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Abstract: A combined acidic fermentation and anaerobic digestion (AD) treatment has been developed on pilot scale for urban bio-waste conversion into volatile fatty acid (VFA) and biogas. The specific waste mixture was composed by the pre-treated organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS), both produced inside the Treviso (northeast Italy) municipality. The effect of temperature (37°C and 55°C) was investigated in both steps. Only the mesophilic fermentation process provided a VFA-rich stream (19.5 g $CODVFA/L$) with stable physical-chemical features, with no need of chemicals addition for pH control. The sludge buffering capacity made this step technically feasible. The AD step was performed on the solid-rich fraction of fermented bio-waste, after dilution with excess WAS. No relevant differences were observed under the two investigated temperature: in the steady state (organic loading rate of 2.5 kg VS/m^3 d), the specific biogas production was 0.40 and 0.45 m^3/kg VS at 37°C and 55°C respectively, with similar CH_4 content (63-64% v/v). The scaled-up version of the system (in an average urban municipality of 170,000 Person Equivalent) revealed that the whole process is thermally sustainable if both reactors are operated at mesophilic temperature: 36% of surplus thermal energy and 13,03 MWh/d of produced electricity, which corresponds to a revenue of 609,605 €/year. In addition, 2,262 kg CODVFA/d are available for parallel purposes, such as the synthesis of bio-products with higher added value than bio-methane (e.g. biopolymers).

Novel routes for urban bio-waste management: a combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production

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Abstract

A combined acidic fermentation and anaerobic digestion (AD) treatment has been developed on pilot scale for urban bio-waste conversion into volatile fatty acid (VFA) and biogas. The specific waste mixture was composed by the pre-treated organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS), both produced inside the Treviso (northeast Italy) municipality. The effect of temperature (37°C and 55°C) was investigated in both steps. Only the mesophilic fermentation process provided a VFA-rich stream (19.5 g COD_{VFA}/L) with stable physical-chemical features, with no need of chemicals addition for pH control. The sludge buffering capacity made this step technically feasible. The AD step was performed on the solid-rich fraction of fermented bio-waste, after dilution with excess WAS. No relevant differences were observed under the two investigated temperature: in the steady state (organic loading rate of 2.5 kg VS/m^3 d), the specific biogas production was 0.40 and 0.45 m^3/kg VS at 37°C and 55°C respectively, with similar CH_4 content (63-64% v/v). The scaled-up version of the system (in an average urban municipality of 170,000 Person Equivalent) revealed that the whole process is thermally sustainable if both reactors are operated at mesophilic temperature: 36% of surplus thermal energy and 13,03 MWh/d of produced electricity, which corresponds to a revenue of 609,605 €/year. In addition, 2,262 kg COD_{VFA}/d are available for parallel purposes, such as the synthesis of bio-products with higher added value than bio-methane (e.g. biopolymers).

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Keywords

Anaerobic digestion; OFMSW; waste activated sludge (WAS); renewable feedstock; biogas; volatile fatty acid (VFA).

Abbreviations

OFMSW, organic fraction of municipal solid waste; WAS, waste activated sludge; AD, anaerobic digestion; WWTP, waste water treatment plant; CSTR, continuous stirred tank reactor; CHP, Combined Heat & Power unit; PE, Person Equivalent; OLR, organic loading rate; HRT, hydraulic retention time; COD, chemical oxygen demand; VFA, volatile fatty acids; COD_{SOL}, soluble COD; TS, Total Solids; VS, Volatile Solid; TKN, Total Kjeldahl Nitrogen; PHA, polyhydroxyalkanoates; MMC, mixed microbial cultures.

1. Introduction

Anaerobic digestion (AD) is currently considered the most feasible technology for biogas production from the treatment of high solid content streams, such as manure, organic fraction of municipal solid waste (OFMSW), energy crops, agro-industrial wastewater etc.. (Tyagi et al., 2018). The oldest and more commonly adopted application of AD is the treatment of biological sludge (Mata-Alvarez et al., 2014), even if minimized with membrane bioreactor (MBR) technology (Sepehri and Sarrafzadeh, 2018). In the two last decades, a particular attention has been devoted to the co-digestion processes, which have been designed for a simultaneous treatment of biological sludge and OFMSW (Mata-Alvarez et al., 2014). With regard to the specific case of bio-waste produced in urban scenario, the increasing of global population has led to new concerns for public authorities dealing with the treatment and disposal of biological sludge produced in municipal wastewater treatment plants (WWTPs) and food waste (Ma et al., 2017).

Through the conventional AD processes, the degradation of organic matter contained in food and sludge waste streams is not even complete, being in the wide range of 35-60% in most cases (Yin et al., 2016a). This performance is strongly affected by the low efficiency of hydrolysis, which is the rate-limiting step for the urban bio-waste anaerobic degradation process (Ma et al., 2018). Many pre-treatment methods have been explored for enhancing bio-solid hydrolysis and improving chemical oxygen demand (COD) solubilisation in order to increase bio-methane yield; among others, thermal hydrolysis (Li et al., 2017) and aerobic micro-aeration (Montalvo et al., 2018) pre-treatment.

Archea have been recognised as main responsible for methane production from volatile fatty acids (VFAs), as well as for ethanol and lactate (Stoyanova et al., 2017) in a typical AD process. However, since different microorganisms with specific biochemical pathways are involved, many other valuable products can be obtained via acidogenic fermentation. These products can have a wide spectrum of possible valorisation. As an example, the VFAs and lactic acid were described as the direct precursors for a particular family of short and medium chain length biopolymers, namely

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polyhydroxyalkanoates (PHAs) (Kourmentza et al., 2017). Alcohols have been also sometimes reported as possible substrate for PHA synthesis (Beccari et al., 2009). This route allows converting platform chemicals in bio-products at higher added value than methane (Pagliano et al., 2017). In fact, PHAs are one of the most promising classes of biopolymers, which can replace part of the synthetic marketed materials, due to their biodegradability and thermoplastic properties. In the frame of mixed microbial culture (MMC) technology (Valentino et al., 2017), the use of complex and low cost substrates is a strategy to accomplish both bio-products production and bio-waste treatment following their acidic fermentation. For this reason, acidogenic fermentation can be considered an option to exploit the organic matter contained in sludge and food waste as renewable feedstock, decreasing the amount of bio-waste to be disposed off (Koller, 2017). The VFAs composition and fermentation yield can vary according to the process parameters and settings. A desirable feature for PHA production is the ratio between odd and even carbons in the VFAs, which determine the composition of PHA co-polymers to a large extent (Bengtsson et al., 2010). Several parameters affected the fermentation process outputs such as inoculum (Yin et al., 2016b), pH (Wu et al., 2017), temperature (Dahiya et al., 2015), hydrogen partial pressure (Agler et al., 2011). A previous study showed that a pH between 5.0 and 6.0 was preferable to reach the highest fermentation process yield (Gottardo et al., 2017). However, the accumulation of VFAs produced in the fermentative metabolism generally causes a pH decreasing below the optimal values. Apart form chemicals additions for pH control, some authors recently proposed the recirculation of the anaerobic digestion effluent, as buffering agent, to control the pH in the fermentative stage. This strategy was applied in the combined two-stage fermentation and anaerobic digestion process (Gottardo et al., 2017). The method was particularly efficient when applied to a high solid content waste such as the pre-treated OFMSW. The high content of putrescible solids facilitate their fermentation even at relatively low hydraulic retention time (HRT < 4.0 days); as a consequence, the high level of VFAs (easily achieved) brings the pH below 5.0, inhibiting a further fermentation activity and making the pH-control strategy strictly necessary.

In the particular case illustrated in this paper, both VFAs and biogas production has been assessed from the anaerobic treatment of the specific cocktail of urban bio-wastes produced in the Treviso (north-east Italy) municipality. The pre-treated OFMSW was mixed with thickened waste activated sludge (WAS) from Treviso WWTP. The produced VFAs from acidogenic fermentation process were separated from the slurry for other potential uses (such as the PHAs synthesis). The solid-rich waste overflow was originated from a solid/liquid separation unit. This fraction was opportunely diluted with the same thickened WAS (collected in Treviso WWTP), and it has been used as secondary waste feedstock for biogas production through AD process. This approach differs from the traditional WAS-OFMSW co-digestion, developed at the Treviso full scale WWTP (Bolzonella et al., 2006), which allows recovering biogas only. In a frame of urban bio-refinery concept, the renewable carbon source may be driven in different routes that include also VFA production followed by biopolymer synthesis in a high load aerobic process (Valentino et al., 2015). The present pilot scale investigation has the scope to verify the process technical feasibility aimed at maximizing the production of VFA and at recovering biogas from the overflows.

