

# Pilot scale comparison of single and double-stage thermophilic anaerobic digestion of food waste

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## Abstract

This study compared the performances of single and two-stage anaerobic digestion processes of food waste. The processes were monitored by taking into account both the steady state process performances and the transient conditions. In addition to a conventional univariate analysis, a multivariate analysis to increase the validity of the results of the comparison study was also performed. The transient states caused peaks due to a high organic loading rate, simulating possible overloading events and the recovery capacity of both processes (resilience). The specific gas production of the methanogenic reactor of the two-stage process was higher (0.88 m<sup>3</sup> biogas/kgVS) than for the single-stage process (0.75 m<sup>3</sup> biogas/kgVS). This finding was related to the increase in the removal efficiency (of 17%). Considering the pilot-scale results, a comparison of mass and energy balance, and costs (assuming the upgrading of the biogas produced) was also discussed.

**Keywords:** Anaerobic digestion, Food waste, Energy comparison, Cluster analysis, Control chart, Statistical analysis

## 1. Introduction

The municipal solid waste produced within the EU was 252 Mt in 2011 (EUROSTAT, 2011) with an average per capita production of 541 kg/inhabitant per year. Up to 40% of this waste was organic material of good quality (high biodegradability and low content of inert material) (Bernstad and la Cour Jansen, 2012). A better quality of the waste was possible due to the successful implementation of separate collection systems in recent years (Cecchi and Cavinato, 2015). The amount of food

wasted is expected to increase by 44% globally between 2005 and 2025 (Messenger, 2016). Landfilling of this type of material can cause an increase in methane emissions from 31 Mt to 43 Mt on a global scale (Messenger, 2016). Organic waste such as segregated food waste, can be conveniently treated via anaerobic digestion (AD). Currently, more than 16,000 AD plants are running within the EU, 20% of which treat organic waste (European-biogas.eu, 2015). Anaerobic digestion performed at the single stage in which four microbiological reactions, hydrolysis, acidogenesis, acetogenesis and methanogenesis, occur concurrently in the same reactor has been extensively studied. Some literature examples are Fernandez-Rodríguez et al. (2013), and Micolucci et al. (2016), treating the organic fraction of the municipal solid waste (OFMSW). Recently, Kastner et al. (2012), have been studied how to define an efficient method for the generation of biogas from organic food waste. Historically, the single-stage reactor has been the most used process for organic waste treatment (Ahamed et al., 2016), Gallert and Winter (1997) studied mesophilic and thermophilic AD of sourcesorted organic wastes, Lissens et al. (2001), studied the process performance for municipal solid waste digestion. Bolzonella et al. (2003) at semi-dry thermophilic conditions. Bouallagui et al. (2009), the improvement of fruit and vegetable waste AD performance and the stability with co-substrates addition. Since the dawn of AD, it is noted that hydrolysis is the ratelimiting step if the substrate is in particulate. Therefore, a further development to enhance the biogas production and to cope the bottleneck of the hydrolysis was to divide the anaerobic dry process into two stages. It has been demonstrated that the two-stage process is a particular valuable option (Ghosh et al., 1985): in these hydrolysis processes, the limiting step of the entire process and acidogenesis are performed in the same (first) reactor (Tagliaferri et al., 2016), whereas methanogenesis is performed in a second, specifically dedicated reactor. This allows for an increase in the organic loading rate and the simultaneous reduction of the hydraulic retention time; thus, an improvement in the performances of these reactors is expected (Cheng et al., 2016). The use of twostage processes for substrates with a low biodegradability, such as waste-activated sludge, has been largely demonstrated as beneficial (Ge et al., 2011).

The use of one-versus two-stage anaerobic digestion processes for food waste has been tested on a laboratory scale by different researchers. Ganesh et al. (2014) performed an AD process in a single-stage reactor and reported a methane yield of 0.45 m<sup>3</sup> CH<sub>4</sub>/kgVS and a volatile solids (VS) removal rate of 83%. AD was also performed in a two-stage system and showed significant reduction in VS; however, the energy and mass balance showed that the single-stage process was 33% superior in terms of biogas production and energy yield compared with the two-phase process. The lower energy yield of the two-phase system was due to the loss of energy during hydrolysis in the first-phase reactor, and the deficit in methane production in the second-phase reactor attributed to the COD loss

due to biomass synthesis and adsorption of slow biodegradable COD onto the flocs. Schievano et al. (2012)

compared the one- and two-stage AD process by applying the same organic loading rate in the two systems and focusing on both chemical and microbiological aspects. The results showed an average methane concentration of 68% and 55% in the two-stage and single-stage systems, respectively. The specific methane production was 351 LCH<sub>4</sub>/kgVS for the two-stage system and 404 LCH<sub>4</sub>/kgVS for the one-stage system. Later, the same authors (Schievano et al., 2014) emphasized that two-stage AD can increase energy recovery from biomass compared with one-stage AD.

This result was evaluated using laboratory-scale reactors with a 300mL operating volume. Nowadays with regard to our knowledge there are no comparative studies at pilot scale.

The aim and the novelty of this study was to verify the process performances of single- and two-phase anaerobic digestion of food on a pilot scale to obtain robust data for comparison. The research considered the mass balances and yields of the two systems. Insights on the start-up phase, transient conditions and steady state conditions were part of the study. A multivariate analysis was performed to support and improve the comparison study.

## **2. Materials and methods**

The research was performed in the experimental hall in the wastewater treatment plant (WWTP) at Treviso (North Italy). Reactors were operated both under steady state conditions (SSC) and at transient conditions, as determined by the hydraulic retention time (HRT) and the variation of the organic loading rate (OLR) to verify the resilience of the systems. Stainless steel CSTRs (AISI-304) were used with a working volume of 230 l for the one-stage digester, whereas the two-stage system included two reactors of a volume of 200 and 760 L. Mechanical anchor agitators ensured mixing occurred to maximize the degree of homogenization inside the reactor (Micolucci et al., 2014). The working temperature was set at 55 °C ± 0.1 and maintained by hotwater recirculation running through an external jacket. The electrical heater was controlled by a PT100 e based thermostatic probe. The reactors are reported in Fig. 1.



Fig. 1 Pictures representing the pilot reactors used in this study, on the left the single-stage reactor, on the centre and right first- and second-stage reactors.

To compare the energy yields of the two systems, a scaled-up version of these processes was evaluated using the analytical data of the pilot-scale experiments. The energy balance analyses and the comparison between the energy yields of the two systems were performed for a treatment plant of 100,000 people equivalent (PE) potential basin. A specific heat request of 1 kcal/kg  $^{\circ}\text{C}$ , a temperature of the feed of 10  $^{\circ}\text{C}$ , an average lower heating value (LHV) for the biogas of 5500 kcal/m<sup>3</sup>, a thermal and electrical yields of the heat and power co-generation unit (CHP) of 50% and 40%, respectively, were used (Leite et al., 2016). A typical specific food waste production of 300 g/PEd for the wet weight of food waste (Bolzonella et al., 2006) and an efficiency of typical mechanical waste pretreatment of 90% were considered. Total heat losses were estimated considering the dimensions of the reactors and the typical construction specifications. Specific yields were determined from the data experimentally obtained.

For the economical evaluation the following parameters were used; the gate fee is 85 V/t waste and the digestate has a cost of 60 V/t when sent for composting (Leite et al., 2016). The revenue of energy production is 130 V/MWh (no incentives) (Leite et al., 2016).

## 2.1. Substrate and inoculum

The anaerobic digested sludge that was used as inoculum for the methanogenic reactors (single-stage and second-phase reactors) was collected in the WWTP where a 2000 m<sup>3</sup> anaerobic digester treats the collected biowaste at 35  $^{\circ}\text{C}$ . The sludge was acclimatized for two weeks to the thermophilic temperature (Bolzonella et al., 2003). The substrate used in these experimental tests was food waste,

which originated from door-to-door collection within Treviso Municipality. The fermentative reactor (first stage) was inoculated with food waste and water and then regularly fed into the reactor to reach the OLR required.

## **2.2. Experimental set-up**

After the initial adaptation step, the two different AD systems were operated by applying an organic loading rate of approximately 3.5 kgTVS/m<sup>3</sup> (single-stage and two-stage) and a hydraulic retention time of 20 days. They were maintained under stable condition for 49 days (RUN I), i.e. more than 2 HRTs. Steady state conditions were checked through biogas production and composition aside total solids and volatile solids, chemical oxygen demand and volatile fatty acid concentration in the reactor, concentration of ammonium nitrogen, total Kjeldahl nitrogen and phosphorous.

