COMPARISON OF SINGLE-STAGE AND TWO-STAGE ANAEROBIC CO-DIGESTION OF FOOD WASTE AND ACTIVATED SLUDGE FOR HYDROGEN AND METHANE PRODUCTION

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ABSTRACT

In this study, the co-digestion of food waste and activated sludge was evaluated in a two-stage anaerobic system and compared to the traditional single-stage process. The two-stage system was composed by two reactors connected in series able to perform the

Abbreviations: AD, anaerobic digestion; AS, activated sludge; BHP, biochemical hydrogen potential; FW, food waste; IA, intermediate alkalinity; HRT, hydraulic retention time; OFMSW, organic fraction of municipal solid waste; OLR, organic loading rate; PA, partial alkalinity, SGP, specific gas production; SHP, specific hydrogen production, SMP, specific methane production, TA, total alkalinity; TS, total solids; TVS, total volatile solids; VFA, volatile fatty acids.

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fermentative and the methanogenic phases separated. Experiments were carried out in semi-continuous mode under mesophilic conditions (37 °C). The two-stage technology achieved an overall improvement of the anaerobic performances. Results highlighted an increase in biogas production and volatile solids degradation of 26% and 9%, respectively. Concerning gas quality, the two-stage system achieved a hydrogen rich biogas in the first fermentative reactor and an improvement of methane content in the second methanogenic digester. The average methane content shifted from 61.2% to 70.1%. The highest methane production of the two-stage process was due to improved substrate hydrolysis, with increased amounts of volatile fatty acids made readily available in the second stage.

Keywords: Hydrogen, Methane, Food Waste, Activated Sludge, Co-digestion, Two-stage process

1. INTRODUCTION

The European Union action plan for the Circular Economy [1] and the Bioeconomy Strategy [2] represent the cornerstones of the European policy to develop a sustainable, low carbon and resource efficient future. The Circular Economy Policy Package aims to close material loops through the recycling and reuse of products, effectively reducing virgin material use and associated environmental pressures. The Bioeconomy Strategy is a research and innovation agenda aimed at enhancing the exploitation of biomaterials in a sustainable way. The two strategies are strictly interrelated since sustainable bioeconomy is the renewable segment of the circular economy turning bio-waste, residues and discards into valuable resources [3]. This new approach has thus focused its attention on municipal waste and wastewater sectors as key fields that can be widely

improved [4]. Wastewater sludge is the major by-product of wastewater treatment plants and anaerobic digestion (AD) is a widespread technology employed for its stabilisation. AD converts the organic matter into biogas, a renewable source of energy, and digestate, a valuable fertilizer and soil conditioner [5, 6]. Despite the positive potentials, most wastewater digesters face problems such as low organic loading rate (OLR) and biogas yield due to the low biodegradability of sludge. To date, the most common disposal approaches are landfilling and incineration, two expensive methods not compatible with the concept of circular economy [7].

Co-digestion of sludge and organic waste is a valuable solution to improve the digestion efficiency and increase the energy output using the spare digestion capacity at wastewater treatment plants [6, 8]. The co-digestion of two or more substrates with complementary characteristics can result in synergistic effects that may lead to improvements in biogas yield, process stability and costs reduction [9, 10]. Concerning organic waste and sludge, both substrates can provide a positive contribution to the anaerobic digestion. Organic waste provides essential carbon to sewage sludge digestion that is necessary for the improvement of digestion performance, mainly because of its influence on the kinetics of the process [6]. Conversely, sludge are protein-rich substrates whose anaerobic degradation releases hydroxide and ammonia ions [11]. Optimal levels of ammonia ions (up to 200 mg L^{-1}) ensure adequate supply of nitrogen as nutrient substance for anaerobic biomass and together with hydroxide ions increase system's buffer capacity, counteracting acidification lead by volatile fatty acid (VFA) production and thus helping to guaranteeing the stability of the process [12, 13].

With the aim of further improving AD efficiency, the two-stage process has been identified as a promising method because it allows a better reduction of organic load and increases the overall energy conversion efficiency by generating two gases with

high combustion power [14]. The traditional AD is split in two reactors connected in series. While the first fermentative phase produces a hydrogen rich biogas and releases volatile fatty acids (VFAs) in the liquid solution, the second phase converts VFAs and the residual biodegradable matter into methane and carbon dioxide [15]. Therefore, the role of the fermentative reactor is twofold: producing a hydrogen-rich biogas and acting as a pretreatment for the methanogenic reactor. Indeed, by degrading the macro-polymers, fermentative bacteria make the substrate more easily accessible to the methanogens, thus improving methane production in the second reactor [16-19]. Furthermore, European Union [20] promotes hydrogen production, as it is a sustainable energy source with no greenhouse gases emissions from its combustion and high-energy content (122 kJ/kg). Such potential benefits are further improved if hydrogen is produced through the biochemical conversion of biodegradable wastes [21].

Previous studies mainly focused on the sequential production of hydrogen and methane employing food waste (FW) as sole substrate [16-19, 22-30]. Other researches mainly focused on the two-stage co-digestion of other substrates than FW and activated sludges (AS). Bertin et al. [31] and Dereioti and Kornaros [32] studied the two-stage co-digestion of cheese whey and cattle manure obtaining a hydrogen-rich biogas in the first reactor and an increase of methane production in the second stage. Similar results were reported by Xiao et al. [33] with the mixture of FW and paper waste. Conversely, information on two-stage anaerobic systems for hydrogen and methane production from the co-digestion of FW and sludge is still scarce and its study needs to be improved.

The objective of the present study is to compare one-stage and two-stage anaerobic co-digestion processes employing a mixture of FW and AS as feeding. In order to have reference scenarios, one-stage and two-stage treatments of the sole FW were also performed. Experiments were carried out in semi-continuous mode under mesophilic

conditions. Process stability was monitored through VFAs, pH and alkalinity. Anaerobic performances were evaluated in terms of production and quality of gas and volatile solids removal efficiency.

2. MATERIALS AND METHODS

2.1 Substrates and inocula

FW was manually sorted from organic fraction of municipal solid waste (OFMSW) collected by means of a kerbside collection system. The domestic FW was collected in an Italian municipality and was mainly composed of pasta, bread, vegetable residues and citrus peels. The sample was shredded in a food processor (Problend 6, Philips, Netherlands) and diluted with tap water. The final FW slurry was stored in a freezer at -20°C.

AS was collected from the aerobic unit of a municipal wastewater treatment plant. The sample was stored in plastic tanks and kept under refrigeration at 4°C.

The substrates were then treated with the aim of obtaining mashes with a total solid (TS) content of 5% by weight, suitable for a wet digestion technology. As for the co-digestion experiments, AS and FW slurry samples were daily removed from storage conditions and mixed in the food processor. The ratio FW slurry:AS was approximately 1:5 by weight. Similarly, the digestion trials were performed by mixing FW slurry and tap water.

The same sample of AS was also used as inoculum for the fermentative reactor [29, 34, 35]. According to previous studies [35, 36], in order to harvest the hydrogen-producing bacteria and inhibit hydrogenotrophic methanogens, the sludge sample was heat-shocked at 105°C for 30 minutes before the start of the experiment. The treatment

was performed in 250 ml beakers placed in a static oven (UM200, Memmert GmbH, Germany). The temperature of the medium was continuously measured with a rigid tip digital thermometer (T1, Testo S.p.A., Italy). After 30 minutes, beakers were removed from the oven and cooled down to ambient air temperature. Tests were carried out when inoculum temperature reached 37°C.

The seed sludge used as inoculum for the methanogenic reactor (IN) was collected from a wet anaerobic reactor treating OFMSW and cattle manure at mesophilic conditions.

The characteristics of FW slurry, AS and IN in terms of TS, Total Volatile Solids (TVS), pH, total alkalinity and carbohydrates, proteins and lipids contents are reported in Table 1. The analytical method of each parameter is presented in Section 2.4.

Parameters	FW slurry	AS	IN
TS (%)	19.9 ± 0.6	2.1 ± 0.0	2.6 ± 0.0
TVS/TS (%)	80.6 ± 0.9	79.3 ± 0.3	61.9 ± 0.4
pH	3.8 ± 0.1	7.1 ± 0.0	8.2 ± 0.1
Carbohydrates (% w/w)	7.4	< 0.1	< 0.1
Proteins (% w/w)	3.9 ± 0.2	0.9 ± 0.1	0.6 ± 0.1
Lipids (% w/w)	3.9 ± 0.2	< 0.3	< 0.3
Fibres (% w/w)	3.0 ± 0.4	0.1 ± 0.0	0.2 ± 0.0
Total alkalinity (mgCaCO3 L^{-1})	1,300 ± 45	5,000 ± 88	7,750 ± 55

Table 1 – Substrates and inoculum characteristics. Values are expressed as average values and related standard deviation.