2. Materials and Methods

2.1. Rationale of the approach

The designed flowchart consisted of: a) hydrolysis and fermentation step for VFA production from the most putrescible matter of the feedstock; b) solid/liquid separation unit for the solids removal from the liquid stream at high VFA content (suitable for further valorisation); c) anaerobic digestion step for biogas production from the solid-rich stream overflow ("solid cake"). Both steps were performed under mesophilic and thermophilic conditions. The whole process was evaluated in terms of yields, namely maximum VFA content for the fermentation process and biogas production for the anaerobic co-digestion processes. A mass and energy balance was made in order to assess the technical feasibility and to understand whether the process is energy efficient and sustainable.

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2.2 Thickened Waste Activated Sludge (WAS) and source-sorted Organic Fraction of Municipal Solid Waste (OFMSW)

The feedstock was weekly collected inside the Treviso municipal WWTP facility. Its composition was representative of influent stream usually treated in AD Treviso full-scale plant: thickened WAS and OFMSW were at volumetric fraction of 65-70% and 30-35% respectively. The WAS came from the static thickener of the WWTP sludge line. Source sorted OFMSW was collected in 50 districts of the Treviso Province, and transferred to the full-scale WWTP after its pre-treatment (squeezing and homogenization) with a screw-press. The WAS and OFMSW volumetric fractions adopted in this study were in the range reported in a recent review (Tyagi et al., 2018), where a comprehensive list of examples of waste sludge-OFMSW mixture co-digestion is provided; in general, the volumetric OFMSW fraction is always lower (10-40% v/v) than WAS fraction (60-90% v/v) (Tyagi et al., 2018).

2.3. Anaerobic pilot units

The acidogenic fermentation unit consisted of a continuous stirred tank reactor (CSTR) of 0.38 m^3 of operative volume, mechanically stirred and under temperature control by using a thermostatic jacket. The hydrolysis and fermentation of the organic content of WAS-OFMSW mixture into VFAs were carried out under both mesophilic (37°C) and thermophilic (55°C) conditions, in two separate trials. All the other operating conditions are listed as follows: hydraulic retention time (HRT) of 6 days; organic loading rate (OLR) 6.5-7.0 kg VS/m^3 d approximately; uncontrolled pH in the range 5.0-5.5. The CSTR fermenter was connected to a solid/liquid separation unit consisting of a coaxial centrifuge, equipped with 5.0 μm porosity nylon filter bag for solid (TS) removal. The solid-rich stream overflow was separated and then diluted with the excess WAS (collected from the full-scale plant) as feeding for anaerobic digester.

Stainless steel CSTR (AISI-304) of 0.23 m^3 operating volume was used as anaerobic digester. Mechanical anchor stirrer ensured mixing and homogenization. Two separated trials were carried-

out at 37°C and 55°C, according to the temperature of the previous fermentation stage. The temperature was maintained by hot water recirculation through external jacket. Anaerobic digested sludge inoculum was collected in the same WWTP, from the 2300 m^3 CSTR anaerobic digester, treating thickened WAS and squeezed OFMSW mixture under mesophilic condition at an OLR of 1.8 – 2.0 kg VS/m^3 d. For both AD trials, the digested sludge was acclimatized for 35-40 days (more than 2 HRTs). Operational parameters in both mesophilic and thermophilic trials were similar: HRT of 15 d and OLR between 2.2 – 2.5 kg VS/m^3 d. The WAS/cake ratio in the feedstock was calculated on the basis of the WAS and OFMSW specific production: 0.3 kg OFMSW/PE day (based on wet weight) and 0.06 kg TS/PE day for WAS (Metcalf & Eddy, 2003).

2.4. Sampling and analytical methods

Feedstock characteristics were determined once per week on fresh sample, immediately after its collection. The reactor effluents were monitored three times per week for total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and phosphorous (P). For the TS determination, a drying temperature of 105°C was adopted (Peces et al., 2014). The process stability parameters, i.e., the pH, volatile fatty acid content and distribution, total and partial alkalinity, ammonia, were measured two/three times per week. All analyses were performed according to the standard methods (APHA/ AWWA/WEF, 1998). The analyses of VFA were conducted using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (T 100°C), a fused silica capillary DB-FFAP (15 m length, 0.53 mm x 0.5 mm film thickness) column, with hydrogen as carrier. The analysis was conducted by increasing the temperature from 80°C to 200°C (10°C/min). Samples were centrifuged at 4000 rpm for five minutes; the supernatant was filtered by using a 0.20 μm cellulose acetate filter before the analysis. The biogas production was monitored using a flow metre (Ritter CompanyTM); methane, carbon dioxide and oxygen in the biogas were continuously determined using a portable infrared gas analyser GA2000TM (Geotechnical InstrumentsTM), and once a day using a gas chromatograph 6890N (Agilent

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TechnologyTM). The analyses were conducted with an HP-PLOT MOLESIEVE column (30 m length, 0.53 mm ID x 25 mm film thickness), using a thermal conductivity detector and argon as carrier (79 ml/min). H_2, CH_4, O_2 and N_2 were analysed using a thermal conductivity detector (TCD) at a temperature of 250°C. The inlet temperature was 120°C, with a constant pressure in the injection port (70 kPa). Samples were taken using a gas-type syringe (200 mL biogas). Once the entire sample was vaporized, separation of the peaks occurred within the column at a constant temperature of 40°C (8 min).

2.5. Calculation

All the parameters characterising reactor performances were calculated after steady states were achieved. Descriptive statistics and exploratory data analysis were performed using the open source program, R (The R Foundation for Statistical Computing, version 3.5.0).

For the fermentation stage, the process was considered sufficiently stable and under steady state after consistent VFA production was detected. Solubilisation and fermentation yield (Y_{VFA}) were determined with respect to the characteristics of initial unfermented feedstock, in particular total COD ($COD_{TOT(0)}$) $TS_{(0)}$ and $VS_{(0)}$. For the anaerobic digestion step, steady state was considered achieved when the biogas production rate (GPR) was constant (below 10% of deviation from average values).

The mass and energy balance, as well as the energy yield, were performed by considering both stages in two combination of temperature: a) fermentation and digester reactors under mesophilic condition; b) fermentation reactor under mesophilic condition and digester reactor under thermophilic condition. The aim of this comparison was to evaluate which configuration is more profitable in terms of surplus thermal energy and electricity production.

All the parameters were adapted to a scaled-up version of both reactors, in an urban municipality context of average size of 170,000 Person Equivalent (PE); reference parameters and boundary

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conditions are given in Table 1. In the energy balance analyses, the price of electrical energy has been assumed to be 130 €/MWh (no incentive; Valentino et al., 2018).

Table 1.

Parameter	Unit	Value
Biogas		
Low Heat Value Biogas	MJ/N m^3	23.012
Combined Heat and Power (CHP)		
Termical Energy yield	-	0.5
Electrical Energy yield	-	0.4
Boundary Conditions		
Operative Temperature Anaerobic Processes	°C	37-55
Water Temperature	°C	15
Air Temperature	°C	20
Ground Temperature	°C	25
Heat Transfer Coefficient		
Outer Concrete Reactor Wall	W/(m^2 °C)	0.7
Inner Concrete Reactor Wall	W/(m^2 °C)	1.2
Floor	W/(m^2 °C)	2.85

3. Results and Discussion

3.1. Bio-waste composition and characteristics

The chemical features of the feedstock used for fermentation reactor were relatively constant during the whole operation period (almost 4 months). The average dry matter was 47 ± 2 g TS/kg, with 83% of VS. The nutrient level was more similar to that of biological sludge (WAS) instead of the typical OFMSW content (usually N- or P-limiting with respect to COD content), since the feedstock largely composed by thickened WAS (65-70% v/v). With regard to the soluble COD, nutrient level was 100/5.7/1.6 as COD/N/P. The monitored features of the feedstock mixture are listed in Table 2 (a more detailed version is given in Supplementary Material, Table S1). The thickened WAS had the following characteristics: 30 ± 2 g TS/kg, 21.5 ± 0.7 g VS/kg (72% of dry matter), 40 ± 1 g N/kg TS, 15.0 ± 0.2 g P/kg TS, 0.15 ± 0.08 g COD$_{SOL}$/L, 48 ± 6 g COD/kg TS.

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Table 2.