After RUN I (steady state conditions), a high organic loading rate (stress tests) was applied to both systems according to the following pattern:

- RUN II: Doubling the OLR for one day;
- RUN III: Doubling the OLR for two consecutive days;
- RUN IV: Doubling the OLR for three consecutive days.

The influent and effluent streams of the processes were monitored during the entire experimentation. Analyses of the parameters and biogas yields were conducted in parallel for the two systems. A comparison was performed that considered the biogas productions in terms of yield and composition and total solid removal (TS), and the system instability via measurements of pH, ammonia, alkalinity and volatile fatty acids (VFAs) was analysed.

## **2.3. Sampling and analysis**

The reactor effluents were monitored 3 times per week for total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorous (P). For the TS determination, a drying temperature of 105 °C was adopted, and no losses were caused (Peces et al., 2014). The process stability parameters, i.e., the pH, volatile fatty acid content and distribution, conductivity, total and partial alkalinity, ammonia nitrogen (N-NH<sub>4</sub>/L) and free ammonia concentration (N-NH<sub>3</sub>/L), were measured daily. All the analyses were performed according to the Standard Methods for Water and Wastewater Analysis (APHA/ AWWA/WEF, 2012). The analysis

of the volatile fatty acids was conducted using a AGILENT 6890 N gas chromatograph equipped with a flame ionization detector (T . 200 \_C), a fused silica capillary column, DB-FFAP (15 m \_ 0.53 mm x 0.5 mm thickness of the film), and hydrogen was the gas carrier. The analysis was conducted by increasing the temperature from 80 \_C to 200 \_C (10 \_C/min). The samples were filtered using a 0.45 mm filter. The biogas production was monitored using a flow metre (Ritter Company™), and methane, carbon dioxide and oxygen in the biogas were determined continuously using a portable infrared gas analyser GA2000™(Geotechnical Instruments™) and once a day using a Gas Chromatograph 6890 N, from Agilent Technology™. It was equipped with an HP-PLOT MOLESIEVE column with a 30 \_ 0.53mmID x 25 mm film using a thermal conductivity detector and argon as the gas carrier (79 ml/min). The H<sub>2</sub>, CH<sub>4</sub>, O<sub>2</sub> and N<sub>2</sub> were analysed using a thermal conductivity detector (TCD) at a temperature of 250 \_C. The injector temperature was 120 \_C. There was a constant pressure in the injection port (70 kPa). Samples were taken using a gas-type syringe in 200 mL biogas amounts. Once the entire sample was vaporized, separation of the peaks occurred within the column at a constant temperature of 40 \_C (8 min). Analysis of the food waste composition was performed. Since unprocessed biowaste is highly heterogeneous material, care was taken to obtain representative samples, eliminating or minimizing sample biasing as reported by Malamis et al. (2015). After appropriate mixing, about 10 kg of a representative sample was collected and stored for further laboratory analyses and commodity class to be performed. The mixture was weighed and transferred to a sorting table where the compositional analysis was performed. The methodology used for manual sorting of the mixture into specified biowaste components until the maximum particle size of the remaining waste particles was less than 15 mm.

#### **2.4. Multivariate data analysis**

For a corrected analysis of the processes, a large amount of information should be considered (e.g. pH, partial alkalinity, VFA etc.). The multivariate analysis is an important approach that can improve analysis than classical univariate analysis. In this work two kind of multivariate analysis were carried out: Cluster Analysis and Multivariate Process Control Chart. The Cluster Analysis was performed using Principal Component Analysis (PCA) and it was the aim to evaluate dissimilarity between the single stage and second stage anaerobic digestion. PCA provides an approximation of a data set bringing back two matrices in reply: a loading and a score matrix. These matrices capture the essential data patterns of the original dataset. A score plot graph is obtained plotting the columns of the score matrix. The relationship between observations is displayed and clusters can be identified. Plotting the

columns of the loading matrix shows a graph named loading plot, in which the relationship between variables is presented. Multivariate statistical process control (MSPC) techniques and charts were applied on data to detect abnormal behaviors and outof- control schemes. For this aim, Shewhart control chart was used. This chart can detect an out-of- control condition not only whenever the sample mean (mean of each week) oversteps the control line, but also it can when the variable shows a non random distribution (Montgomery, 2009). A non-random distribution may indicate that process variability is not linked to the process itself but to external causes. Since Shewhart control chart is an univariate chart, dimensionality reduction was accomplished with the PCA model conducted on the standardized matrix of variables (zero mean and unit variance). Descriptive statistics and exploratory data analysis were performed using the open-source program, R (The R Foundation for Statistical Computing, version 3.1.3). Datasets for both experiments, including the results of the analytical procedures, were obtained three times per week (Monday, Wednesday and Friday). The statistical process control chart is defined as a group of methods that evaluate whether a singular process remains efficient and not susceptible to specific problems, which can change and jeopardize the entire course of the process (Mastrangelo and Montgomery, 1995; Mason et al., 1996; Woodall and Montgomery, 1999). For an acceptable region that is limited by an upper (UCL) and a low (LCL) control limit, a control statistic should be calculated and tested to accept or reject the null hypothesis ( $H_0$ : process control) with a certain probability of obtaining a Type I error.

### 3. Results and discussion

#### 3.1. Composition and characterization of the food waste used in this study

An analysis of the composition of the food waste is showed in Table 1. The table shows a food fraction (fruit, vegetable and other organic waste) greater than 85% for the wet weight of the food waste, whereas the remaining percentage is mainly composed of paper (approximately 4%), which is still anaerobically biodegradable, and inert material (approximately 3%).

Table. 1 Composition of the food waste collected in Treviso (Italy).

Fractions	Wet weight %
Fruit and Vegetable	56.3–66.9
Bread and Bakery	4.6–7.0
Meals	0.6–1.9
Fish and Meat	12.8–18.3
Pasta, Rice, Flour, Cereals	9.3–12.8
Paper and Cardboard	1.9–4.6
Inert and Unclassified Materials	0.8–3.8

The fraction of fruit and vegetable was approximately 60% of the overall organic waste. Comparing Treviso values with values from reference (Cavinato et al., 2012), in this study, the fraction of fruit and vegetable was lower, whereas the fraction due to other organic waste (e.g., bread, pasta, dairy, etc.) was higher. Because of these differences in the composition, the total solid content of the organic waste fraction used in this study (Table 2) was higher than that used in reference (Cavinato et al., 2012). The observed results are, however, typical of the Mediterranean Region, as confirmed by data reported in a Greek study (Malamis et al., 2015) in which the organic fraction (fruits, vegetable and other organic waste) was 86.2%, with vegetable and fruits at approximately 60%. These values are in agreement with our study. Compositional analysis of different organic wastes from UK, Finland, Portugal, Greek and Italy was also evaluated for making a comparison. The data comparison is based on the results compiled in the report of the Deliverable “compositional analysis of food waste from study sites in geographically distinct regions of Europe” of the FP7 EU project “Valorisation of food waste to biogas” (Zhang et al., 2013), Treviso (Italy) (Micolucci et al., 2015) and Kifissia, Athens and Tinos (Greece) data (Malamis et al., 2015). A variety of categorisation systems exists for the main components of waste streams, including organic fraction of municipal solid waste, source segregated organic waste or food waste from households.

The comparison of the 5 Countries showed that “Fruit and vegetables wastes” fraction is the largest proportion, with an average 45%e63% of the total wet weight in each case. The fraction of “Meat and fish” was similar in all countries. It is an important aspect due to the fact that this category is likely to make a major contribution to the high protein and nitrogen content of foodwaste, which might lead to stability problems in anaerobic digestion. Despite some variation in the waste compositions, the values for key analytical parameters have a high degree of similarity. While food preferences and cuisine may vary from region to region, the fundamental requirements of human diet and therefore of domestic food waste are likely to remain similar. This is essential if we want to asses this study adaptable in every European Country. With specific reference to data reported in Table 2, it is clear that this material was particularly suitable for the AD process. The VS/TS ratio was 90%, and the COD:N ratio was an average value of 35.

Table. 2 Food waste characterization.

