation and washout [18,19]. On the other hand, it has been widely reported that high LCFA concentrations can destabilise anaerobic digesters due to inhibition of methanogenic bacteria by possible damage to cellular membrane [20,21]. On that point, however, different values of inhibition concentration for different LCFA are reported. Thus, concentrations of inhibition are in the range of 30-300 $mg \cdot l^{-1}$ for oleic acid [4,22-24], 100-300 $mg \cdot l^{-1}$ for stearic acid [23,24] or 30

$mg \cdot l^{-1}$ for linoleic acid [17]. The variability of the reported inhibition values for LCFAs on anaerobic digestion is often attributed to the different characteristics of the anaerobic population (granular or suspended). Nevertheless, adaptation of microorganisms to high loads of LCFA to degrade concentrations well above the inhibition limits are also reported [22,25]. However, to our knowledge, there is no information about the transition and adaptation of the anaerobic population to a sudden change in the composition of a co-digested lipid (and thus in the LCFA profile to be degraded).

The objectives of this work are: i) to study the suitability of anaerobic digestion to treat a simulated organic fraction of municipal solid wastes (OFMSW) with a high percentage of lipids, determining the optimal and stable organic loading and the operational conditions at the steady state; ii) to investigate the co-digestion of simulated OFMSW and increasing amounts of fats of animal origin (mainly composed of palmitic, stearic and oleic acid); and iii) to determine the effects of changing to a fat of vegetable origin (mainly composed of lauric, myristic and palmitic acid).

2. Materials and Methods

2.1 Materials

Three different substrates were used in the anaerobic digestion: simulated synthetic OFMSW, which consisted of diluted dry pet food (Dog Menu, Affinity Pet Care, Barcelona, Spain), and two kinds of residual fats used as co-substrates. Pet food was chosen as a basic substrate because of its nutritional similarity with OFMSW (fibre, protein and fat content) [26,27]. Pet food was diluted with distilled water according to the organic loading applied

to the digester. Residual fats were: animal fat from the food industry (lard), the composition of which was very similar to that of simulated OFMSW especially in the LCFA profile, and commercial vegetable (coconut) oil. The main properties of the substrates used are shown in Tables 1 and 2.

2.2. Anaerobic digester configuration and operation

A semi-continuous, completely mixed liquid reactor with a working volume of 14 l was used for the anaerobic digestion of wastes. The volume was maintained by means of a level control. The digester is cylindrical and its dimensions are: 20 cm diameter, 58 cm height. Stirring rate during all the experiments was 24 rpm with 4 disc turbines with 6 flat blades. The digester was inoculated with sludge from an industrial-scale anaerobic digestion plant treating municipal solid wastes (Barcelona, Spain). The initial concentration of total volatile solids (TVS) of the reactor was 1.56 $g \cdot l^{-1}$. The digester was inside a chamber where temperature was maintained to 37ºC by a heating device. The substrate was fed 4 times a day using a time-controlled pump. Feed influent was prepared twice a week. A recirculation system was periodically working 6 hours a day to ensure a good homogenization of the digester liquid by pumping a flow of 150 $ml \cdot min^{-1}$. The hydraulic retention time of the digester (HRT) was maintained to 17 days during all the experiments. Biogas produced in the digester was collected and measured daily. A detailed scheme of the digester is presented in Figure 1.

During start-up, the influent organic loading was increased from 0.55 to 2.57 $kgTVS \cdot m^{-3} \cdot day^{-1}$, using diluted pet food, to find the optimal value. After seven HRT under stable conditions, the co-digestion process was started using animal fat. Animal fat was

gradually increased up to 28% of the organic loading. At this point, animal fat was substituted by vegetable fat (coconut oil) maintaining the organic loading. Fats were mixed previously with the simulated OFMSW in the feed influent tank. For each experimental condition, the reactor was continuously operated for at least two HRT to ensure a situation of steady state. Calcium bicarbonate was added occasionally to maintain the alkalinity level.

