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Enhancing volatile fatty acids (VFA) production from food waste in a two-phases pilot-scale anaerobic digestion process

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ABSTRACT

The bio-based production of added-value products and energy from waste streams while minimizing environmental impacts is a crucial aspect within the circular economy's principles. A two-phases anaerobic digestion process to produce volatile fatty acids (VFA) and biogas production in an energetically feasible manner from the organic fraction of municipal solid waste (OFMSW) has been investigated. Mesophilic temperature (T) coupled to 5.0 days as hydraulic retention time (HRT) gave a VFA-rich stream with an overall concentration of 24.4 ± 0.2 g COD_{VFA}/L, dominated by propionic and valeric acids, and high acidification yield, being VFA close to 90% of the soluble COD. The pH was maintained around 7.0 by the digestate recirculation from the second methanation reactor (HRT of 20 days) where biogas production was quantified under mesophilic and thermophilic T. The assessment of energy balance showed the benefits to carry out the second methanation stage under thermophilic condition, having more income from electricity generation without losing the thermal sustainability of the two-phases process as a whole.

1. Introduction

The sustainable growth is the main aspect of the ambitious plan adopted by the European Union (EU) 2030 strategy regarding the "Circular Economy" methods and their application [1]. This plan allows to take measures for the improvement of the life cycle of products, with benefits for environment, economy and society. Hence, in this context, the research lines need to adopt those approaches which foreseen a real conservative use of resources. Among others, carbon and nutrients recovery from organic wastes is still an actual and pivotal concept. In particular, the organic waste produced within an urban scenario (at the best case fully separated or source sorted collected) constitutes a valuable resource. Its biodegradability is the main characteristic which allows to apply anaerobic processes in order to recover platform chemicals and/or bioenergy [2]. The anaerobic digestion (AD) is one of the most widespread and well-known technologies in Europe and, in the last decade, it has been adapted for the treatment of a combination of different organic wastes [3,4] in view of eco-design process for a better resources' utilisation. The consideration of a treatment plant as a real production process within a context of an integrated cycle is the background idea for the application of the circular economy concept [5]. For this purpose, the actual facilities need to be newly designed or integrated in order to develop a biorefinery platform [6], leading to several well-defined economic and environmental advantages deriving from: a) a differentiation of the obtainable products and, b) the possibility to adapt/use the existing facilities.

The actual AD full scale plants are devoted to the treatment of organic waste for energy recovery, which is still the most attractive and applied strategy [7]. From the metabolic point of view, the AD pathways can be divided into two main routes: the first is represented by the acidogenic fermentation, and the second is represented by the methanogenesis pathways. The knowledge of this combined process suggests the possibility to produce volatile fatty acids (VFA) and hydrogen from the first fermentative stage and methane from the second one; in practice by dividing the two different process in a two-stages configuration [8]. However, the AD plants that have been installed in Europe are almost in favour of the one stage [9], despite of the better connection of the two-stages AD with the biorefinery concept. In fact, apart from the biogas production, this innovation in the AD process allows to obtain added value streams rich in VFA, which can be further utilized as

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building blocks for the chemical industry or as precursors of reduced chemicals and derivatives (esters, ketones, aldehydes, alcohols and alkanes) in conventional organic chemistry [10,11].

There are several risks which can compromise the efficiency of the anaerobic treatment of the organic wastes, especially if VFA production is one of the main objectives; among others, pH inhibition phenomena or metabolic changes of the fermentation process towards less appetite by-products (such as lactic acid and alcohols) with negatively affected hydrogen and VFA production [8]. It is of primary importance to control and maintain the fermentation process parameters much stable as possible. As main target, it is well known that the pH in the fermenter needs to be kept over 5.0 to promote the fermentative activity, but below 7.0 to avoid the proliferation of methanogenic bacteria [5]. In order to control the pH in the fermentation process and consequently favouring VFA production, the recirculation of the digested effluent from the methanation stage of a two-stages AD process has been recently investigated as an eco-friendly solution [8]. The buffering capacity of the anaerobic digestated is connected to its alkalinity. At the same time, the method of digestate recirculation requires particular attention in order to avoid an excessive accumulation of ammonia and the consequent inhibition of the fermentative bacteria [12]. It has been also demonstrated that the VFA production was negatively affected when ammonia concentration was higher or equal to 1.5 g $N\text{-}NH_4^+$/L [13,14].

A two-phases anaerobic process has been designed for food waste valorisation, in particular the organic fraction of municipal solid waste (OFMSW) after source sorted collection and screw-press as mechanical pre-treatment. The digestate recirculation flow rate, opportunely modelled and set according to the demands and the reactors' evolution on time, was suggested to guarantee process stability for long term period for both fermentation and methanation stages [8]. Despite of this stability and relatively high yield in biomethane production, the combined anaerobic process did not exhibit the maximum potentiality in terms of VFA production, being the biohythane production the privileged route for the valorisation of this waste [13]. In fact, under the fixed investigated process conditions, the VFA concentration did not exceed 16–18 g/L (as chemical oxygen demand; COD), with a fermentation yield around 0.30 kg COD_{VFA}/kg COD_{in} [8,15].

This work aims to increase the conversion yield of the carbon source (the screw pressed OFMSW) mainly into VFA by gradually increase the organic loading rate (OLR) of the 1st phase fermentation reactor (and simultaneously decrease the hydraulic retention time; HRT) under mesophilic condition (37 °C) in order to find the best condition for a stable acidification and high VFA production. Two digestion reactors (2nd phase) were in parallel maintained under mesophilic (37 °C) and thermophilic (55 °C) conditions for biogas production comparison.

Hence, this pilot-scale study aims to further increase the knowledge on the possible and more profitable routes of organic waste valorisation, in particular the OFMSW produced in the urban context, a ubiquitous resource with an economic value still not totally fulfilled [14].

2. Material and methods

2.1. The screw pressed OFMSW characteristics

The OFMSW that has been used in this study came from the door-to-door collection of Treviso municipality (northeast Italy) and it was conferred in the pilot experimental area within the wastewater treatment plant (WWTP) of the same city after its mechanical pre-treatment. The latter was performed in a dedicated plant, outside the WWTP facilities. The screw pressed OFMSW was analysed every week, immediately after its sampling. In terms of chemical and physical characteristics, the OFMSW showed an average dry matter content of 120 ± 14 g TS/kg (Total Solids) and 107 ± 7 g VS/kg (Volatile Solids). The volatile organic matter accounted for almost 90%, meaning that the putrescible and fermentable fraction was high enough for the fermentation process. The COD was 857 ± 42 g COD/kg TS; nitrogen (as TKN) and phosphorus were 26 ± 3 g N/kg TS and 6 ± 1 g P/kg TS on average, respectively.