Parameter	Unfermented feedstock	Fermented feedstock	
		Mesophilic	Thermophilic
Total Solid (TS, g/kg)	47 ± 2	44 ± 1	41.2 ± 0.8
Volatile Solid (VS, g/kg)	39 ± 1	34.5 ± 0.9	33 ± 2
COD_{SOL} (g O_2/L)	24.4 ± 0.8	26.8 ± 0.4	39 ± 4
VFA (g COD/L)	2.8 ± 0.2	19.5 ± 0.3	23 ± 2
pH range	4.8 - 5.2	4.8 - 5.7	3.4 - 5.7
COD_{VFA}/COD_{SOL}	0.09 ± 0.03	0.72 ± 0.01	0.59 ± 0.05
Total Kjeldahl Nitrogen (TKN; g N/kg TS)	23.1 ± 0.9	30 ± 1	34 ± 3
Phosphorus (P; g P/kg TS)	2.7 ± 0.2	4.7 ± 0.2	5.5 ± 0.6

3.2. Acidogenic Fermentation

The WAS-OFMSW fermentation was performed without pH control using the sludge buffering capacity (Cabbai et al., 2016), and thereby avoiding process costs associated with the addition of chemicals. The process conducted at 37°C produced a fermented stream with stable chemical characteristics starting from the 20th days (about 3.5 HRT) of operation (Figure 1A). After this initial period, VFA level was constantly maintained close to 20 g COD_{VFA}/L; correspondingly, pH trend did not show relevant fluctuations, being minimum values close to 5.0 and maximum values around 5.7 (Figure 1B). Indeed, the WAS-OFMSW mesophilic fermentation did not require any strategy for pH control since the feedstock buffering capacity (402 ± 24 mg $CaCO_3$/L, pH 5.7) counterbalanced the unavoidable pH decrease due to the stable VFA production. This was a relevant aspect, which demonstrated the robustness and simplicity of process operation at the same time. The combination of two parameters such as the relative WAS percentage in the mixture and the mesophilic temperature made the fermentation process technically feasible.

Figure 1.
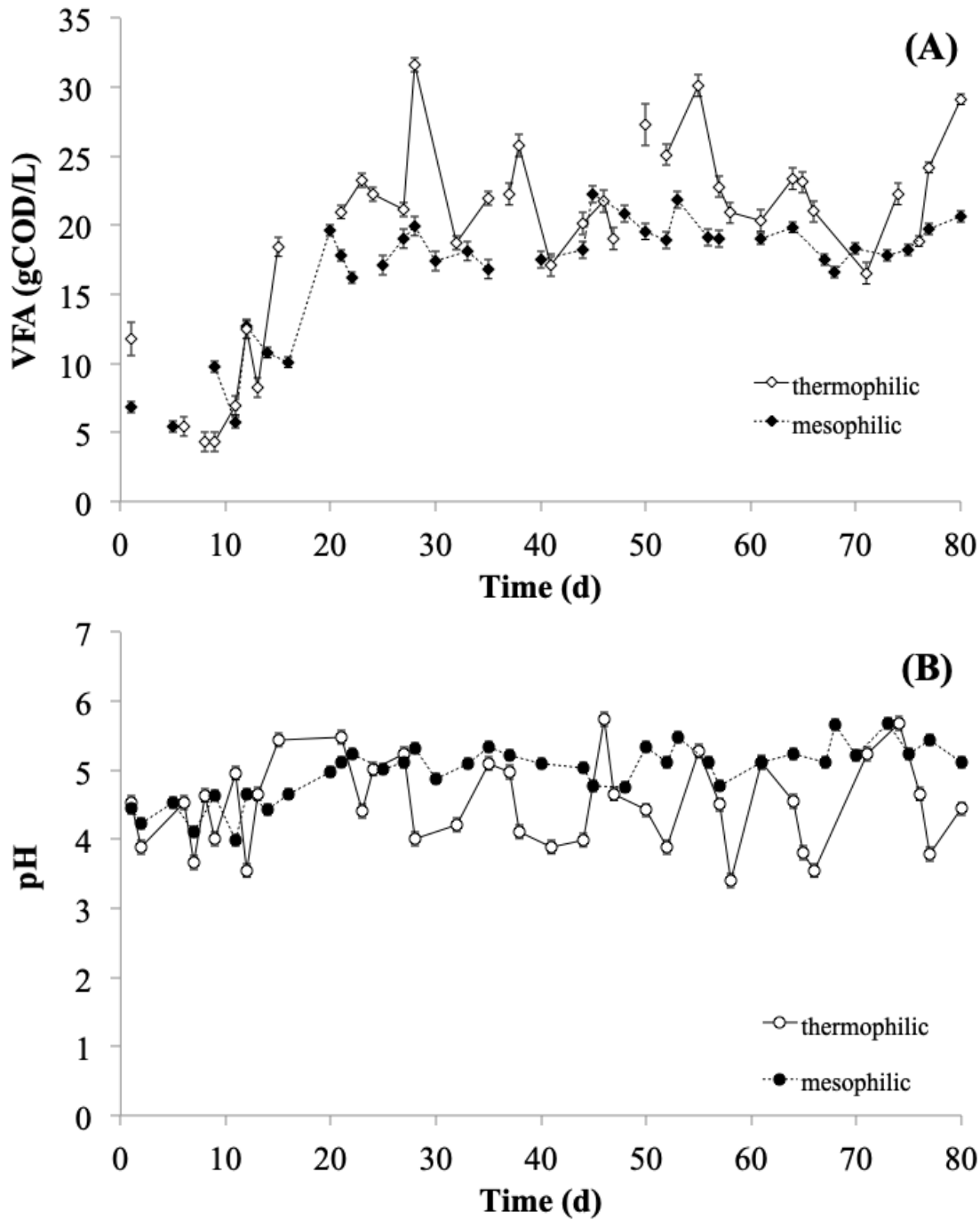
Thermophilic fermentation was characterized by a shorted acclimation period (2.5 HRT) compared

to mesophilic process. The VFA level in this fermented stream was generally higher than those

measured in mesophilic condition, but frequent fluctuations in VFA concentration trend were

observed. In addition, the VFA concentrations widely changed over the course of process operation,

in a range from 16.5 to 31.6 g COD_{VFA}/L. This profile reflected the equally frequent pH decrease,

due to a fast hydrolyzation and fermentation activity (supported by the higher T), not enough

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buffered by the alkalinity of the feedstock itself. In practice, WAS-OFMSW mixture fermentation at 55 °C appeared more difficult in terms of process control and VFA production stability. Fermentation performances as well as physical-chemical characteristics were affected by this process instability as indicated by the standard deviations of average values (sometimes one order of magnitude higher than those under mesophilic temperature) reported in Table 2.

Higher temperatures solubilised more solids and increased the COD_{SOL} and nutrient release in the mixed liquor. The VFA concentration also increased above 20 g COD_{VFA}/L; however, a not negligible fraction of COD_{SOL} remained unconverted into VFA, being the average COD_{VFA}/COD_{SOL} ratio equal to 0.59. Feedstock fermentability at 37°C was found to be less performing for solids and COD solubilisation (0.41 ± 0.02 and 0.26 ± 0.01 on initial VS and COD_{TOT} respectively) even though VFA yields were comparable to those measured at 55 °C.

The acidic WAS-OFMSW fermentation was found to be more performing to that WAS only, independently from the chosen process temperature. The VFA yields obtained in this work (0.41-0.44 g COD_{VFA}/g $VS_{(0)}$, 0.26-0.28 g COD_{VFA}/g $COD_{TOT(0)}$; Table 1, Supplementary Material) are higher than yields reported for WAS at 37°C (0.01-0.06 g COD_{VFA}/g $VS_{(0)}$, Ucisik and Henze, 2008; 0.06–0.14 g COD_{VFA}/g $COD_{TOT(0)}$, Yuan et al., 2009), and at 42°C (0.27 g COD_{VFA}/g $VS_{(0)}$, Morgan-Sagastume et al., 2014). The presence of the OFMSW in the feedstock may have provided a more fermentable bio-waste and explained differences to the literature values. As a consequence, the level of VFA obtained here was higher (roughly double) if compared to those reported in the literature for WAS (6.0-9.4 g COD_{VFA}/L; Morgan-Sagastume et al., 2015) or primary sludge-WAS mixture (7.5-13.5 g COD_{VFA}/L; Pittmann and Steinmetz, 2017), and similar to those obtained with thermally pre-treated sludge (18.5-19.9 g COD_{VFA}/L; Morgan-Sagastume et al., 2010).

