An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas

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ABSTRACT

This study focuses on the application of the concept of circular economy, with the creation of added-value marketable products and energy from organic waste while minimizing environmental impacts. Within this purpose, an urban biorefinery technology chain has been developed at pilot scale in the territorial context of the Treviso municipality (northeast Italy) for the production of biopolymers (pol-yhydroxyalkanoates, PHAs) and biogas from waste of urban origin. The piloting system (100−380 L) comprised the following units: a) acidogenic fermentation of the organic fraction of municipal solid waste (OFMSW) and biological sludge; b) two solid/liquid separation steps consisting of a coaxial centrifuge and a tubular membrane (0.2 μm porosity); c) a Sequencing Batch Reactor (SBR) for aerobic PHA-storing biomass production; d) aerobic fed-batch PHA accumulation reactor and e) Anaerobic co-digestion (ACoD). The thermal pre-treatment (72 °C, 48 h) of the feedstock enhanced the solubiliza-tion of the organic matter, which was converted into volatile fatty acids (VFAs) in batch mode under mesophilic fermentation conditions (37 °C). The VFA content increased up to 30 ± 3 g COD/L (overall yield 0.65 ± 0.04 g COD_{VFA}/g $VS_{(0)}$), with high COD_{VFA}/COD_{SOL} (0.86 ± 0.05). The high COD_{VFA}/COD_{SOL} ratio enhanced the PHA-storing biomass selection in the SBR by limiting the growth of the non-storing mi-crobial population. Under fully aerobic feast-famine regime, the selection reactor was continuously operated for 6 months at an average organic loading rate (OLR) of 4.4 ± 0.6 g COD/L d and hydraulic retention time (HRT) of 1 day (equal to SRT). The ACoD process (HRT 15 days, OLR 3.0−3.5 kg VS/m^3 d) allowed to recover the residual solid-rich overflows generated by the two solid/liquid separation units with the production of biogas (SGP 0.44−0.51 m^3/kg VS) and digestate. An overall yield of 7.6% wt PHA/$VS_{(0)}$ has been estimated from the mass balance. In addition, a preliminary insight into potential social acceptance and barriers regarding organic waste-derived products was obtained.

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1. Introduction

Some of the most challenging problems which governments and populations have to face nowadays are natural resources depletion, waste generation, air, soil and water pollution, climate change and increasing global energy demand. The use of non-renewable feed-stock has led to a progressive consumption of natural environments and increase in greenhouse gas (GHG) emissions (Hassan et al., 2019). This approach can no longer be the answer to growing product and energy demand, and the need to seek new solutions promoted sustainable and resource-efficient policies (Moncada et al., 2016). Recent strategies in the European Union (EU) set as a priority the transition towards a circular economy by adopting the "closing the loop" approach to industrial production systems (Maina et al., 2017). One of the objectives of this strategy is to maintain the value of products, materials and resources in the economy for the longest time possible, together with the objective of waste minimi-zation (European Commission, 2015). The conversion of low value products into higher value products, fully consistent with the EU approach to the circular economy, can be realized with the

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Abbreviations

3HB	3-hydroxybutyrate
HV	3-hydroxyvalerate
ACoD	anaerobic co-digestion
BNR	biological nutrient removal
CAPEX	capital expenditure
CHP	combined heat & power unit
COD	chemical oxygen demand
COD_{SOL}	soluble COD
COD_{TOT}	total COD
COD_{VFA}	volatile fatty acids expressed as COD
COD_{Xa}	active biomass expressed as COD
CSTR	continuous stirred tank reactor
DO	dissolved oxygen
EE	electric energy
EoW	end of waste
EU	European Union
GC	gas chromatograph
GHG	greenhouse gas
GPR	gas production rate
GSI	green self-identity
HRT	hydraulic retention time
MMC	mixed microbial cultures
OFMSW	organic fraction of municipal solid waste
OLR	organic loading rate
OPEX	operating expense
PE	person equivalent
PHA	polyhydroxyalkanoates
PLC	programmable logic controller
SBR	sequencing batch reactor
SGP	specific gas production
TE	thermal energy
TKN	total Kjeldahl nitrogen
TRL	technology readiness level
TS	total solids
TSS	total suspended solids
VFA	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
WAS	waste activated sludge
WWTP	wastewater treatment plant
X_A	active biomass

application of a biorefinery technology chain. The biorefinery represents a way to approach resource valorization, aiming to use available renewable substrates in order to provide high value marketable products while minimizing energy consumption and waste generation (Andersen et al., 2018). The use of renewable resources such as biomass or even organic waste can be challenging due to the competition with economically inexpensive fossil fuel energy. End-product identification and cost-effecting processing schemes are key elements in the biorefinery design, allowing the development of competitive technologies (Zabaniotou, Kamaterou, 2019). In order to realistically assess any technology chain, some indexes such as the technology readiness level (TRL) were defined. Higher TRLs, up to level 9, are characterized by lower complexity and lower technical and economic risks due to the scaling up and verification of the technology (European Commission, 2017). The creation of a biorefinery using organic waste as starting substrate can contribute to both waste disposal problems and renewable resources valorization. In urban contexts, two of the most available organic substrates are food waste and wastewater sludge. Food waste production in Europe accounts for about 87.6 million tons annually (Colombo et al., 2017). Over past years, this organic waste was sent to landfill, without even any treatment, causing severe air, groundwater and soil contamination due to its high biodegradability and leachate formation (Zhou et al., 2018). The need for new environmentally friendly solutions led to food waste treatment with composting, allowing the production of high-quality compost for agricultural applications (Bolzonella et al., 2006). However, this process is highly energy consuming and requires the occupation of large land quantities with possible GHG formation (Lawal-Akinlami, Palaniyandi, 2017). Similar disposal problems are encountered with other organic waste produced in urban areas, especially wastewater sludge generated from biological wastewater treatment processes (Li et al., 2018). Some of the most common processes applied for biological sludge disposal are composting or anaerobic digestion. Anaerobic digestion of one or more organic substrates is a process currently applied also in existing wastewater treatment plants (WWTPs), in order to stabilize organic matter along with the production of biogas (Mata-Alvarez et al., 2014). The application of a

biorefinery technology chain in urban scenarios, where organic substrates are always available, can foster the combination and valorization of different substrates into added-value marketable products other than biogas alone (Valentino et al., 2018). The creation of this new value chain may unlock new job positions and market opportunities and needs to be thoroughly investigated (Philippidis et al., 2019). Also, waste derived products need to end their waste status and to be accepted as alternative products by consumers, meaning that producing bioplastics from food waste and sludge determines a strong social impact along the entire value chain. Considering that bio-based materials are produced from heterogeneous organic waste by using brand new technologies, the development of quality standards is a prerequisite to increase the confidence of market operators, social acceptance and to fill regulatory gap based on adequate knowledge. In the present study, an urban biorefinery technology chain was developed at pilot scale inside the Treviso WWTP (northern Italy) with a TRL 5. The Treviso province showed optimum conditions for the application of such a technology chain, since waste separated collection is very efficient. The recycling and separate collection percentage on the total municipal solid waste collected in urban districts is equal to 87.9%, giving a highly sourced food waste (ISPRA, 2017). Organic fraction of municipal solid waste (OFMSW) and biological sludge from the municipal WWTP were combined together for polyhydroxyalkanoates (PHAs) from mixed microbial cultures (MMCs) and biogas production. The performances of each single unit were determined and the whole technology chain was assessed with a full mass and energy balance. An experimental study was carried out to also measure the possible acceptance of consumers for waste derived products by means of a specific survey. Very less is known about the acceptance from consumers on bio-based products, including how companies might consider these products. Thus, successfully marketing these products is relevant not only to companies considering whether to invest in this new market, but also to identify the potential drivers or barriers to the acceptance of urban waste derived products. Consumers' switch to products derived from closed-loop processes would indeed provide a much less expensive source of raw materials compared to fossil fuel.