2.2. The two-phases anaerobic system

The fermentation reactor (F) was filled up with the OFMSW, mixed with digestate (from the full-scale AD plant located in Treviso WWTP) and tap water, in order to obtain a TS content close to 7–8% w/w. The fermentative bacteria already existing in the substrate allowed to convert the organic matter into VFA, which was the main goal of the study; hence, no anaerobic inoculum was used [16]. Differently from previous experiments where short HRT (close to 3.0 d), thermophilic temperature (55 °C) and slightly acid pH values (5.0–5.5) were used in favour of hydrogen production [8,17], in this case, longer HRT (5 d), mesophilic temperature (37 °C) and pH in the range 6.5–7.5 were set as operating parameters. Over the course of operation, the fermentation reactor was daily fed with a liquid mixture of the OFMSW, digestate from the mesophilic methanogenic reactor (AD_1) and dilution water in order to reach the required OLR.

The two methanogenic reactors, AD_1 and AD_2, were inoculated with the anaerobic digested sludge coming from the full-scale digester of Treviso wastewater treatment plant (WWTP) and maintained at 37 °C and 55 °C respectively. Both methanogenic reactors were daily fed with VFA-rich effluent from the fermentation reactor. AD_1 and AD_2 were maintained in parallel for a representative comparison of the biogas production, quantified in terms of specific gas production (SGP) and gas production rate (GPR).

Three pilot stainless steel CSTR reactors (AISI 304) were used for the first fermentation stage (F; working volume 0.2 m^3) and for the second digestion stage (AD_1 and AD_2; working volume 0.2 m^3). The three reactors were heated by a hot water recirculation system and maintained at the chosen temperature (37 °C for F and AD_1; 55 °C for AD_2) using electrical heater controlled by a PT100-based thermostatic probe. Fig. 1 shows the flow diagram of the pilot scale anaerobic reactors system described above.

The digestate from AD_1 was daily recirculated through a control method based on inputs from the online probes: the pH meter in F, pH meter and a conductivity probe in AD_1. This digestate recirculation method has been deeply described elsewhere [8].

The fermentation process was maintained in operation for about 180 days. The second AD phase was started after a remarkable fermentation activity was observed in F; both AD_1 and AD_2 were approximately maintained for 140 days. The basic idea of the process configuration is that a portion of VFA-rich mass flow produced in F has to be removed from the anaerobic system to be used for other purposes.

Table 1 summarizes the operating conditions of the three reactors after the steady-state was reached. The first fermentation stage had an HRT of 5 d and an OLR in the range 14–15 kg VS/(m^3d). In the methanogenic stage, the HRT and OLR were set at 20 d and 2.6–2.9 kg VS/(m^3d) respectively, in both AD_1 and AD_2.

2.3. Analytical methods

The reactors' effluent was monitored two times per week, for total and volatile solids content, COD, ammonia, TKN, total phosphorus, total and partial alkalinity. The pH and VFA (both concentration and distribution) were checked three times per week. With the exception for VFA, all the analyses were carried out in accordance with the Standard Methods [18]. The quantification of the VFA was conducted using AGILENT 6890N gas chromatograph equipped with a flame ionization detector (with T of 200 °C), a fused silica capillary column, DB-FFAP (15 m x 0.53 mm × 0.5 μm thickness of the film), and hydrogen was the gas carrier. The chromatographic run was conducted by increasing the temperature from 80 °C to 200 °C at 10 °C/min. The samples were analysed before being centrifuged and then filtered with a 0.20 μm filter.

Biogas production was quantified by a flow meter (Ritter

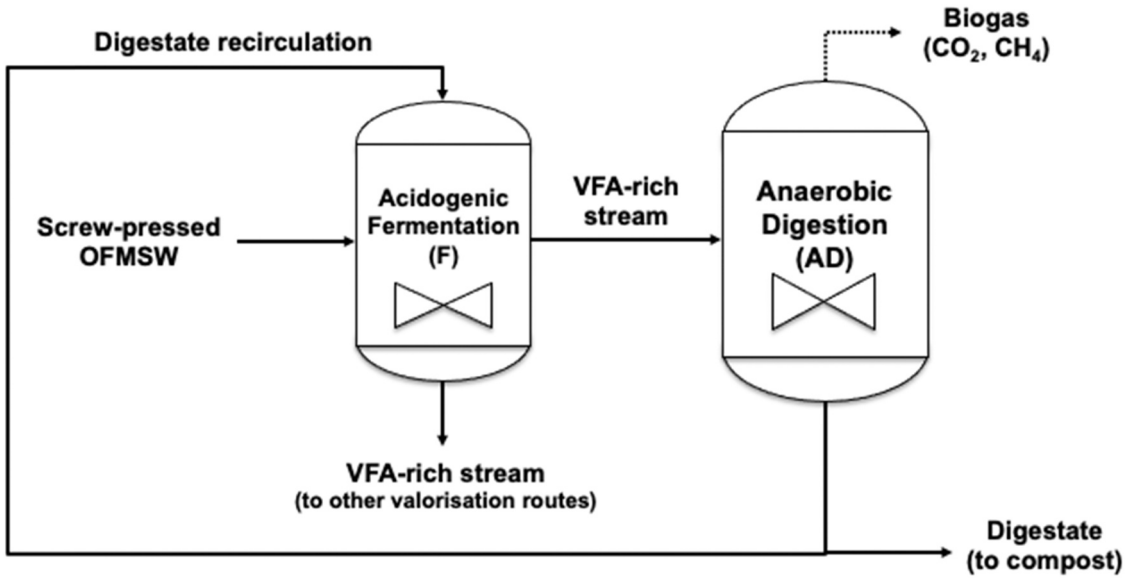

Fig. 1. Two-phases anaerobic process configuration for biogas and VFA production.

Table 1
Operating conditions of the three anaerobic reactors in the two-phases anaerobic digestion pilot platform.