2.3. Biochemical methane potential assays

Biochemical methane potential (BMP) was assayed to determine the potential toxicity of fats in the co-digestion process. 150 ml of mixed liquor from the digester was placed in 300 ml bottles, previously sealed and purged with N_2. Bottles were incubated at 37ºC and the biogas and methane production were analyzed in batch experiments during approximately 70 hours (when a plateau in the biogas production was reached). In experiments with fats, these were previously diluted with diethylether (DE, 10 $g \cdot l^{-1}$) due to the low solubility of the fats (Lalman and Bagley, 2000). BMP was calculated according to the rate of methane generation per kg of TVS. A blank experiment with only simulated OFMSW was carried out, jointly with a control experiment with simulated OFMSW and DE. Inhibition caused by fats was calculated as the percentage of decrease in the methane generation in relation to that of the control experiment. BMP was statistically analysed by the Tukey's method at 5% level of probability.

2.4. Lipolytic activity

Lipolytic activity was determined using a commercial kit (Roche/Hitachi LIP num. 1821792). Briefly, samples (adjusted to pH=8.0 with a Tris-HCl buffer 400 mM) were

mixed with Triton X-100 (1%) to extract the lipases [30]. After extraction, samples were centrifuged (30 min, 3500g) and filtered (0.45 µm) to remove biomass and solids. This sample is then used for lipolytic activity determination according to manufacturer's instructions.

2.5. Analytical methods

Routine analysis: The feed and digester content were analyzed twice a week for routine parameters. Water content, Total Solids (TS), Total Volatile Solids (TVS), pH and alkalinity were determined according to the Standard Methods [28].

Fat content: The quantification of the total lipid content was carried out using a standard Soxhlet method using n-heptane (99% purity, Panreac, Spain) as organic solvent [29].

Biogas composition: Methane and CO_2 content in 200-µl biogas samples collected from the headspace of the reactor were analyzed by gas chromatography (Perkin-Elmer AutoSystem XL Gas Chromatograph) with a thermal conductivity detector (TCD) and using a column Hayesep 3m 1/8" 100/120. The carrier gas was He in splitless mode (column flow: 19 ml min^{-1}). Oven temperature was maintained at 40ºC during analysis. Injector and detector temperatures were 150ºC and 250ºC, respectively. The system was calibrated with pure samples of methane and CO_2 (99.9% purity, Carburos Metálicos, Spain).

Volatile fatty acid (VFA) analysis: 50 µl of sulphuric acid (98%) were added to 0.6 ml of sample. The acidified sample was then centrifuged (30 min, 3500g) and the resulting supernatant filtrated through a Millipore Millex-FGS filter (0.2 µm). This sample was used for VFA determination by gas chromatography. A Perkin-Elmer AutoSystem XL

Gas Chromatograph with a flame ionization detector (FID) and a HP Innowax 30 m x 0.25 mm x 0.25 µm column was used. The carrier gas was He and with a split ratio of 13 (column flow: 5 ml min^{-1}). An initial oven temperature of 120ºC was maintained for 1 min; then, it was increased to 245ºC at 10ºC min^{-1}, and maintained at that temperature for 2 min. Injector and detector temperatures were 250ºC and 300ºC, respectively. The system was calibrated with different dilutions of a standard mixture of VFAs (including acetic, propionic, butyric, isovaleric, isobutyric, valeric and isocaproic acid from Sigma, Spain) of concentrations in the range of 0-100 mg l^{-1}. Detection limit for VFA analysis was 5 mg l^{-1}.

LCFA analysis: 5 ml of n-heptane (99% purity, Panreac, Spain) were added to 5 ml of sample and mixed for 30 min to extract LCFA. The suspension was then centrifuged (30 min, 3500g) and the resulting supernatant filtrated through a Millipore Millex-FGS filter (0.2 µm). This extract was used for free LCFA determination by gas chromatography. A Perkin-Elmer AutoSystem XL Gas Chromatograph with a flame ionization detector (FID) and a HP Innowax 30 m x 0.25 mm x 0.25 µm column was used. The carrier gas was He and with a split ratio of 13 (column flow: 5 ml min^{-1}). An initial oven temperature of 120ºC was maintained for 1 min; then, it was increased to 250ºC at 8ºC min^{-1}, and maintained at this temperature for 7 min. Injector and detector temperatures were 250ºC and 275ºC, respectively The system was calibrated with different dilutions of a standard mixture of LCFAs (including lauric, mystiric, palmitic, stearic, oleic and linoleic acid from Sigma, Spain) of concentrations in the range of 0-100 mg l^{-1}. Detection limit for LCFA analysis was 5 mg l^{-1}.