The process stability is also an important aspect affecting the technical process feasibility: mesophilic fermentation appeared more technically attractive since the VFA-rich stream features were more stable over time and the COD_{VFA}/COD_{SOL} ratio was higher (t − test, alpha value = 0.01) than value obtained in thermophilic fermentation (Figure 2).

Figure 2.
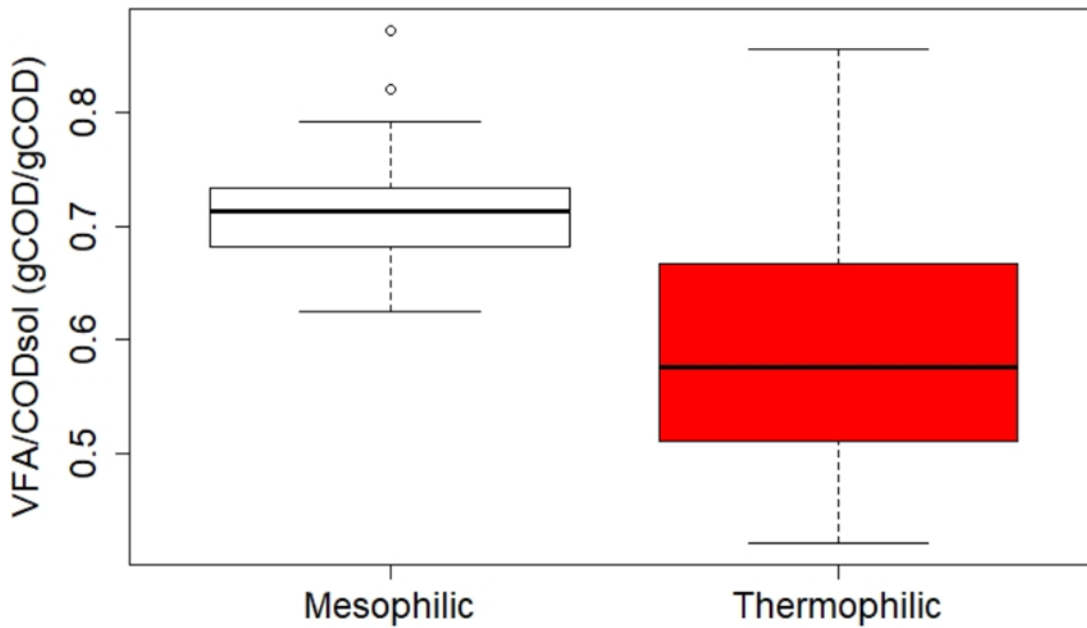
A VFA-rich stream with consistent composition is also a desirable feature for PHA production since the balance of odd and even carbons in the VFA determines the polymer composition (Bengtsson et al., 2010). Fermentation products in mesophilic and thermophilic conditions were similar and dominated by butyric (41-42%, COD basis), acetic (22-23%, COD basis), propionic (11-10%), valeric (12-11%), caproic (9-8%), eptanoic (4-6%), isovaleric (2-3%) and isobutyric (1-0.8%) acids. The predominance of butyric was probably related to the presence of OFMSW in the feedstock, as reported elsewhere (Girotto et al., 2017), since acetic acid was usually the most abundant in fermentation process performed with sludge only (Morgan-Sagastume et al., 2010, 2011). The molar fraction of VFA with odd number of C-atoms if compared to the total $[C_3/(C_3+C_2)]_{VFA}$ was also similar (Table S1; Supplementary Material), most likely dependent on feedstock characteristics and not affected by fermentation temperature.

Regarding the solid cake (SC), the Table 3 summarizes the main characteristics of both SC overflows, obtained from mesophilic and thermophilic fermentation process. In spite of the observed differences for the features of mesophilic and thermophilic VFA-rich streams, the working

temperature did not show any relevant effect on the main characteristics of the two SC (t – test, alpha value = 0.01).

Table 3.

Parameter	Solid-rich cake overflow*	
	Mesophilic	**Thermophilic**
Total Solid (TS, g/kg)	235 ± 21	220 ± 21
Volatile Solid (VS, g/kg)	189 ± 10	177 ± 8
VS/TS (%)	80 ± 2	81 ± 1
COD (g O_2/kg TS)	740 ± 61	755 ± 43
Total Kjeldahl Nitrogen (TKN; g N/kg TS)	29 ± 1	33 ± 1
Phosphorus (P; g P/kg TS)	5.0 ± 0.6	6.0 ± 0.7

* Solid-rich cake produced after fermentation process

3.3. Anaerobic Digestion

Mesophilic and termophilic processes were started at relatively low OLR (0.7-0.8 kg VS/m^3 d) by using a feed solution mainly composed by thickened WAS. The SC content was gradually increased in order to achieve an OLR of 2.3 - 2.5 kg VS/m^3 d approximately. The SC content strongly affected the operative OLR since it was characterized by TS of one order of magnitude higher than the thickened WAS.

In mesophilic condition, steady state was achieved in 40 days (roughly 2 HRTs). In the start up period, no relevant pH drop was observed even at the highest VFA concentration detected (close to 1.0 g COD/L at day 10th after inoculum). Most of the readily fermentable COD was already converted into VFA in the previous hydrolysis/fermentation step; as a consequence, the process was easily controlled even in the transient conditions when the risk of methanogenic bacteria inhibition is usually high. Average pH value of steady state period was 7.6 ± 0.2, advantageous for the growth of methanogens (Gottardo et al., 2017). The pH remained constant because of the high buffering capacity of the system as indicated by the partial alkalinity (1,738 ± 138 mg CaCO$_3$/L) and by the VFA/Partial Alkalinity (P.Alk) ratio (0.13 ± 0.04 mg acetic acid/mg CaCO$_3$) (Figure 3A). In particular VFA/P.Alk ratio showed a maximum peak (1.14) in the first week after inoculum and then it decreased over the course of operation. In the steady state period, VFA/P.Alk ratio was constantly below 0.20 indicating high process stability and the negligible VFA accumulation. In

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fact, it is widely recognized that VFA/P.AlK value greater than 0.30 may indicate a possible process upset or instability usually caused by organic matter overload and a consequently VFA concentration increase (Bolzonella et al., 2003). Ammonia release from proteins degradation led to an increase of total ammonium in the liquid phase from 0.46 ± 0.07 to 0.79 ± 0.06 g $N\text{-}NH_4^+/L$. However, this value was abundantly below 3.0-3.5 g $N\text{-}NH_4^+/L$, which is the typical critical value for the inhibition of metanogenic bacteria (Chen et al., 2008).

The AD step under thermophilic conditions reached the steady state in 30 days after inoculum, approximately 0.5 HRT less than mesophilic process. Also in this case, the process was easily controlled since no relevant VFA accumulation was detected (maximum value 1.01 g COD/L, day 13) and pH was easily maintained above 7.5. Starting from day 22, VFA/P.Alk ratio was permanently below 0.20 mg acetic acid/mg $CaCO_3$. Figure 3B shows the trends of total-partial alkalinity and VFA/P.Alk ratio along the experimentation: when the steady state was achieved, partial and total alkalinity had average concentrations of $2,524 \pm 279$ and $3,788 \pm 314$ mg $CaCO_3/L$; VFA/P.Alk ratio was equal to 0.12 ± 0.03.

Figure 3C shows the biogas production rate (GPR) and methane percentage in the produced biogas during AD mesophilic trial. Despite the fast increase of methane content (above 60% v/v after two weeks of operation or 1.0 HRT approximately), the GPR trend showed a slower increase in the course of process stabilization and in parallel to the progressive increase of the applied OLR (roughly up to 1.5 kg VS/m^3 d) during the start up phase. At the steady state, GPR was 1.38 ± 0.08 m^3 biogas/m^3 r d; the percentage of methane detected in the biogas was $63 \pm 3\%$; most of remaining part was CO_2 ($32 \pm 3\%$). The specific gas production (SGP) was found to be equal to 0.40 ± 0.02 m^3 biogas/kg VS.