Fermentation reactor (F)			Digestion Reactor (AD_1)			Digestion Reactor (AD_2)		
HRT d	OLR kg VS/(m^3d)	T °C	HRT d	OLR kg VS/(m^3d)	T °C	HRT d	OLR kg VS/(m^3d)	T °C
5	14–15	37	20	2.6–2.9	37	20	2.6–2.9	55

Company™), while CO_2 percentage in the biogas was determined through a portable infrared gas analyzer GA2000TM (Geotechnical Instruments™). The percentage of CH_4 and H_2 were determined by a gas chromatograph GC Agilent Technology 6890 N™ equipped with a column HP-PLOT MOLESIEVE™ (30 m x 0.53 m ID x 25 μm thickness of the film), using a thermal conductivity detector (TCD) at 250 °C. The injector T was 120 °C and a constant pressure of 70 kPa in the injection port was maintained. Samples were taken using a gas-type syringe in 200 μL biogas amounts. The analyses were conducted at a constant T of 40 °C for 8 min, by using Argon as gas carrier.

2.4. Calculations

All the parameters characterising F, AD_1 and AD_2 performances were calculated after the achievement of the steady states. The fermentation process was considered under steady state after consistent VFA production was observed. Solubilisation and fermentation yield (Y_{VFA}) were quantified based on the characteristics of the unfermented screw pressed OFMSW, in particular the initial total and soluble COD (COD_{TOT} and COD_{SOLin}) and initial VS (VS_{in}):

$$Solubilization = \frac{g\ (CODsol - CODsol\ in)}{g\ VSin}$$

$$Fermentation\ \ yield\ \ (Yvfa) = \frac{g\ (CODvfa - CODvfa\ \ in)}{g\ \ CODtot}$$

The steady state in the second anaerobic digestion step was considered achieved when the biogas production rate (GPR) was constant (below 10% of deviation from average values).

The mass and energy balance were assessed in a real scenario of 100,000 person equivalent (PE), which is an average potentiality plant like the full-scale WWTP of Treviso. Both "F-AD_1" and "F-AD_2" conditions were evaluated. Boundary conditions and reference parameters used for the thermal balance are listed in Table S1, including the thermal and electrical yields of a Combined Heat & Power unit (CHP) for electricity and thermal energy generation. The price of electric energy, without incentive, was assumed to be 130 €/MWh [19]. The disposal cost of the digestate (including dewatering treatment) was assumed to be 100 €/ton [20]. A biogas upgrade cost of 0.25 €/m^3 was considered for biomethane production (98% CH_4) with the pressure swing adsorption (PSA) technique [17]. A biomethane selling price of 0.70 €/kg has been considered [21]. The OFMSW inlet flow rate was quantified from a specific production of 0.25 kg OFMSW/PE day, based on wet weight [22].

3. Results and discussion

3.1. The first acidogenic fermentation stage

3.1.1. Process implementation and stability

In the first 50 days, the OFMSW fermentation was conducted without pH control. This approach was used in the process start up, before reaching the steady state of AD_1. Afterwards, the AD_1 digestate was used to control the pH of fermentation reactor, through the application of the recirculation method recently described [8]. The OFMSW fermentation start-up was characterised by a gradual increase of the OLR in order to maintain an appropriate equilibrium between the alkalinity and the acidification (due to VFA production). The high fermentability of the screw pressed OFMSW was related to the collection system within the municipality. The separate door-to-door collection increased the VS/TS content up to 90% w/w, meaning that the putrescible and fermentable matters were in large amount. Hence, from the perspective of acidification and process control, the initial OLR was set at very low values, around 3.0–3.5 kg VS/(m^3 d), with an HRT higher than 10 days. The initial pH of the fermentation reactor was close to 8.0, due to the digestate addition. The initial low-rate process caused a quite long start-up phase, which was not usual for the fermentation reactors treating organic wastes [23]. A substantial fermentation activity was observed after the 37th day from the inoculum, when the VFA concentration started to increase above 5.0–7.0 g COD/L (Fig. 2A) and the OLR was almost doubled compared to the initial value. The partial alkalinity was quantified at pH 5.0, since it was expected that with the increase of VFA content, the pH values could decrease below 6.0. Even though the alkalinity content showed remarkable variations over the course of the fermentation process, it was never below 1600 mg$CaCO_3$/L (Fig. 2B). As a consequence, the pH of the fermentation reactor was always and abundantly above 6.0, despite of the progressive increase of both OLR

