

Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge

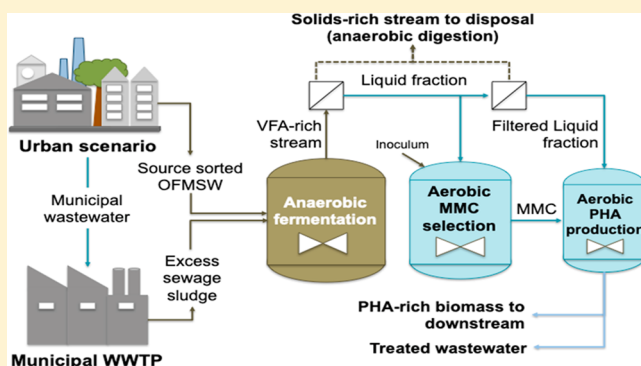
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ABSTRACT: Although the organic fraction of municipal solid waste (OFMSW) and sewage sludge (SS) originate from the same urban area and contain similar organic matter, they are collected separately and handled with different technologies. In this work, a combined treatment of OFMSW–SS mixture was investigated at pilot scale, by using a three-step mixed microbial culture (MMC) process in order to produce polyhydroxyalkanoate (PHA) as final high value biobased product. Biomass selection efficiency was quantified by PHA-specific storage rate that was 258 mg COD_{PHA}/g COD_{Xa}/h under the optimized process condition. In fed-batch tests, PHA-storing MMC was able to accumulate up to 46 wt % PHA. In the perspective of a full-scale application and taking into account the mass flows in each process step, an overall yield of 65 g PHA/kg TVS was estimated.



1. INTRODUCTION

In the frame of applied microbiology and bioprocess engineering, the use of mixed microbial cultures (MMCs) is attracting remarkable attention for the development of novel bioprocesses that respond to changing environmental frameworks. A well-established example of MMC application consists of the production of a particular family of polyester, namely, polyhydroxyalkanoates (PHA). These biopolymers are synthesized as intracellular carbon and energy source mainly by numerous species of Gram-positive and/or Gram-negative microorganisms but also by anaerobic photosynthetic bacteria and Archea.¹ Since they are completely biodegradable and have thermoplastic properties, the interest in these biopolymers is progressively increasing, also in consideration of the possibility to use renewable resources (instead of fossil fuels) as carbon source for their synthesis.^{2,3}

However, more investments are necessary to promote feasibility studies, possibly at a pilot-scale level, in order to quantify a reasonable range of PHA productivity and final polymer quality. These are crucial aspects for MMC derived PHA commercialization and relative market scenario, which presently does not exist yet.⁴

Nowadays, industrial-scale PHA production focuses on pure-culture systems, which are based on refined feedstock and sterile cultivation conditions. Both features entail a high-energy requirement that strongly increases the PHA production cost.⁵ This is why the PHA production from microorganisms'

cultivation accounts for only a small percentage of global plastic production so far.

The possibility to integrate MMC–PHA production into infrastructures typically adopted for biowaste residuals and wastewater treatment could make this technology economically and environmentally sustainable. In the past decade, this basic concept has led to the scaling-up of the process from laboratory- to pilot-scale facilities with demonstrative plants.² The examples that have been reported in the literature show the integration of MMC–PHA production with municipal wastewater treatment,^{6,7} excess sludge fermentation liquid,⁸ and dairy and food-processing wastewater.^{9,10}

Independent from the possible configurations, consistently explained in recent reviews,^{2,4} MMC technology provides for the use of activated sludge as inoculum, the microbial composition of which needs to be enriched in bacteria with high polymer accumulation capacity. This approach is known as “feast–famine” selection strategy and is realized by applying dynamic feeding to microorganisms in sequencing reactors.¹¹ Under these process conditions, the microorganisms are submitted to consecutive periods of external substrate excess and lack. Those species able to store the substrate in the form

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of PHA during the feast phase have a competitive advantage over the others, because they can use the stored polymer as an intracellular carbon source when the substrate is no more available (famine phase). Fermented waste feedstock rich in volatile fatty acid (VFA) are usually the election substrates for this purpose.