2. Materials and methods

2.1. Biorefinery process scheme

The biorefinery process scheme is represented in Fig. 1. A mixture of OFMSW and biological sludge was fed to the acidogenic fermenter (a) in 30% and 70% volumetric ratio, respectively. The fermented stream, enriched in volatile fatty acids (VFAs), was sent to the solid-liquid separation unit (b) for further refining before being fed to the sequencing batch reactor (SBR) for PHA selection (c) and production (d). The overflows, namely the solid fraction from the centrifuge and the retained phase from the membrane both produced in stage (b), were sent to anaerobic co-digestion together with biological sludge from the WWTP (e).

Activated sludge from the full-scale plant was used as inoculum for the SBR and the biomass was selected using an aerobic dynamic feeding strategy, known as the feast famine regime (Reis et al., 2011). The surplus biomass was then directed to the production reactor. Here, the PHA accumulation within the microbial cells was maximized by feeding the MMC with the same VFA-rich permeate that was fed in the selection reactor.

2.2. Organic substrates

The substrates treated using this process were biological sludge and OFMSW, both available inside the Treviso WWTP. The biological sludge came from the static thickener after the biological nutrient removal (BNR) process was applied in the Treviso WWTP water line. OFMSW came from the source separate collection of 50 districts in the Treviso province. After collection, waste is transferred into a dedicated plant for a solid-liquid separation stage achieved with a mechanical screw-press. The liquid fraction (ca. 15% total solids basis) is sent to anaerobic co-digestion with biological sludge in the Treviso full-scale WWTP and the solid fraction is sent to composting. The two substrates were collected weekly inside the Treviso WWTP and characterized in terms of total solids and volatile solids (TS and VS), soluble chemical oxygen demand (COD_{SOL}), VFAs (COD_{VFA}), total COD (COD_{TOT}), nitrogen as ammonium ($N-NH_4^+$), phosphorus as orthophosphate ($P-PO_4^{3-}$), Total Kjeldahl Nitrogen (TKN) and organic phosphorus (P). Substrates characterization is reported in Table 1.

2.3. Pilot reactors

The anaerobic line consisted of the fermentation and digestion reactors. The anaerobic fermentation (a) was carried out in batch mode in a 380 L reactor equipped with a mechanical stirrer and a temperature control system, controlled by means of a thermostatic jacket. The reactor was operated at 37 °C, with and without a thermal pre-treatment for the enhancement of the organic matter solubilization. The thermal pre-treatment applied in the second fermentation run consisted of the application of a high temperature (72 °C) for 48 h to the feedstock mixture inside the fermentation reactor. After this time, the reactor temperature was decreased and maintained at 37 °C for four days. The solid/liquid separation stage (b) consisted of a coaxial centrifuge with 5.0 μm porosity nylon filter bag for solids removal, followed by a 0.2 μm porosity ultra-filtration membrane. Anaerobic co-digestion (e) was conducted in a 230 L working volume stainless steel CSTR (AISI-304). The reactor was equipped with a mechanical anchor stirrer and the temperature was maintained at 37 °C and 55 °C by hot water recirculation through an external jacket. The inoculum was collected from the 2000 m³ full-scale digester of the WWTP and it was acclimatized for at least 2 hydraulic retention times (HRTs).

In the aerobic line, the PHA MMC selection was conducted in a 100 L working volume reactor (c). Dissolved oxygen (DO)
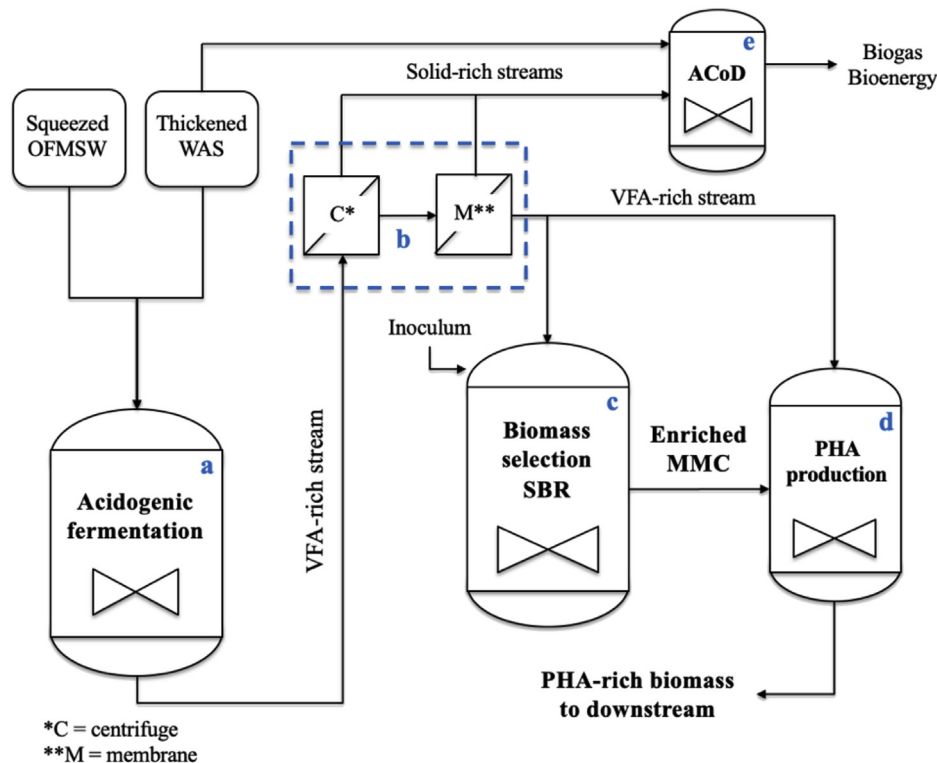

Fig. 1. Biorefinery process scheme. (a): Acidogenic fermenter reactor; (b): Solid-liquid separation stage; (c): Aerobic PHA selection SBR; (d): Aerobic PHA accumulation reactor; (e): Anaerobic digester.

Table 1
Characteristics of squeezed OFMSW, biological sludge and 30%−70% mixture fed to the acidogenic fermenter.

Parameter	Squeezed OFMSW	Biological sludge	30% OFMSW - 70% Biological sludge mixture
TS (g TS/kg)	150 ± 3	30 ± 2	64 ± 2
VS (g VS/kg)	132 ± 3	21 ± 2	51 ± 2
VS/TS (%)	88 ± 1	70 ± 1	80 ± 1
COD_{SOL} (g COD/L)	40 ± 2	−	20 ± 1
COD_{VFA} (g COD/L)	8 ± 1	−	3.5 ± 0.5
COD_{TOT} (g COD/L)	95 ± 6	50 ± 3	62 ± 4
Ammonium (mg $N-NH_4^+$/L)	212 ± 57	489 ± 68	342 ± 28
Phosphate (mg $P-PO_4^{3-}$/L)	78 ± 4	150 ± 9	113 ± 7
TKN (g N/kg TS)	23 ± 5	50 ± 8	25 ± 3
P (g P/kg TS)	1.8 ± 0.1	2.5 ± 0.1	2.3 ± 0.1

concentration was maintained at a maximum of 8.0 mg O_2/L with linear membrane blowers (Bibus EL-S-250), which also allowed the complete stirring of the mixed liquor. DO concentration, oxidation reduction potential (ORP), pH and temperature were constantly monitored in real time by immersion probes (Hamilton®) and online signals were acquired through a programmable logic controller (PLC) (myRio Labview by National Instrument®). Temperature was regulated by an immersion heater and maintained between 25 and 28 °C in both aerobic (c-d) units. Selection SBR operating cycles were automated and controlled by the PLC. The PHA accumulation reactor (d) was operated in fed-batch mode with the same equipment as the selection reactor, with a working volume ranging between 80 L and 120 L. Selected biomass (X) at the end of the feast phase was discharged from the SBR to the accumulation batch. VFA rich permeate liquid was fed by multi-spike strategy with an initial VFA/X ratio of <2.0 on a COD basis, in order to prevent substrate or pH inhibition phenomena (Valentino et al., 2015a). Operating conditions of all pilot units are listed in Table 2.