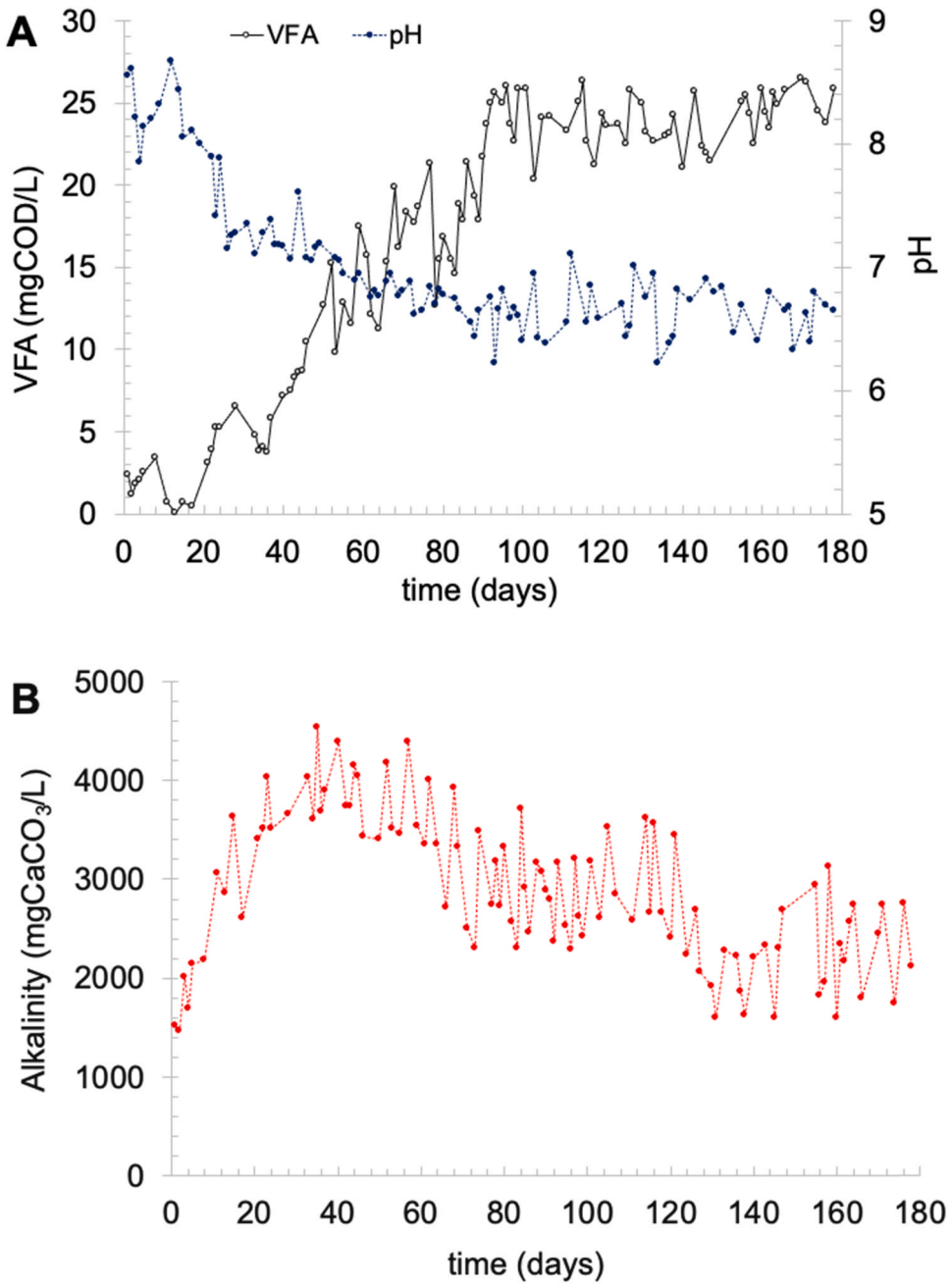

Fig. 2. Trends of VFA and pH (A), and alkalinity (B) in the first acidogenic fermentation phase.

and VFA concentration. In the first window time of 80–90 days (roughly), the OLR was increased up to the chosen value of 14–15 kg VS/(m^3 d). Similarly, the VFA concentration showed an increasing trend in the first 90 days; then, the VFA level showed a stable trend with an average concentration of 24.4 ± 0.2 g COD_{VFA}/L. The pH trend reflected the increase of VFA level, and it was stable on the average value of 6.6 ± 0.2 for the whole stability period (days 90–180). The alkalinity trend showed an initial increase when the HRT was maintained at relatively high values (>10 days); the increase of VFA concentration after 40 days progressively consumed the alkalinity, which was also stable (2478 ± 80 mg $CaCO_3$/L) when the stationary condition was achieved. In practice, the mesophilic process produced a fermented stream with stable chemical characteristics starting from the 90th days of operation. Such long period was a consequence of the chosen initial low-rate (HRT > 10 d), which was necessary to control the pH of the system, before that the digestate recirculation from AD_1 was applied. The relatively high and stable content of VFA in the steady state period was achieved based on three factors, not previously adopted on the two-phases anaerobic process for OFMSW treatment:

a) the mesophilic T caused lower acidification kinetic compared to previous thermophilic T [5], avoiding sudden drops of the pH (followed by a fermentative activity inhibition [24]);
b) higher HRT (5 d) and lower OLR (14–15 kg VS/(m^3 d)) compared to those usually applied in the two-phases system (HRT 3 d; OLR 17–20 kg VS/(m^3 d) [5,15], made the fermentation process more stable despite of the higher VFA content;
c) higher pH (6.6 ± 0.2) compared to previous studies (5.0–5.5, [5,8]) reduced the risk of inhibition from acidic environment.

In a previous study [5], the fermenter HRT was set at 3.3 d and the OLR was equal to 17 kg VS/(m^3 d); the latter was increased up to 20 kg

VS/(m^3 d) in a following study [15] where the maximum VFA content achieved was 16.0 ± 0.7 g COD_{VFA}/L, with a fermentation yield of 0.31 ± 0.02 COD_{VFA}/COD_{TOT}. Similar fermentation yield (0.31–0.32 COD_{VFA}/COD_{TOT}) was obtained in a previous study [8], where the digestate recirculation method was optimized in order to ensure stable VFA and high H_2 production (up to 40% v/v in the gas phase of F reactor) in a slightly acid fermentation environment (pH 5.0–5.2) under thermophilic condition. The operating parameters adopted in this study were not optimal for H_2 production, whose content was below 15% v/v in the gas phase. However, the VFA in the liquid stream represented a substantial fraction of the soluble COD (0.90 ± 0.03 COD_{VFA}/COD_{SOL}) and the corresponding fermentation yield was 0.49 ± 0.03 COD_{VFA}/COD_{TOT}, above those obtained in previous investigations where higher T, OLR and lower pH were applied [2,5,8,15]. Fermentation performances as well as physical-chemical characteristics of the fermented stream in the steady state period are reported in Table 2.

The low standard deviations of average values for both fermentation performances and physical-chemical characteristics indicated the high process stability under mesophilic conditions, as previously observed for other feedstock mixture containing food waste [19]. This is a pivotal aspect affecting the robustness and the technical process feasibility.

Compared to higher T examples [25], OFMSW fermentability at 37 °C was found to be less performing for COD and solids solubilisation (0.49 ± 0.02 and 0.33 ± 0.01 on COD_{TOT} and initial VS respectively). However, the obtained high conversion VFA yield led to a high VFA content on soluble COD. Previous experiments based on OFMSW fermentation reported higher VFA concentrations (up to 29.0 g COD_{VFA}/L [25], and 60 g COD_{VFA}/L [26]). However, such high values were obtained in batch test and presumably, effective strategies for pH control are necessary in a CSTR process. In addition, a substantial part of the COD_{SOL} remained unconverted (0.27–0.54 COD_{VFA}/COD_{SOL} [25]) compared to this investigation (0.90 ± 0.03 COD_{VFA}/COD_{SOL}), making the economic valorisation of this stream only partially fulfilled.