Since our food supply chain has recently been recognized as being inefficient, producing large quantities of waste,¹² the need to both avoid waste production and find new renewable resources has led to the use of food waste as a renewable feedstock. Food waste includes the organic waste produced at household level or by the food service sector, and the waste produced at retail level. This source can be valorised with available practises and technologies (including composting, landfilling, anaerobic digestion, bioenergy production). In addition, the interest for biopolymer production from urban organic waste is relatively recent. In fact, few studies described different methods for PHA production from the organic fraction of municipal solid waste (OFMSW),¹³ and related sources such as leachate,¹⁴ percolate,¹⁵ mixture of OFMSW, and primary sludge (PS).¹⁶ In the context of a possible integration in the existing wastewater treatment plant (WWTP), pilot-scale demonstrations are of primary importance.

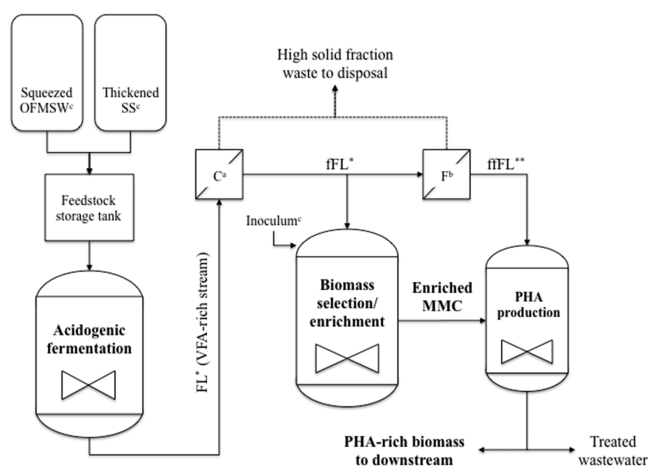
The study proposed herein describes PHA production by MMC using pretreated OFMSW and excess thickened secondary sludge (SS), applying the feast–famine approach in the traditional three-step process scheme, developed at pilot scale. The paper is focused on the treatment of these two relevant organic urban wastes since they have the same origin but are still separately collected and handled with different technologies. In this work, a single process technology has been proposed for the simultaneous treatment of both streams, collected in Treviso (northeast of Italy) municipality and presently valorized in a full-scale anaerobic digestion plant. If compared to the conventional anaerobic digestion technology, then PHA biobased products can offer a more profitable way to recover the organic matter, with the concrete option of retrofitting existing facilities of waste or wastewater treatment. The piloting facilities for PHA production from organic urban biowaste are located in the Treviso municipal WWTP.

2. MATERIALS AND METHODS

2.1. Pilot Plant Experimental Platform.

A process schematic of the units installed at the Treviso WWTP is presented in Figure 1. A fermentation reactor produced a hydrolyzed and fermented OFMSW–SS mixture rich in VFA. The fermented feedstock was subjected to a first solid/liquid separation unit (C) for suspended solid removal before feeding in the first aerobic reactor (sequencing batch reactor, SBR). The SBR stimulated the storage response of initial activated sludge (inoculum), selecting a PHA-accumulating consortium. This selection was performed by applying a dynamic feeding condition, also known as feast–famine regime.¹¹ The second aerobic reactor (PHA production) produced PHA inside the harvested surplus biomass from SBR, when fed with VFA-rich stream after second solid/liquid separation unit (F).

The start-up of the pilot units was realized in close times: acidogenic fermenter was started up in January 2017, and the SBR commenced biomass production from the end of March 2017. The first accumulation trials were made after pseudo steady state was achieved in the selection reactor. The operation of reactors is automated and controlled via a



*fFL = Centrifuged-filtered Fermentation Liquid; **fFL = Refined-filtered Fermentation Liquid

^aCentrifuge unit for first solid/liquid separation

^bFilter press for further FL refining

^cCarbon source and biomass inoculum provided by Treviso WWTP (Italy)

Figure 1. Process scheme developed at pilot scale for OFMSW and SS valorization toward PHA production.

programmable logic controller (myRio Labview by National Instrument) except for the transfer of biomass from the selection/enrichment step to accumulation, and the treatment of VFA-rich stream in the second solid/liquid separation unit, which are manually operated/conducted.