2.2 Reactors configuration

Two stainless steel (AISI 316) reactors of 6 and 20 L (working volumes of 3 L and 12 L) were adopted as continuously stirred tank reactors (CSTR)for the fermentative and methanogenic phases, respectively. Continuous mixing inside the reactors was ensured by mixing blades connected to electric gear motors (COAX MR 615 30Q

1/256, Unitec s.r.l., Italy). Warm water heated by a thermostatic bath (FA90, Falc Instruments s.r.l., Italy) passed through each reactor cladding in order to keep the temperature constant at mesophilic conditions (37 ± 0.1 °C). pH was continuously measured by pH probes (InPro4260i, Mettler Toledo, Italy). The volume of the produced gas during the tests was measured by using volumetric counters connected to the upper side of the reactors through a 3-way valve. Each counter was composed of two concentric cylinders partially filled with water: when the gas flowed from the reactor to the external side of the counter, the water rose through the internal cylinder up to the level of an electrode. The electrode activated a 3-way valve, which connected the counter to a 10 L multilayer foil bag (SupelTM, Merck KGaA, Germany) that collected the gas. After bag filling, the water level in the counter dropped to a second electrode, which reconnected the counters to the reactors and the gas restarted to enter into them. Each impulse was related to a gas volume of 0.07 L. In order to convert gas volume data at normal conditions, a pressure transducer (HD 9908T Baro, Delta Ohm S.r.l., Italy) and a T-type thermocouple (PT100, Delta Ohm S.r.l., Italy) measured ambient pressure and temperature respectively. All signals coming from the reactors were acquired by a cRIO 9030 controller (National Instruments, USA) and were processed by a software specifically developed in Labview® environment. As for the fermentative reactor, the acquisition system and the software were used also to control a peristaltic pump (Reglo ICC, Ismatec, Germany) dedicated to the dosage of NaOH 2M solution for pH control. 3 ml of solution were automatically added when the pH decreased under the set value in order to constantly keep the pH in the range of ±0.1 all through the tests. This pH control strategy was adopted on the basis of previous works that tested the efficacy of pH control through the automatic addition of an alkaline solution [29, 34, 35, 37]. The communication between the acquisition device and the pump occurred via a serial RS-

232 connection. After filling, the reactors were flushed with nitrogen for a few minutes to ensure anaerobic conditions.

2.3 Operational conditions

Experiments were carried out with FW and mixtures of FW and AS as substrates. Mashes were daily fed to the reactors by means of a syringe. Both trials were characterized by two scenarios (Figure 1). In the first scenario (S1), the methanogenic reactor was run alone aiming at evaluating the traditional one-stage AD. Simultaneously, the fermentative reactor was also fed in order to reach steady state conditions. In the second scenario (S2), the two digesters were connected in series aiming at evaluating the two-stage process. Each scenario was performed for three HRTs of the methanogenic reactor: 51 days S1 and 36 days S2. As for the methanogenic reactors, the first 34 and 24 days of S1 and S2 respectively were considered as the acclimatization phase (equal to two HRT), while the last HRT of each scenario (from day 35 to day 51 and from day 25 to day 36) was considered as the steady state and its data were used for comparison. As for the fermentative reactors, the whole S1 was considered as a trial stage, while S2 was entirely considered as steady. Both scenarios were characterized by an OLR of the methanogenic reactor of 2.5 kgTVS $m^{-3}d^{-1}$. This value was selected as the optimum value for wet digestion technologies and mesophilic conditions [38] and in the range of previous studies [18]. Consequently, similarly to other works [24-26, 39] the HRT was approximately 17 days for S1 and 12 days for S2. As for the fermentative reactor, the HRT was set to 3.0 days based on previous studies [25, 26]. The related OLR was then calculated to be approximately 14 kgTVS $m^{-3}d^{-1}$.

183 Table 2 summarizes the operational conditions applied to the reactors during the
184 tests.

185

	Digestion (FW)		Co-digestion (FW + AS)	
	Fermentative reactor	Methanogenic reactor	Fermentative reactor	Methanogenic reactor
HRT S1 (d)	-	17	-	17
OLR S1 (kgTVS $m^{-3}d^{-1}$)	-	2.5	-	2.5
HRT S2 (d)	3	12.8	3	11.9
OLR S2 (kgTVS $m^{-3}d^{-1}$)	14.2	2.5	14.6	2.5

186 **Table 2 – Operational conditions applied during the experimental tests.**

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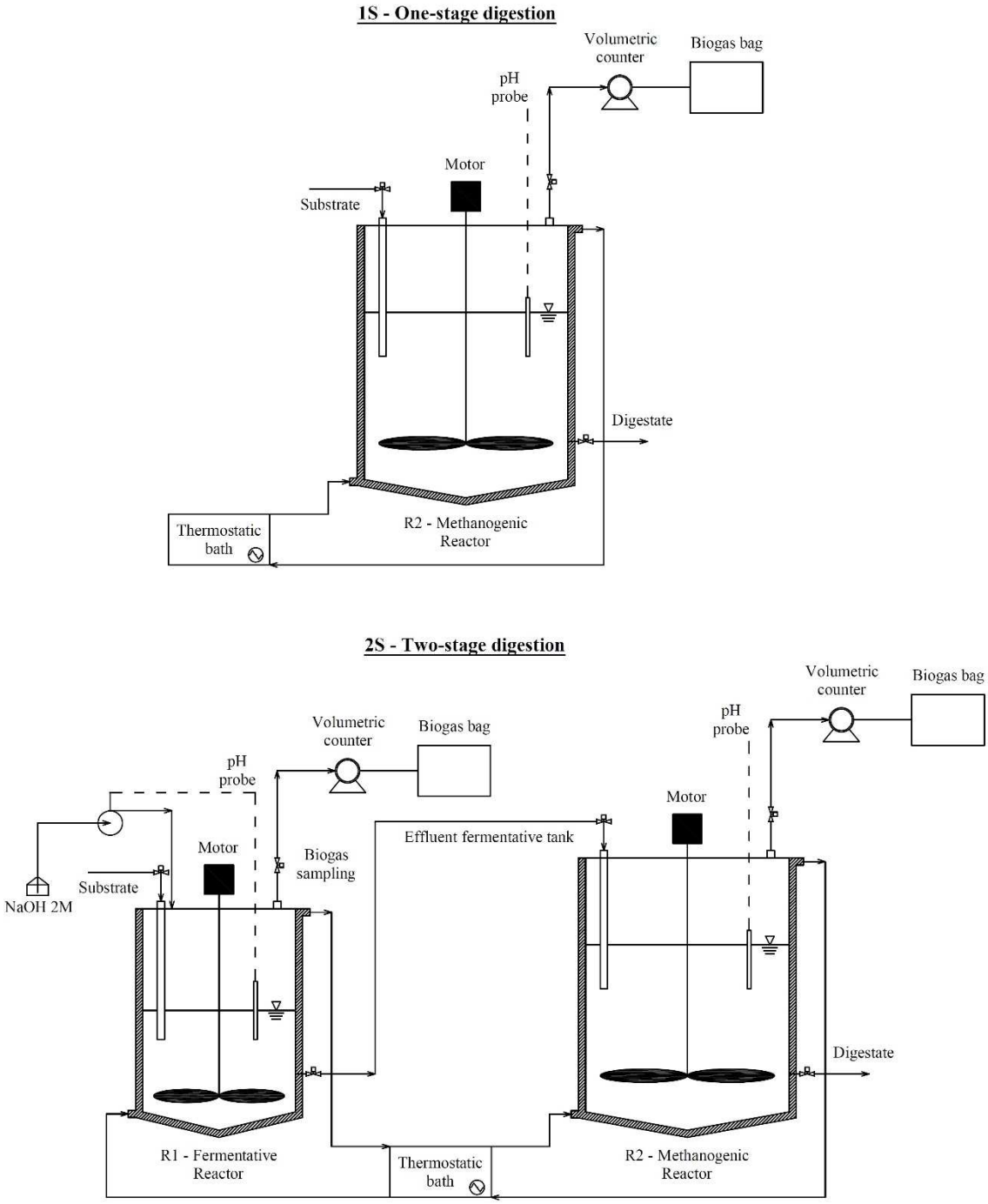

Figure 1. Schematic diagrams of one-stage and two-stage tests.