3.1.2. VFA composition

The average composition of produced VFA in the stability period is reported in Fig. 3 and it refers to "Case1" (this study). On a COD basis, the 56% of VFA was represented by propionic and valeric acids. The VFA composition is a pivotal characteristic for a VFA-rich stream in a perspective of its possible utilization besides bio-methane production. As an example, the microbial utilization of VFA to synthetize biopolymers (namely polyhydroxyalkanoates or PHA [27]) is an emerging technology which allows to recover an added-value product from different kind of renewable resources, after the conversion of their soluble COD into VFA. In particular, the balance of odd and even carbons in the VFA determines the polymer composition [28]. If the VFA to PHA productive line is foreseen, the VFA-rich stream obtained in this study would lead to a higher fraction of hydroxyvalerate (HV) monomers in the PHA chains, compared to the hydroxybutyrate (HB) content [29]. This chemical feature gives a copolymer (PHBV) with particular properties of thermal resistance, elasticity and hardness, which in turn could expand the market applications of this material [30]. In previous works indicated as "Case2" [15] and "Case3" [8] in Fig. 3, acetic and butyric acids accounted for almost 60% of total VFA (on a COD basis) produced in the OFMSW fermentation process. Such difference could be due to several factors, such as the different HRT, OLR, pH or T that have been used in the three experiments. Other authors did not observe relevant differences in VFA composition by changing the HRT (4–6 d) and OLR (7.7–11.3 kg VS/m^3 d) in a CSTR fermentation process [31] carried out with a feedstock mixture composed by OFMSW and sewage sludge. The difference in VFA distribution between "Case1" and "Case2–3" was probably related to the different pH (6.6 and 5.0–5.2 respectively) and T (37 °C and 55 °C respectively). In support of this hypothesis, other authors [32] demonstrated how the operating conditions of "Case2–3" (pH and T in particular) stimulated the hydrogenase enzymes activity for the optimization of H_2 production, with acetic and butyric acids as main by-products. For higher pH and under mesophilic regime (similar to "Case1" of this study), the same authors demonstrated how the hydrogenase activity was less favoured. The recent literature studies showed that neutral and/or alkaline environment (pH 7.0–9.0) led to higher fraction of VFA with even carbons (propionic and valeric in particular) compared to acidic conditions (pH 5.0): this was demonstrated with a mixture of OFMSW and sewage sludge [31] and with cellulosic primary sludge [33].

The fermentation products in this study were dominated by propionic and valeric acid (28.6% and 27.2% COD basis, respectively), followed by lower amount of butyric and acetic acids (15.4% and 14.4% respectively). The residual 14.4% was represented by the other VFA with odd carbons (caproic, isobutyric) and a smaller portion of isovaleric acid. The molar fraction of VFA with odd number of C-atoms ($[C_3/(C_3+C_2)]_{VFA}$) related to the total was equal to 0.52 mol/mol, at least doubled if compared to the previous "Case2" [15] and "Case3" [8].

3.2. The second anaerobic digestion stage

Both mesophilic (AD_1) and thermophilic (AD_2) processes were started at relatively low OLR (1.2–1.5 kg VS/m^3 d) by diluting the F effluent with sewage sludge, coming from the adjacent WWTP. The F effluent amount was gradually increased in order to achieve the set OLR range of 2.6–2.9 kg VS/m^3 d. The gradual increase of the F effluent was necessary in order to prevent possible pH drop in AD reactors.

In mesophilic condition, steady state was achieved in 50 days (roughly 2 HRTs). In the start-up period, when the risk of methanogenic bacteria inhibition is usually high, the OLR was increased from 1.2 to 2.3 kg VS/m^3 d approximately; no relevant pH drop was observed since the VFA concentration was steadily maintained below 0.7–0.8 g COD_{VFA}/L. Average pH value of AD_1 steady state period was 8.1 ± 0.2. The process stability was guaranteed by the high buffering capacity of

Table 2
Performances of the three pilot reactors (F, AD_1 and AD_2) and physical-chemical features of the fermented OFMSW (F effluent) and mesophilic and thermophilic digestate (respectively AD_1 effluent and AD_2 effluent).

Acidogenic Fermentation (F)		Anaerobic Digestion (AD_1)		Anaerobic Digestion (AD_2)	
Parameter	**Average value**	**Parameter**	**Average value**	**Parameter**	**Average value**
TS (g/kg)	71 ± 2	*TS (g/kg)*	34 ± 2	*TS (g/kg)*	29 ± 2
VS (g/kg)	62 ± 3	*VS (g/kg)*	27 ± 2	*VS (g/kg)*	22 ± 4
pH	6.6 ± 0.2	pH	8.1 ± 0.2	pH	7.8 ± 0.3
COD_{VFA} (g/L)	24.4 ± 0.2	*COD_{VFA} (g/L)*	0.75 ± 0.3	*COD_{VFA} (g/L)*	0.87 ± 0.2
COD_{SOL} (g/L)	27.2 ± 0.3				
Alkalinity (pH 5) ($mgCaCO_3$/L)	2478 ± 80	*Alkalinity (pH 5) ($mgCaCO_3$/L)*	3558 ± 141	*Alkalinity (pH 5) ($mgCaCO_3$/L)*	3921 ± 172
TKN (g N/kg TS)	–	*TKN (g N/kg TS)*	29 ± 2	*TKN (g N/kg TS)*	33 ± 2
P (g P/kg TS)	–	*P (g P/kg TS)*	12 ± 1	*P (g P/kg TS)*	15 ± 1
Yield (g COD_{VFA}/g COD_{TOT})	0.49 ± 0.03	*SGP (m^3/k VS)*	0.66 ± 0.03	*SGP (m^3/k VS)*	0.76 ± 0.02
Solubilisation (g COD_{SOL}/g VS_{in})	0.33 ± 0.01	*GPR (m^3/m^3 d)*	2.20 ± 0.04	*GPR (m^3/m^3 d)*	2.45 ± 0.05
		%CH_4 (v/v)	63 ± 2	*%CH_4 (v/v)*	64 ± 1

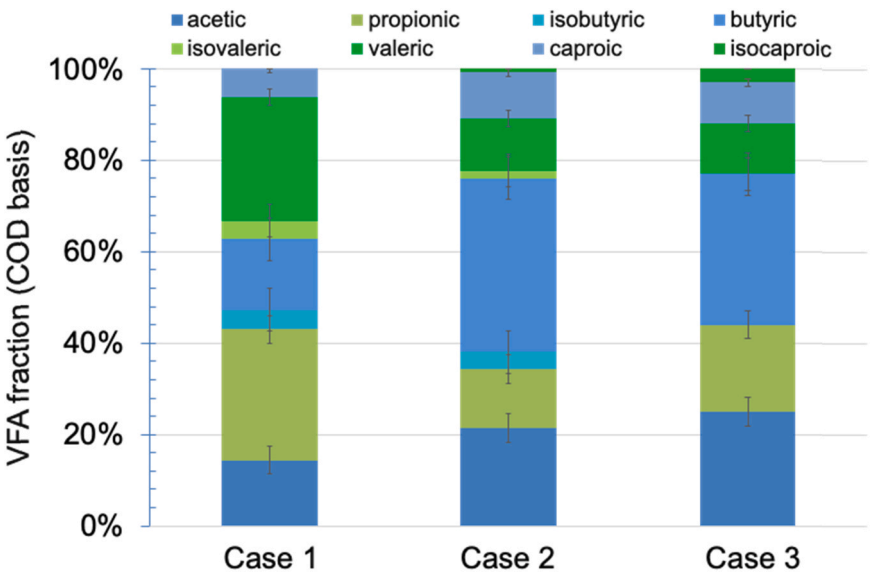

Fig. 3. VFA composition of the fermented feedstock obtained in this study (Case1), in Valentino et al., 2018 (Case2) [15] and Micolucci et al., 2020 (Case3) [8], with OFMSW produced from the same urban origin under the same process configuration.