Parameters	Units	Average $\pm$ S.D.	Min	Max
TS	g/kg <sub>w.w.</sub>	298.2 $\pm$ 44.2	244.4	332.3
VS	g/kg <sub>w.w.</sub>	267.6 $\pm$ 32.5	239.8	299.5
VS/TS	%	89.8 $\pm$ 3	87.2	92.8
COD	g O <sub>2</sub> /kg <sub>TS</sub>	960 $\pm$ 141	915	996
TKN	g N/kg <sub>TS</sub>	27 $\pm$ 4	25	31
P	g P/kg <sub>TS</sub>	4.0 $\pm$ 0.2	3.7	4.2

w.w. wet weight.

### 3.2. Comparison of the single-vs. two-stage system for steady state conditions

The comparison of the processes was conducted by considering the data obtained during approximately 50 days of stable operating conditions. Table 3 shows the single-stage (SS) and first- (F1) and second stage (F2) effluents and respective gas yields. The comparison between Boxplots of pH, partial alkalinity, VFA, SCOD, SGP and SMP (for single stage and second stage respectively) is shown in Fig. 2.

Table 3 Process parameter data.

Parameter	Unit	SS	F1	F2
TS	g/kg <sub>w.w.</sub>	27 $\pm$ 1		15 $\pm$ 5
VS	g/kg <sub>w.w.</sub>	17 $\pm$ 1		10 $\pm$ 4
COD	gO <sub>2</sub> /kg <sub>TS</sub>	806 $\pm$ 74	950 $\pm$ 55	754 $\pm$ 42
Total Nitrogen	gN/kg <sub>TS</sub>	44 $\pm$ 1		33 $\pm$ 7
Total Phosphorus	gP/kg <sub>TS</sub>	12 $\pm$ 2		17 $\pm$ 2
pH	–	8.0 $\pm$ 0.1	4.6 $\pm$ 0.3	8.0 $\pm$ 0.1
Partial Alkalinity	mgCaCO <sub>3</sub> /L	3414 $\pm$ 91	–	2715 $\pm$ 113
Total Alkalinity	mgCaCO <sub>3</sub> /L	5311 $\pm$ 117		4943 $\pm$ 241
Free Ammonia	mgNH <sub>3</sub> -N/L	285 $\pm$ 35		385 $\pm$ 28
VFAs	mgCOD/L	892 $\pm$ 68	9997 $\pm$ 3962	548 $\pm$ 96
Specific Gas Production (SGP)	m <sup>3</sup> <sub>biogas</sub> /kg <sub>VS</sub>	0.75 $\pm$ 0.1	–	0.88 $\pm$ 0.01
Specific Methane Production (SMP)	m <sup>3</sup> <sub>CH<sub>4</sub></sub> /kg <sub>VS</sub>	0.45 $\pm$ 0.02	–	0.55 $\pm$ 0.01
Gas Production Rate (GPR)	m <sup>3</sup> <sub>biogas</sub> /(m <sup>3</sup> <sub>reactor</sub> d)	2.5 $\pm$ 0.2	–	2.6 $\pm$ 0.3

SS: single stage.

F1: first stage.

F2: second stage.

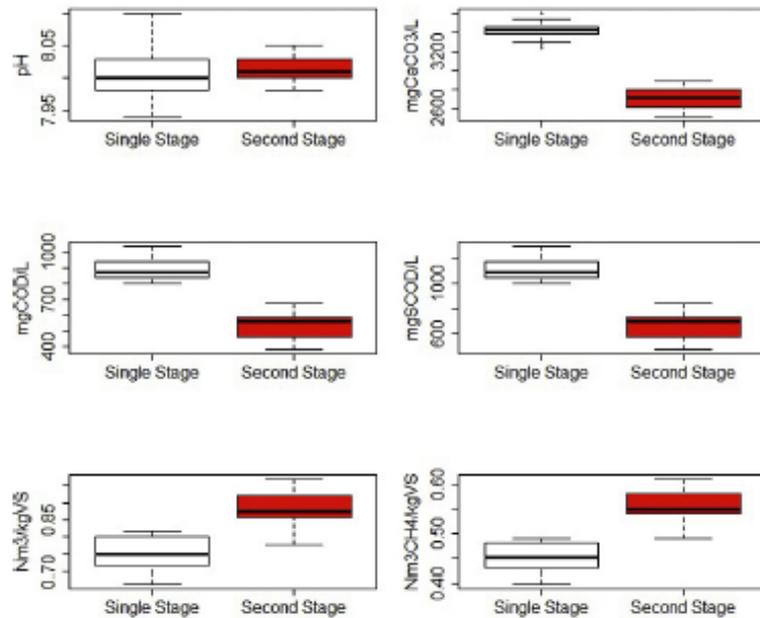
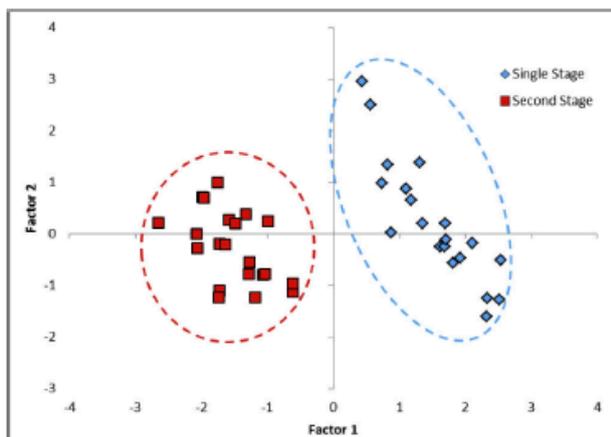


Figure 2 Boxplot of pH, Partial Alkalinity, VFA, SCOD, SGP and SMP for single stage and second stage respectively.

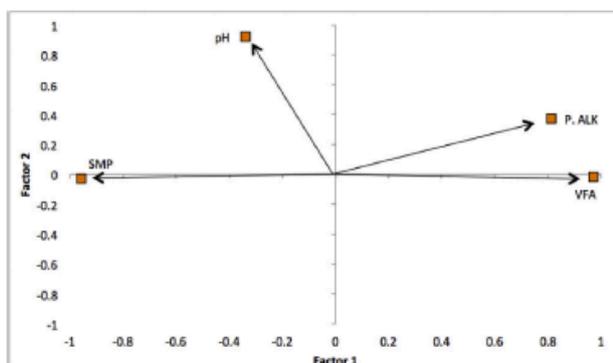
The univariate analysis (Table 3 and Fig. 2) of the single stage and second stage processes shows how, during the steady state condition, the performance in term of biogas and biomethane production was better for the second stage than single stage: both SGP and SMP for second stage were higher (t-test,  $p < 0.01$ ) than for the single stage. Thus, the fermentation played the role in “pretreatment” of the food waste, which was designed to increase the conversion efficiency of food waste to biogas. For the other parameters used to characterize the methanogenic process (pH, partial alkalinity, VFA and SCOD), Table 3 and Fig. 2 show the buffering capacity for the second stage on average was lower than for the single stage (t-test,  $p < 0.01$ ). This evidence can be ascribable to the characteristics of fermentate, particularly the pH value which was always below 5 for the overall period of experiment. Gottardo et al. (2017) studied the effect of recirculation ratio in a two stage anaerobic digestion process, showed how the partial alkalinity of the second stage depends from the pH value of the coupled fermenter: less is the pH value in the fermenter, less is the partial alkalinity in the second stage.

Regardless, the VFA concentration and SCOD for the second stage were on average higher than for the single stage (t-test,  $p < 0.01$ ), and the average pH values were similar for both experiments (t-test,  $p = 0.35$ ). No inhibition phenomenon of the methanogenic activity was individuated during the stable state period for both experiment; although the free ammonia concentration was higher in the second phase than single stage, this parameter never exceeded 600 mg N-NH<sub>4</sub>/L.

Absence of inhibition was also demonstrated by the biogas composition in both experiments. In fact, the single-stage reactor and the second stage exhibited an average percentage of methane that was detected in the SSC of  $56 \pm 2 \%CH_4$  and  $62 \pm 2 \%CH_4$ , respectively, and they were almost constant during the 50-day trial. The decrease in the overall production of biogas and the increase in the percentage of  $CO_2$  could have been caused by the presence of inhibition phenomena that was detrimental to the methanogenic component, for example, due to the excessive presence of volatile fatty acids (Cecchi et al., 2015). During steady state conditions to evaluate if the two systems may be distinguished into two clusters, multivariate Principal Component Analysis was adopted. Considering pH, VFA, partial alkalinity and SMP the cluster analysis showed that the two experimental performances were divided in two different clusters (Fig. 3a of the score plot). As shown in Fig. 3b (loading plot), pH contributes less significantly than the other variables to the division of the two classes, whereas the VFA and SMP exhibited the inverse correlation, suggesting that the second stage had a lower VFA and a higher SMP than the single stage. In contrast, the VFA concentration and partial alkalinity showed a direct correlation; therefore, the second stage exhibited a lower buffer capacity than the single stage.



a



b

Figure 3 a) Score plot and b) loading plot.