2.4. Analytical methods

OFMSW and biological sludge mixture was characterized once per week after fresh sample collection. Fermentation liquid from unit (a) was collected two times per week for characterization analyses. Liquid and solid streams coming from the centrifuge and membrane separation stage (b) were characterized once per week. Biomass from the selection SBR (c) was collected twice per week for total suspended solids (TSS), volatile suspended solids (VSS), PHA, ammonium and phosphate characterization. All analyses were performed according to Standard Methods (APHA, AWWA, WEF, 1998), except for VFA and PHA. VFAs were determined by means of an Agilent 6890 N gas chromatograph (GC) equipped with a flame ionization detector (FID) (T = 230 °C) and an Agilent J&W DB-FFAP fused silica capillary column (15 m length, 0.53 mm i.D.

0.5 mm film) using hydrogen as carrier. The inlet (T = 220 °C) was working in split mode with a split ratio of 20:1. The instrument operated with a ramp temperature from 80 °C to 200 °C with 2-ethyl butyric acid used as internal standard for peak identification. Sample preparation before GC analysis included centrifugation at 4500 rpm for 5 min, subsequent filtration of the supernatant through 0.2 μm acetate cellulose syringe filters (Whatman®) and acidification at pH 2 with orthophosphoric acid. PHA samples with 5.0 mL of unfiltered mixed liquor were treated with 1.0 mL NaClO solution (5% active Cl_2) and then stored at −4 °C for following analysis. The polymer was extracted, hydrolysed and esterified to 3-hydroxyacyl methyl esters and quantified by a GC method (Braunegg et al., 1978). 3-Hydroxybutyric (3HB) methyl ester and 3-hydroxyvaleric (3HV) methyl ester monomers were quantified using P (3HB-co-3HV) Sigma-Aldrich standard polymer at 5 wt% HV content as a reference.

2.5. Calculations

Process performances were assessed after reaching a pseudo steady state or steady state conditions in every reactor, characterized by stable VFA, PHA and biogas production. In the fermentation unit, solubilization was calculated as the ratio between final COD_{SOL} (net concentration after subtracting the initial COD_{SOL} at time zero, $COD_{SOL(0)}$) and feedstock VS at time zero ($VS_{(0)}$), as follows:

$$Solubilization = \frac{(COD_{SOL} - COD_{SOL(0)})}{VS_{(0)}}$$

Fermentation yield (Y_{VFA}) was determined as the ratio between net VFA concentration (after subtracting the initial feedstock concentration, $COD_{VFA(0)}$) and the feedstock VS at time zero:

$$Y_{VFA} = \frac{(COD_{VFA} - COD_{VFA(0)})}{VS_{(0)}}$$

Table 2
Operating conditions of all pilot units and different runs operated.

Anaerobic Fermentation (AnF)					
Run	**HRT (d)**	**SRT (d)**	**OLR (kg VS/m³ d)**	**Temperature (°C)**	
AnF1	5	5	12−15	37	
AnF2	5	5	12−15	37−72	
Aerobic PHA line (Ae)					
Run	**HRT (d)**	**SRT (d)**	**OLR (g COD/L d)**	**Temperature (°C)**	**Cycle Length (d)**
Ae	1	1	3.1−5.9	25−28	0.25
Anaerobic Co-Digestion (ACoD)					
Run	**HRT (d)**	**SRT (d)**	**OLR (kg VS/m³ d)**	**Temperature (°C)**	
ACoD1	15	15	3.0−3.5	37	
ACoD2	15	15	3.0−3.5	55	

In the aerobic PHA selection unit, pseudo steady state conditions were reached when the feast phase length was quite constant, with 5% deviation from average for 10 SRTs (Valentino et al., 2014). Active biomass (X_A), expressed in g/L, was determined as difference between the VSS and PHA content in g/L at the end of each SBR cycle:

$$X_A = VSS - PHA$$

The PHA content within the biomass, expressed as g PHA/g VSS, was calculated as ratio between the PHA concentration and the VSS concentration at the end of the feast phase. Specific substrate uptake rate ($-qS^{feast}$), expressed as mg COD_{SOL}/g X_A h, was calculated as the ratio between VFA consumed during the feast phase and the length of the feast phase (t) per unit of X_A:

$$-qS^{feast} = \Delta COD_{SOL}/t \cdot X_A$$

The PHA production rate during the feast phase (qP^{feast}) was determined as the ratio between the stored PHA and the length of the feast phase per unit of X_A, expressed as mg PHA/g X_A h:

$$qP^{feast} = \Delta PHA/t \cdot X_A$$

PHA production yield ($Y_{P/S}^{feast}$) was calculated as ratio between the PHA produced and the COD_{SOL} consumed during the feast phase:

$$Y_{P/S}^{feast} = \Delta PHA/\Delta COD_{SOL}$$

The average observed growth yield of the selection reactor (Y_{OBS}^{SBR}) was calculated as a ratio between the active biomass (X_A) expressed as COD and the OLR and HRT applied to the reactor:

$$Y_{OBS}^{SBR} = X_A/(OLR \cdot HRT)$$

All mass values of X_A and PHA were converted on COD basis (g COD) considering the conversion factors from oxidation stoichiometry (Valentino et al., 2014).

The mass and energy balance for the anaerobic line was assessed on a 70,000 person equivalent (PE) scenario, which corresponds to the maximum potential of the Treviso WWTP, where an ACoD reactor is currently operating for biogas production from the same OFMSW and biological sludge mixture used in this study. Reference parameters and boundary conditions used for the balance are reported in Table S1. Thermal and electrical yields of the Combined Heat & Power unit (CHP) are also listed. The price of electric energy was assumed to be 130 €/MWh (no incentive; Valentino et al., 2018). The OFMSW and waste activated sludge (WAS) inlet flow rate was calculated on the basis of their specific production: 0.3 kg OFMSW/PE day (wet weight) and 0.06 kg TS/PE day for WAS (Tchobanoglous et al., 2003).

2.6. End of waste definition and social acceptance survey

Legislative barriers and social acceptance of bio-based products potentially hindering the implementation of the final product were preliminarily investigated. The End of Waste (EoW) status of waste-derived products was preliminarily evaluated and the social acceptance of hypothetical products tested. All participants received the same information about the product they are supposed to purchase. The panel composition represented all age ranges (18−65+) and all genders. The final sample was composed of 230 consumers from the United Kingdom. A specific focus was dedicated to the production of chairs made of waste derived bio-plastic, represented by two pictures of modern chairs. The descriptions were inspired by actual high fashion designer furniture

and mass-production convenience furniture, respectively. A different scenario was deemed to be helpful to understand the role of product involvement (designer versus convenience chair) on consumers' acceptance and perception. Product involvement is commonly defined as a consumer's enduring perception of the importance of the product category based on the consumer's inherent needs, values, and interests (Lin and Chen, 2006). The ensuing questionnaire assessed consumers' attitude toward the product (Hazen et al., 2017), purchase intention (Mugge et al., 2017), green self-identity (GSI; Barbarossa et al., 2016), switching intention (Hazen et al., 2017), perceived value (Lin et al., 2012) and perceived risk (Wang and Hazen, 2016) of bio-based products, as well as likelihood of switching behavior to bio-based products (Hazen et al., 2017) and awareness of recycling (Mugge et al., 2017). All items ranged from 1 ("strongly disagree") to 7 ("strongly agree") based on a Likert scale. The customer acceptance is increasing linearly along this scale from 1 to 7. Furthermore, participants were asked their willingness-to-pay for and past purchase experience regarding eco-products. A Multivariate Analysis of Variance was run with willingness-to-pay, intention to switch and purchase intention as dependent variables. More in-depth information regarding the study methodology and the statistics applied can be found in the Supplementary material. Another preliminary survey was then carried out with participants from other countries, namely USA, Spain and Poland. The preliminary results obtained are useful to show feeling and perceptions with other products, that are both very relevant to understand where to direct further investigations. More information regarding this preliminary survey can be found in the Supplementary material.