2.6. Data analysis

Statistical significance of values of different parameters obtained for consecutive organic loadings applied to the digester was carried out by means of F-test (variance analysis) and Student's t-test (mean analysis) both at 5% level of probability.

3. Results and discussion

3.1. Digestion of simulated OFMSW

Anaerobic digestion of simulated OFMSW was carried out in two steps. Initially, the organic loading of the reactor was progressively increased to find the maximum value under stable conditions. In the second step, the steady state reached was maintained for 7 hydraulic residence times to obtain the main operational parameters for anaerobic digestion of simulated OFMSW. Both periods are shown in Figure 2.

Start-up period was characterized by a parallel increase of the biogas production and TVS removal as the organic loading increased, showing a good acclimation of sludge to simulated OFMSW. However, from day 60, total VFA concentration reached values above 1 $g \cdot l^{-1}$. Although literature values for inhibition caused by VFA are lower than those found in the start-up period [31], the decrease in the biogas production and TVS removal confirmed that the maximum organic loading had been reached (Figure 2). The relatively low values of VFA inhibition in this process were probably caused by the type of VFA detected. In Figure 3, it can be observed that although acetic acid was by far the dominant VFA produced, propionic acid and isovaleric acid were also detected. These acids are reported to be inhibitory for methanogenic bacteria even at trace concentrations [32].

To avoid problems of reactor instability and to permit a stable co-digestion process, organic loading was fixed to a low value of 0.97 kg $TVS \cdot m^{-3} \cdot d^{-1}$. The reactor was then maintained under these conditions during 7 consecutive HRTs showing a highly stable operation (Figure 2), with values of total VFA concentration below 0.1 $g \cdot l^{-1}$ and undetected levels of acetic acid, propionic acid and other VFA. The main results corresponding to the steady state period are presented in Table 3. Values of methane content in biogas and biogas and methane yields were similar to those found for mesophilic anaerobic digesters [33], whereas TVS removal can be considered high when compared with values found for other organic wastes [33], which is probably due to the high biodegradability of simulated OFMSW. Nevertheless, it must be pointed that literature data shows some dispersion in the values obtained for typical operational parameters of anaerobic digesters, especially with organic solid wastes.

3.2. Inhibition of biochemical methane potential by fats

Inhibition experiments were carried out previously to the co-digestion process. Thus, anaerobic sludge from the reactor was incubated with different concentrations of animal fat (lard), the composition of which was very similar to that of simulated OFMSW (Table 2) being palmitic, stearic and oleic acid the dominant LCFAs. Results showed a percentage of inhibition within the range of 10-20% calculated for fat additions corresponding to increments of 10 to 25% of the organic loading at steady state. However, statistical analysis indicated that there were no significant differences among treatments. Nevertheless, it could be concluded that inhibition of BMP by animal fat should be low or inexistent and the co-digestion process could be initiated. Additionally, some authors have

reported studies on acclimation of anaerobic sludge to several inhibitors, including fats and LCFAs [4,22,25], which might be expected to occur in the anaerobic reactor.

3.3. Co-digestion with animal fat

Anaerobic co-digestion was started from the steady state. The first co-substrate used was animal fat (lard), which LCFA profile was very similar to that of simulated OFMSW (Table 2), with palmitic, stearic, oleic and linoleic acids accounting for the 91% of the total LCFA content. Fat content in the influent of the reactor was progressively increased by a percentage of 4, 7, 14, 21 and 28% of the organic loading at steady state. In Figure 4, the main results for this period are presented.

Throughout the co-digestion process, levels of VFA were negligible (total content below 0.1 $g \cdot l^{-1}$) indicating a high stability of the reactor, which was confirmed by the presence of steady profiles of pH and alkalinity of the reactor (data not shown). On the other hand, LCFA content was very low during the co-digestion with animal fat, with only traces of oleic acid detected (below 12 $mg \cdot l^{-1}$). These values of LCFA content are quite lower than those reported in other studies, where higher contents cause inhibition to methanogenic microorganisms [4,17,22-24]. Additionally, no accumulation of fat was detected in the reactor, and total fat removal was within the range of 88% and 94%, whereas TVS removal was within the range of 70-80%. It is likely that the similar composition between animal fat and fat from simulated OFMSW provoked both the animal fat fast hydrolysis and the quick consumption of hydrolysis products (glycerol and LCFA) [34]. To confirm this, the lipolytic activity of the reactor was determined (28% animal fat period). However, no lipolytic activity was detected throughout the co-digestion process.