2.1.1. Sewage Sludge (SS) and Organic Fraction of Municipal Solid Waste (OFMSW) Collection.

The feedstock was collected weekly inside the Treviso municipal WWTP facility. The source-sorted collection of OFMSW was made throughout the whole Treviso municipality and transferred to the full-scale WWTP after its squeezing and homogenization in a dedicated plant. The squeezed OFMSW was then mixed with SS produced in the WWTP. The chemical features of the feedstock changed as a function of relative volume percentage of the substrates and mixed in two different ratios as it follows: OFMSW and SS at a volumetric fraction of 40–45% and 60–55%, respectively; OFMSW and SS at a volumetric fraction of 30–35% and 70–65%, respectively. Both mixtures were characterized in terms of total and volatile solids (TS and TVS), Total Kjeldahl nitrogen (TKN), and organic phosphorus (P), ammonium and phosphate, total and soluble chemical oxygen demand (COD_{TOT}, COD_{SOL}), and VFA. The main features of both mixtures are summarized in Table 1.

2.1.2. Pilot Units.

The acidogenic fermentation unit included an anaerobic batch reactor ($V = 380$ L) and a filter-bag equipped centrifuge (unit “C”). The anaerobic reactor consisted of a continuous stirred tank reactor (CSTR), mechanically stirred and under temperature control by using a thermostatic jacket. The hydraulic retention time (HRT) was set at 6 days (equal to sludge retention time, SRT). The pH (5.0–5.5) was not controlled since the alkalinity of the feedstock ensured enough buffer capacity. A centrifugation unit was used for solids/liquid separation after fermentation: a coaxial centrifuge equipped with 5.0 μm porosity nylon filter bag for solids removal. This step was crucial for obtaining a cleaner filtered fermented stream (fFL), with a suspended solid level below 10.0 g/L, to be used for the following aerobic selection step after dilution with tap water.

Acidogenic fermentation has been conducted in continuous mode, by investigating different mixture composition and

Table 1. Physical–Chemical Features of the Two Influent Mixtures of Urban Origin

parameter	unfermented feedstock	
	OFMSW 40–45%/ SS 60–55% ^a	OFMSW 30–35%/ SS 70–65% ^a
TS (g/kg)	92 ± 5	56 ± 3
TVS (g/kg)	75 ± 4	44 ± 3
COD _{TOT} (g/L)	97 ± 8	62 ± 5
COD _{SOL} (g/L)	38 ± 2	20 ± 1
COD _{VFA} (g/L)	3.1 ± 0.9	2.5 ± 0.5
TKN (g N/kg TS)	24 ± 2	25 ± 3
phosphorus (P; g P/kg TS)	2.0 ± 0.1	2.3 ± 0.1
ammonia (N–NH ₄ ⁺ ; mg/L)	327 ± 35	342 ± 28
phosphate (P–PO ₄ ³⁻ ; mg/L)	163 ± 6	113 ± 7

^aEach percentage refers to a volumetric fraction.

temperature (T); more specifically, different fermentation liquids (FL) were produced according to the following parameters: (FL-I) OFMSW 40–45% v/v, SS 55–60% v/v mixture, T 55 °C; (FL-II) OFMSW 30–35% v/v, SS 65–70% v/v mixture, T 55 °C; (FL-III) OFMSW 30–35% v/v, SS 65–70% v/v mixture, T 42 °C.

The three conditions have been tested without interruption of the fermentation process, by changing the OFMSW content first (from I to II) and then by changing the T from thermophilic (II) to mesophilic (III) conditions. The first trial tested condition I up to day 120, condition II from day 121 to 185, and condition III from day 185 to 255. The applied OLR was different between the three experimental conditions and strongly affected by the content of OFMSW in the feedstock: 12.2 ± 0.3 (condition I) and 6.6 ± 0.1 g TVS/L day (conditions II and III).

A SBR (working volume 120 L) was utilized for the PHA-accumulating biomass production. The SBR was inoculated with activated sludge from the Treviso full-scale WWTP. The aerobic run was conducted for approximately 7 months, at 1.0 day of HRT (equal to SRT) and a cycle length of 6 h. Each operating cycle was divided into four aerobic phases: biomass withdrawal (0.5 min), first aeration (10 min), feeding (0.5 min), and second aeration (349 min). The reactor was aerated by means of linear membrane blowers (Bibus EL-S-250), which allowed stirring the system. The temperature (22–25 °C, maintained with immersion heater) and pH (8.0–9.0) were continuously measured but not controlled. The applied OLR was set at 4.0 g COD_{SOL}/L day. In order to maintain such OLR, the fFLs were 6- to 10-fold diluted with tap water. Feedstock shifts were made at day 93 (from fFL-I to fFL-II) and day 160 (from fFL-II to fFL-III). The SBR performance was monitored by measurement of biomass concentration, as volatile suspended solid (VSS), PHA, and COD_{SOL}.