the system; the partial alkalinity was 3558 ± 141 mg $CaCO_3$/L and the VFA/Partial Alkalinity (P.Alk) ratio was 0.21 ± 0.01 mg acetic acid/mg $CaCO_3$ (Fig. 4A). Despite of the initial unbalance, due to the high VFA content in the feed (VFA/P.Alk ratio showed a maximum peak of 0.88 mg acetic acid/mg $CaCO_3$), the steady state period was characterized by a VFA/P.Alk ratio constantly below 0.30, indicating a negligible VFA accumulation. This value is widely recognized as the threshold, beyond which the system could face with the risk of instability due to the excessive OLR and/or uncontrolled increase of VFA concentration [34]. Another factor that could be detrimental for the methanogenic bacteria is the accumulation of ammonium, which usually occurs from the proteins' degradation during high HRT processes. In AD_1, the ammonium in the liquid phase in the steady state was 0.87 ± 0.05 g $N–NH_4^+$/L, abundantly below 3.0 g $N–NH_4^+$/L, which is the critical value inhibiting methanogenic populations [34]. In AD_2 process, the steady state was reached in similar timescale to AD_1. No relevant VFA accumulation was detected with the exception of the first 30 days (1.5 HRTs approximately), when the VFA/P.Alk ratio was permanently above 0.30 (maximum value 1.04 mg acetic acid/mg $CaCO_3$, day 12) (Fig. 4B). In the steady state period, the average pH value was 7.8 ± 0.3 and the VFA/P.Alk ratio was always below 0.30 (specifically 0.22 ± 0.01 mg acetic acid/mg $CaCO_3$).

Fig. 4 A shows the biogas production rate (GPR) and the OLR, along with VFA/P.Alk ratio in AD_1. As expected, the GPR consistently increased with the progressive increase of the OLR in the course of process stabilization. The initial GPR was quite low (0.80–1.00 m^3/m^3 d) due to the high content of VFA in the feed and the system was critically under unstable conditions. At the steady state, GPR was 2.20 ± 0.04 $m^3/(m^3$ d) and the percentage of methane detected in the biogas was 63 ± 2%; most of remaining part was CO_2 (35 ± 3%). The GPR value was lower than that previously reported (2.6–2.8 m^3/m^3 d [17] in a two-phases process with OFMSW as substrate. However, compared to the previous case [17], the configuration adopted in this study foresaw a lower rate process (e.g. lower OLR and higher HRT) in order to better control the methanogenic phase and to avoid any risks associated to the use of the highly VFA concentrated stream from the first phase (24.4 ± 0.2 g COD_{VFA}/L). The specific gas production (SGP) in the steady state was equal to 0.66 ± 0.03 m^3/kg VS, in line with similar values obtained by the anaerobic treatment of the OFMSW [5, 15].

In AD_2, the progressive increase of the OLR reflected the GPR trend, according to what observed in AD_1 (Fig. 4B). The steady state GPR was 2.45 ± 0.05 $m^3/(m^3$ d), while methane biogas content was 64 ± 1%. As for the GPR, also the SGP in AD_2 (0.76 ± 0.02 m^3/kg VS) was higher than SGP in AD_1, as observed in high T anaerobic digestion processes where higher solid abatement (compared to mesophilic conditions) usually occurred [35].

As a whole, both AD trials were technically feasible; no relevant accumulation of VFA in the reaction media was detected (at least in the steady-state) and the digestate recirculation did not remove such high level of alkalinity to compromise the process stability and the balance with the high VFA-concentrated influent. In fact, both reactors maintained stable pH values around 8.0. Biogas production and composition confirmed the robustness of the process in AD_1 and AD_2. Table 2 summarizes the parameters and the quantified performances obtained in the steady state of both AD processes.

3.3. Mass and energy balance

The mass balance has been evaluated for both process configurations (F-AD_1 and F-AD_2) and could represent a realistic approach to be applied in urban areas where the food waste is separately collected and mechanically pre-treated. Each data related to the performances of F, AD_1 and AD_2 reactors as well as the characterization of the process influents/effluents and produced biogas have been taken into account (Fig. 5). The OFMSW mechanical pre-treatment produces two streams with a different VS concentration. The portion named as "Liquid Fraction" in Fig. 5 represents the screw pressed OFMSW that has been used in this study. The OFMSW mass flow in the two-phases system was approximately 21,250 kg/d. Assuming the average VS concentration measured in this study (112 ± 24 gVS/kg), the VS flow rate was 2380 kgVS/d. The OLR applied to the first reactor (F) was assumed to be 15 kgVS/m^3d, with a related HRT of 5 d. Based on input data (SGP 0.15 m^3/kgVS), the biogas produced from F reactor was 357 m^3/d (CO_2, H_2 and CH_4 at 78%, 14% and 8% respectively). The fermentation effluent flow rate was 1878 kgVS/d (31,250 kg/L) and, based on the methodology applied at pilot scale, this VFA-rich stream was equally split in order to recover biogas and digestate through the 2nd methanation stage, and to sustain a parallel valorisation route [36]. This stream was highly enriched in VFA compared to the unconverted COD_{SOL} (0.90 ± 0.03 COD_{VFA}/COD_{SOL}). In the perspective of VFA utilization for PHA synthesis by mixed microbial culture (MMC), a high COD_{VFA}/COD_{SOL} is particularly desired.