2.4 Analytical methods

The effluent of both the reactors was monitored daily in terms of TS, TVS, pH, alkalinity and VFAs.

193 TS, TVS and pH were determined according to standard methods [40]. Based on the
194 volatile solids content of the effluent (TVS_{OUT}) and the incoming substrate (TVS_{IN}), the
195 daily volatile solids removal efficiency (η_{TVS}) was calculated as follows (Eq. (1)):
196

$$_{TVS} = \frac{TVS_{IN} - TVS_{OUT}}{TVS_{IN}} \times 100 \quad (1)$$

197

198 Alkalinity was measured according to Martín-González et al. [41]. The measurement
199 consisted in a two-end point titration methodology to monitor VFAs/alkalinity ratio
200 leading to obtain total alkalinity (TA) and partial alkalinity (PA). The former included
201 both VFA and bicarbonate alkalinity and the latter was roughly related only to
202 bicarbonate alkalinity. The difference, defined as intermediate alkalinity (IA), was
203 related only to VFA alkalinity. Several studies have included alkalinity ratios as
204 monitoring parameters. For instance, the pilot scale digester was daily monitored
205 through the ratios intermediate/partial alkalinity (IA PA^{-1}).
206 Hydrogen, methane, carbon dioxide, nitrogen, oxygen and hydrogen sulphide
207 contents in biogas were analysed using a gas chromatograph (3000 Micro GC,
208 INFICON, Switzerland) equipped with a thermal conductivity detector. Carbon dioxide
209 and hydrogen sulphide passed through a PLOTQ column (10 μm / 320 μm / 8 m) using
210 helium as gas carrier at temperature of 55°C. The other gas passed through a Molsieve
211 column (30 μm / 320 μm / 10 m) using argon as gas carrier at a temperature of 50°C.
212 VFAs, including acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic
213 acids were measured using a gas chromatograph (7890B, Agilent Technology, US) with
214 hydrogen as gas carrier, equipped with a CPFFAP column (0.25 mm / 0.5 μm / 30 m)
215 and with a flame ionization detector (250°C). The temperature during the analysis

started from 60°C and reached 250°C with a rate of 20 °C/min. Samples were centrifuged (30 minutes, 13,500 rpm) and filtrated on a 0.45 μm membrane. 500μL of filtrate were mixed with isoamyl alcohol (1.00179, Merck KGaA, Germany) in a volumetric ratio of 1:1, 200 μL of phosphate buffer solution (pH 2.1), sodium chloride and 10 μL of hexanoic-D11 acid solution (10.000 ppm) used as internal standard. The blend was mixed with a Mortexer™ Multi-Head vortexer (Z755613-1EA, Merck KGaA, Germany) for 10 minutes. The liquid suspension of the sample was then inserted in the gas chromatograph by means of an auto-sampler.

As presented in Table 1, substrates and the methanogenic inoculum were also characterized in their carbohydrate, protein, lipid and fibre content. Proteins, lipids and fibres were obtained following the European Commission Regulation 2009/152/EC of 27 January 2009 [42]. Total carbohydrates were determined by subtracting the contents of humidity, ashes, proteins, lipids and fibres from the total amount.

3. RESULTS

Results are firstly presented by analysing process stability through pH, alkalinity and VFAs. Subsequently, single-stage and two-stage processes are compared by their anaerobic performances through biogas production, biogas quality and volatile solids removal efficiency.

3.1 Process stability

The average results of pH, IA, TA and total VFAs obtained from the two experimental set-ups are reported in Table 3.

Digestion (FW)			
	S1	S2	
Parameters	Methanogenic reactor	Fermentative reactor	Methanogenic reactor
pH	7.33 ± 0.02	5.52 ± 0.02	7.43 ± 0.02
TA ($mgCaCO_3\ L^{-1}$)	10,557 ± 424	6,459 ± 627	12,995 ± 298
IA ($mgCaCO_3\ L^{-1}$)	1,976 ± 307	-	1,840 ± 303
Total VFAs ($mg\ L^{-1}$)	1,022 ± 273	8,172 ± 651	1,033 ± 340
Co-digestion (FW+AS)			
	S1	S2	
Parameters	Methanogenic reactor	Fermentative reactor	Methanogenic reactor
pH	7.02 ± 0.03	5.54 ± 0.02	7.35 ± 0.03
TA ($mgCaCO_3\ L^{-1}$)	6,186 ± 488	8,785 ± 1,235	14,691 ± 679
IA ($mgCaCO_3\ L^{-1}$)	1,115 ± 238	-	1,877 ± 412
Total VFAs ($mg\ L^{-1}$)	267 ± 21	8,204 ± 828	364 ± 124

Table 3 –Process stability indicators. Results are expressed in terms of averages and standard deviations.

In the fermentative stage pH was constantly kept around 5.5 all through both experimentations due to the addition of NaOH solution. Such pH value was set according to previous studies that defined 5.5 as the optimum for hydrogen production [25, 43, 44]. The external control of pH was necessary to avoid the drop to values below 4 which could significantly suppress the hydrogenase activity [39]. Concerning the methanogenic stage, pH highlighted more neutral values (7.0-7.6), typical of a proper AD process [38].

Figure 2 and Figure 3 show the VFA content in the fermentative and methanogenic

reactors during the digestion of FW and the co-digestion of FW and AS, respectively. Figures represent the three main released organic acids: acetate, propionate and butyrate. Concerning the methanogenic reactor, the IA PA^{-1} ratio is also represented and used as indicator of process stability. Indeed, according to Martín-González et al. [41], an IA PA^{-1} ratio below 0.3 is recommended to achieve stable reactor performance.

As for the digestion of FW, IA PA^{-1} ratio below 0.3 was reached after 28 days. This is attributable to a larger release of VFAs in the first phase of the digestion experiment with a maximum concentration that reached 3,689 mg L^{-1} on day 14. During this phase, propionic acid was the main product. According to Wang et al. [45], the conversion rates of VFAs to methane vary in the order of acetic acid > butyric acid > propionic acid and an accumulation of the latter can result in a failure of methanogenesis. According to Martín-González et al. [41], a total VFA concentration above 3,500 mg L^{-1} is considered the threshold limit for process imbalance. After day 18, propionate production dropped, and stable state conditions were definitively achieved after day 28. Such change in the metabolic pathway may be attributable to a change of methanogenic bacteria species together with a progressive adaption to the substrate as the experiment proceeded [45]. Conversely, in the co-digestion trial, IA PA^{-1} ratio was always found to be lower than 0.3 with a total concentration of VFAs in the range of 200-800 mg L^{-1}.

As for the two-stage scenarios, the methanogenic digesters observed a pH increase (Table 3) together with a progressive decrease of the IA PA^{-1} ratio. These results may be attributable to both the stabilisation of VFA production and to a continuous increase of TA caused by an accumulation of NaOH in the reactor. As abovementioned, during the fermentative phase a 2M NaOH solution was used to avoid pH drop to values inhibiting the hydrogenase activity. Once the reactors were connected in series, the saline solution was also conveyed to the second reactor, thus increasing pH and total alkalinity.

As expected, fermentative reactors highlighted a significant production of VFAs. The average concentrations of the two experimentations showed comparable results of approximately 8,000 mg L^{-1}. Similarly to previous studies [16, 17, 24], the prevalent acid released was butyrate, followed by acetate. This result is an indication of a proper hydrogenase activity since acetate and butyrate pathways are recognized to maximise hydrogen production yields [15].

In conclusion, after an initial unstable phase, both trials were characterized by process stability. The indicators (pH, IA PA^{-1} ratio, VFAs) were consistent with other works showing stable performances and absence of inhibitory phenomena. Process stability was therefore also guaranteed during the periods considered as steady state, thus confirming the proper use of their data for the comparison of the scenarios.