The accumulation potential of the biomass was exploited through fed-batch tests ($V = 50$ – 70 L), performed with both synthetic (acetic acid with no nutrient addition) and filtered fFL (fFL; specifically fFL-II and fFL-III). The fFLs were further filtered with a stainless-steel filter press (unit “F”) equipped with 0.2 μm paper filter (CKP V28), in order to remove all the suspended solids and to obtain a clear feeding solution. The biomass (X) was loaded from the SBR at the end of the 6 h cycle and the initial fFL/ X ratio was chosen to be

≤ 2.0 COD basis, in order to prevent any possible substrate or pH inhibition phenomena.¹⁷

2.2. Sampling and Analytical Methods. Feedstock mixture was characterized after each collection (weekly basis). The analysis were performed on dry solid material at first, specifically for TS and TVS, COD, TKN, and P. COD_{SOL}, VFA, alkalinity, ammonium, and phosphate were measured on filtered (0.2 μm) samples. The same approach has been followed for fermented feedstock characterization even though with more frequent analysis (at least twice a week). In the aerobic reactors, the quantified parameters were total and volatile suspended solids (TSS and VSS), COD_{SOL}, ammonium, phosphate, VFA, and PHA. In the SBR, the mixed liquor was sampled at the end of the cycle (for COD_{SOL}, ammonium, phosphate, and PHA quantification) and at the end of the feast phase (for TSS, VSS, COD_{SOL}, and PHA quantification), according to a previous laboratory-scale study.¹⁸ In the batch reactor, the mixed liquor was sampled at the beginning of each test and every hour (for VFA and PHA quantification). The COD_{SOL} and VSS were analyzed at the beginning and at the end of each test. All the analyses, except for VFA and PHA, were carried out in accordance with the standard methods.¹⁹

VFA were quantified using a gas chromatography (GC) method (Carlo Erba instruments) with hydrogen as gas carrier. The GC was equipped with a Fused Silica Capillary Column (Supelco Nukol TM, 15 m \times 0.53 mm \times 0.5 mm film thickness) and with a flame ionization detector (200 °C). During analysis, the temperature (T) increased from 80 to 200 °C with a rate of 10 °C/min.

For PHA measurements, 5.0 mL of unfiltered mixed liquor were treated with 1.0 mL of NaClO solution (5% active Cl₂) and then stored at -4 °C for following analysis. In accordance to Braunegg et al.,²⁰ the polymer was extracted, hydrolyzed, and esterified to 3-hydroxyacyl methyl esters and quantified by GC method. The abundance of hydroxybutyric (HB) and hydroxyvaleric (HV) monomers were quantified using P(3HB-co-3HV) Sigma-Aldrich standard polymer at 5 wt % HV content.

2.3. Calculations. All the parameters characterizing reactor performances were calculated after steady states or pseudosteady state (for SBR) was achieved.

The fermentation process was considered under steady-state when a stable VFA distribution was observed, after 3.0 SRTs approximately (at day 20). Solubilization and fermentation yield (Y_{VFA}) were quantified with respect to the features of initial unfermented feedstock, in particular total COD (COD_{TOT(0)}) and TVS₍₀₎. COD_{TOT} was quantified as sum of separated COD analysis on dry matter and filtered liquid sample as explained in the above paragraph.

In the SBR, the pseudo steady state was recognized when the feast phase length remained approximately constant (within a 5% deviation from average) for at least 10 SRTs.¹⁸ In each SBR cycle, the dissolved oxygen (DO) was continuously monitored in order to identify the period in which the readily biodegradable substrates (mainly VFA) were exhausted (end of feast phase), as indicated by a sudden increase of the O₂ concentration. The nonpolymer biomass (or active biomass, X_A) was calculated as the difference between VSS and PHA concentrations: $X_A = \text{VSS} - \text{PHA}$. The biomass PHA content (g PHA/g VSS) was defined as the ratio between the PHA and VSS concentrations. For rates and yields calculation, the mass values of X_A , PHA and VFA were converted into COD units by using the relative conversion