Similarly to mesophilic condition, in the AD thermophilic trial the OLR was increased from 0.8 to 1.5-1.8 kg VS/m^3 d during the start up, and then it was maintained close to 2.5 kg VS/m^3 d over the course of the run. The progressive increase of the OLR reflected the trend of GPR, which was 1.80

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± 0.09 m³ biogas/m³ r d in the steady state, while methane biogas content exceeded 60% in 0.5 HRT
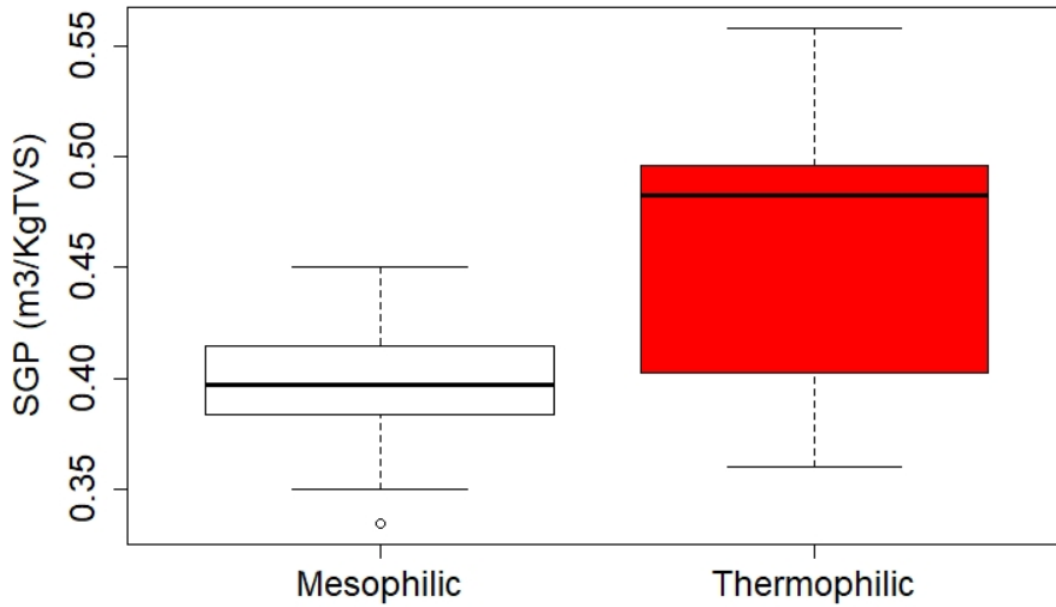
approximately (Figure 3D).

Figure 3.

(C)

(D)
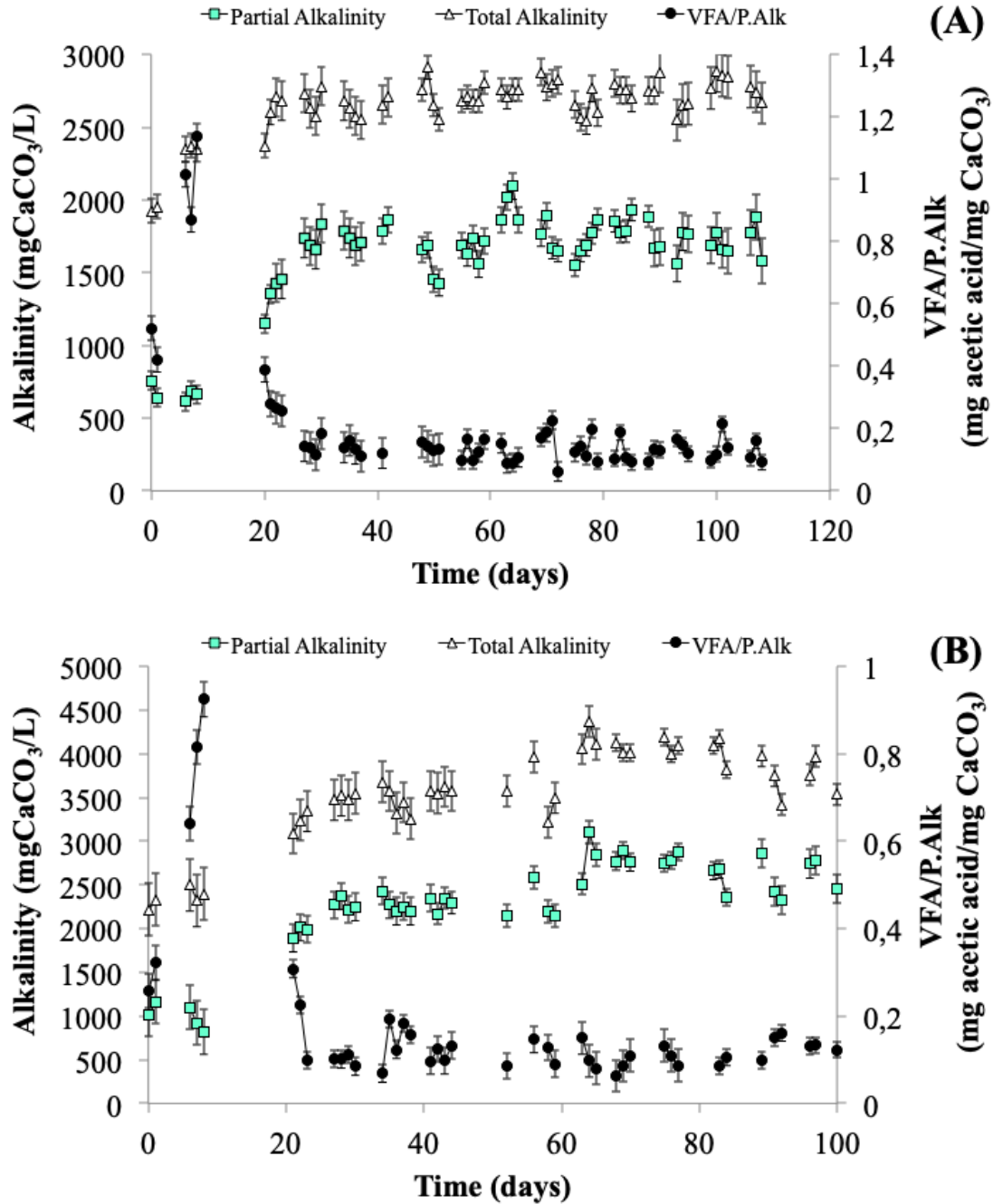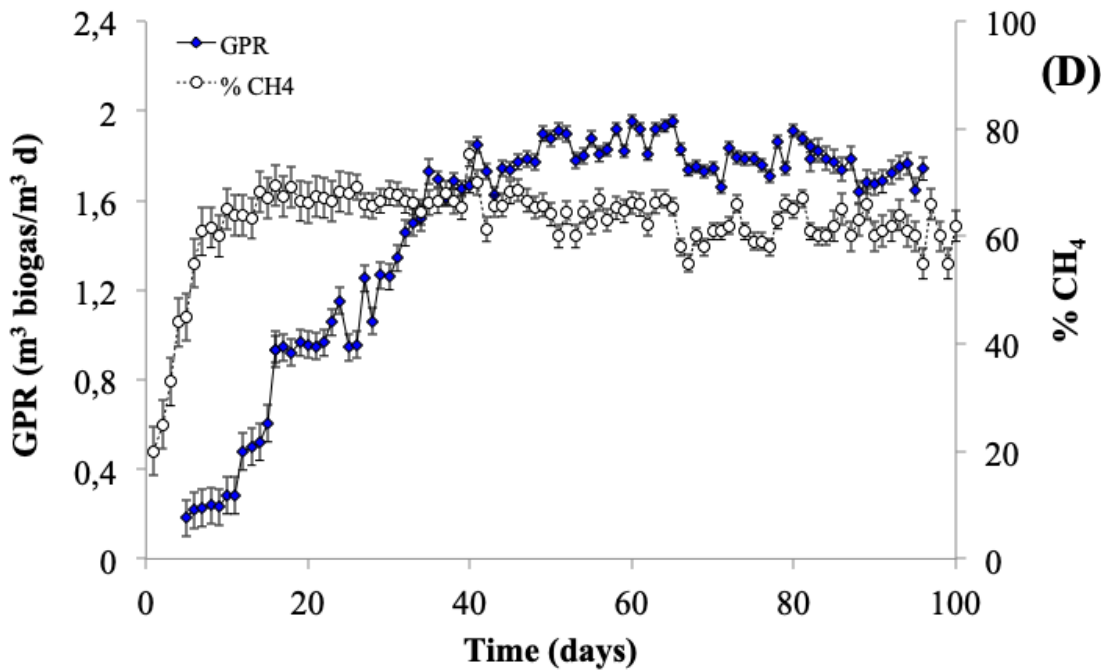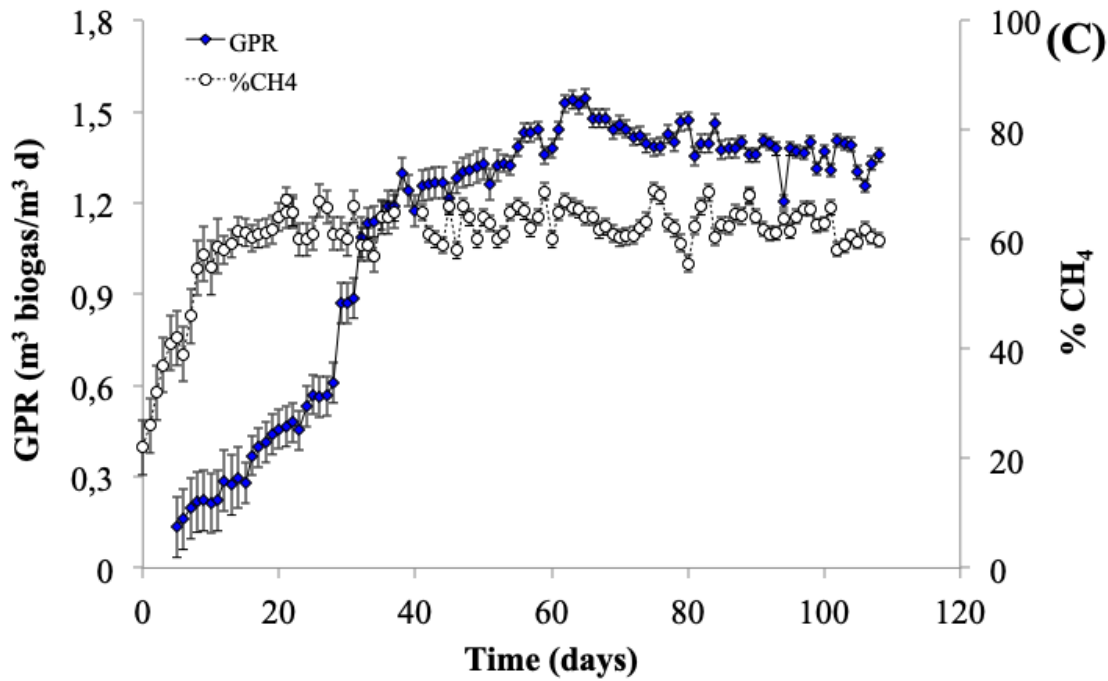
As for the GPR, also the SGP at 55 °C was higher (Figure 4; t – test, alpha value = 0.01) than SGP quantified at 37 °C, and equal to 0.45 ± 0.06 m³ biogas/kg VS. This was due to the higher solid abatement, which accounted for $71 \pm 5\%$ and $74 \pm 4\%$ for TS and VS at 55 °C, and $43 \pm 4\%$ and $45 \pm 4\%$ for TS and VS at 37 °C.