The score plot described 91% of the information via the first factor (67%) and the second factor (24%). Ultimately, the two experiments showed that employing a two-stage rather than a single stage configuration significantly affects the methanogenic process.

This is mainly due to the different chemical-physical characteristics of the food waste fed into the single stage digester and the fermented waste fed into the second stage digester.

### 3.3. Stress tests: process-monitoring results

Upon observing the variation in the VFAs concentration in the single stage system after the first OLR increased (day 50th, RUN II), the system reported an initial accumulation of VFAs, and in particular, propionic acid, possibly indicating a potential change in the metabolic pathways (Giuliano et al., 2013). This was followed by a fast biodegradation of the rapidly hydrolysable material; the volatile fatty acid concentration decreased from 666 mgCOD/L to less than 200 mgCOD/L. Conditions far from stability were also subsequently monitored during the following increases of OLR during RUN III. During this period and after two consecutive days of overloading (58th day, RUN III), the soluble chemical oxygen demand (SCOD) content rose to 2289 mgCOD/L, although during the following days the system showed clear signs of rapid degradation of the organic fraction with a decreased concentration of approximately 1000 mgCOD/L. The system, therefore, did not report critically unstable conditions, and maintained average values for VFA and SCOD of 232 mgCOD/L and 1030 mgCOD/L, respectively.

While observing the variations in the SCOD concentration in the single stage system (Fig. 4) during the first OLR increase (day 50th, RUN II), the system did not report a significant accumulation of SCOD.

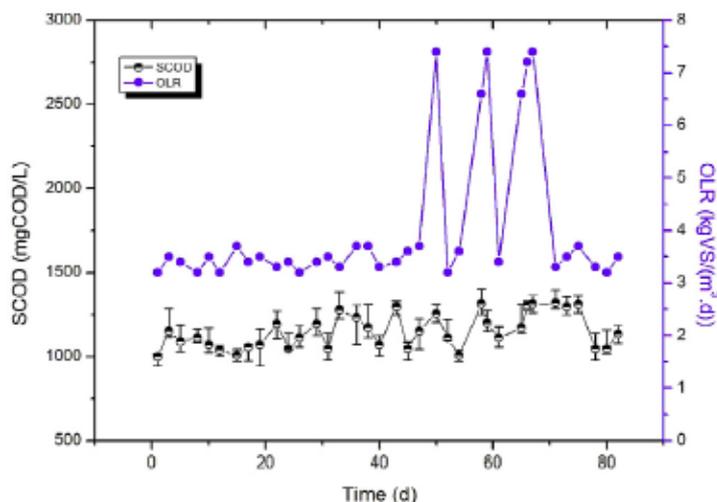


Figure 4 SCOD single stage.

Instead, during this period an increase in biogas production was detected (Fig. 5), and the SGP was near the average value that was determined during the stable period. During the two and three consecutive days of overloading (58th and 59th day, RUN III, 65th, 66th and 67th day, RUN IV) the SCOD trend showed a small increase than previous trend. As in the latter overloading, biogas production increased but the SGP was slightly lower than average value determined in the stable period. The average percentage of methane was  $59\% \pm 4$ , and there were no substantial fluctuations in the CH<sub>4</sub> percentage. Fig. 5 shows that the system increased its GPR consistently with every increase in organic load, thus exhibiting variations in the percentage of methane. Although not entirely critical in the long term, this outlines how the reactor was resistant to perturbations. The total ammonia in the methanogenic reactor also was below the potential inhibition value at an average of 403 mgNH<sub>3</sub>-N/L. In the two stage system, the effect of the fermentation step on the solubilisation of the organic matter was evaluated.

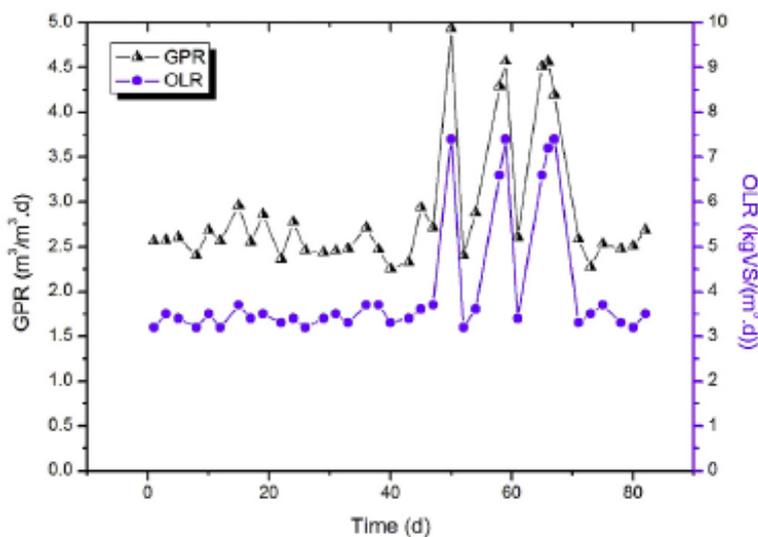


Figure. 5 GPR during the single stage trial.

The VFA concentrations increased up to 14 gCOD/L (during RUN I) with a dominance of acetic and butyric acid. The first stage exhibited significant changes in the VFA and SCOD concentrations due to the waste variability, but this fermentation step withstands a high OLR and acts as a real “buffer system” for the methanogenesis process. The maximum VFA concentration was detected after two consecutive days (RUN III) at approximately 15,890 mgCOD/L.

For the methanogenic step, during transient conditions, the system reported no critical stability issues, and it maintained average SCOD and SGP values close to the averages determined during the stable

period (see Fig. 6). By analysing these RUNs in particular, it was found that during the days when the organic load was doubled up to  $6 \times 10^7$  kgVS/m<sup>3</sup>d, the methanogenic reactor found no substantial increases of the SCOD concentration. The organic fraction that was fed was well degraded and converted to biogas without accumulation in the reactor, maintaining an average VFA and SCOD of 219 mgVFA/L and 844 mgCOD/L, respectively. Low VFA concentration detection brings an important implication.

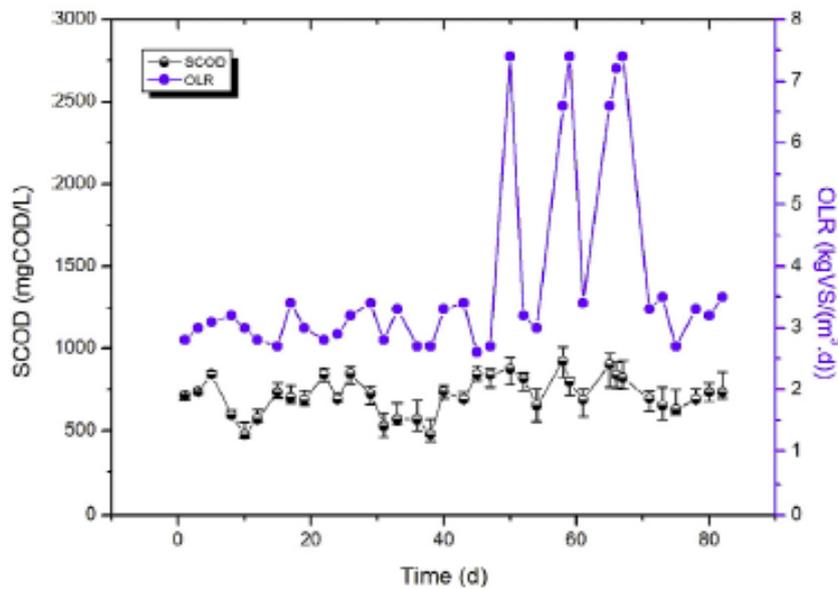


Figure 6 SCOD in the second stage reactor.