factor from oxidation stoichiometry.²¹ The specific VFA uptake rate was calculated as ratio between the removed VFA and the length of feast phase (t), per unit of X_A : $(-qVFA^{feast}) = \Delta VFA / (t \cdot X_A)$. The specific COD_{SOL} removal rate was similarly calculated: $(-qS^{feast}) = \Delta COD_{SOL} / (t \cdot X_A)$. The PHA production rate was defined by the produced PHA divided by the feast phase length (t), per unit of X_A : $qP^{feast} = \Delta PHA / (t \cdot X_A)$. The storage yields were defined by the ratio between the produced PHA and the consumed COD_{SOL} or VFA in the feast phase: $Y_{P/S}^{feast} = \Delta PHA / \Delta COD_{SOL}$; $Y_{P/VFA}^{feast} = \Delta PHA / \Delta VFA$. The observed yield was calculated as it follows: $Y_{OBS}^{SBR} = VSS / (OLR \cdot HRT)$. In fed-batch accumulations, the specific PHA production rate (qP^{batch}) was calculated by linear regression of data versus time. Only the initial period (2.0 h from the beginning of each test) at constant rate was considered. The volumetric rate was transformed into specific rate using the X_A concentration at the beginning of the test. The growth yield was calculated as the ratio between the produced X_A and the removed VFA ($Y_{X/VFA}^{batch}$) or COD_{SOL} ($Y_{X/S}^{batch}$) at the end of each test. The storage yield was calculated as the ratio between the produced PHA and the removed VFA ($Y_{P/VFA}^{batch}$) or COD_{SOL} ($Y_{P/S}^{batch}$) at the end of the test. The final PHA content in the biomass was calculated at the end of each test as explained above.

3. RESULTS AND DISCUSSION

3.1. Fermentation of Feedstock for PHA Production.

Acidogenic fermentation was feasible to good extent under each condition tested, even though some variations of performance were observed, particularly associated with the system stability.

Figure 2A shows the evolution of VFA production, COD_{SOL} and pH from the process start-up; the three different periods at different feedstock composition and/or temperature are separated by vertical axis. Average data are reported in Table 2.

In the FL-I, the higher OLR (due to the higher OFMSW content in the feedstock) resulted in lower conversion yield of total COD into COD_{SOL} and VFA, in accordance to what reported by Hao et al.²² In spite of this, both higher COD_{SOL} and VFA concentration were observed. In particular, VFA trend was characterized by significant fluctuation of VFA concentration with maximum value up to 35 g COD_{VFA}/L . As a consequence, the pH was lower than in the following periods, on average, and often below 5.0. Moreover, even though both VFA concentration and COD_{SOL} were higher in FL-I (23 ± 6 g COD_{VFA}/L and 45 ± 8 g COD_{SOL}/L), the COD_{VFA}/COD_{SOL} ratio was consistently lower (0.53 ± 0.11), meaning that a significant fraction of solubilized COD (or TVS) was not converted into VFA.

By decreasing the OFMSW content (and correspondingly the OLR), a more stable VFA production was observed, even with lower average value 21 ± 3 (FL-II). Girotto and co-workers also observed lower VFA production by decreasing food waste load in batch acidogenic fermentation test.²³ The authors negatively highlighted this fact; however, in continuous process configuration as used in this platform, lower OLR allowed for a better control of acidogenic activity since the pH of the system was easily maintained slightly above 5.0. Thermophilic conditions led to a significant COD_{TOT} and TVS solubilization, even higher than those measured in condition I, which performances were strongly affected by system stability. Higher COD and TVS conversion into VFA were also observed (COD_{VFA}/COD_{SOL} ratio 0.64 ± 0.07).

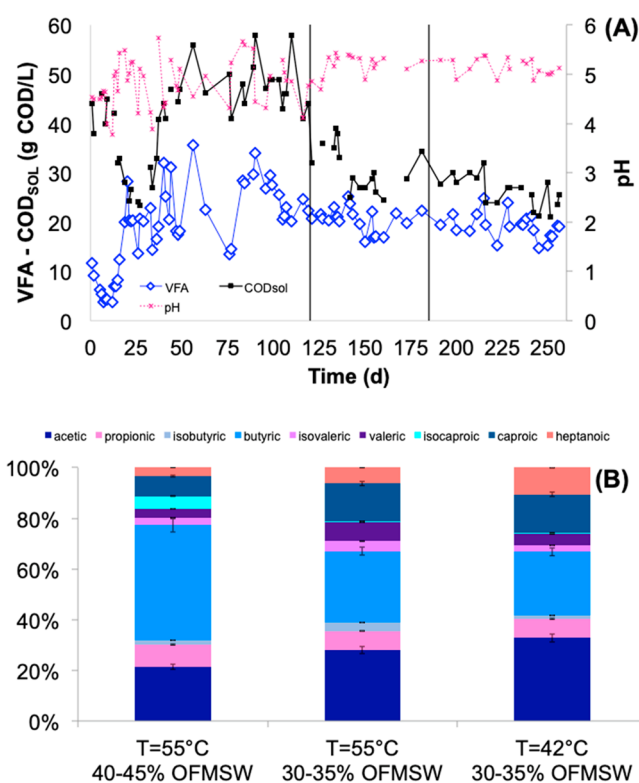


Figure 2. VFA, COD_{SOL} , and pH evolution in the fermented feedstock (A); VFA distribution under the three different conditions tested (B).