Figure 4.

Overall, both mesophilic and termophilic digestions of SC overflow diluted with thickened WAS were demonstrated to be technically feasible and robust with reference to the process parameters (pH, partial alkalinity, VFA concentration, biogas production and composition). Table 4 summarizes the main monitored parameters and quantified performances in the steady state of the two AD trials.

Table 4.

Parameter	AD reactors	
	Mesophilic AD	**Thermophilic AD**
Total Solid (TS, g/kg)	30 ± 1	18 ± 2
Volatile Solid (VS, g/kg)	25 ± 1	12 ± 1
COD (g O_2/kg TS)	607 ± 23	547 ± 48
VFA (mg/L)	244 ± 46	313 ± 57
pH	7.6 ± 0.2	7.9 ± 0.2
Partial Alkalinity (mg $CaCO_3$/L)	$1,738 \pm 138$	$2,524 \pm 279$
Total Alkalinity (mg $CaCO_3$/L)	$2,731 \pm 94$	$3,788 \pm 314$
VFA/P.Alk (mg acetic acid/mg $CaCO_3$)	0.13 ± 0.04	0.12 ± 0.03
Total Kjeldahl Nitrogen (TKN; g N/kg TS)	34 ± 2	37.7 ± 0.9
Phosphorus (P; g P/kg TS)	15.9 ± 0.9	18 ± 2
Ammonia (N-NH_4^+; g/L)	0.79 ± 0.06	0.85 ± 0.04
Performances	**Mesophilic AD**	**Thermophilic AD**
CH_4 % (v/v)	63 ± 3	64 ± 3
GPR (N m^3 biogas/m^3 d)	1.38 ± 0.08	1.79 ± 0.09
SGP (N m^3 biogas/kg VS)	0.40 ± 0.02	0.45 ± 0.06
SMP (N m^3 CH_4/kg VS)	0.25 ± 0.01	0.29 ± 0.03

Compared to literature values, the performances obtained herein were similar to those reported for mixed sludge digestion rather than sludge-OFMSW mixture co-digestion. For the mesophilic treatment of mixed sludge, Mattioli and co-workers (2017) quantified 0.45 Nm^3 biogas/kg VS (SGP), similar to the average value of thermophilic AD in this study. The authors reported a higher SGP, 0.50 Nm^3 biogas/kg VS, for sludge-OFMSW co-digestion. Similarly, Koch et al. (2016) reported 0.31 and 0.39 Nm^3 CH_4/kg VS, as specific methane production (SMP) for raw sludge and sludge-OFMSW mesophilic digestion respectively. Liu et al. (2012) obtained a much higher SGP value, 0.72 Nm^3 biogas/kg VS, in the co-digestion of a feedstock with more than 50% v/v OFMSW and a lower fraction of sludge. In the AD trials of this study, part of the most putrescible material (supplied by the OFMSW), was converted into VFA and not conveyed to the AD step. As a consequence, the AD performances were strongly affected by the solids of excess WAS rather than the slowly biodegradable COD which was residual in the SC itself.

3.4. Mass and energy balance

The mass balance discussed in this paragraph is illustrated in detail in Figure S1 (Supplementary Material). The data analysis of each separate pilot reactor was transferred to an industrial plant of average size, identified as 170,000 PE. Based on the specific production reported in the paragraph 2.3, the inlet amount of WAS corresponds to 10,200 kg TS/d. A smaller amount is driven to the fermentation reactor (around 24%) and the rest to the AD step (76%) in order to dilute the SC overflow. The inlet amount of the pre-treated OFMSW is 6,120 kg TS/d, based on a dry matter of 15% and a recovery of 80% of TS from the OFMSW screw-press pre-treatment (Majone et al., 2017).

For the fermentation process, only the mesophilic condition was considered technically feasible and the relative yield of 0.41 g COD_{VFA}/g $VS_{(0)}$ was taken into account. The bio-waste mixture is conveyed to the mesophilic fermenter with a TS and VS level of 6.3% and 5.2% w/w. As a

consequence, the applied OLR was approximately 10 kg VS/m^3 d. The gaseous effluent flow rate out of the acidogenic fermenter is 712 Nm^3/d, corresponding to 1,153 kg VS/d, roughly equal to the 16% of the VS influent amount. The solid/liquid separation unit allows obtaining two streams: the liquid fraction and the SC overflow. The liquid stream has a volumetric flow rate of 105,946 kg/d (almost 80% of the total), corresponding to 2,262 kg COD_{VFA}/d, to be used for the synthesis of bio-products with higher added value than methane (Tamis and Joosse, 2015). In fact, a production of 0.54 tons of marketable biopolymer (PHA) per year is potentially feasible, based on average yield of 0.40 COD_{PHA}/COD_{VFA}, typically quantified in the aerobic MMC-PHA production technology (Valentino et al., 2017).

The SC overflow has a flow rate of 30,054 kg/d, which is mixed with 297,108 kg/d of excess thickened WAS. This dilution is necessary to decrease the solid content of the SC overflow, approximately from 23% w/w to 4.5% w/w (TS basin), before being fed into the final AD step.

The first scenario provides for the AD step in mesophilic condition (experimental SGP of 0.40 Nm^3/kg VS); the mesophilic AD step produces 4,379 Nm^3/d of biogas. The heat requirements and energy balance analyses were assessed for a working volume of 712 m^3 and 5,213 m^3 for fermentation and methanogenic reactors respectively. Considering the thermal yield of the Combined Heat & Power unit (CHP) of 0.5 (Valentino et al., 2018), the thermal energy produced is 58,577 MJ/d, if AD is performed at 37°C. Indeed, the thermal balance is closed positively because the estimated produced thermal energy overcomes the consumed thermal energy (for heating and heat dispersion balance) of about 36% (Figure 6). In thermophilic conditions (SGP of 0.45 Nm^3/kg VS), the biogas production was higher and equal to 4,927 Nm^3/d; however, the thermal energy consumed for process operation (65,108 MJ/d) is similar to the produced thermal energy (64,875 MJ/d) (Figure 5). In practise, the similarity does not guarantee the process self-sustainability, making this configuration not scalable at full scale.