3. Results & discussion

3.1. Anaerobic line

3.1.1. Acidogenic fermentation

The acidogenic fermentation process was performed in batch mode, testing two different conditions, AnF1 and AnF2 as listed in Table 2. The experiments were carried out in order to achieve the maximum VFA concentrations possible with the highest COD_{VFA}/COD_{SOL} ratio. With nutrient rich carbon sources, a high COD_{VFA}/COD_{SOL} ratio has been identified as a key element for an effective PHA MMC selection process (Albuquerque et al., 2010). The main difference consisted of the application of a thermal pre-treatment (72 °C; 48 h) in run AnF2, followed by 4 days in the mesophilic range, while run AnF1 was maintained at 37 °C for 6 days. During both runs, the process showed quite stable performance and the pH
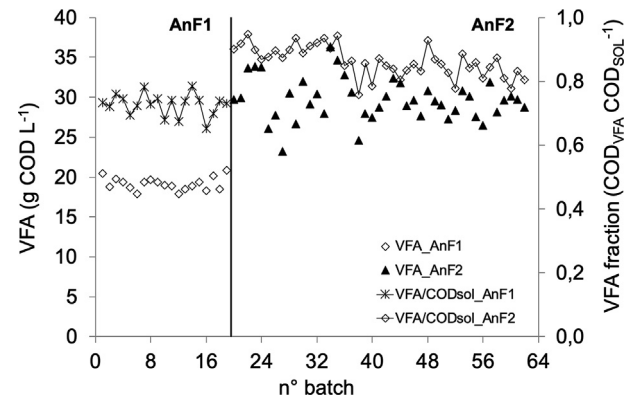

Fig. 2. VFA concentrations and CODVFA/CODSOL ratio obtained during the two fermentation runs.

maintained itself between 5.0 and 5.5, due to the biological sludge buffering capacity (Cabbai et al., 2016). In Fig. 2 are reported the VFA production and the COD_{VFA}/COD_{SOL} ratios obtained at the end of every fermentation batch.

AnF1 showed a stable VFA production in every batch, ranging between 17.8 and 20.8 g COD/L. The COD_{VFA}/COD_{SOL} ratio values fluctuated from a minimum of 0.65 up to 0.79. AnF2 was characterized by higher VFA production and COD_{VFA}/COD_{SOL} ratios. However, both parameters showed broader fluctuations, especially in the first batches (from 19 to 38). The minimum VFA concentration obtained was equal to 23 g COD/L and the maximum one was equal to 36 g COD/L. The COD_{VFA}/COD_{SOL} ratio ranged between a minimum of 0.76 and a maximum of 0.95. The overall yields in terms of VFA production were 0.37 and 0.65 g COD_{VFA}/g $VS_{(0)}$ in runs AnF1 and AnF2, respectively. Table 3 reports the average fermented stream characteristics obtained in both runs.

For the aim of this work, run AnF2 showed optimum results. This proved the crucial role played by the application of a thermal pre-treatment stage before actual mesophilic fermentation, since other parameters remained constant. Recent studies (Moretto et al., 2019a) have demonstrated that the fermentation process of a similar starting substrate can be further optimized for the subsequent PHA MMC selection process, achieving higher VFA concentrations, higher COD_{VFA}/COD_{SOL} ratios and a higher proportion of 3HV precursors (i.e. higher odd numbered acids concentrations). These performances were obtained with the application of a thermal pre-treatment (at 72 °C for 72 h) followed by alkaline fermentation (pH 9) in batch tests and in a continuous CSTR process, both at lab scale. Targeting the subsequent applications of the fermented stream is necessary in order to adopt one process rather than the other. In this study, the pilot scale fermentation process was conducted without pH control, based on a chemicals and cost saving perspective. The AnF2 fermented stream was utilized for the subsequent PHA selection and accumulation processes, after undergoing two solid/liquid separation steps. The aim of the solid/liquid separation units (centrifuge and membrane) was the removal of all coarse solids, in order to obtain a clear VFA-rich stream. This refining stage is crucial in order to avoid possible impurities transfer to the final polymer. Impurities will eventually lead to problems in the downstream (PHA extraction and purification), raising also legislative barriers preventing the PHA marketing.

3.1.2. Anaerobic co-digestion

Anaerobic co-digestion process was performed on a mixture of excess biological sludge, centrifuge solid fraction (solid cake, SC) and membrane retained phase (MRP). The process was started up by feeding biological sludge only, almost for 1.0 HRT. Then, the feeding with SC and MRP started by gradually increasing their content in the feed mixture up to an OLR of 3.5 kg VS/m³ d approximately. The fed mixture was composed by 66% of

Table 3
Average characteristics of the fermented stream obtained in run AnF1 and AnF2.

Parameter	Unit	AnF1	AnF2
COD_{SOL}	g COD/L	26 ± 2	34 ± 3
COD_{VFA}	g COD/L	19 ± 1	30 ± 3
COD_{TOT}	g COD/L	65 ± 2	70 ± 3
COD_{VFA}/COD_{SOL}	COD/COD	0.73 ± 0.03	0.86 ± 0.05
[3HVp/(3HB + 3HV)p][a]	mol/mol	0.20 ± 0.01	0.25 ± 0.02
TKN	g N/kg TS	26 ± 2	27 ± 3
Organic Phosphorus (P)	g P/kg TS	2.7 ± 0.5	2.8 ± 0.5
Solubilization	g COD_{SOL}/g $VS_{(0)}$	0.12 ± 0.02	0.20 ± 0.02
Yield (Y_{VFA})	g COD_{VFA}/g $VS_{(0)}$	0.37 ± 0.03	0.65 ± 0.04

[a] Ratio between odd numbered acids precursors (3HVp) and the sum of even and odd numbered acids precursors (3HB + 3HV)p.

Table 4
Stability parameters of the anaerobic co-digestion processes (ACoD1 and ACoD2).

Parameter	Unit	ACoD1	ACoD2
pH	–	7.6 ± 0.6	7.2 ± 0.4
Partial Alkalinity	mg CaCO₃/L	1890 ± 129	1598 ± 119
Total Alkalinity	mg CaCO₃/L	2903 ± 201	3192 ± 223
COD_{VFA}	g COD/L	0.30 ± 0.04	0.43 ± 0.07
Ammonium	mgN-NH₄⁺/L	670 ± 101	879 ± 192
VFA/P.Alk	mg Acetic acid/mg CaCO₃	0.14 ± 0.03	0.16 ± 0.05

biological sludge, 8% of SC and 26% of MRP in terms of volumetric percentages. The OLR was kept at a maximum of 3.5 kg VS/m³ d for technical reasons, since the availability of SC and MRP was dependent on the centrifuge and membrane unit operation and volumetric capacity. The OLR was strongly influenced by the SC contribution, due to the higher solids content (235 g TS/kg, 80% VS/TS). MRP, with an average solid content of 20 g TS/kg, contributed to a minor extent to OLR in terms of TS. The mixture fed to the digester was characterized by an average TS and VS content of 44 g TS/kg and 33 g VS/kg respectively. Operating conditions applied to the process were based on a previous study, in which a mixture of biological sludge and SC only was co-digested both in mesophilic and thermophilic conditions (Valentino et al., 2019). In this previous study, the co-digestion process was carried out before the implementation of the membrane as a second solid/liquid separation unit. The mesophilic run (37 °C) was operated for 139 days and the thermophilic run (55 °C) was operated for 108 days. Results obtained indicated that both processes under steady state showed a good stability and robust performances. Partial alkalinity concentration ensured a good buffer to the system, avoiding VFAs accumulation in the reactor with a possible pH drop and subsequent inhibition of methanogenic activity (Zahedi et al., 2016). The stability parameters monitored are reported in Table 4.