Although the reason for this is not clear, it may be hypothesized that lipolytic activity was beyond the detection level or that lipases were used as substrate as soon as they hydrolyzed the fats. Moreover, some authors point out that the extraction of lipases from sludge is not easy and that lipase activity remains immobilized on the organic particulate matter and/or intracellular membrane or cell-wall bound [30,35].

Biogas production, biogas and methane yields are presented in Figure 4 for each period of animal fat with increasing concentration. Data in Figure 4 for each period was analysed for statistical significance. Periods that are statistically different are shown in Figure 4 with different letters. A significant decrease ($p<0.05$) was observed for all the parameters when animal fat co-digestion was initiated, which was probably due to a partial inhibition of the methanogenic activity as it was also observed in the BMP assays. The inhibition observed in the reactor was in the range of 25% for the studied parameters. However, when the fat loading was progressively increased, biogas and methane yields recovered the steady state levels and biogas production increased according to an increase in the total organic loading of the reactor. These results seemed to confirm that sludge was progressively acclimated to the new co-substrate, as it has been reported in other works [22,25].

During the co-digestion with animal fats, methane content in biogas slightly increased from 58% (steady state) to 61% (28% animal fat period). However, in the case of our study, the low increase in the methane content did not rise significantly the methane yield (Figure 4). In other works, although methane yield increases when lipids are used as co-substrates, the theoretical methane yield calculated according the beta-oxidation of

LCFA is not usually reached, which is often explained by some degree of inhibition caused by LCFA [15,16].

3.4. Co-digestion with vegetable fat

Animal fat (28% period) was suddenly substituted by vegetable fat (coconut oil) maintaining fat concentration and the rest of operational conditions of the reactor. Results for this last period of co-digestion are also presented in Figure 4. It is worthwhile to notice that LCFA profile for vegetable fat is completely different from that of animal fat and simulated OFMSW (Table 2) being short-chain saturated LFCA the most predominant (lauric acid, mystiric acid and palmitic acid accounting for the 74% of the total LCFA content). Despite this fact, only a slight increase in the VFA concentration was observed during the first days of vegetable fat feeding (data not shown), whereas LCFA concentration and other routine parameters (pH and alkalinity) remained steady. There were other operational parameters, such as methane yield, where there was not statistical difference ($p<0.05$) from that found on of animal fat, whereas total fat removal was very high (97%). These results showed that an acclimatized anaerobic sludge can degrade fats from different sources (and chemical composition). Besides, it seems that no important metabolic changes are implied in the hydrolysis of different fats and in the degradation of their LCFAs.

4. Conclusions

A pilot size digester was operating for nearly two years treating wastes with high lipid content. From this period of study, the main conclusion is that no important differences in the performance of the anaerobic co-digestion were observed when a fat from

animal origin was suddenly changed by a fat of vegetable origin with a completely different LCFA profile. This may indicate that no important metabolic changes are implied in the degradation of different LCFAs with an acclimatized sludge. This is of special interest in the industrial application of co-digestion process, where the co-substrates may be variable in composition and, thus, in expected inhibition.

Additionally, other relevant conclusions from this work are:

- During the steady state of the digester, treating a simulated OFMSW, high percentages of TVS removal were obtained (73%), jointly with typical yields of biogas and methane generation (0.8 m^3 biogas per kg of TVS degraded, 0.5 m^3 methane per kg of TVS degraded, 58% of methane in biogas).
- After an adaptation period, the co-digestion with fat increases the amount of biogas produced according to the applied organic loading. The yields of biogas and methane generated per kg of TVS degraded are similar to those found with OFMSW, being the methane content slightly higher in the presence of fats.
- Both fats from animal or vegetal origin were almost completely degraded in high percentages (94% for animal fat and 97% for vegetal fat), which confirmed that anaerobic digestion is a suitable technology for treatment of these wastes.

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Tables

Table 1: Main properties of the simulated OFMSW (dry pet food) according to manufacturer's data.