The system reported no disturbances to its internal stability, and it exhibited a good tolerance to transient conditions and a high removal efficiency. The average biogas percentage did not substantially change during the period of increased OLR. Contrary to what is observed for the single stage process, during the transition period in the second stage process the concentration of VFA and SCOD did not increase as during the stability period. This indicates that the process exhibited a good tolerance to transient conditions and a high removal efficiency.

During the transient days (RUN IIeIIIeIV), the average percentage of methane was  $61\% \pm 2$ . The biogas production confirmed that the system was able to recover for a hypothetical organic overload, which is relevant from the point-of-view of the upgrading process to a full-scale process (see Fig. 7).

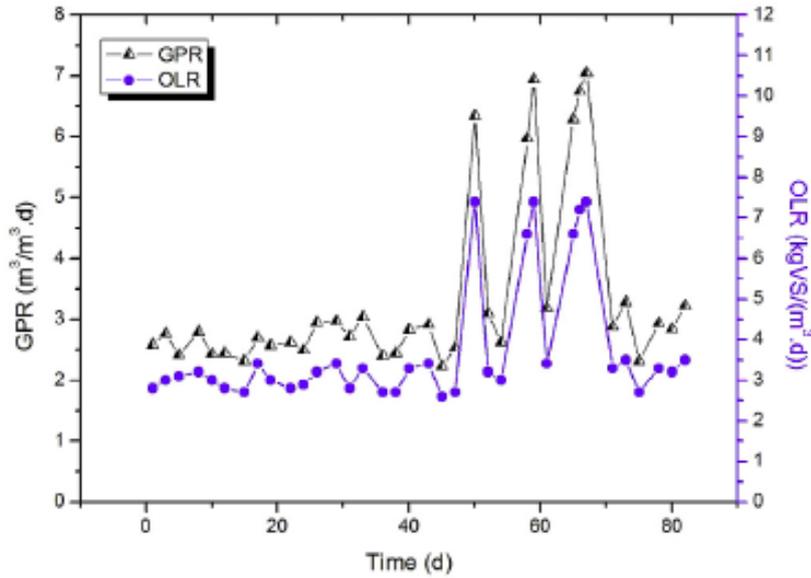


Figure 7 GPR compared with OLR in the second stage reactor.

During the long activity of a full-scale reactor, there may be periods of alteration, for instance due to maintenance times in which the reactor may not be running or used with lower organic loads and then quickly brought to normal feeding condition due to the impossibility of storing the waste. The total ammonia in the fermentation reactor has typically been reported below the inhibition limit with an average value of 505 mgNH<sub>4</sub>-N/L during transient conditions. The value of free ammonia depends on the temperature and pH of the system, and thermophilic conditions could cause problems, but this concentration is well below the level of inhibition (Chen et al., 2008). The total ammonia in the methanogenic reactor has typically been reported below the potential inhibition value at an average value of 300 mgNH<sub>3</sub>-N/L. No evidence of instability was observed during the transient days (RUN IIeIIIeIV) for both experiments, as demonstrated by the VFA and partial alkalinity ratio (Figs. 8 and 9). The term “instability” means that the observed process shows an anomalous behaviour that could indicate a shift of the internal equilibrium to the process.

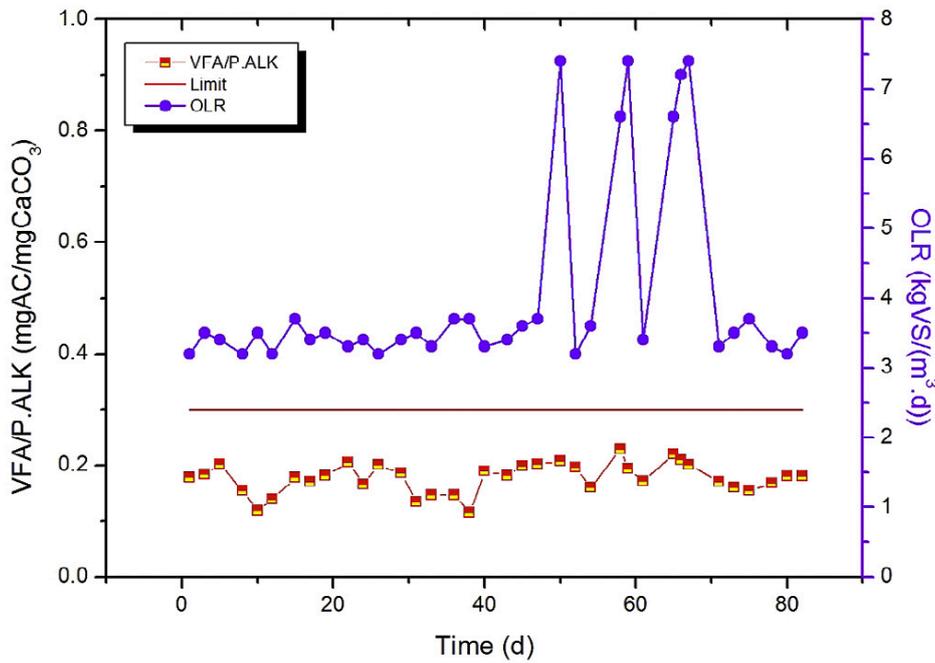


Figure 8 Single stage VFA/alkalinity ratio.

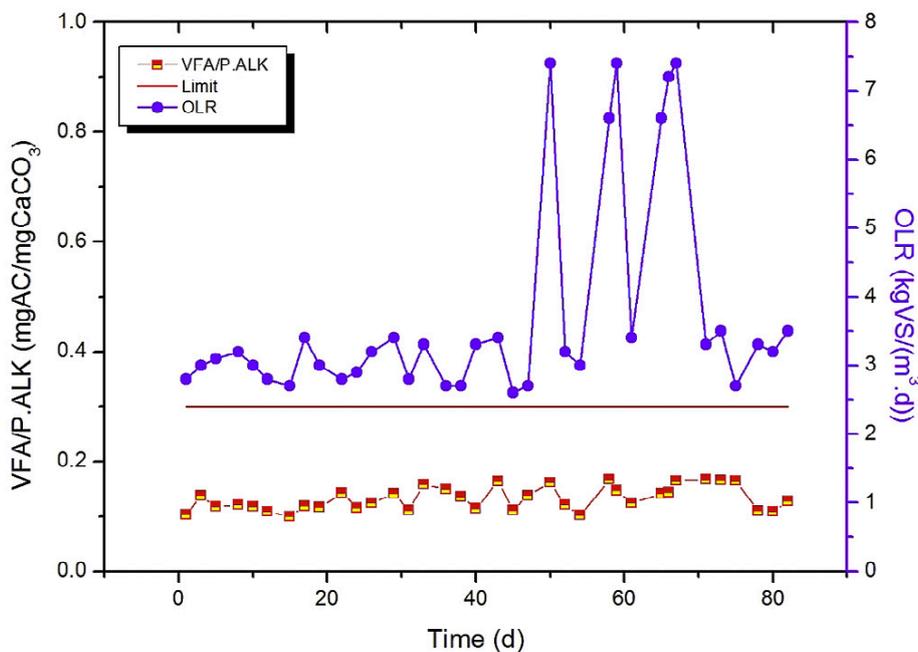


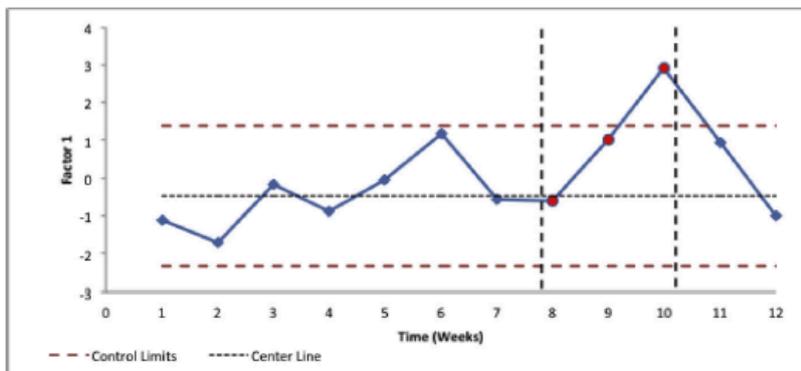
Figure 9 Double stage VFA/alkalinity ratio.