As expected, the thermophilic condition (ACoD2) guaranteed higher yields in terms of specific gas production (SGP) and methane production as compared to mesophilic operation (ACoD1). In ACoD2, the steady state period was characterized by an average gas production rate (GPR) of 2.1 ± 0.1 m³_biogas/m³ d and an SGP equal to 0.51 ± 0.02 m³_biogas/kg VS. In ACoD1, the GPR and SGP were equal to 1.5 ± 0.1 m³_biogas/m³ d and 0.44 ± 0.02 m³_biogas/kg VS, respectively. In both runs, biogas was composed of 62−63% CH_4, 35−37% CO_2 and 1−2% other trace gases (e.g. H_2S). In Fig. 3 are shown the GPR and CH_4 content trends during the whole monitoring period.

The GPR trends reflected the progressive increase in OLR in the two conditions, as expected. During ACoD1 start-up phase, the GPR showed a rapid increase, especially from day 15 (0.33 m³_biogas/m³ d) to day 32 (1.34 m³_biogas/m³ d), when the SC and MRP were added stepwise to the mixture fed to the digester. The methane content in the biogas showed a similar trend to those of the GPR. A rapid increase in methane content can be noticed from day 12 (39%) to day 22 (57%), in correspondence to the rapid increase in GPR. Both parameters showed stable trends with narrow fluctuation ranges during steady state conditions. Similar trends were observed in the ACoD2 run. In this case, the higher temperature allowed steady state to be reached in a shorter time compared to ACoD1.

Literature studies generally report higher yields obtained with a similarly pressed OFMSW single digestion, up to 0.65 and 0.82 m³_biogas/kg VS with applied OLRs equal to 4.3 and 4.5 kg VS/m³ d, respectively (Micolucci et al., 2016; Novarino and Zanetti, 2012). When the pre-treated OFMSW was mixed with biological sludge, similar yields were obtained, at around 0.5 m³_biogas/kg VS as SGP (Mattioli et al., 2017; Moretto et al., 2019b). In those cases, a higher SGP could be potentially achievable since the applied OLRs were much lower (1.38−1.74 kg VS/m³ d) than the one adopted in this
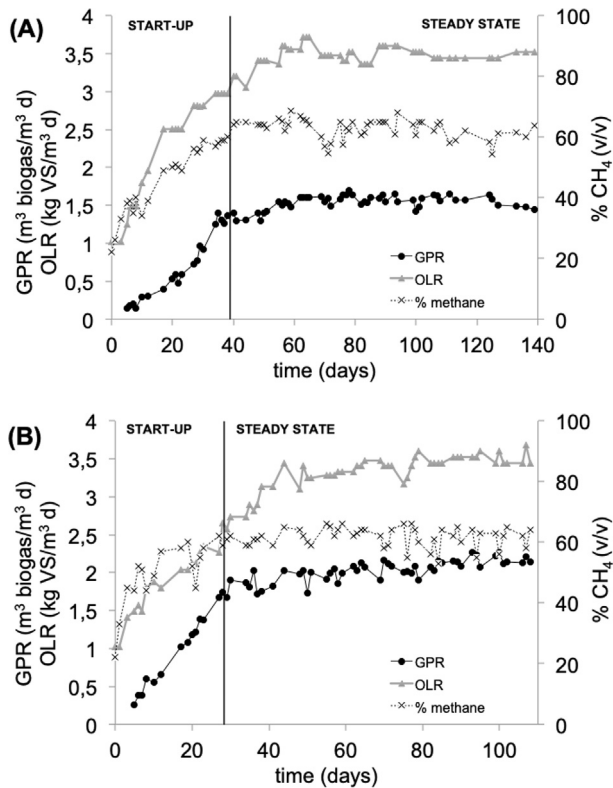
Fig. 3. OLR, GPR and methane percentage trends of biological sludge, SC and MRP anaerobic co-digestion in mesophilic (A) and thermophilic (B) conditions.

study. The lower performances compared to the previous studies are most likely due to the partition of the major part of the carbon source into the PHA line. Since only a part of the fermented stream produced is directed to anaerobic co-digestion, performances are consequently lower than those obtained directly from a highly biodegradable substrate (Mata Alvarez et al., 2014). In a biorefinery technology chain where PHAs are the core product, all waste streams produced can be conveyed to the anaerobic co-digestion process, which has been demonstrated to be technically feasible.

3.1.3. Anaerobic line mass and energy considerations

In general, the addition of another valuable product, such as biogas, is perfectly in line with the approach of "closing the loop" in industrial production systems. However, the technical feasibility needs to be supported by the energy balance, which has been evaluated for the two following process configurations: AnF2-ACoD1 and AnF2-ACoD2, both applied in a full-scale platform of an urban scenario of 70,000 PE. The inlet amount of WAS corresponds to 155,556 kg/d, of which 25% is driven to AnF2 step and the rest to the ACoD step for the dilution of the SC and MRP overflows. The whole OFMSW flow rate (16,800 kg/d) is driven to AnF2. Only the AnF2 step was considered to be technically feasible for the purpose of the biorefinery, and the relative yield of 0.65 kg COD_{VFA}/kg VS was taken into account. Three different streams from the solid/liquid separation units were obtained: the SC (9677 kg/d; 20% w/v of VS), the MRP (13,545 kg/d; 4% VS), at a higher solid content level, and the permeate liquid stream (34,742 kg/d; 1175 kg COD_{VFA}/d) for the aerobic PHA line. The SC and MRP overflows are diluted with 116,356 kg/d of excess thickened WAS, generating an inlet ACoD flow rate of 139,930 kg/d with a 3.4% VS content approximately (Fig. 4).

Heat requirements and produced energy were assessed for

reactors with a working volume of 296 m^3 (AnF) and 2133 m^3 (ACoD). In the AnF2-ACoD2 approach, the biogas production (2689 m^3/d) was higher than for the AnF2-ACoD1 process (2361 m^3/d). The use of biogas in the CHP unit allows to generate 30,945 MJ/d of thermal energy (TE). This value is clearly close to the required energy (31,237 MJ/d) for heating and heat dispersion balance. Despite the lower biogas production, the AnF2-ACoD1 configuration is preferable to the other when comparing the thermal energy consumption and the generated energy. With the AnF2-ACoD1 approach, the thermal balance is positively closed, since 70% of the produced energy is enough to cover the energy demand of both reactors (Fig. 5).

This surplus guarantees the anaerobic line self-sustainability. In turn, a production of 6.04 MWh/d of electric energy (EE) is possible. From the sale of generated EE, it has been estimated that a potential income of 286,626 €/year could be achieved, which makes this approach more attractive if compared to the traditional AD process (single or two-phases) for the treatment of excess sludge only (Leite et al., 2016).