Parameter	Value
Moisture (%)	9.0
Total Volatile Solids (%, dry basis)	91.0
Total Protein (%, dry basis)	21.0
Total Fat (%, dry basis)	10.0
Total Cellulose (%, dry basis)	3.3
Ashes (%, dry basis)	7.0

Table 2: Percentage of the main LCFAs found in the fats from different substrates.

Substrate	Lauric (C12:0)	Mystiric (C14:0)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Other LCFA (<1%)
Pet food	0.2	2.2	32.9	14.9	33.8	5.6	10.4
Animal fat	ND*	3.0	30.0	17.0	38.0	6.0	6.0
Vegetable fat	45.5	18.5	10.4	3.3	8.7	2.2	11.4

*ND: not detected

Table 3: Operational parameters for the digestion of simulated OFMSW at steady state.

Parameter	Value	Standard deviation
Organic loading (kg TVS$\cdot m^{-3}\cdot d^{-1}$)	0.97	-
Total VFA concentration ($g\cdot l^{-1}$)	< 0.1	-
Biogas production ($l\cdot d^{-1}$)	8.0	1.5
TVS removal (%)	73	4
Biogas yield ($m^3\cdot$ kg TVS removed^{-1})	0.8	0.3
Methane yield ($m^3\cdot$ kg TVS removed^{-1})	0.5	0.2
Methane in biogas (%)	58	2
Total alkalinity (meq. $CO_3^{2-}\cdot l^{-1}$)	50	5
pH	8.0	0.2

Legends to Figures

Figure 1: Scheme of the digester.

Figure 2: Organic loading, Total VFA concentration, Biogas production and TVS removal during the period of digestion of simulated OFMSW.

Figure 3: Concentrations of different VFAs during the period of digestion of simulated OFMSW.

Figure 4: Biogas production, Biogas and Methane yield for the different treatments in the co-digestion of simulated OFMSW and fats. Solid bars correspond to average values and vertical lines correspond to standard deviation. Consecutive treatments with different letters are statistically different.

Fig. 1: Fernández et al.