The VFAs and alkalinity are two parameters that show a rapid variation when the AD system gradually moves away from stable conditions. The volatile fatty acids concentration tends to increase and the alkalinity tends to decrease, thus, a useful parameter to consider is the ratio of these two amounts (Chen et al., 2015). In general, a ratio of approximately 0.3e0.4 indicates stable operation of the digester (Cecchi et al., 2015), whereas higher values may indicate the onset of instability issues. During the transient days of our experiments (RUNS II, III, IV), the ratio mentioned above never went above the threshold value; therefore, the systems showed no signs of instability (Figs. 8 and 9).

To understand if the increase in OLR was due to the natural variability of the processes, a control chart (Shewhart control chart coupled with principal component analysis) was used. The principal components included the four variables used for the cluster analysis described previously (pH, VFA, SMP, partial alkalinity). Using several Rank analysis approaches (Scree plot, Kaiser Guttan criterion, corrected average eigenvalue criterion), the first principal component (factor) was determined to be significant. Moreover, the model formed using the first factor was evaluated to be correct via residual analysis (Jackson, 1993).

The control limits were calculated using the steady state period data from RUN I (49 days equal to 2.3 HRTs). By analysing the control chart for the single stage system (Fig. 10a), extraneous data during RUN IV was observed. The extraneous point indicates the anomalous variability of the process; this anomalous variability is due to external causes, specifically, the increase in OLR. In fact, by observing the loading variables for the first factor (pH  $-0.74$ , P. Alk  $-0.83$ , VFA  $.0.94$ , SMP  $e0.89$ ), an increase in the VFAs concentration and decrease in the remaining variables during the transient period was clearly observed, which may be due to a possible imbalance at the start of the system in favour of an acidogenic process. Instead, concerning the second stage system, no extraneous data were observed (Fig. 10b); therefore, the increases in OLR produced no change in the natural variability of the process.

a



b

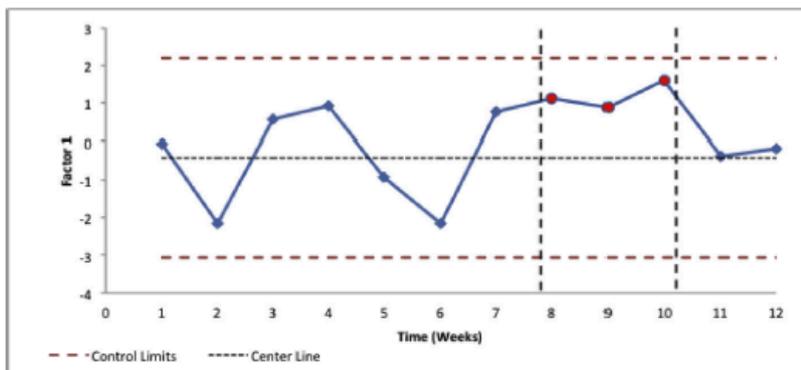


Figure 10 Control charts; a) single stage, b) double stage.

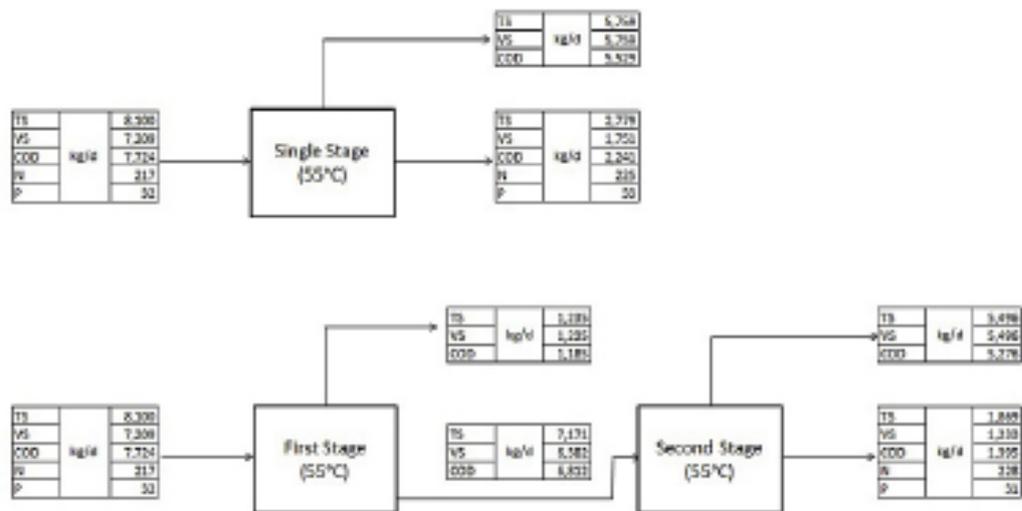


Figure 11 Mass balance.

The mass and energy balance comparison between the two systems were performed for a 100,000 PE potential basin treatment plant (see Fig. 11).

Mass flow of food waste in the AD system was approximately 27,000 kg food waste/d. The total volatile solids in the food waste, assuming the concentration obtained from these experiments (0.267 kg VS/kg food waste) was 7209 kg VS/d. The OLR relative to the one-stage reactor was 3.5 kg VS/m<sup>3</sup>d, i.e., 13 kg food waste/m<sup>3</sup>d. By applying a hydraulic retention time of 20 d, the reactor volume was 2060 m<sup>3</sup>. The total flow rate into the one-stage reactor was 103 m<sup>3</sup>/d of feed. Assuming that the specific gas production of the system is 0.76 m<sup>3</sup>/kg VS, the biogas production in a full scaled-up plant could be 5479 m<sup>3</sup>/d.

Based on the same input data, the two-stage process had a biogas production of 865 m<sup>3</sup>/d (SGP 0.12 m<sup>3</sup>/kg VS), which was obtained during the fermentation stage; this flow rate, on a VS basis, was calculated to be 1235 kg VS/d. Applying a hydraulic retention time of 3.3 d and 16.7 d for the first and second stage, respectively, the volumes of the reactors were 405 m<sup>3</sup> and 2060 m<sup>3</sup>.

The fermentation effluent flow rate was 6381 kg VS/d (7170 kg TS/d) and was feed for the second stage. The methanogenic reactor had an SGP of 0.88 m<sup>3</sup>/kg VS, hence a biogas production of 5551 m<sup>3</sup>/d. The thermal and electrical yields of the combined heat and power unit (CHP) had an overall efficiency of 0.9 (0.5 Heat efficiency and 0.4 Electrical efficiency) (Zamalloa et al., 2011), and the rest was lost.

Specific yields were determined from the experimental data. The thermal energy that could be produced from the single stage system through the CPH was approximately 63,039 MJ/d, and an electric energy of 50,432 MJ/d could have been possible, which corresponds to 14 MWh/d. The

energy request for the single stage reactor corresponds to 23,463 MJ/d. Approximately 83% of the total thermal energy request came from the heating power for the organic waste by pre-heating from 10 °C to 55 °C for the thermophilic process. The remaining 17% was due to the 9.5 °C added to the 55 °C to support the heat dissipation phenomena. Therefore, the thermal balance had a net production of 39,577 MJ/d.

### 3.4. Solid removal efficiency and economical evaluation

The mass balance exhibited an 11% increase for VS removal efficiency for the second stage and single stage. Considering all results from the two-stage system, an efficiency removal increase of 16% compared to the single stage system was determined. In accordance with the higher VS removal efficiency, a higher ammonification rate was revealed. In fact, the ammonification rate in the second phase was 4.4% higher than for the single-stage system (62.0% for the single stage, 66.4% for the double stage). Taken into account that the biogas production from full scaled up plant can be 5479 m<sup>3</sup>/d, it means that 5531 kgVS/d are removed (77% removal efficiency). Therefore, the flow rate of the digestate coming from the single stage was 1750 kgVS/d (2779 kgTS/d). During the fermentation stage, the flow rate was calculated to be 1235 kgVS/d, with a VS removal of 17%. The fermentation effluent flow rate was feed for the second stage. The methanogenic reactor had an efficiency removal of 86% of the VS that were fed as fermentate; hence, the transformation of the biogas produced into VS removed was 5495 kgVS/d. The two reactors, therefore, converted into biogas 6730 kgVS/d of the total input amount of 7209 kgVS/d fed with a substrate removal efficiency of 93%. The output digestate flow rate from the second stage was 1233 kgVS/d (1868 kgTS/d). Comparing the two AD systems, it was observed: 1) the two-stage system had a removal efficiency that was 17% higher than the single stage system, and 2) taking into account a digestate dewatering post-treatment, the two-stage system had 33% less sludge for disposal.