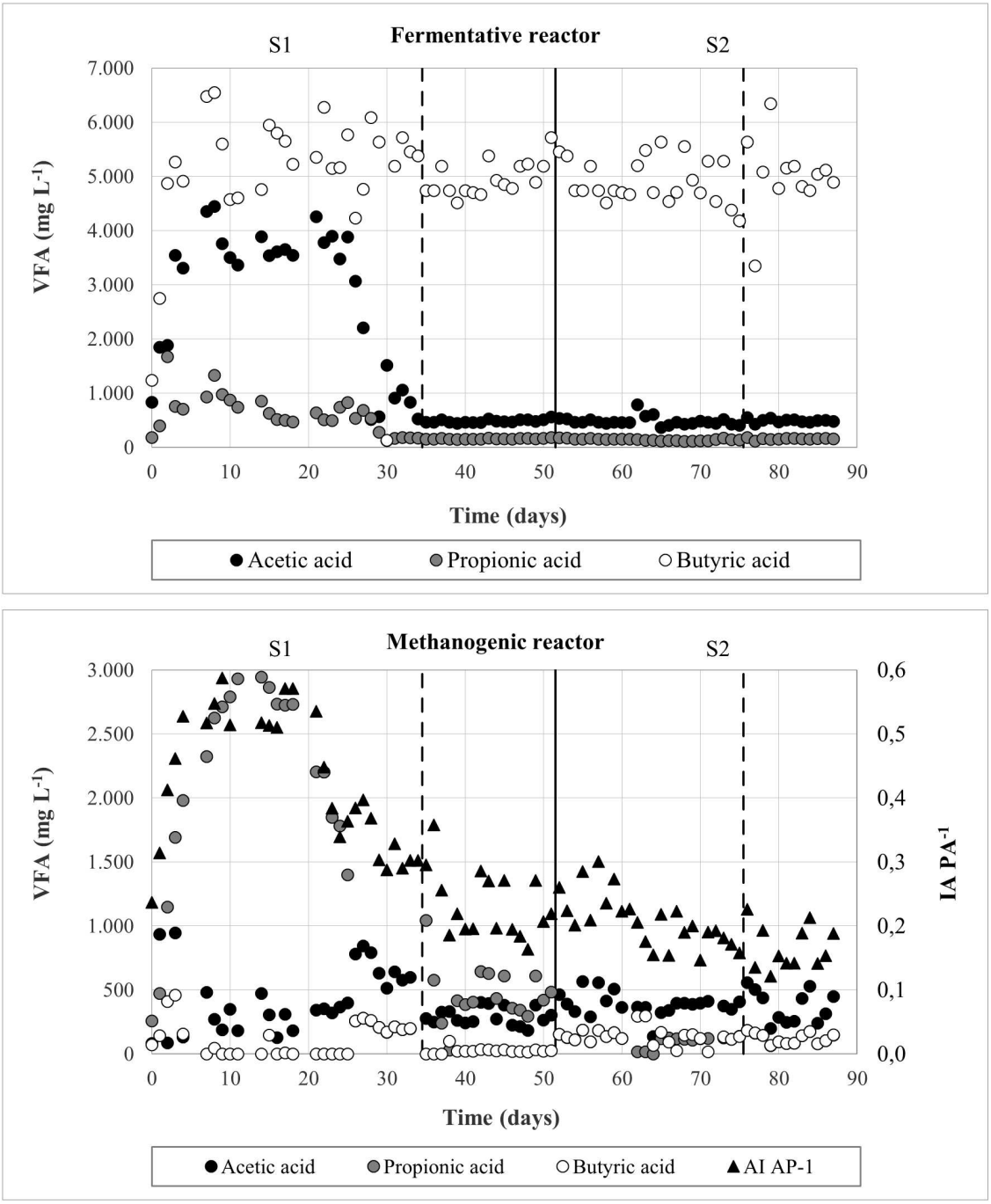

Figure 2. Volatile fatty acid content in the fermentative and methanogenic reactors during the digestion of FW. As for the methanogenic reactor, the ratio AI AP^{-1} is also represented.

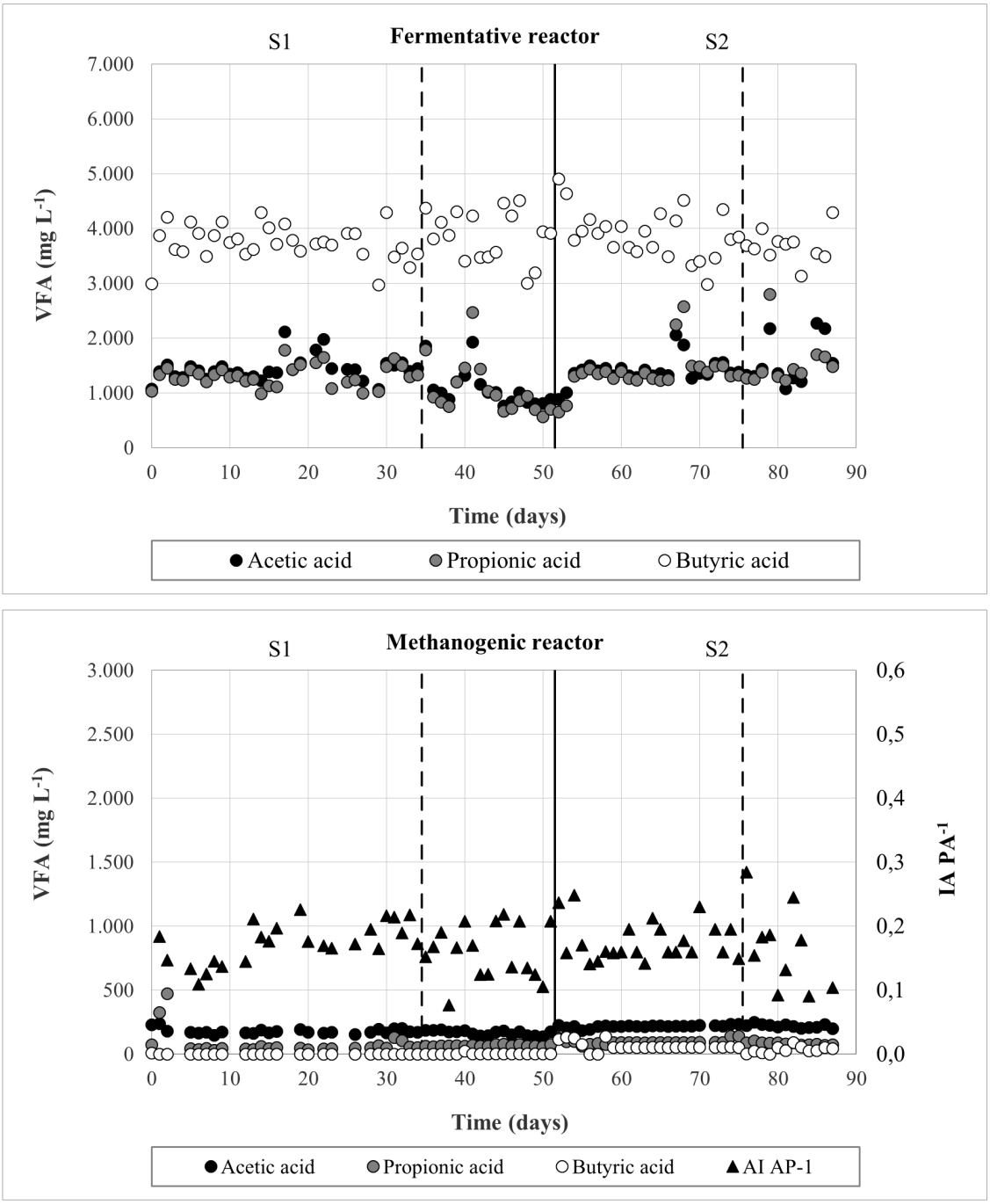

Figure 3. Volatile fatty acid content in the fermentative and methanogenic reactors during the co-digestion of FW and AS. As for the methanogenic reactor, the ratio IA PA^{-1} is also represented.

3.2 Anaerobic performances of single-stage and two-stage processes

The average results of specific gas production (SGP), hydrogen and methane content, specific hydrogen production (SHP), specific methane production (SMP) and η_{TVS} obtained from the two experimental set-ups are reported in Table 4. The complementary gas in the biogas produced by both reactors was mainly carbon dioxide. Figure 4 shows the composition of biogas in terms of methane and hydrogen contents over time.