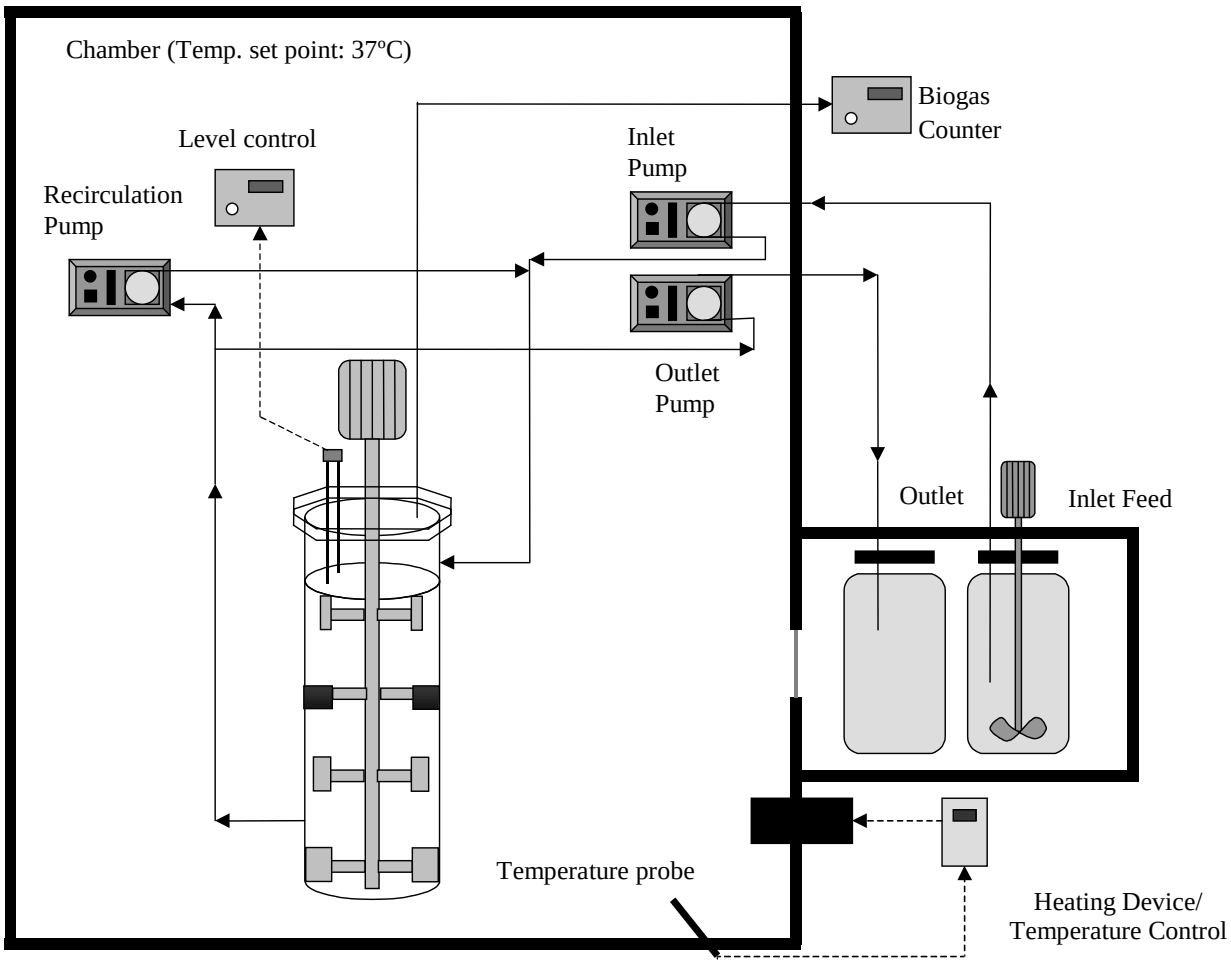

Fig. 2: Fernández et al.