3.2. Aerobic PHA line

3.2.1. Biomass selection/enrichment and PHA accumulation performances

A single long-term SBR run was performed in order to validate the performances of the enrichment of PHA-storing biomass under a high-rate selection strategy (HRT and SRT equal to 1.0 day). Activated sludge from the Treviso full-scale WWTP was used as inoculum. A clear feast phase was observed after one or two days following the inoculation, with the establishment of a stable feast-famine regime within two weeks (at a feast to cycle length ratio equal to 0.06−0.12 for most part of the run) (Fig. S1). After the achievement of a steady state, the reactor was continuously operated for a sufficiently long period (approximately six months) in order to allow the characterization of the biomass storage properties and maximal accumulation capacity. As usually observed in a typical full-aerobic feast-famine regime, the simultaneous consumption of the VFA-rich carbon source, ammonium and phosphorus during the feast phase led to both PHA synthesis and cellular growth (Rodriguez-Perez et al., 2018). In the following famine phase, stored PHA consumption was observed since it was used for further biomass synthesis. The robust fermentation performances achieved in run AnF2, with high COD_{VFA} (30 ± 3 g COD/L) concentrations and a high COD_{VFA}/COD_{SOL} ratio (0.86 ± 0.05), ensured a good selective pressure by minimizing the impact of non PHA-storing biomass growth, usually associated with the COD_{SOL} fraction other than VFA. The enrichment in PHA-accumulating microorganisms in the mixed culture was confirmed by the average specific storage rate obtained during the feast phase (qP^{feast}), equal to 253 mg COD_{PHA}/g COD_{Xa} h. The solid/liquid separation stages after the fermentation stage removed most of the slowly biodegradable COD (sbCOD), further enhancing the specific substrate uptake rate (617 mg COD_{SOL}/g COD_{Xa} h) and the rapid establishment of famine conditions after substrate depletion. The average storage yield ($Y_{P/S}^{feast}$) and average observed yield (Y_{OBS}^{SBR}) obtained were equal to 0.43 COD_{PHA}/COD_{SOL} and 0.57 COD_{VSS}/COD_{SOL} respectively. The importance of the carbon source quality plays a key role in determining the efficiency of the selection process, and consequently the performances obtained in the accumulation stage (Kourmentza et al., 2017). Korkakaki et al. (2016) observed a negative effect of OFMSW leachate on microbial populations during the selection stage, which translated into low VFA uptake and low PHA production. The mixture of fermented and refined biological sludge and pre-treated OFMSW was suitable also for accumulation batches, avoiding pH inhibition phenomena
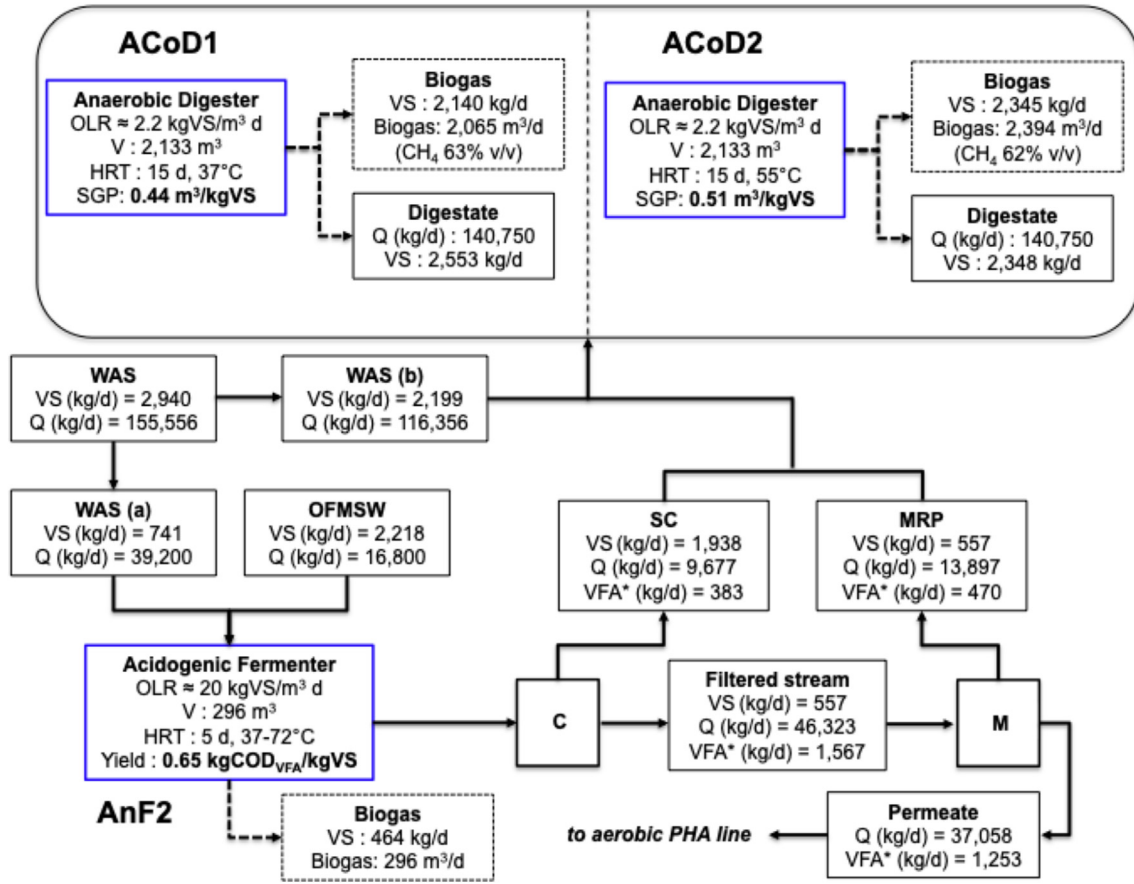
Fig. 4. Flow rates and mass balance of the scaled up anaerobic process: mesophilic acidogenic fermentation (AnF2) followed by anaerobic co-digestion in mesophilic (ACoD1) and thermophilic (ACoD2) condition. *COD based.

through a multi-spike strategy (Montiel-Jarrillo et al., 2017). Trends of PHA concentration and intracellular content, consumed VFA, produced X_A and consumed ammonium obtained during a typical accumulation batch are reported in Fig. 6.

An intracellular PHA content of 0.51 g PHA/g VSS was obtained after 7 h and did not increase during the following hour, suggesting that the biomass reached its maximum PHA accumulation potential under these conditions. Both growth and storage response were observed during accumulation, due to the utilization of a carbon source not deficient in nutrients. However, the storage mechanism was better stimulated and overcame the growth mechanism of

substrate consumption. This was particularly verified at the beginning of the accumulations, since the growth response could be quantified after the 2nd hour based on the produced PHA, produced X_A and ammonium trends. These trends can be connected to the good selection strategy applied in the SBR, which stimulated the PHA storage response more than the growth one (Albuquerque et al., 2010). The growth response in the accumulation batches increased quite linearly over time after the second hour due to the further increase in nutrient concentrations. The produced X_A trend followed quite closely the consumed ammonium one, confirming the nutrient utilization for biomass growth.
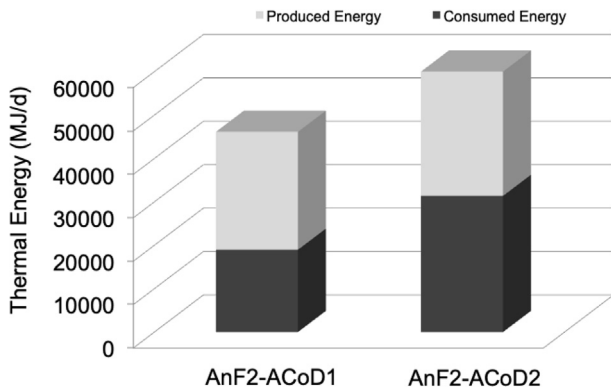

Fig. 5. Thermal energy balance of the two anaerobic processes configurations AnF2-ACoD1 and AnF2-ACoD2.
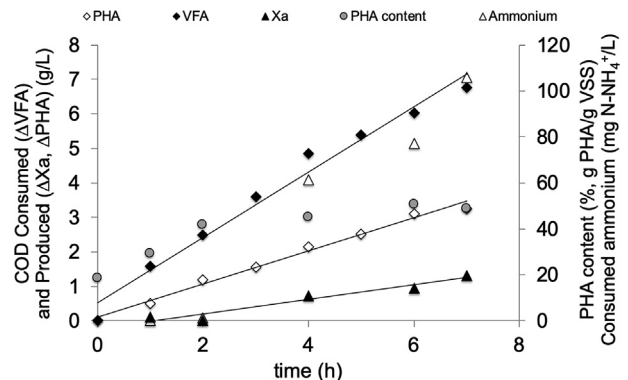

Fig. 6. Trends of PHA concentration and content, consumed VFA and produced active biomass during a typical accumulation batch.