Despite the lowest average VFA concentration (19 ± 2), FL-III seemed to be more suitable, giving the highest COD_{VFA}/COD_{SOL} ratio at 0.75 ± 0.09 , a crucial parameter for an efficient selection/enrichment of a PHA-accumulating consortium.^{6,18} The temperature of 42 °C led to lower COD solubilization (0.10 g COD_{SOL}/g $COD_{TOT(0)}$ and 0.15 g COD_{SOL}/g $TVS_{(0)}$) but comparable VFA yields to thermophilic condition (FL-II). The measured yield was 0.40 g COD_{VFA}/g $TVS_{(0)}$, lower than those reported in some literature examples for OFMSW, 0.5 – 0.9 g COD_{VFA}/g TVS .²⁴ This difference could be reasonably attributed to the presence of sewage sludge in the mixture.

In terms of composition, butyric acid was predominant in FL-I (46% of total VFA, COD basis), followed by acetic (22%) and propionic (9%) acids. Other VFAs with higher molecular weight (up to C7) had lower concentrations (Figure 2B). By decreasing the OFMSW content and OLR in FL-II, a remarkable increase of acetic acid was quantified (28%), whereas the amount of butyric acid was considerably reduced to 28%. In addition, the content of caproic acid increased up to 15%, as well as for heptanoic acid (6%). A further increase of acetic (33%) and heptanoic (11%) acids was obtained in FL-III. On the contrary, butyric acid was further reduced to 25%. Despite of these differences in concentration, the VFA composition in FL-III was more similar to that of FL-II rather than FL-I. This similarity suggested that temperature did not have relevant effect on the final acids distribution (at least in the explored range).

For the three conditions tested, despite some differences in VFA distribution, the molar fraction of acids containing an odd number of carbon atoms (propionic, valeric, isovaleric and heptanoic etc.) with respect to total VFA [$C_3/(C_3 + C_2)_{VFA}$]

Table 2. Fermented Feedstock Characteristics and Related Fermentation Performances

parameter		FL-I	FL-II	FL-III
fermented feedstock characteristics				
TS (g/kg)		82 ± 6	56 ± 4	57 ± 3
TVS (g/kg)		68 ± 4	46 ± 4	46 ± 2
COD _{TOT} (g/L)		98 ± 10	61 ± 8	60 ± 4
COD _{SOL} (g/L)		45 ± 8	32 ± 5	26 ± 3
COD _{VFA} (g/L)		23 ± 6	21 ± 3	19 ± 2
COD _{VFA} /COD _{SOL}		0.53 ± 0.11	0.64 ± 0.07	0.75 ± 0.09
alkalinity (mg CaCO ₃ /L)		5910 ± 469	4811 ± 741	4451 ± 498
TKN (g N/kg TS)		2.8 ± 4	23 ± 4	20 ± 2
phosphorus (P; g P/kg TS)		2.0 ± 0.3	2.2 ± 0.4	2.1 ± 0.3
ammonia (N-NH ₄ ⁺ ; mg/L)		698 ± 55	724 ± 138	562 ± 44
phosphate (P-PO ₄ ³⁻ ; mg/L)		155 ± 28	127 ± 22	110 ± 9
COD _{SOL} /N/P		100/5.3/0.4	100/4.1/0.4	100/4.4/0.5
[C ₃ /(C ₃ +C ₂)] _{VFA} (mol/mol)		0.15 ± 0.03	0.17 ± 0.02	0.16 ± 0.02
fermentation performance				
solubilization	g COD _{SOL} /g COD _{TOT(0)}	0.08 ± 0.05	0.17 ± 0.02	0.10 ± 0.03
	g COD _{SOL} /g TVS ₍₀₎	0.10 ± 0.06	0.26 ± 0.02	0.15 ± 0.03
yield (Y _{VFA})	g COD _{VFA} /g COD _{TOT(0)}	0.23 ± 0.04	0.27 ± 0.03	0.25 ± 0.02
	g COD _{VFA} /g TVS ₍₀₎	0.24 ± 0.06	0.39 ± 0.04	0.40 ± 0.03

was similar and strongly oriented to the predominance of acids with even number of C atoms (0.17 ± 0.02 mol/mol at maximum in FL-II). It is well-known that this parameter can be used to characterize complex VFA mixtures and to predict polymer composition in terms of monomers stoichiometry.^{6,25}