Digestion (FW)			
	S1	S2	
Parameters	Methanogenic reactor	Fermentative reactor	Methanogenic Reactor
SGP (NL $kgTVS^{-1}d^{-1}$)	694.4 ± 24.6	43.1 ± 12.8	704.6 ± 28.5
H_2 (%)	-	22.9 ± 5.5	-
CH_4 (%)	65.2 ± 1.9	-	68.4 ± 1.1
SHP ($NLH_2\,kgTVS^{-1}d^{-1}$)	-	12.6 ± 5.0	-
SMP ($NLCH_4\,kgTVS^{-1}d^{-1}$)	453.1 ± 28.2	-	482.1 ± 24.0
η_{TVS} (%)	67.0 ± 2.0	23.5 ± 4.0	62.5 ± 2.7
Co-digestion (FW+AS)			
	S1	S2	
Parameters	Methanogenic reactor	Fermentative reactor	Methanogenic Reactor
SGP (NL $kgTVS^{-1}d^{-1}$)	485.9 ± 25.8	44.8 ± 12.6	611.0 ± 45.4
H_2 (%)	-	18.4 ± 6.3	-
CH_4 (%)	61.2 ± 2.2	-	70.1 ± 1.6
SHP ($NLH_2\,kgTVS^{-1}d^{-1}$)	-	8.6 ± 4.8	-
SMP ($NLCH_4\,kgTVS^{-1}d^{-1}$)	298.0 ± 24.5	-	428.3 ± 30.9
η_{TVS} (%)	61.0 ± 2.2	32.3 ± 4.4	54.5 ± 4.1

Table 4 - Yields of the process. Results are expressed in terms of averages and standard deviations.

As previously shown in Figure 2 and Figure 3, in the two-stage process, methanogenesis almost completely degraded the organic acids produced in the fermentative stage. The utilization ratios of acetate and butyrate were beyond 52.5% and

97.0% in the digestion trials, and beyond 84.5% and 99.0% in the co-digestion trials, respectively. These significant degradations were consistent with previous works [16, 19]. De Gioannis et al. [19] obtained a VFA removal in the second stage of 97.0%, while Lee et al. [16] reported utilization ratios in the range of 80.5%-99.9%. Such degradations were strictly linked to an increase in biogas production and in methane content that was generated following the acetoclastic pathway. During S2, methane content gradually increased with time with peaks of 70.7% for the digestion trial and 76.3% for the co-digestion experiment. The two-stage process enabled an average enrichment of methane by respectively 3.2% and 8.9% when compared to the traditional one-stage system. This is consistent with Voelklein et al. [18] and De Gioannis et al. [19], who stated that an acidogenic digester might serve as a carbon dioxide stripping step, thus reducing the potential costs for upgrading the biogas to biomethane. This higher methane production is essentially due to the improved hydrolysis of substrates in the first stage, with the production of relevant amounts of volatile fatty acids which were readily available to methanogens in the second stage [19].

As for the fermentative reactor, methane was never detected. The initial thermal treatment of inoculum and process conditions, such as acid pH and low HRT, were therefore efficient in the inhibition of hydrogenotrophic methanogens. The average hydrogen content in biogas was 22.9% and 18.4% with peaks of 42.1% and 37.0% for the digestion and the co-digestion trials, respectively. Such concentrations are comparable to previous studies. Cavinato et al. [24] highlighted hydrogen concentrations in the range of 19-37% while Micolucci et al. [26] reported an average content of 25 ± 9% using FW as substrate.

Figure 5 illustrates the time course of biogas production in the two configurations of digestion and co-digestion. After a first unstable phase, biogas was continuously

generated in both reactors without inhibition problems. This result was achieved due to an overall process stability previously evaluated in terms of VFAs, alkalinity and pH.

Comparing the two scenarios, the two-stage improvement in methane content was accompanied by an increase in biogas generation. The methanogenic reactor highlighted a slight improvement for the digestion study (+1.4%), while in the co-digestion experiment the average increase was around 26%. Considering the whole two-stage system, i.e. the sum of the biogas productions of the first and the second digester, these percentages increased up to 7.7% and 35.0%. As for the digestion of FW, SGP and SMP results were in the range of results of previous works adopting the two-stage technology. Chinellato et al. [25] observed a SGP of 728 $NLkgTVS^{-1}d^{-1}$ and a SMP of 484 $NLCH_4\,kgTVS^{-1}d^{-1}$ using HRTs of 3 d and 12 d and OLRs of 15 kgTVS $m^{-3}d^{-1}$ and 3 kgTVS $m^{-3}d^{-1}$ for the fermentative and the methanogenic reactor, respectively. Similarly, Cavinato et al. [27] obtained an SGP of 640 $NLkgTVS^{-1}d^{-1}$ with an average methane content of 65%. In this case, the two-stage technology was performed using HRTs of 3.3 d and 12.6 d and OLRs of 16 kgTVS $m^{-3}d^{-1}$ and 4 kgTVS $m^{-3}d^{-1}$ for the fermentative and the methanogenic reactor, respectively. Regarding the single-stage co-digestion of FW and AS, the review study of Iacovidou et al. [6] highlighted SMP in the range of 186-346 $NLCH_4\ kgTVS^{-1}d^{-1}$, thus concluding that methane production is directly related to the amount of FW in the mixture.

As for the fermentative tank, the SGP was found to be significantly lower than the methanogenic reactor, with the two experiments showing comparable results of about 45 NL $kgTVS^{-1}\ d^{-1}$. In the matter of hydrogen generation, the co-digestion tests showed lower productions than the digestion trial. This may be attributable to the lower content of carbohydrates in the mixture FW+AS than in the FW mash. Indeed, as highlighted from Table 1 and previous studies, FW is a carbohydrate-rich substrate [6, 37], while

AS is mainly composed of proteins [37, 46]. The correlation between hydrogen production and the carbohydrates content of the substrate was studied by Alibardi et al. [36], who found a linear relation between the two variables. Conversely, the same study highlighted that proteins and lipids did not produce significant contributions to hydrogen generation. The two final SHP values were in the same order of magnitude of hydrogen yields of other studies using similar reactor conditions. As such, SHP values of 1, 51.2 and 66.7 NLH_2 $kgTVS^{-1}$ d^{-1} were obtained by Chinellato et al. [25], Cavinato et al. [27], and Cavinato et al. [24], respectively. Conversely, Chu et al. [47] using an HRT of 1.3 d, obtained a SHP of 205 NLH_2 $kgTVS^{-1}$ d^{-1}, thus suggesting that the use of low HRT can optimize hydrogen production.

Concerning η_{TVS}, Table 4 and Figure 6 show an overall reduction of degradation of the organic matter in the methanogenic reactor. More specifically, the average value decreased from 67.0% to 62.5% and from 61.0% to 54.5% for the digestion and the co-digestion study, respectively. This was due to the volatile solids content of the incoming substrate of the methanogenic reactor. Indeed, while during S1 the reactor was fed with the pure substrates (FW and FW+AS mashes), during S2 it was fed with the outgoing digestate of the fermentative tank that was already partially degraded. Indeed, while FW mash and the mixture FW+AS had a TVS content of approximately 4% w/w, the outgoing digestate of the fermentative tank presented an average TVS content of around 3% w/w. Taking into account the whole two-stage process, i.e. considering TVS_{IN} as the volatile content of the incoming substrate of the first reactor and TVS_{OUT} as the volatile substance of the outgoing digestate of the second tank, the two final η_{TVS} values of S2 were calculated to be 69.4% and 71.5%, 6.8% and 8.4% more than S1.