Besides the complete energetic sustainability of the two steps performed at 37°C, the revenues from sale of generated electricity (13.03 MWh/d, from electrical CHP yield of 0.4; Valentino et al., 2018)

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has been quantified to 609,605 €/year. This potential economic income makes this approach more attractive than the traditional or the two-phase AD process treating excess sludge only (Leite et al., 2016).

Figure 5.
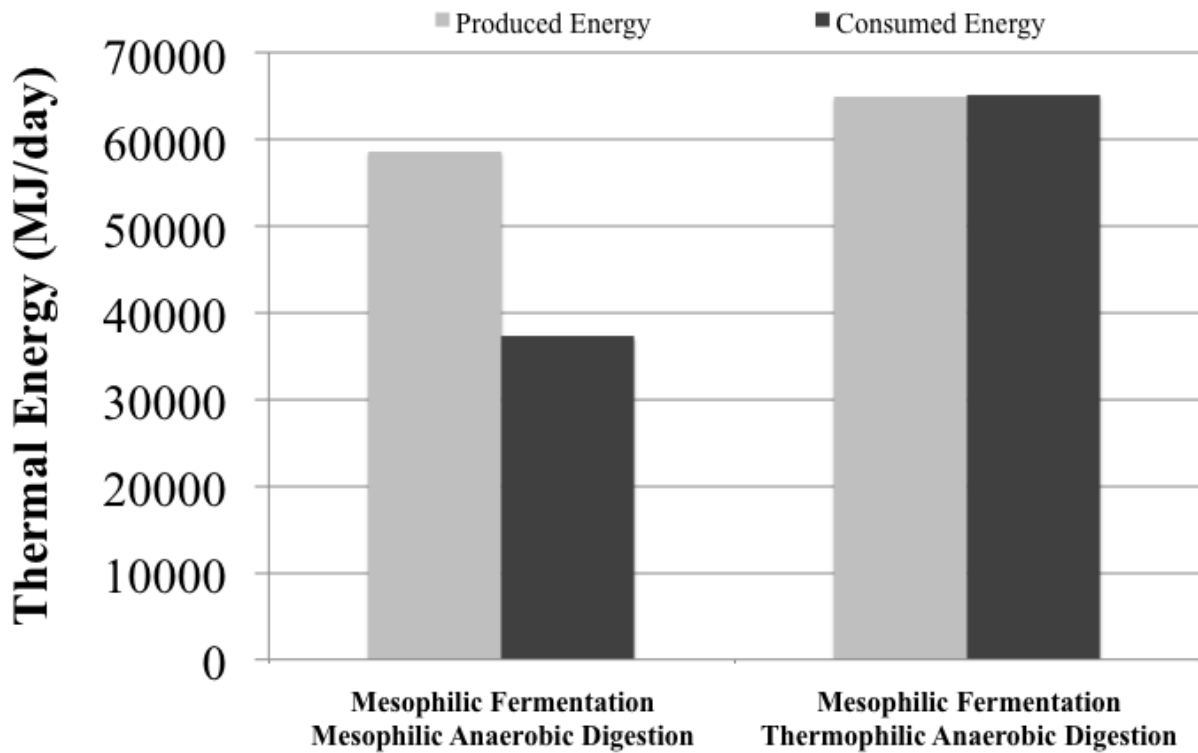
4. Conclusion

This work demonstrated that biogas production is not the only option for anaerobic treatment of WAS-OFMSW mixture produced in an urban scenario. If separated from AD process, the fermentation step allows recovering a consistent amount of solubilized COD, in form of VFA, that can be used as platform chemicals for the synthesis of other bio-products with higher market value than biogas. The mass and energy balances of the anaerobic fermentation-digestion reactors revealed that the overall process was thermally self-sustaining if both stages were performed at mesophilic temperature (37°C). Under this adopted condition, the scaled-up system (170,000 PE) had the 36% surplus of thermal energy (21,360 MJ/d), and a potential economic income from the

produced electricity (13.03 MWh/d) of 609,605 €/year. The production of 2,262 kg COD_{VFA}/d makes this approach even more attractive since the VFA-rich valuable stream can be used in other exploitable routes. In fact, this approach represents the application of circular economy concept, focused on the secondary carbon streams treatment process, designed for bioenergy recovery and bio-products creation. Indeed, future works need to be addressed to the development of a new multi-utility bio-refinery, possibly integrated in the existing facilities of an urban context, where each process unit is separately optimized for a specific target, leading to relevant economic and environmental advantages.

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Figure Captions

Figure 1. VFA (A) and pH (B) trends during mesophilic and thermophilic acidogenic fermentation stage.

Figure 2: Boxplot of VFA/COD_{SOL} for mesophilic and thermophilic fermentation trials.

Figure 3. Alkalinity and VFA/P.Alk ratio during mesophilic (A) and thermophilic (B) anaerobic digestion stage; GPR and CH_4 percentage in the biogas during mesophilic (C) and thermophilic (D) anaerobic digestion stage.

Figure 4. Boxplot of SGP for mesophilic and thermophilic Anaerobic Digestion trials.

Figure 5. Consumed thermal energy and produced thermal energy for the two investigated process configurations.

Table Captions

Table 1. Reference parameters and boundary conditions for energy balance.

Table 2. Physical-chemical features of unfermented and fermented feedstock (OFMSW-WAS mixture).

Table 3. Physical-chemical features of the solid-rich cake overflows

Table 4. Physical-chemical features of AD effluents and process performances

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Supplementary Material

Novel routes for urban bio-waste management: a combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production

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Materials and Methods

Mass and energy balance assessment

The calculation for the assessment of mass and energy balance were made by considering the size of the plant (170,000 Person Equivalent), and the WAS and OFMSW specific production: 0.3 kg OFMSW/PE day (based on wet weight) and 0.06 kg TS/PE day for WAS (Metcalf & Eddy, 2003). From these inputs, it has been calculated the initial TS flow rates of WAS and OFMSW:

- (170,000 PE • 0.3 kgTS/PE • 0.15 kgTS/kg • 0.8 kgTS/kg) = 6,120 kgTS/d (for the OFMSW)

where [0.15 kgTS/kg] is the OFMSW solid fraction and [0.8 kgTS/kg] is the recovery yield after the pre-treatment;

- (170,000 PE • 0.06 kgTS/PE) = 10,200 kgTS/d (for WAS)

After the TS flow rate, it has been defined the mass flow rate and the TS flow rate:

- (6,120 kgTS/d) / (0.15 kgTS/kg) = 40,800 kg/d OFMSW

- (6,120 kgTS/d) • (0.88 kgVS/kgTS) = 5,386 kgVS/d OFMSW

- (10,200 kgTS/d) / (0.026 kgTS/kg) = 392,308 kg/d WAS

- (10,200 kgTS/d) • (0.70 kgVS/kgTS) = 7,140 kgVS/d WAS

The distribution of these two streams in the fermentation and AD step was defined as experimentally made, as well as for the relative volumetric portions (the VFA-rich liquid fraction and the solid cake fraction) after the solid/liquid separation unit. The OFMSW stream was assumed to be totally conveyed to the acidogenic fermentation step, together with the 24% of the WAS stream. Indeed, the volumetric percentage (as shown in Figure S1) were: 30% v/v OFMSW and 70% v/v WAS as feedstock for the fermentation step. The rest of WAS (76%) was used for the dilution of the solid cake, recovered from the solid/liquid separation unit. The solid cake (30,054 kg/d) roughly corresponds to the 22% of the total influent acidogenic fermentation flow rate (136,000 kg/d). Indeed, the influent of AD step (327,161 kg/d) was the sum of the solid cake and excess WAS flow rates.

The VFA production as well as biogas production was quantified based on the experimental data obtained: specifically the fermentation yield ($gCOD_{VFA}/gVS_{(0)}$) and the specific gas production (SGP, m^3 biogas/kgVS).