The produced PHA and produced X_A reached a maximum of 3.21 g COD_{PHA}/L and 1.35 g COD_{Xa}/L respectively after 8 h. Overall, both selection and accumulation processes reached higher yields compared to other aerobic processes with lower carbon and nutrient load (Morgan-Sagastume et al., 2015). These results suggest that the exploitation of organic waste easily available especially in urban contexts, such as OFMSW, can be integrated in WWTPs with the utilization of biological sludge. Despite the sufficiently high nutrient load, the optimization of the previous steps can allow the valorization of urban waste into PHA production without compromising the performances of selection and accumulation processes.

3.2.2. Overall PHA process yield and preliminary economic evaluation

For each pilot unit, data analysis related to the yields and flow rate partitions have been taken into account for the quantification of the PHA yield over the whole platform. The overall yield is expressed as the amount of VS necessary for the production of 1.0 kg of PHA (roughly 1.7 kg COD_{PHA}). The PHA-related mass flow diagram is given in Fig. 7.

A backward calculation began with the final PHA biomass content achieved at the end of accumulation (0.52 ± 0.04 g PHA/g VSS). As a consequence, the produced active biomass was 0.92 kg X_A (or 1.31 kg COD_{Xa}). Hence, for both produced PHA and X_A, the required VFA amount is equal to 5.44 kg COD_{VFA}, as a sum of two separate contributions: 2.88 kg COD_{VFA} for PHA synthesis ($Y_{P/VFA}^{batch} = 0.59 ± 0.04$ COD_{PHA}/COD_{VFA}), and 2.56 kg COD_{VFA} for X_A growth ($Y_{Xa/VFA}^{SBR} = 0.51 ± 0.02$ COD_{Xa}/COD_{VFA}). The two yields have been quantified as previously reported (Valentino et al., 2018). The use of the two units for the solid/liquid separation process reduced the VFA-rich stream flow rate to about 30% v/v (as shown in Fig. 4), meaning that the amount of VFA required for the production of 1.0 kg PHA had to be increased up to 7.77 kg of COD_{VFA}. By considering a concentration of 30 ± 3 g COD_{VFA}/L, an amount of 259 L of VFA-rich stream is necessary for the production of 1.0 kg PHA. In turn, based on the feedstock VS level (51 ± 2 g/kg; before AnF2 step), 13.2 kg of VS is required, which corresponds to an overall yield of 76 g PHA/kg VS. This analysis revealed a significant improvement compared to a previous study where only the OFMSW was used as feedstock (Valentino et al., 2018), where an overall yield of 37 g PHA/kg VS was obtained. The approach herein described thus has doubled the previous yields, highlighting the real possibility for the implementation of a biorefinery technology chain to treat simultaneously two major organic streams produced in an urban scenario. The overall yield determines the economy of the whole process. This obtained yield leaves space for some possible improvements, especially considering the VFA-rich stream loss encountered in the solid/liquid separation stage. The available process units for pilot scale plants are quite limited and not perfectly optimized compared to the ones available for industrial full-scale scale plants. Hence, the adoption of an optimized solid/liquid separation stage should foster the recovery of a major part of the COD, which will consequently lead to a higher overall yield. Diverting the major part of the COD to the aerobic PHA line instead of converting all COD to methane is indeed an interesting point that should be evaluated. The proposed approach was compared to an ACoD scenario reported in a recent study (Moretto et al., 2019b) in which OFMSW and WAS from the same urban context were co-digested in the Treviso WWTP full-scale digester (2000 m^3). Even considering the best case of a maximum SGP (0.69 m$^3_{biogas}$/kg VS), the calculated revenue obtained from EE production is equal to 426,133 €/y. In the biorefinery approach, a production of 81 tons PHA/y can be estimated, based on the VS partition reported in Fig. 4 (2959 kg VS/d sent to the PHA line) and the overall yield calculated above (76 g PHA/kg VS). Assuming an average market price of 4 €/kg PHA (Rodriguez-Perez et al., 2018), the total estimated revenue is equal to 552,031 €/y, given by PHA production (324,543 €/y) and net EE (125,898 €/y), considering the best anaerobic process configuration (AnF2-ACoD1). Net EE considers both biogas production and oxygen consumption from the PHA aerobic line (Table S1). Revenues from the present scenario are 23% higher than revenues from simply co-digesting organic waste, giving an interesting perspective for possible investments. It has to be taken into account that digested sludge disposal cost and possible incentives from biogas production were not considered. Additional
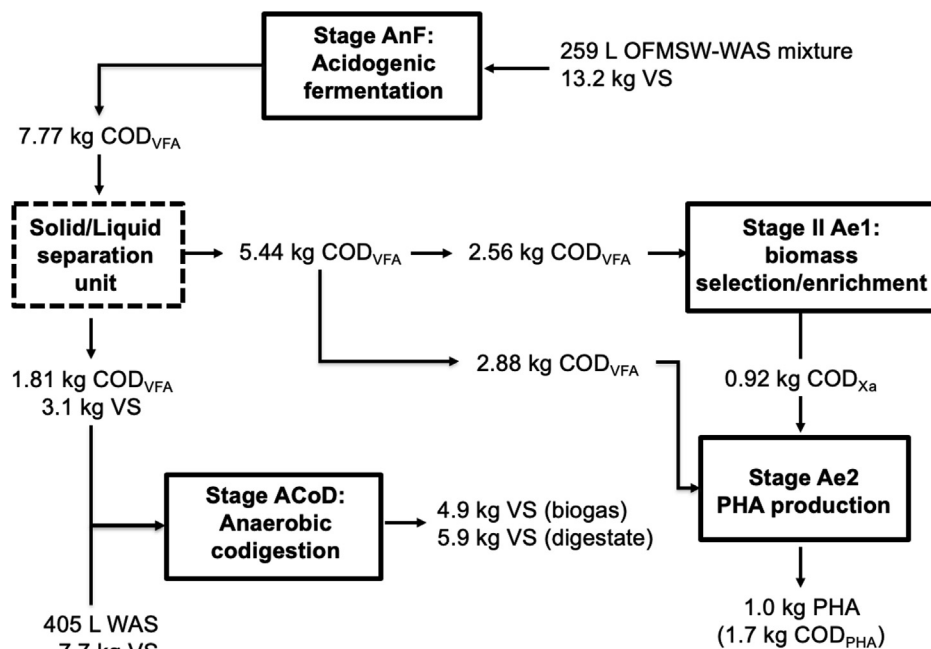

Fig. 7. Mass balance of the whole biorefinery technology chain.

preliminary economic evaluations for each of the compared scenarios can be found in Table S2 (Supplementary material). An in-depth economic evaluation would require more detailed analyses also in terms of capital expenditure (CAPEX) and operating expense (OPEX), which is beyond the scope of the present work.

3.3. Definition of the end of waste status

Within the biorefinery scenario, the combination of heterogeneous raw material flows requires a careful consideration of the regulatory gaps and a clear definition of the "End-of-Waste" (EoW) status. Its definition is of fundamental importance in the field of bio-based compounds production (Battista et al., 2019). With specific reference to the conversion of urban organic waste into intermediates like VFAs and derived biopolymers, it was found that both REACH and ECHA registration do not apply to these substances (A Circular Economy for Plastics, EU, 2019). The Directive 2018/851 (European Commission, 2018), the new Waste Directive, suggests the need to define the EoW status case by case, considering a single type of waste instead of considering waste in general. A specific decree will be defined by the competent Ministry or Agency for a specific bio-based product.