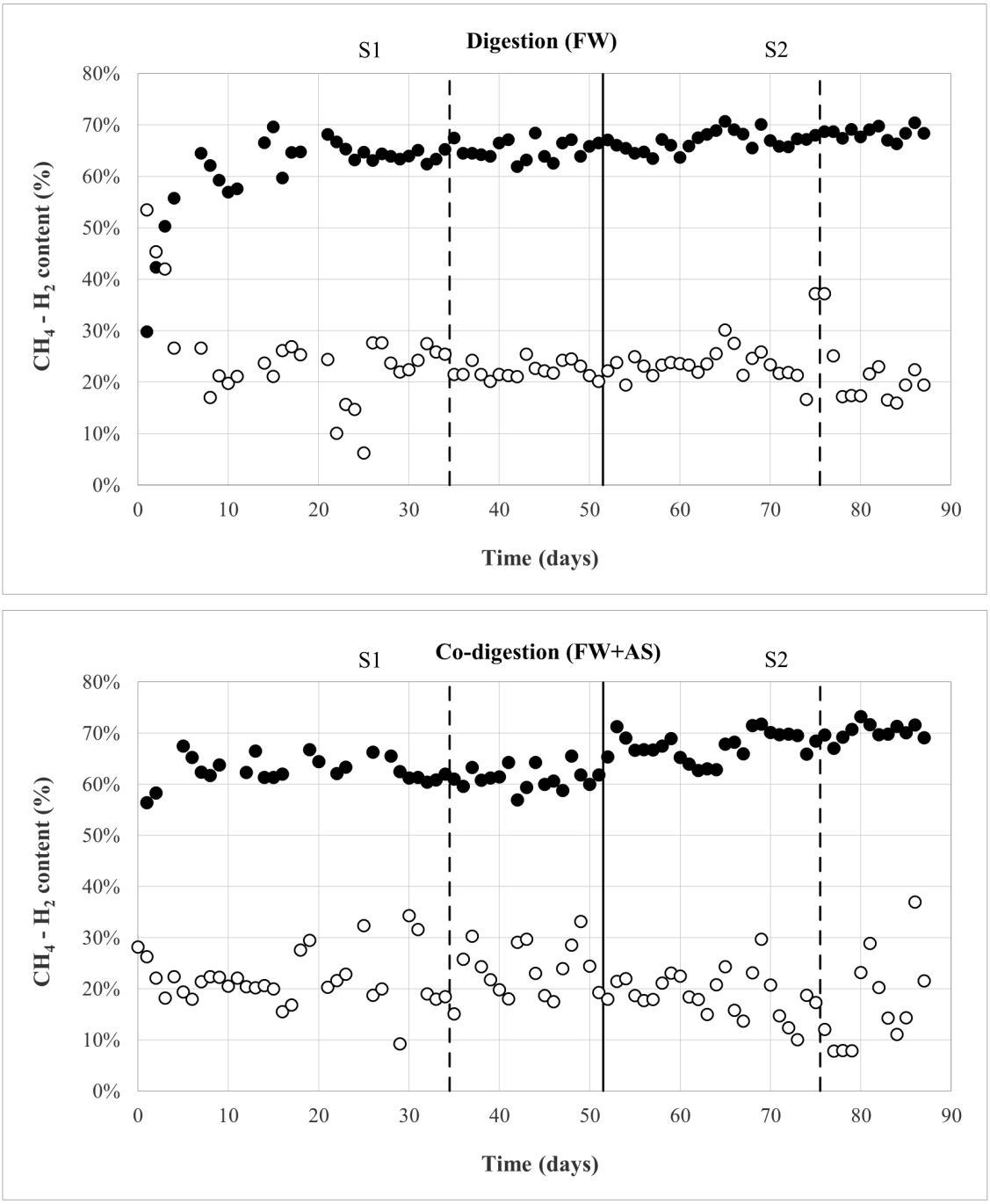

Figure 4. Hydrogen (○) and methane (●) content in the fermentative and in the methanogenic reactor, respectively.

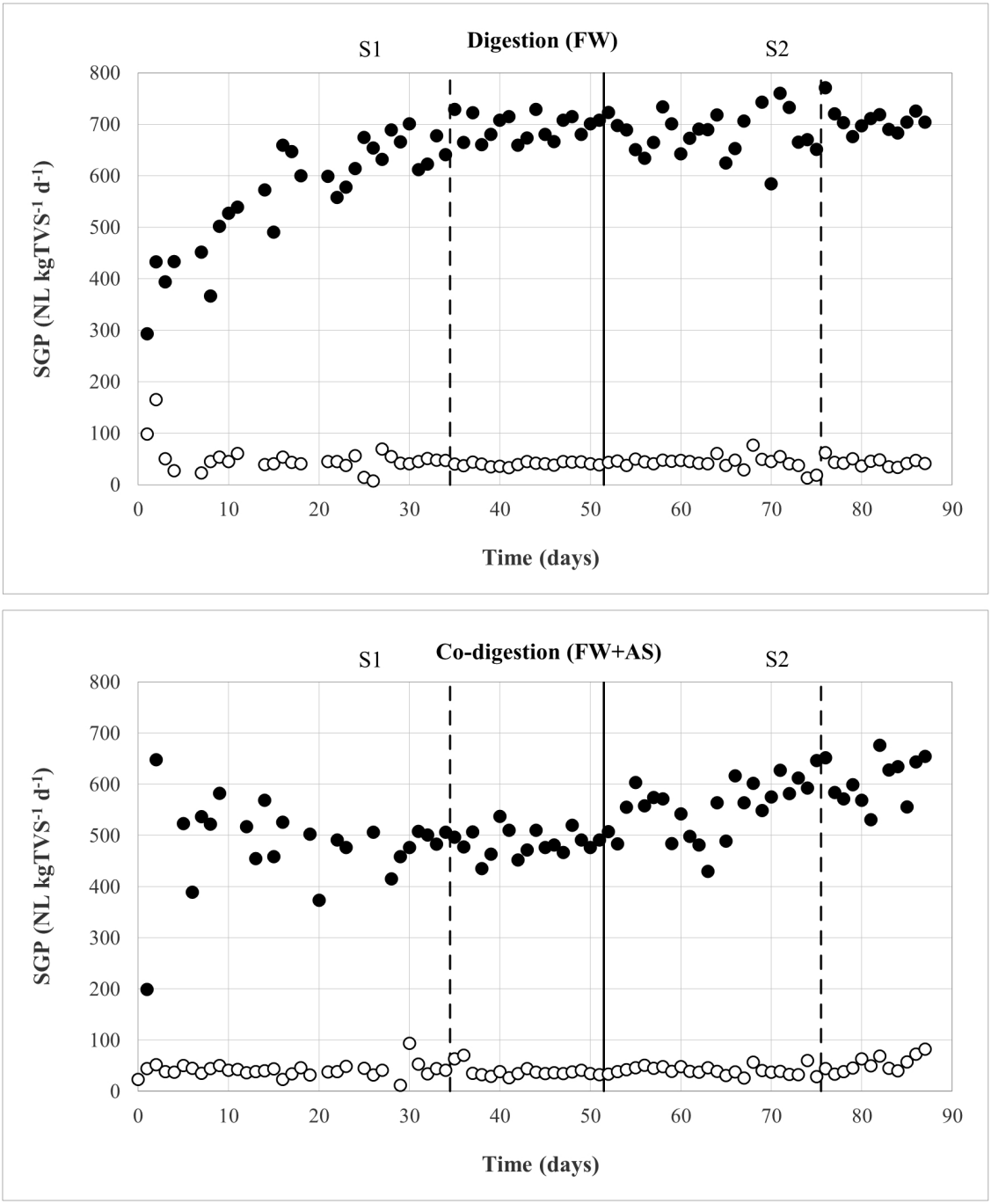

Figure 5. Specific Gas Production (SGP) obtained for the fermentative (○) and the methanogenic reactor (●).