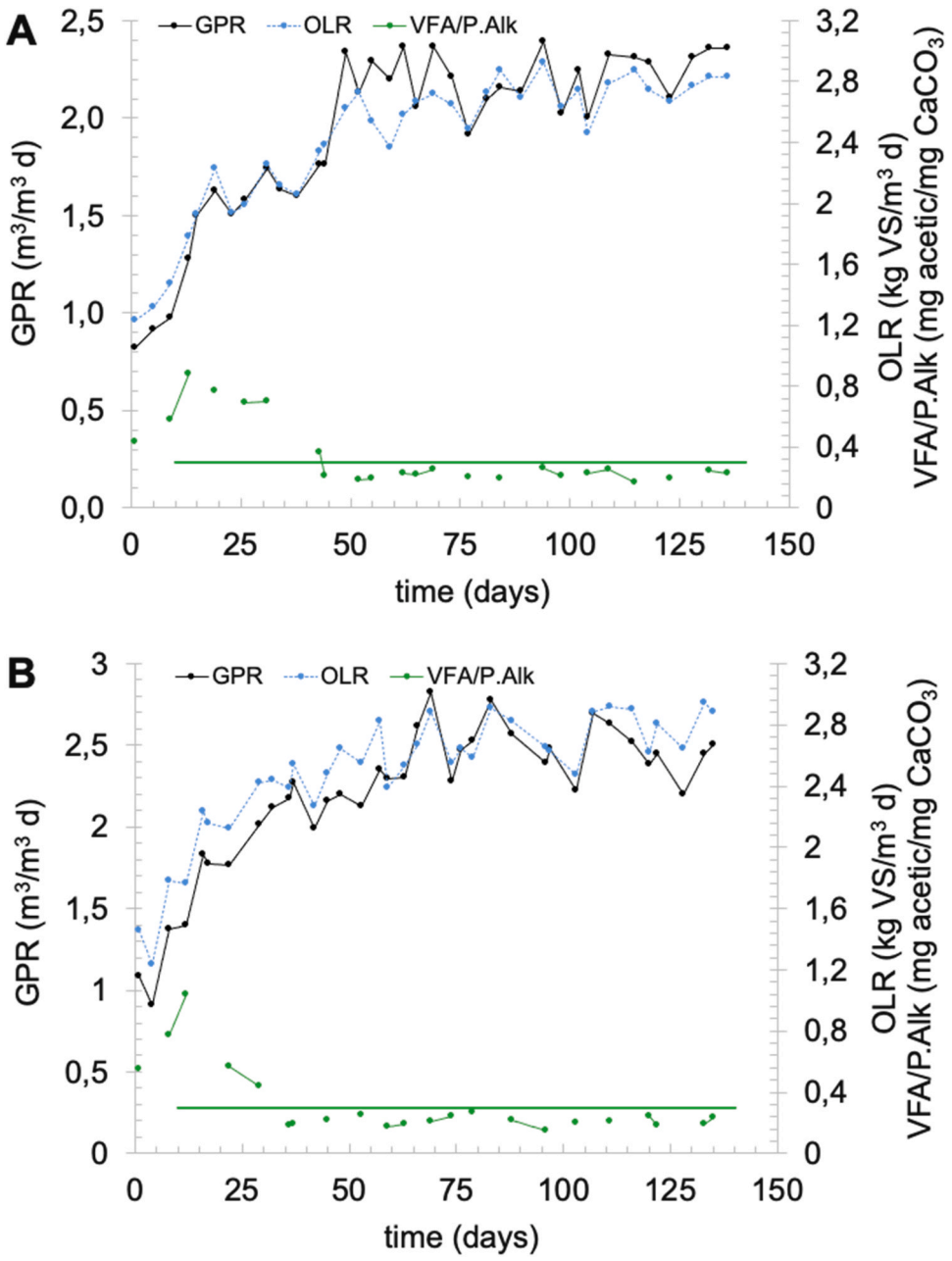

Fig. 4. Trends of GPR, OLR and VFA/P.Alk in mesophilic (AD_1) (A) and thermophilic (AD_2) (B) process.

Literature data reported a PHA production yield between 0.35 and 0.40 COD_{PHA}/COD_{VFA} [37], through a combination of a two-stages aerobic processes for PHA accumulation within cellular walls. Apart from the necessary downstream processing for the extraction and purification of PHA, the approach described in this study could potentially sustain a production of 150 kg $COD_{Polymer}$/d, in addition to biogas and digestate from the methanation phase. The two digestors were fed with the concentrated VFA solution at medium OLR (3.0 kg VS/(m^3 d)) and high HRT (20 d). Both AD_1 and AD_2 had similar biogas composition (in particular CH_4 content was 63–64% v/v). As expected, the thermophilic trial produced more biogas compared to mesophilic (based on the experimental SGP values), being 713 and 619 m^3/d in AD_2 and AD_1 respectively.

The thermal and electrical yields of the CHP had an overall efficiency of 0.9 (0.5 and 0.4 for heat and electrical efficiency respectively; [19]). Hence, in F-AD_1 configuration, the CHP allowed to produce 11, 232 MJ/d and 2.5 MWh/d as thermal and electrical energy respectively. On the other hand, the required thermal energy corresponded to 2574 MJ/d. Therefore, the thermal balance had a net production of 8659 MJ/d. Almost 80% of the required thermal energy was necessary for the OFMSW pre-heating (up to 37 °C) before the mesophilic fermentation. In this calculation, the effect of the AD_1 digestate recirculation was considered. The other lower part was a consequence of supporting the heat dissipation phenomena. From the sale of generated electricity (2.5 MWh/d), a yearly revenue of 116,892.00 €/y has been quantified. In the fermentation stage, the VS flow rate was reduced from 2380 to 1878 kgVS/d; in AD_1, from 939 to 296 kgVS/d. As a whole, the two reactors (F-AD_1) converted 1146 kgVS/d into biogas, with a VS removal efficiency of 87%. The output digestate flow rate was 296 kgVS/d (or 15,625 kg/d), of which approximately 60% was recirculated into F reactor. Taking into account the digestate recirculation, the F influent flow rate (21,250 kg/d) and the HRT (5.0 d), the F volume was 156 m^3. Similarly, the AD_1 reactor volume was 313 m^3. The not recirculated digestate stream had a cost of 29,427.00 €/y. In summary, given