With waste treatment facilities, such as AD facilities, a gate fee offsets the operation, maintenance, labour costs, capital costs of the facility and any profits and the final disposal costs of any unusable residues. The gate fee (or tipping fee) is the charge charged upon a given quantity of waste received at a waste processing facility. Currently, the gate fee is 85 V/twaste. However, digestate has a cost of 60 V/t when sent for composting. Assuming only 130 V/MWh (no incentives) (Leite et al., 2016), the annual increased revenues from electricity (IRE) could be 656,050 V/y. The digestate (after dewatering treatment, approximately 25% TS) disposal costs, assuming 100 V/t (Leite et al., 2016), are estimated at 400,173 V/y. Hence, the single stage system can produce a net profit of 255,877 V/y.

As heat losses, the amount of energy per day dissipated through the reactor wall was assumed. Total heat losses were estimated considering the dimension of the two reactors and the typical construction specifications. Specific yields were determined from experimental data. The thermal energy produced from the system through the CPH was approximately 73,822 MJ/d, and electric energy of 59,058 MJ/d was produced, which corresponds to 16,4 MWh/d. The thermal energy request for the two-stage reactors corresponds to 28,557 MJ/d. From the total thermal energy request, 81% comes from the heating power for the food waste by preheating from 10 °C to 55 °C for the thermophilic process. The remaining 9% is due to the 10.6 °C added to the 55 °C to support the heat dissipation phenomena. Therefore, the thermal balance has a net production of 45,265 MJ/d. Assuming 130 V/MWh (no incentives), the annual increased revenues from electricity (IRE) could be 768,269 V/y. The digestate (after dewatering treatment, approximately 25% TS) disposal costs for 100 V/t for the two-stage system are 272,835 V/y. Hence, the two-stage system can produce a net profit of 495,434 V/y. Capex costs are also required. The cost to actuate a first fermentation reactor with a necessary volume of 405 m<sup>3</sup> is 347,325 V (Leite et al., 2016) (750 V/m<sup>3</sup> reactor plus heat exchanger and pumps/piping costs). The payback time should be 1.45 years.

The final consideration for the single and two-stage comparison is that the two-stage reactor has a net 55% higher energy production compared with the single stage reactor. This amount is due to the higher removal efficiency of the two-stage system that leads to a higher biogas production and some reduced total solid concentration of the resulting digestate.

The upgrading process has been evaluated to achieve biomethane production at a concentration above 98% (Hullu et al., 2008) for the automotive sector.

**Single stage:** The energy required for AD is 5,607,764 kcal/d for the single stage (substrate heating), and as specified above, the boiler efficiency is approximately 0.9. This leads to a net biogas production of 4346 m<sup>3</sup>/d, i.e., 181 m<sup>3</sup> biogas/h. Assuming a cost to upgrade of 0.25 V/m<sup>3</sup> biomethane produced (98% CH<sub>4</sub>) using the pressure swing adsorption (PSA) technique (Hullu et al., 2008), the expenses needed per day are 437 V/d with a biomethane production of 102 m<sup>3</sup>/h (1750 kg CH<sub>4</sub>/d). Assuming a price of 0.99 V/kg, the annual net income (for 360 d/y) would be 466,182 V/y.

**Two-stage:**

The energy required for anaerobic digestion would be 6,825,284 kcal/d for the double stage (substrate heating), and as specified above, the boiler efficiency is approximately 0.9. This leads to a net biogas production of 5037 Nm<sup>3</sup>/d, i.e., 181 Nm<sup>3</sup> biogas/h. Assuming the cost to upgrade is 0.25 V/Nm<sup>3</sup> biomethane produced (98% CH<sub>4</sub>) using the pressure swing adsorption (PSA) technique, the expenses

needed per day would be 508 V/d with a biomethane production of 118 Nm<sup>3</sup>/h (2030 kgCH<sub>4</sub>/d). Assuming a price of 0,99 V/kg, the annual net income (for 360 d/y) could be 540,874 V/y.

#### 4. Conclusions

This study determined how the anaerobic digestion of an organic substrate with a COD content of approximately 1 g/L of food waste or organic fraction of municipal solid waste could be utilized in both single-stage and double-stage approaches. The systems showed resilience and high biogas yields, although interesting issues for possible instability prevention have emerged via our multivariate analysis.

-By comparing the two AD systems, it was observed that the two-stage has a removal efficiency that is 17% higher than the single stage, and using a digestate dewatering post-treatment, the two-stage system has 33% less sludge for disposal.

-The SGP in the second phase was higher (0.88 m<sup>3</sup> biogas/kgVS) than in the single stage reactor (0.75 m<sup>3</sup> biogas/kgVS).

-The overall double-stage system efficiency for removal augmentation was 16% more than for the single stage system.

-Employing a two-phase rather than single stage configuration process significantly affects the methanogenic process. Fermentation played the role of “pretreatment” for the food waste; it promotes the conversion efficiency of the volatile fraction to biogas. The payback time for introducing a fermenter is less than 1.5 year.

-Generally, no evidence of instability was observed during the transient conditions for both experiments, even though upon analysing the control vs. the single stage system, it was observed extraneous data during RUN IV. This extraneous data indicates the anomalous variability of the process.

In spite of what is happening in Europe, where the single stage (De Baere and Mattheeuws, 2015) is generally preferred, implementing separate stages of anaerobic digestion for the organic fraction of the municipal waste treatment is an approach that ensures significant improvements. The use of the dark fermentation is an important stage to produce VFA, platform chemicals for biorefinery approaches. The fermentation step becomes the ultimate substrate production process to create the building blocks of different products, from plastics to methyl esters. The design of new integrated multi-utility facilities can be considered as a parallelism with the bio-refinery widespread concept, directly leading to well-defined economic and environmental advantages deriving from the possibility to use main

existing facilities. A small-scale fermenter before the digester will be capable to extract platform chemicals from the secondary carbon streams.

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### **References**

- Ahamed, A., Yin, K., Ng, B.J.H., Ren, F., Chang, V.W.C., Wang, J.Y., 2016. Life cycle assessment of the present and proposed food waste management technologies from environmental and economic impact perspectives. *J. Clean. Prod.* 131, 607e614. <https://doi.org/10.1016/j.jclepro.2016.04.127>.
- APHA/AWWA/WEF, 2012. Standard Methods for the Examination of Water and Wastewater. Standard Methods.
- Bernstad, A., la Cour Jansen, J., 2012. Separate collection of household food waste for anaerobic degradation e comparison of different techniques from a systems perspective. *Waste Manag.* 32, 806e815. <https://doi.org/10.1016/j.wasman.2012.01.008>.
- Bolzonella, D., Battistoni, P., Susini, C., Cecchi, F., 2006. Anaerobic codigestion of waste activated sludge and OFMSW: the experiences of Viareggio and Treviso plants (Italy). *Water Sci. Technol.* 53, 203e211. <https://doi.org/10.2166/wst.2006.251>.
- Bolzonella, D., Innocenti, L., Pavan, P., Traverso, P., Cecchi, F., 2003. Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: focusing on the start-up phase. *Bioresour. Technol.* 86, 123e129. [https://doi.org/10.1016/S0960-8524\(02\)00161-X](https://doi.org/10.1016/S0960-8524(02)00161-X).
- Bouallagui, H., Lahdheb, H., Ben Romdan, E., Rachdi, B., Hamdi, M., 2009. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. *J. Environ. Manage* 90, 1844e1849. <https://doi.org/10.1016/j.jenvman.2008.12.002>.
- Cavinato, C., Giuliano, A., Bolzonella, D., Pavan, P., Cecchi, F., 2012. Bio-hythane production from food waste by dark fermentation coupled with anaerobic digestion process: a long-term pilot scale

experience. *Int. J. Hydrogen Energy* 37, 11549e11555.  
<https://doi.org/10.1016/j.ijhydene.2012.03.065>.

Cecchi, F., Battistoni, P., Pavan, P., Bolzonella, D., Innocenti, L., 2015. Digestione anaerobica della frazione organica dei rifiuti solidi. Manuali linee Guid 13. Cecchi, F., Cavinato, C., 2015. Anaerobic digestion of bio-waste: a mini-review focusing on territorial and environmental aspects. *Waste Manag. Res.* 33, 429e438. <https://doi.org/10.1177/0734242X14568610>.