The energy balance was assessed taking into account the parameters reported below:

a)   the volume of both reactors, defined by the ratio between the VS flow rate (kgVS/d) and the fixed OLR ($kgVS/m^3.d$). The OLRs were: 10 kg $VS/m^3.d$ and 2.1 $kgVS/m^3.d$, respectively for the fermenter and the digester. The volumes were 712 m^3 (acidic fermenter) and 5,213 m^3 (digester);

b)   the reference parameters and boundary conditions defined in Table 1 (Metcalf & Eddy, 2003); all the parameters in this table were necessary for the calculation of each energy item (heating water, thermal dispersion, thermal and electrical energy produced from CHP) summarized in Table S2 (Supplementary Material)

Figure S1. Flow rates and mass balance of the scaled up anaerobic process: mesophilic acidogenic fermentation followed by mesophilic or thermophilic anaerobic digestion.
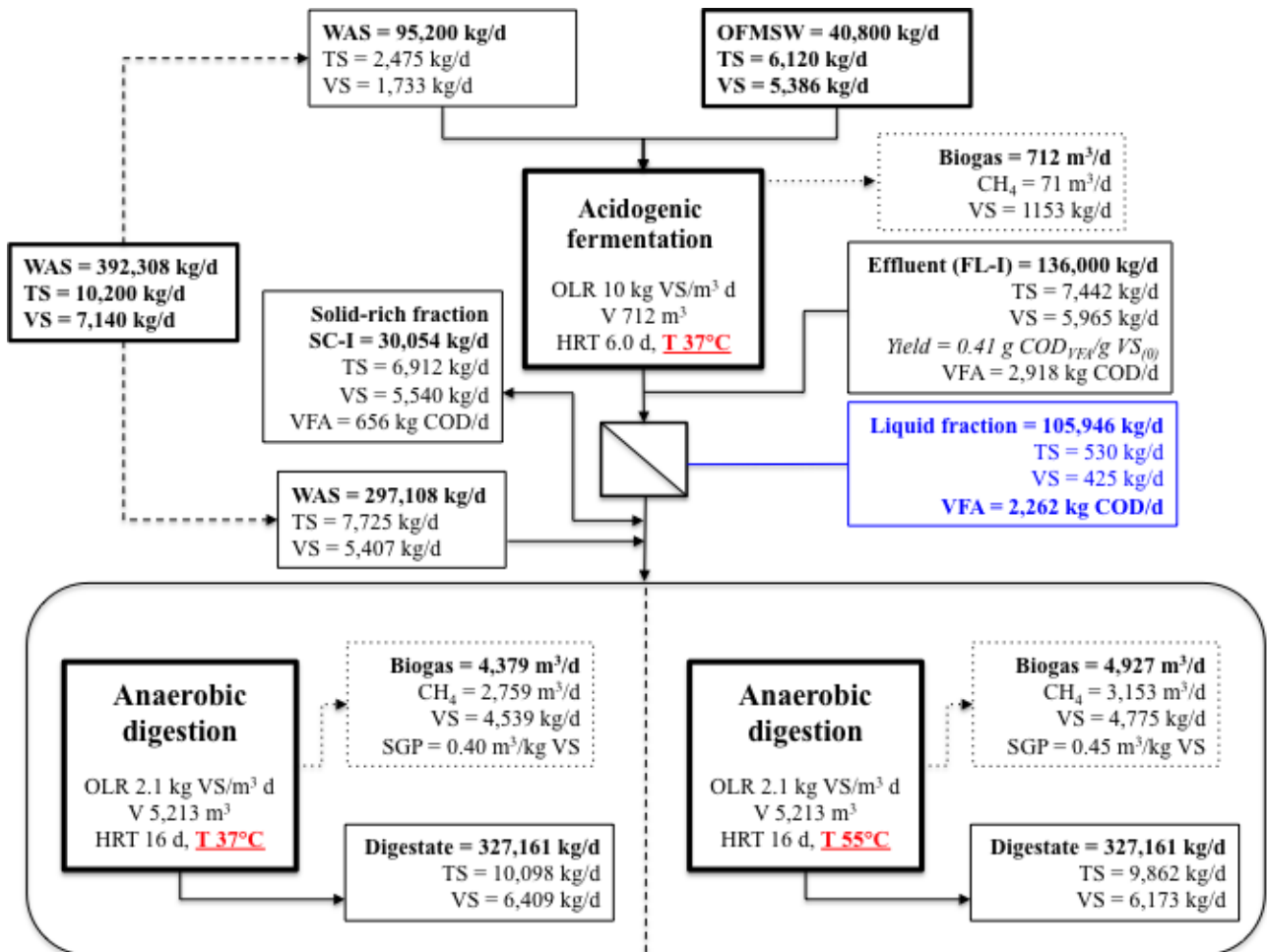
Table S1. Physical-chemical features of unfermented and fermented feedstock (OFMSW-WAS mixture).

Parameter	Unfermented feedstock	Fermented feedstock	
		Mesophilic	Thermophilic
Total Solid (TS, g/kg)	47 ± 2	44 ± 1	41.2 ± 0.8
Volatile Solid (VS, g/kg)	39 ± 1	34.5 ± 0.9	33 ± 2
COD_{TOT} (g/L)	63 ± 2	58 ± 2	72 ± 3
COD_{SOL} (g/L)	24.4 ± 0.8	26.8 ± 0.4	39 ± 4
VFA (g COD/L)	2.8 ± 0.2	19.5 ± 0.3	23 ± 2
Acetic acid (g COD/L)	1.0 ± 0.1	4.3 ± 0.1	5.1 ± 0.2
Propionic acid (g COD/L)	0.31 ± 0.05	2.1 ± 0.1	2.2 ± 0.2
Isobutyric acid (g COD/L)	-	0.18 ± 0.05	0.17 ± 0.05
Butyric acid (g COD/L)	1.3 ± 0.2	7.8 ± 0.3	9.7 ± 0.8
Isovaleric acid (g COD/L)	1.3 ± 0.2	0.31 ± 0.08	0.6 ± 0.1
Valeric acid (g COD/L)	-	1.9 ± 0.2	2.0 ± 0.6
Caproic acid (g COD/L)	-	1.6 ± 0.2	1.8 ± 0.5
Eptanoic acid (g COD/L)	0.55 ± 0.04	0.7 ± 0.1	1.4 ± 0.3
pH range	4.8 - 5.2	4.8 - 5.7	3.4 - 5.7
COD_{VFA}/COD_{SOL}	0.09 ± 0.03	0.72 ± 0.01	0.59 ± 0.05
Total Kjeldahl Nitrogen (TKN; g N/kg TS)	23.1 ± 0.9	30 ± 1	34 ± 3
Phosphorus (P; g P/kg TS)	2.7 ± 0.2	4.7 ± 0.2	5.5 ± 0.6
Ammonia (N-NH_4^+; g/L)	0.41 ± 0.02	0.60 ± 0.02	0.68 ± 0.07
Phosphate (P-PO_4^{3-}; mg/L)	0.30 ± 0.02	0.39 ± 0.02	0.48 ± 0.06
COD_{SOL}/N/P (g)	100/5.7/1.6	100/7.3/2.3	100/5.5/1.8

Parameter		Fermentation performances	
		Mesophilic	Thermophilic
Solubilization	g COD_{SOL}/g $COD_{TOT(0)}$	0.04 ± 0.01	0.22 ± 0.04
	g COD_{SOL}/g $VS_{(0)}$	0.06 ± 0.01	0.34 ± 0.03
Yield (Y_{VFA})	g COD_{VFA}/g $COD_{TOT(0)}$	0.26 ± 0.01	0.28 ± 0.05
	g COD_{VFA}/g $VS_{(0)}$	0.41 ± 0.02	0.44 ± 0.03
$[C_3/(C_3+C_2)]_{VFA}$	mol/mol	0.20 ± 0.02	0.20 ± 0.01

Table S2. Assessment of the energy balance for the two anaerobic platform configurations investigated: mesophilic fermentation and mesophilic anaerobic digestion (A); mesophilic fermentation and thermophilic anaerobic digestion (B).

Process Configuration	Energy and Process Unit		Temperature	Item	Value (MJ/d)
A	**Consumed Thermal Energy**	Fermentation reactor	37°C	Heating Water	9,673
				Thermal Dispersion	895
		Anaerobic Digester	37°C	Heating Water	23,270
				Thermal Dispersion	3,377
		Whole anaerobic platform	37°C	Total	37,215
	Produced Thermal Energy from CHP		-	Thermal Energy	58,577
				Electrical Energy	13,026*
B	**Consumed Thermal Energy**	Fermentation Reactor	37°C	Heating Water	9,673
				Thermal Dispersion	895
		Anaerobic Digester	55°C	Heating Water	47,909
				Thermal Dispersion	6,630
		Whole anaerobic platform	37-55°C	Total	65,107
	Produced Thermal Energy from CHP		-	Thermal Energy	64,875
				Electrical Energy	14,426*

*KWh/d (unit of the electrical energy produced from CHP)