In particular, the specific Decree for the definition of the EoW for a given substance at National level should be based on the following points:

1) Definition of the waste type (with an indication of the European Catalogue Code) and its characteristics including the acceptability definitions/standards;
2) Technical parameters in terms of characteristics and definition of limits of the resulting "new" material/product;
3) Definition of the specific use and market for the new waste-derived material.

The completed dossier and relative notification will appear in the specific databases of the DG Growth at European level. In this case, waste types and their characteristics are well known and defined, although clearly variable (Battista et al., 2019), while the characteristics of the final products need to be defined. Very interestingly, previous studies have demonstrated that specific pollutants such as heavy metals and organic micropollutants do not migrate into the final products (Valentino et al., 2015b). This aspect is clearly related to the specific features of the process proposed. In the present study, the following steps are considered:

i) An acidogenic step for the conversion of organic matter into VFAs;
ii) A solid/liquid separation step for the fermented stream "cleaning and refining";
iii) A two-stage biological process for mixed culture selection and subsequent PHA production and intracellular storage starting from fed VFAs;
iv) An extraction and purification chemical process for PHA recovery.

All these steps, globally, determine a barrier to the translocation of pollutants into the final products. In the present study, the solid/liquid separation step by means of a double stage (centrifuge and ultrafiltration membrane) could enhance pollutants removal since most of the coarse solids are retained. Once this dossier is created, it will be used by public bodies (Ministries, Regional or Province Governments) to declare the EoW status for a given bio-based product.

3.4. Social acceptance survey of bio-based products

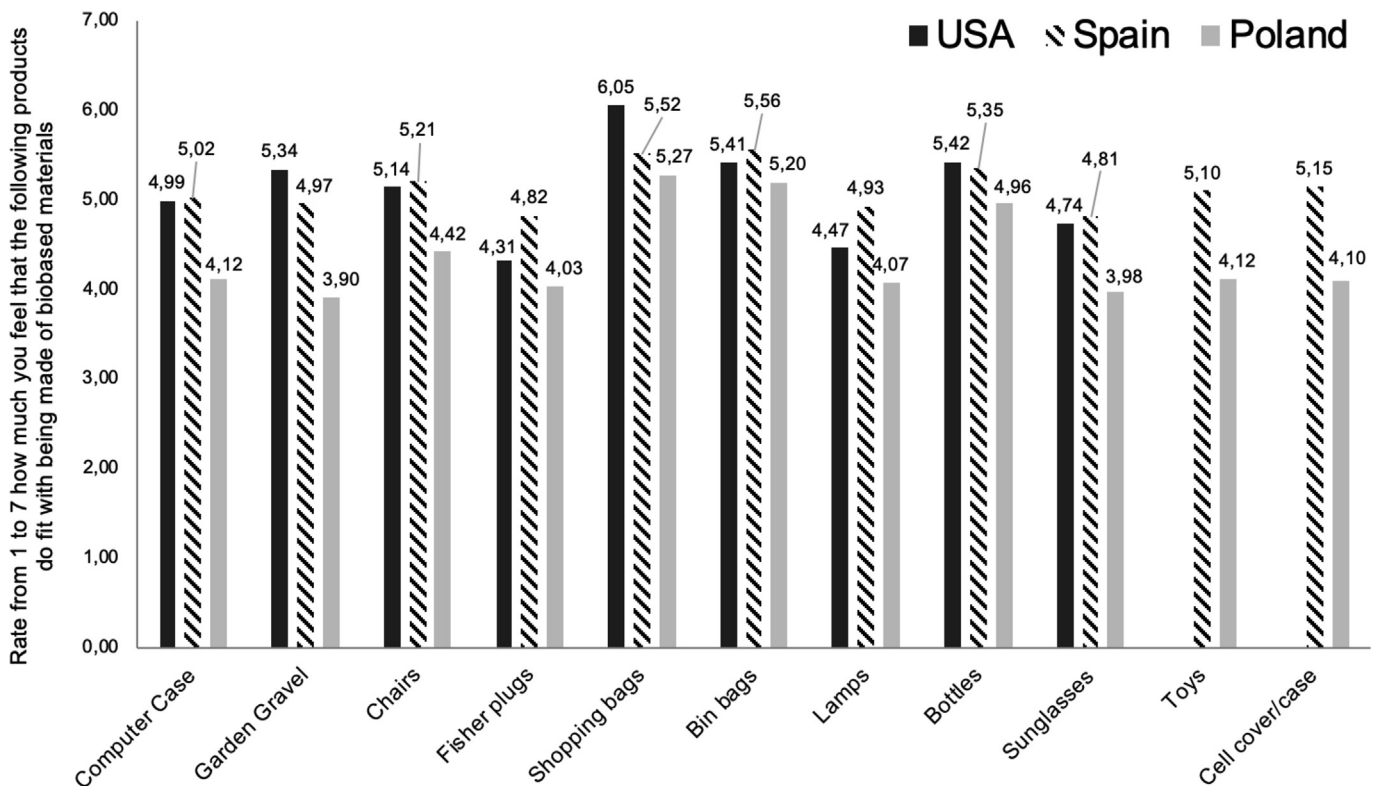One of the first outcomes from the social acceptance survey was

Fig. 8. Outcomes from the survey regarding the possible most suitable application for bio-based products derived from waste.

that product involvement does not affect the purchase intention for bio-based products. The green self-identity (GSI) partially mediates the relationship between the attitude toward bio-based products and the intention to purchase and to switch to bio-based products. However, GSI fully mediates the relationship between attitude toward bio-based products and willingness-to-pay. Consumers' demographics highlight that the perception of bio-based product is mostly affected by age and past purchases rather than gender. A Manova interaction effect show that senior age leads to a higher willingness to pay, especially when the GSI is high. A younger age instead leads to lower willingness to pay, especially when the GSI is low. Consumers who already purchased eco-products had higher intentions to purchase and switch to bio-based products. Both perceived value and GSI lead to higher behavioral intentions and previous purchase of eco-products positively impacts all behavioral outcomes. These outcomes show a general consumer acceptance in specific market segments (category of consumers) for those specific products able to replace traditional plastic. Another survey including also other products has been made, with different levels of involvement and physical contact. Different consumers from USA, Spain and Poland gave "their feeling and perception with other products", such as eyeglasses, toys, shopping bags, bin bags, bottles, lamps, garden gravel, computer case and fisher plugs. Opinions ranged in a scale from 1 (does not fit with being made from organic waste) to 7 (fits perfectly with being made from organic waste). The outcomes obtained from the survey are reported in Fig. 8.

Bin bags gave good results in all three countries, representing an interesting option of the "closing the loop" approach since food waste is used to produce bin bags used to contain food waste.

4. Conclusions

The biorefinery developed in this study allows the production of high added-value compounds (e.g. PHA) along with biogas. The platform gives interesting and useful data, especially related to the overall PHA yield (7.6% wt PHA/VS$_{(0)}$) and preliminary economic evaluations. Overall, this approach demonstrated the potential to shift into an integrated approach where an urban biorefinery is producing bio-based product of good economic and environmental value. However, each urban scenario needs to be specifically evaluated in terms of production and composition of organic waste, since the adaptability of an eventual existing facility for the implementation of a similar technology chain is another key point that should be thoroughly assessed. Regulatory gaps and consumers' acceptance evaluations will offer an important contribution about the shift on a production system based on organic waste recycling as a source of new added-value bio-based products (Russo et al., 2019). A deeper study on legislative barriers, consumers' different attitude and marketing strategies is of the utmost importance in order to foster the future creation of an economically sustainable market (Confente et al., 2019).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: *Persons not to be used as reviewer*:1) Maria Reis, New University of Lisbon (Portugal), amr@fct.unl.pt2) Alan Werker, Wetsus.nl (The Netherlands), alan.werker@wetsus.nlA possible conflict of interest may occur with both persons, since they are involved in the same project, which funded the research for this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.115371.

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