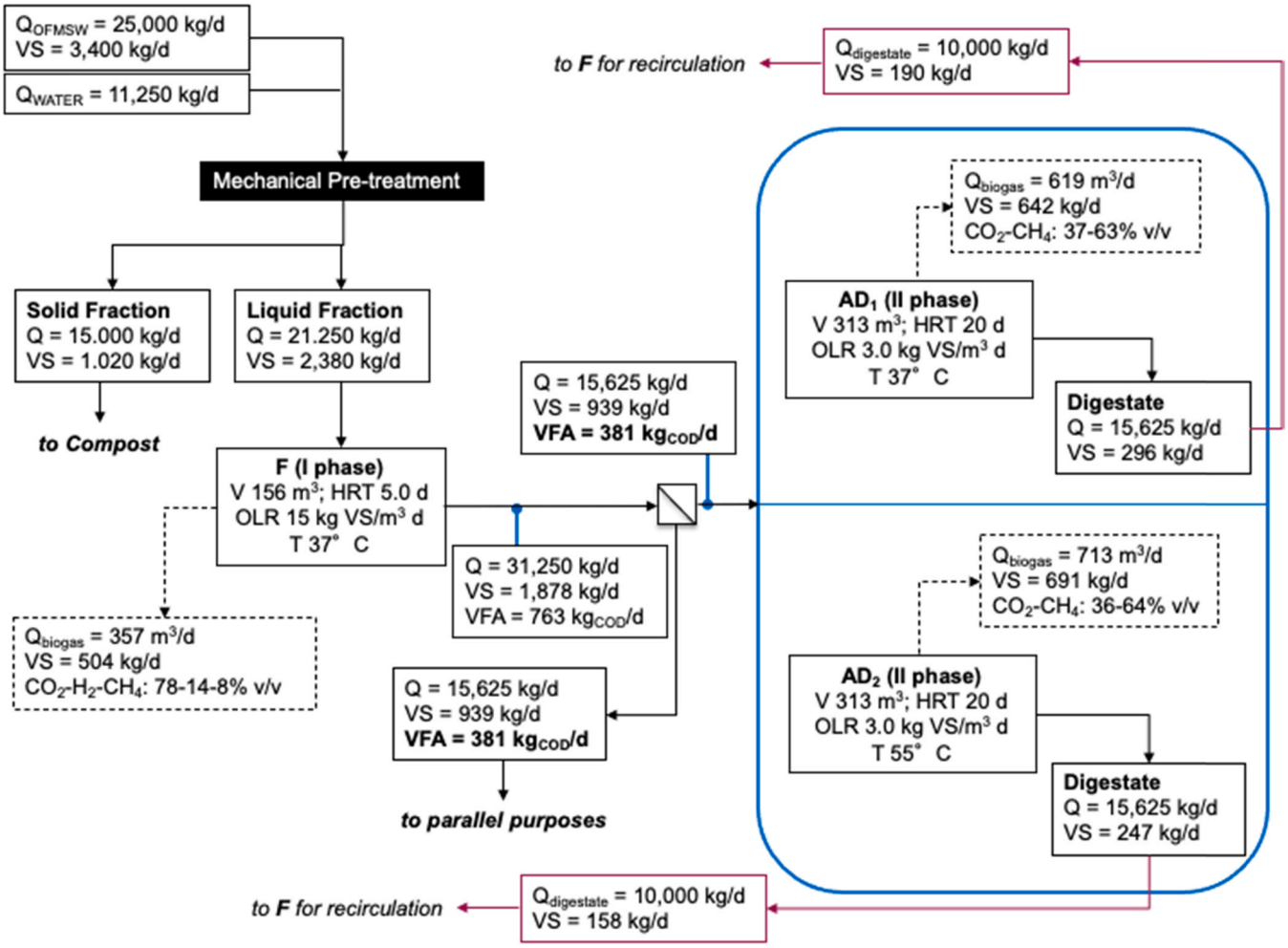

Fig. 5. Mass balance of the whole anaerobic two-phases process including both configurations F-AD_1 and F-AD_2.

the income from electricity and the cost associated to the digestate disposal, the net revenue for the F-AD_1 system has been estimated to be 87,465.00 €/y.

Following the same demonstration approach, the F-AD_2 system showed a similarly favourable thermal balance (or process self-sustainability in terms of thermal energy). However, the application of thermophilic temperature in the methanation stage led to 10% higher biogas production (1070 m^3/d) and 8% higher electricity generation (2.7 MWh/d), and 9% lower digestate to be disposed of (187 kgTS/d). Hence, the net revenue for the F-AD_2 system has been estimated to be 101,254.00 €/y, almost 16% higher than F-AD_1 system. All terms of produced and required energy, as well as the economic incomes and outcomes are summarized in Table S2.

The biogas upgrading process has been also evaluated, in order to achieve biomethane production at 98% v/v for the automotive sector. Independently from the operative T in the methanation stage, the automotive scenario was less economically attractive compared to electricity generation. The whole F-AD_1 system produced 976 m^3/d of biogas at 43% v/v of CH_4 content. Assuming an upgrading cost of 0.25 €/m^3 biomethane [17], the annual needed expenses were 38,202.00 €/y, with a biomethane production of 419 m^3/d (293 $kgCH_4$/d). At the price of 0.90 €/kg, the annual net income from biomethane production was 58,067.00 €/y. In the F-AD_2 system, given the higher biogas production, the net income was almost 16% higher (67,254.00 €/y).

In order to shift from the most applied single stage AD to two-phases process, capex costs are also required, and their quantification is essential for the estimation of the payback time. A comprehensive evaluation needs to take into account also the produced VFA-rich stream, its potential utilization and the market value of VFA, which is higher than methane value [38].

Therefore, the F-AD_2 configuration represented the best option for the anaerobic OFMSW valorisation. The low T in the first reactor ensured stability in the fermentation process conducted with highly fermentable feedstock and characterized by high VFA content; the high T in the second reactor maximize the electrical energy generation without negatively affecting the thermal balance.

4. Conclusions

The designed two-phases anaerobic process enhanced the VFA production to high concentration (24.4 ± 0.2 g COD_{VFA}/L), maximizing the acidification of the COD_{SOL} contained in the screw pressed OFMSW. The conduction of mesophilic OFMSW fermentation at intermediate HRT (5 d) also led to a stable acidification activity, sustained by the digestate recirculation from the methanogenic reactor.

The thermophilic condition applied on the 2nd methanation stage increased the biogas recovery and the benefits from electricity generation without negative impact on the thermal balance of the process (as a whole). Besides biogas and compost, the availability of a VFA-rich stream with stable characteristics, especially the distribution of the short-chain molecules, gives the possibility to recover bio-products with high market potential. The removal of the 50% of the COD_{SOL} (in form of VFA) for parallel purposes (as made in this study) is not mandatory and it can be manageable, provided that the AD operating conditions are

guaranteed. The amount of COD_{SOL} to be used for other scopes needs to be related to the market choice, which could be represented by VFA their self as building blocks for chemical industry, or by other VFA-derived bioproducts such as microbially synthetized biopolymers (such as PHA). This choice has to be also evaluated in terms of technical and economic feasibility in a realistic full-scale scenario.

CRediT authorship contribution statement

Francesco Valentino: Conceptualization, Methodology, Data Curation, Writing – original draft, Writing – review & editing. **Gianluca Munarin**: Investigation, Formal Analysis, Data Curation. **Marco Biasiolo**: Investigation, Data Curation. **Cristina Cavinato**: Visualization. **David Bolzonella**: Validation, Project Administration. **Paolo Pavan**: Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2021.106062.

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