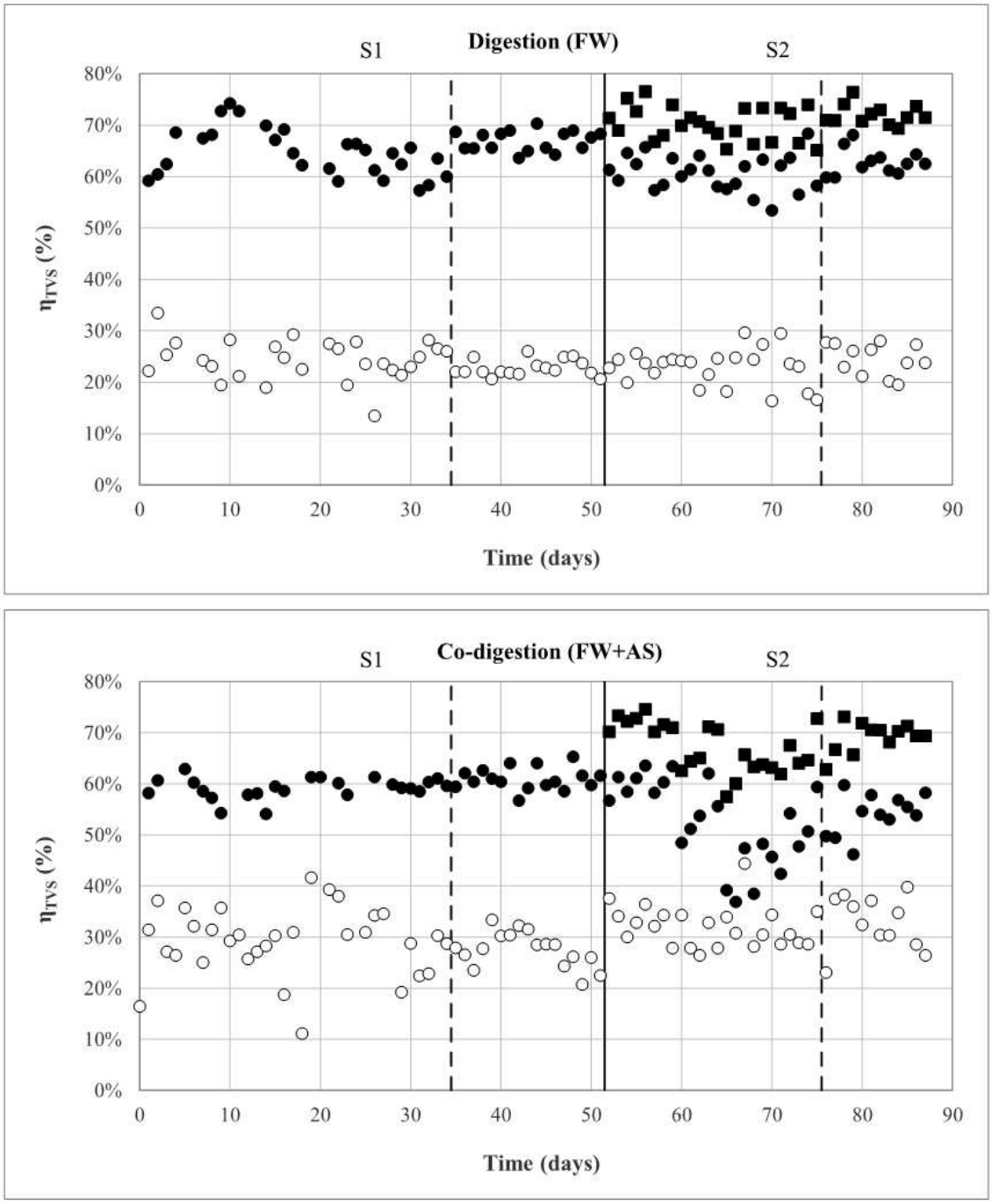

Figure 6. Volatile solids removal efficiency (η_{TVS}) obtained for the fermentative (○) and the methanogenic reactor (●). (■) represents the total efficiency in the second scenario.

The present study highlighted two important results: the confirmation of the improvement of the anaerobic digestion of FW using a two-stage technology and the evidence that this technology can be successfully used also for the co-digestion of FW

and AS. As expected, biogas yield and volatile solids removal efficiencies of the co-digestion experiment were found to be lower than what obtained for the digestion of FW. This is mainly due to a lower biodegradability of the mixture of FW and AS than the mash of pure FW. Nevertheless, the improvement of the two-stage technology compared to the traditional one-stage system was more effective on the co-digestion trial than the single digestion of FW. Another relevant result achieved in the co-digestion test was a better process stability than in the digestion study. Indeed, in the fermentative reactor, a lower average daily volume of NaOH solution was used to balance pH (31.6 mL d^{-1} vs 40.2 mL d^{-1}). As for the methanogenic reactor, conversely to the digestion trial, the IA PA^{-1} ratio was always found to be lower than 0.3. This fact is attributable to the high alkalinity and buffer capacity of AS (Table 1). As stated by several authors [11-13], the fermentation of this protein-rich substrate (Table 1) is characterized by the release of a large amount of hydroxide ions together with ammonia ions helping to mitigate pH drop and thus consuming less external saline solution.

4. CONCLUSIONS

The two-stage co-digestion of food waste and activated sludge efficiently improved the traditional single-stage process. The enhancement of the anaerobic performances in terms of biogas production, biogas quality and volatile solids removal were even higher than the two-stage digestion of the sole food waste, thus highlighting the viability of this technology also for the mixture of food waste and activated sludge. Furthermore, the co-digestion configuration observed a better process stability.

Results showed an increase in biogas production and volatile solids removal by 26% and 9%, respectively. Concerning gas quality, the two-stage system observed a hydrogen rich biogas in the first fermentative reactor and an improvement of methane

content in the second methanogenic digester. The average methane content shifted from 61.2% to 70.1%. The highest methane production of the two-stage process was due to improved substrate hydrolysis, with increased amounts of volatile fatty acids being readily available in the second stage. Other additional advantages of the two-stage process are associated to the overall reduction of the hydraulic retention time and the higher removal of volatile solids. As such, the reduction of the HRT implies a reduction of digester volume and investment costs while the increase in volatile solids removal is associated to a higher degree of digestate stabilisation, which is a relevant issue when considering its final disposal.

AKNOWLEDGEMENTS

This work was supported by the Bio2Energy project, a project funded by MIUR-Regione Toscana DGRT 1208/2012 and MIUR-MISE-Regione Toscana DGRT 758/2013 PAR FAS 2007-2013 in sub-programme FAR-FAS 2014 (Linea d'Azione 1.1).

REFERENCES

[1] European Commission, 2015. Report from the Commission to the European Parliament, the Council, the European economic and social committee and the committee of the regions, closing the loop – An EU action plan for the Circular Economy, COM (2015) 614 final, 1-21.

[2] European Commission, 2018. A sustainable bioeconomy for Europe: strengthening the connection between economy, society and the environment – updated bioeconomy strategy. ISBN 978-92-79-94145-0, https://doi.org/10.2777/478385

[3] EEA, 2018. The circular economy and the bioeconomy. Partners in sustainability.

445 EEA Report, 8/2018. ISBN 978-92-9213-974-2, https://doi.org/10.2800/02937
446 [4] European Commission, 2017. Report from the Commission to the European
447 Parliament, the Council, the European economic and social committee and the
448 committee of the regions, On the implementation of the Circular Economy Action
449 Plan, COM (2017) 33 final, pag. 1-14.
450 [5] Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S.,
451 Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from
452 anaerobic digestion through a comparative study with digested sludge and compost.
453 Chemosphere 81, 577-583. https://doi.org/10.1016/j.chemosphere.2010.08.034
454 [6] Iacovidou, E., Ohandja, D., Voulvoulis, N., 2012. Food waste co-digestion with
455 sewage sludge – realising its potential in the UK. J. Environ. Manage. 112, 267-274.
456 https://doi.org/10.1016/j.jenvman.2012.07.029
457 [7] Nghiem, L. D., Koch, K., Bolzonella, D., Drewes, J. E., 2017. Full scale co-
458 digestion of wastewater sludge and food waste: bottleneck and possibilities. Renew.
459 Sust. Ener. Rev. 72, 354-362. https://doi.org/10.1016/j.rser.2017.01.062
460 [8] Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Williems, K.,
461 JVanImpe, J., Dewil, R., 2011. Anaerobic digestion in global bio-energy production:
462 potential and research challenges. Renew. Sust. Ener. Rev. 15, 4295-4301.
463 https://doi.org/10.1016/j.rser.2011.07.121
464 [9] Wu, L.J., Kobayashi, T., Kuramochi, H., Li, Y.Y., Xu, K.Q., 2016. Improved biogas
465 production from food waste by co-digestion with de-oiled grease trap waste.
466 Bioresour. Technol. 201, 237–244. https://doi.org/10.1016/j.biortech.2015.11.061
467 [10] Xie, S., Wickham, R., Nghiem, L.D., 2017. Synergistic effect from anaerobic
468 codigestion of sewage sludge and organic wastes. Int. Biodeterior. Biodegradation
469 116, 191–197. https://doi.org/10.1016/j.ibiod.2016.10.037

[11] Jung K.-W., Kim, D.-H., Kim, S.-H., Shin, H.-S., 2011. Bioreactor design for continuous dark fermentative hydrogen production. Bioresour. Technol. 102, 8612-8620. http://dx.doi.org/10.1016/j.biortech.2011.03.056

[12] Polizzi, C., Alatriste-Mondragón, F., Munz, G., 2018. The role of organic load and ammonia inhibition in anaerobic digestion of tannery fleshing. Water Resources and Industry 19, 25-34. https://doi.org/10.1016/j.wri.2017.12.001

[13] Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632-641. https://doi.org/10.1016/j.biortech.2013.06.030

[14] Liu, X., Li, R., Ji, M., Han, L., 2013. Hydrogen and methane production by co-digestion of waste activated sludge and food waste in the two-stage fermentation process: substrate conversion and energy yield. Bioresour. Technol. 146, 317-323. http://dx.doi.org/10.1016/j.biortech.2013.07.096

[15] De Gioannis, G., Muntoni, A., Polettini, A. and Pomi, R., 2013. A review of dark fermentative hydrogen production from biodegradable municipal waste fractions. Waste Manage. 33, 1345-1361. http://dx.doi.org/10.1016/j.wasman.2013.02.019

[16] Lee, D.-Y., Ebie, Y., Xu, K.-Q., Li, Y.-Y., Inamori, Y., 2010. Continuous H_2 and CH_4 production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge. Bioresour. Technol. 101, 542-547. http://dx.doi.org/10.1016/j.biortech.2009.03.037