Chen, X., Yuan, H., Zou, D., Liu, Y., Zhu, B., Chufo, A., Jaffar, M., Li, X., 2015. Improving biomethane yield by controlling fermentation type of acidogenic phase in two phase anaerobic co-digestion of food waste and rice straw. *Chem. Eng. J.* <https://doi.org/10.1016/j.cej.2015.03.067>.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresour. Technol.* <https://doi.org/10.1016/j.biortech.2007.01.057>.

Cheng, J., Ding, L., Lin, R., Yue, L., Liu, J., Zhou, J., Cen, K., 2016. Fermentative biohydrogen and biomethane co-production from mixture of food waste and sewage sludge: effects of physiochemical properties and mix ratios on fermentation performance. *Appl. Energy* 184, 1e8. <https://doi.org/10.1016/j.apenergy.2016.10.003>.

De Baere, L., Mattheeuws, B., 2015. State of the art of anaerobic digestion of municipal solid waste in Europe. *Int. Conf. Solid Waste 2011-Mov. Towar. Sustain. Resour. Manag.* 416.

European-biogas.eu [WWW Document], 2015. <http://european-biogas.eu/2015/12/16/biogasreport2015/>.

EUROSTAT, n.d. Eurostat 2011 [WWW Document]. <http://ec.europa.eu/eurostat>, <http://ec.europa.eu/eurostat/web/products-datasets/-/ten00110>. Fern\_andez-Rodríguez, J., P\_erez, M., Romero, L.I., 2013. Comparison of mesophilic and thermophilic dry anaerobic digestion of OFMSW: kinetic analysis. *Chem. Eng. J.* <https://doi.org/10.1016/j.cej.2013.07.066>.

Gallert, C., Winter, J., 1997. Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: effect of ammonia on glucose degradation and methane production. *Appl. Microbiol. Biotechnol.* 48, 405e410. <https://doi.org/10.1007/s002530051071>.

Ganesh, R., Torrijos, M., Sousbie, P., Lugardon, A., Steyer, J.P., Delgenes, J.P., 2014. Single-phase and two-phase anaerobic digestion of fruit and vegetable waste: comparison of start-up, reactor stability and process performance. *Waste Manag.* 34, 875e885. <https://doi.org/10.1016/j.wasman.2014.02.023>.

Ge, H., Jensen, P.D., Batstone, D.J., 2011. Increased temperature in the thermophilic stage in temperature phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. *J. Hazard. Mater* 187, 355e361. <https://doi.org/10.1016/j.jhazmat.2011.01.032>.

Ghosh, S., Ombregt, J.P., Pipyn, P., 1985. Methane production from industrial wastes by two-phase anaerobic digestion. *Water Res.* 19, 1083e1088. [https://doi.org/10.1016/0043-1354\(85\)90343-4](https://doi.org/10.1016/0043-1354(85)90343-4).

Giuliano, A., Bolzonella, D., Pavan, P., Cavinato, C., Cecchi, F., 2013. Co-digestion of livestock effluents, energy crops and agro-waste: feeding and process optimization in mesophilic and thermophilic conditions. *Bioresour. Technol.* 128, 612e618. <https://doi.org/10.1016/j.biortech.2012.11.002>.

Gottardo, M., Micolucci, F., Bolzonella, D., Uellendahl, H., Pavan, P., 2017. Pilot scale fermentation coupled with anaerobic digestion of food waste - effect of dynamic digestate recirculation. *Renew. Energy* 114. <https://doi.org/10.1016/j.renene.2017.07.047>.

Hullu, J., De Meel, P. a Van, Shazad, S., Bini, L., 2008. Comparing different biogas upgrading techniques. *Comp. Differ. Biogas Upgrad. Tech* 2, 25. Jackson, D.A., 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 74, 2204e2214. <https://doi.org/10.2307/1939574>.

Kastner, V., Somitsch, W., Schnitzhofer, W., 2012. The anaerobic fermentation of food waste: a comparison of two bioreactor systems. *J. Clean. Prod.* 34, 82e90. <https://doi.org/10.1016/j.jclepro.2012.03.017>.

Leite, W.R.M., Gottardo, M., Pavan, P., Belli Filho, P., Bolzonella, D., 2016. Performance and energy aspects of single and two phase thermophilic anaerobic digestion of waste activated sludge. *Renew. Energy* 86, 1324e1331. <https://doi.org/10.1016/j.renene.2015.09.069>.

Lissens, G., Vandevivere, P., De Baere, L., Biey, E.M., Verstrae, W., 2001. Solid waste digestors: process performance and practice for municipal solid waste digestion. *Water Sci. Technol.* 44, 91e102.

Malamis, D., Moustakas, K., Bourka, A., Valta, K., Papadaskalopoulou, C., Panaretou, V., Skiadi, O., Sotiropoulos, A., 2015. Compositional analysis of biowaste from study sites in Greek municipalities. *Waste Biomass Valorization* 6, 637e646. <https://doi.org/10.1007/s12649-015-9406-z>.

Mason, R.L., Tracy, N.D., Young, J.C., 1996. Monitoring a multivariate step process. *J. Qual. Technol.* 28, 39e50.

Mastrangelo, C.M., Montgomery, D.C., 1995. SPC with correlated observations for the chemical and process industries. *Qual. Reliab. Eng. Int.* 11, 79e89. <https://doi.org/10.1002/qre.4680110203>.

Messenger, B., 2016. *Waste Management World*. <https://waste-management-world.com/a/waste-management,Consult.2016/16/03>.

Micolucci, F., Gottardo, M., Bolzonella, D., Pavan, P., 2014. Automatic process control for stable bio-hythane production in two-phase thermophilic anaerobic digestion of food waste. *Int. J. Hydrogen Energy* 39. <https://doi.org/10.1016/j.ijhydene.2014.08.136>.

Micolucci, F., Gottardo, M., Cavinato, C., Pavan, P., Bolzonella, D., 2016. Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed biowaste for high energy yields recovery. *Waste Manag.* 48 <https://doi.org/10.1016/j.wasman.2015.09.031>.

Micolucci, F., Gottardo, M., Malamis, D., Bolzonella, D., Pavan, P., Cecchi, F., 2015. Analysis of meso/Thermo AD process applied to pressed biowaste. *Waste Biomass Valorization*. <https://doi.org/10.1007/s12649-015-9407-y>.

Montgomery, D.C., 2009. *Introduction to Statistical Quality Control*. John Wiley & Sons Inc. [https://doi.org/10.1002/1521-3773\(20010316\)40:6<9823::AIDANIE9823>3.3.CO;2-C](https://doi.org/10.1002/1521-3773(20010316)40:6<9823::AIDANIE9823>3.3.CO;2-C).

Peces, M., Astals, S., Mata-Alvarez, J., 2014. Assessing total and volatile solids in municipal solid waste samples. *Environ. Technol.* 1e6. [tps://doi.org/10.1080/09593330.2014.929182](https://doi.org/10.1080/09593330.2014.929182).

Schievano, A., Tenca, A., Lonati, S., Manzini, E., Adani, F., 2014. Can two-stage instead of one-stage anaerobic digestion really increase energy recovery from biomass? *Appl. Energy* 124, 335e342. <https://doi.org/10.1016/j.apenergy.2014.03.024>.

Schievano, A., Tenca, A., Scaglia, B., Merlino, G., Rizzi, A., Daffonchio, D., Oberti, R., Adani, F., 2012. Two-stage vs single-stage thermophilic anaerobic digestion: comparison of energy production and biodegradation efficiencies. *Environ. Sci. Technol.* 46, 8502e8510. <https://doi.org/10.1021/es301376n>.

Tagliaferri, C., Evangelisti, S., Clift, R., Lettieri, P., Chapman, C., Taylor, R., 2016. Life cycle assessment of conventional and advanced two-stage energy-from-waste technologies for methane production. *J. Clean. Prod.* 129, 144e158. <https://doi.org/10.1016/j.jclepro.2016.04.092>.

Woodall, W.H., Montgomery, D.C., 1999. Research issues and ideas in statistical process control. *J. Qual. Technol.* 31, 11.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. *Bioresour. Technol.* 102, 1149e1158. <https://doi.org/10.1016/j.biortech.2010.09.017>.

Zhang, Y., Arnold, R., Paavola, T., Vaz, F., 2013. Compositional analysis of food waste entering the source segregation stream in four European regions and implications for valorisation via anaerobic digestion. *Fourteenth Int. Waste Manag. Landfill Symp.*