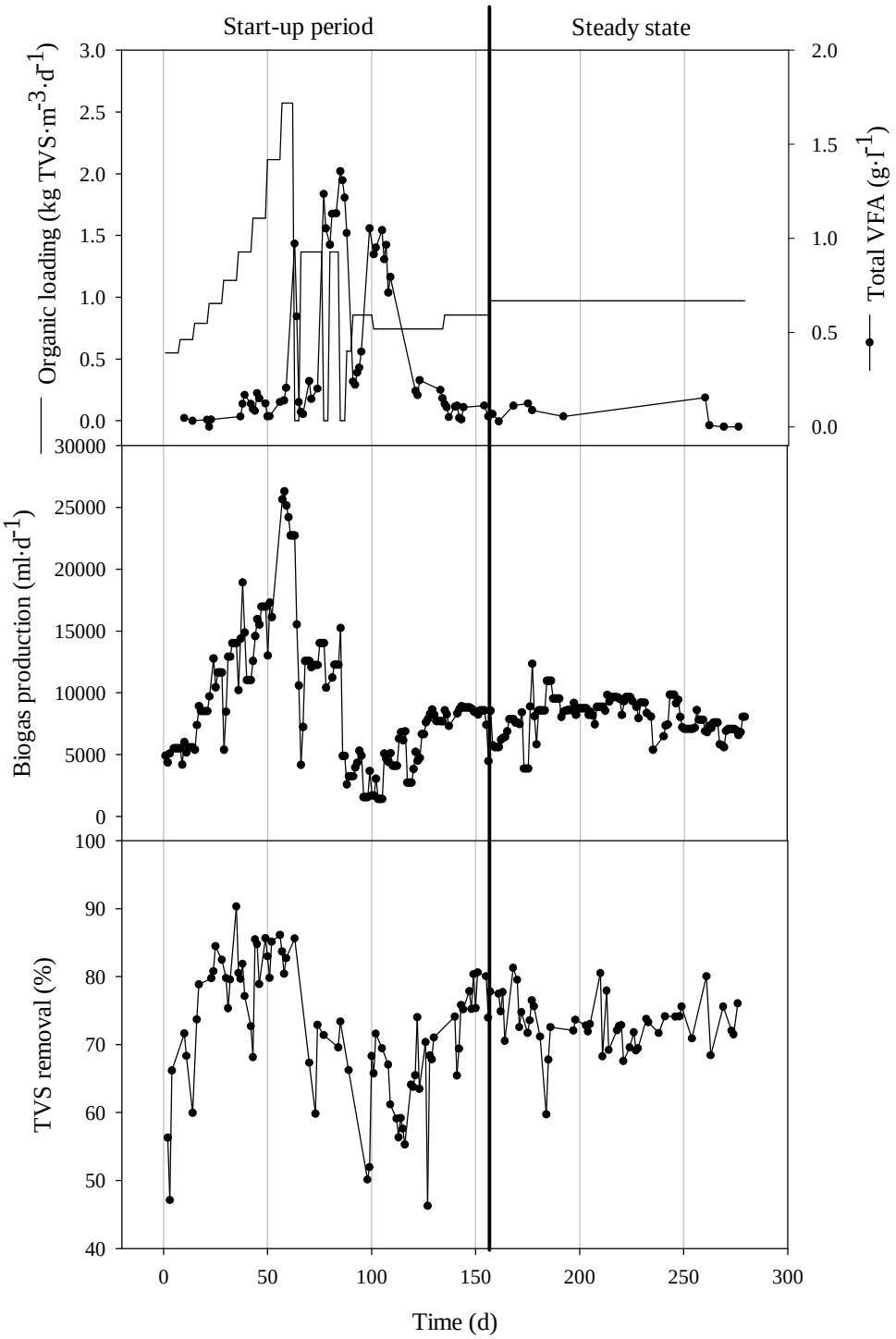

Fig. 3: Fernández et al.

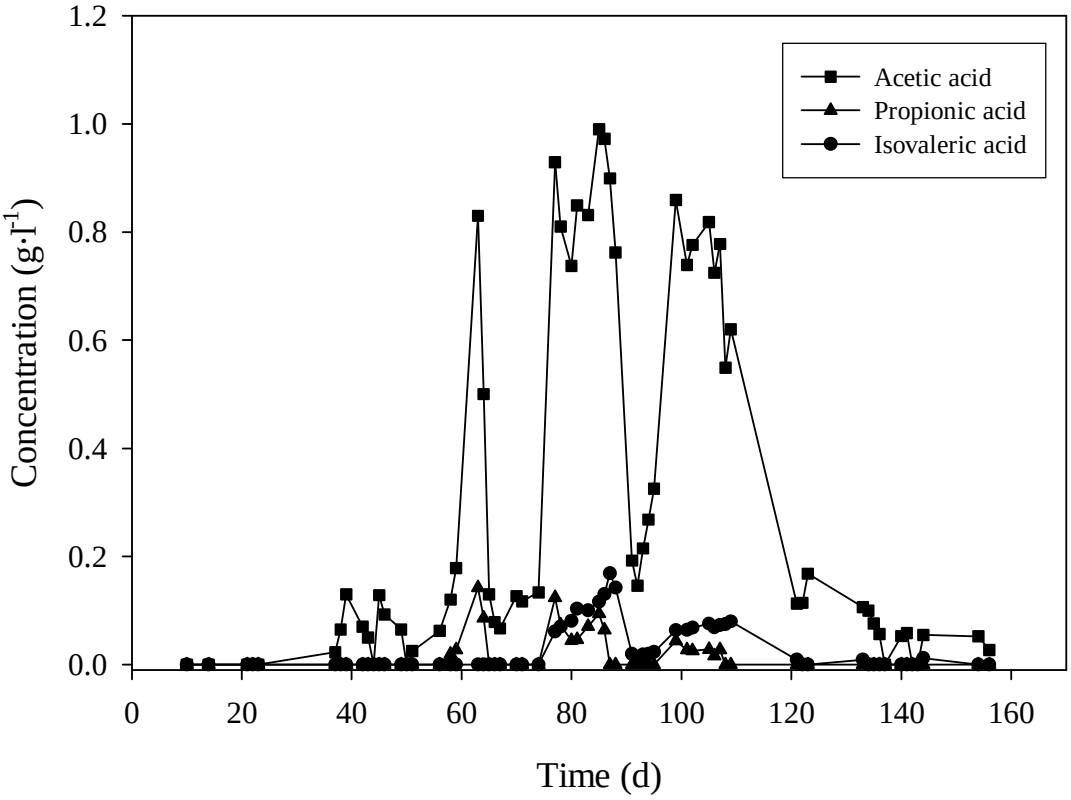

Fig. 4: Fernández et al.