[17] Luo, G., Xie, L., Zhou, Q., Angelidaki, I., 2011. Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production. Bioresour. Technol. 102, 8700-8706. http://dx.doi.org/10.1016/j.biortech.2011.02.012

[18] Voelklein, M.A., Jacob, A., O' Shea, R., Murphy, J.D., 2016. Assessment of increasing loading rate on two-stage digestion of food waste. Bioresour. Technol. 202, 172–180. http://dx.doi.org/10.1016/j.biortech.2015.12.001

[19] De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., Spiga, D., 2017. Energy recovery from one- and two-stage anaerobic digerstion of food waste. Waste manage 68, 595-602. https://doi.org/10.1016/j.wasman.2017.06.013

[20] European Commission, 2003. Hydrogen energy and fuel cells. A vision of future. Final report of the high level group. Revisita de la investigacion Europea. http://ec.europa.eu/research/fch/pdf/hlg_vision_report_en.pdf

[21] Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P. N. L., Esposito, G., 2015. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Appl. Energ. 144, 73-95. http://dx.doi.org/10.1016/j.apenergy.2015.01.045

[22] Lee, Y.-W., J., Chung, 2010. Bioproduction of hydrogen from food waste by pilot-scale combined hydrogen/methane fermentation, Int. J. Hydrogen Energy 35, 11746-11755. https://doi.org/10.1016/j.ijhydene.2010.08.093

[23] Chu, C.-F., Ebie, Y., Xu, K.-Q., Li, Y.-y., Inamori, Y., 2010. Characterization of microbial community in the two-satage process for hydrogen and methane production from food waste. Int. J. Hydrogen Energy 35, 8253-8261. http://dx.doi.org/10.1016/j.ijhydene.2009.12.021

[24] 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, 11549-11555. http://dx.doi.org/10.1016/j.ijhydene.2012.03.065

[25] Chinellato, G., Cavinato, C., Bolzonella, D., Heaven, S., Banks, C.J., 2013.

520 Biohydrogen production from food waste in batch and semi-continuous conditions:
521 evaluations of a two-phase approach with digestate recirculation for pH control. Int.
522 J. Hydrogen Energy 38, 4351-4360. http://dx.doi.org/10.1016/j.ijhydene.2013.01.078
523 [26] Micolucci, F., Gottardo, M., Bolzonella, D., Pavan, P., 2014. Automatic process
524 control for stable bio-hythane production in two-phase thermophilic anaerobic
525 digestion of food waste. Int. J. Hydrogen Energy 39, 17563-17572.
526 http://dx.doi.org/10.1016/j.ijhydene.2014.08.136
527 [27] Cavinato, C., Bolzonella, D., Fatone, F., Cecchi, F., Pavan, P., 2011. Optimizazion
528 of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and
529 methane production through reject water recirculation. Bioresour. Technol. 102,
530 8605-8611. http://dx.doi.org/10.1016/j.biortech.2011.03.084
531 [28] Yeshanew, M. M., Frunzo, L., Pirozzi, F., Lens, P. N. L., Esposito, G., 2016.
532 Production of biohythane from food waste via an integrated system of continuously
533 stirred tank and anaerobic fixed bed reactors. Bioresour. Technol. 220, 312-322.
534 http://dx.doi.org/10.1016/j.biortech.2016.08.078
535 [29] De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., Spiga, D., 2017. Energy
536 recovery from one- and two-stage anaerobic digestion of food waste. Waste Manage.
537 68, 595-602. https://doi.org/10.1016/j.wasman.2017.06.013
538 [30] Xiao, B., Qin, Y., Wu, J., Chen, H., Yu, P., Liu, J., Li, Y.-Y., 2018. Comparison of
539 single-stage and two-stage thermophilic anaerobic digestion of food waste:
540 Performance, energy balance and reaction process. Energy Convers. Manag. 156,
541 215-223. https://doi.org/10.1016/j.enconman.2017.10.092
542 [31] Bertin, L., Grilli, S., Spagni, A., Fava, F., 2013. Innovative two-stage anaerobic
543 process for effective codigestion of cheese whey and cattle manure. Bioresour.
544 Technol. 128, 779-783. http://dx.doi.org/10.1016/j.biortech.2012.10.118

545 [32] Dareioti, M. A., Kornaros, M., 2015. Anaerobic mesophilic co-digestion of ensiled
546 sorghum, cheese whey and liquid cow manure in a two-stage CSTR system: Effect of
547 the hydraulic retention time. Bioresour. Technol. 175, 553-562.
548 http://dx.doi.org/10.1016/j.biortech.2014.10.102
549 [33] Xiao, B., Zhang., W., Yi, H., Qin, Y., Wu, J., Liu, J., Li, Y.-Y., 2019. Biogas
550 production by two-stage thermophilic co-digestion of food waste and paper waste:
551 Effect of paper waste ratio. Renew. Energ. 132, 1301-1309.
552 https://doi.org/10.1016/j.renene.2018.09.030
553 [34] Akhlaghi, M., Boni, M. R., De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R.,
554 Rossi, A., Spiga, D., 2017. A parametric response surface study of fermentative
555 hydrogen production from cheese whey. Bioresour. Technol. 244 (1), 473-483.
556 https://doi.org/10.1016/j.biortech.2017.07.158
557 [35] Cappai, G., De Gioannis, G., Friargiu, M., Massi, E., Muntoni, A., Polettini, A.,
558 Pomi, R., Spiga, D., 2014. An experimental study on fermentative H_2 production
559 from food waste as affected by pH. Waste Manage. 34, 1510-1519.
560 http://dx.doi.org/10.1016/j.wasman.2014.04.014
561 [36] Alibardi, L., Cossu, R., 2016. Effects of Carbohydrate, protein and lipid content of
562 organic waste on hydrogen production and fermentation products. Waste Manage.
563 47, 69-77. http://dx.doi.org/10.1016/j.wasman.2015.07.049
564 [37] Pecorini, I., Baldi, F., Iannelli, R., 2018. Biochemical hydrogen potential tests
565 using different inocula. Sustainability (submitted, under review)
566 [38] APAT, 2005. Digestione anaerobica della frazione organica dei rifiuti solidi –
567 Aspetti fondamentali, progettuali, gestionali, di impatto ambientale ed integrazione
568 con la depurazione delle acque reflue. Manuali e linee guida 13/2005.
569 [39] Zhu, H., Parker, W., Conidi, D., Basnar, R., Seto, P., 2011. Eliminating

methanogenic activity in hydrogen reactor to improve biogas production in two-stage anaerobic digestion process co-digesting municipal food waste and sewage sludge. Bioresour. Technol. 102, 7086-7092. http://dx.doi.org/10.1016/j.biortech.2011.04.047

[40] APHA, 2006. Standard Methods for the Examination of Water and Wastewater, Eighteenth ed. American Public Health Association, 2006, Washington, DC.

[41] Martín-González, L., Font, X., Vicent, T., 2013. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochem. Eng. J. 76, 1-5. http://dx.doi.org/10.1016/j.bej.2013.03.016

[42] European Commission, 2009. European Commission Regulation 2009/152/EC of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union, 1–54.

[43] Favaro, L., Alibardi, L., Lavagnolo, M.C., Casella, S., Basaglia M., 2013. Effects of inoculum and indigenous microflora on hydrogen production from the organic fraction of municipal solid waste. Int. J. Hydrogen Energy 38, 11774-11779. https://doi.org/10.1016/j.ijhydene.2013.06.137

[44] Alibardi, L., Cossu, R., 2015. Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. Waste Manage. 36, 147-155. http://dx.doi.org/10.1016/j.wasman.2014.11.019

[45] Wang, Y., Zhang, Y., Wang, J., Meng, L., 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Bioresour. Technol. 33, 848-853. http://dx.doi.org/10.1016/j.biombioe.2009.01.007

[46] Wilson, C.A., Novak, J.T., 2008. Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water

595 Res. 43, 4489–4498. http://dx.doi.org/10.1016/j.watres.2009.07.022
596 [47] Chu, C.-F., Li, Y.-Y., Xu, K.-Q., Ebie, Y., Inamori, Y., Kong, H.-N., 2008.A pH-
597 and temperature-phased two-stage process for hydrogen and methane production
598 from food waste. Int. J. Hydrogen Energy 33, 4739-4746.
599 http://dx.doi.org/10.1016/j.ijhydene.2008.06.060