In terms of continuous process operation, the process stability was strongly affected by the relative OFMSW and SS content (and consequent change of OLR); in particular, OFMSW volumetric percentage between 40 and 45% led to frequent spots of high VFA content not balanced by mixture buffering capacity. This caused an unstable VFA production for both concentration and composition with possible difficulties in the operations of the two following aerobic stages and likely affecting final polymer composition.²⁶ By decreasing OFMSW content to 30–35%, the OLR decreased, whereas the buffer capacity increased. As a result, VFA production was steadily maintained for the whole operation period and, importantly, the effluent presented a lower fraction of COD_{SOL} other than VFA. As mentioned above, a centrifuge filter has been used to obtain a filtered VFA-rich stream for selection and production of PHA-accumulating biomass.

The characterization of the fermented effluent after filtration (fFL) indicated a high volumetric recovery of filtrate (90–95% v/v of the feeding slurry) having TSS level between 5 and 8 g/L in the filtrate. Soluble COD and VFA were not affected by the filtration at all as well as for soluble nutrient level.

3.2. Biomass Selection/Enrichment under Aerobic Dynamic Feeding. After the inoculum, the adaptation of the biomass to the imposed dynamic feeding was easily evaluated by monitoring the dissolved oxygen (DO) concentration as illustrated in the representative example of Figure 3A. The DO concentration decreased to around 2.0 mg/L at the beginning of the cycle (fFL feeding), as a consequence of the increase of the metabolic activity.²⁷ After VFA (or most of COD_{SOL}) depletion, DO concentration started to increase and it stabilized approximately at 6.0 mg/L and slowly returned back to the initial value during the slowly biodegradable COD consumption. Indeed, the sharp increase of DO concentration was due to the consumption of the readily biodegradable COD, mainly consisting of VFA. This point has been

recognized as “feast phase length”. PHA concentration profile confirmed the occurrence of feast–famine regime: The maximum PHA level was quantified at the end the feast phase (with almost complete VFA exhaustion) and then degraded over the course of remaining part of the cycle (Figure 3A).

The ratio “feast phase/cycle length” was regularly measured and used to monitor biomass adaptation and process stability. From Figure 3B, acclimating biomass needed less than 1 week of operation ($\sim 4/5$ SRT) to achieve a stable response to the imposed dynamic feeding condition. Despite of the different COD_{VFA}/COD_{SOL} ratio of fFL-I, -II, and -III, the biomass behavior showed a certain stability as indicated by the low feast/cycle time ratio during the whole SBR run, with a maximum of 0.22 h/h, (with the exception of the first day after inoculum) and the short fluctuations casually recorded were no more than 1 day long or even less (Figure 3B). This indicated that the process was technically feasible for long-term operation, despite the presence of changing inputs. Average values of feast phase were 0.14 ± 0.05 , 0.11 ± 0.02 , and 0.10 ± 0.02 h/h in runs FL-I, FL-II, and FL-III, respectively, abundantly lower than largely recognized threshold of 0.2 h/h, necessary to achieve an efficient biomass selective pressure.^{4,11} A slight decrease of F/F ratio was observed when moving from FL-I to FL-III, which can be likely linked to the corresponding increase of VFA/COD_{SOL} ratio in the feed.

The storage response was consequently expected to be strong and robust, and it was verified especially in run FL-II and FL-III where the difference between PHA concentration at the end of feast and the end of famine were higher and characterized by less variability with respect to run FL-I (Figure 3C).

Consequently, based on increasing effectiveness of dynamic feeding regime, biomass storage properties progressively increased as also indicated by the evolution of specific storage rate and yield (Figure 3D).²⁸ It is reasonable to suppose that the higher stability of biomass storage response was primarily favored by the equally more stable fermentation performance (in particular yield and VFA concentration), which made easier the operation of SBR. In addition, it was observed from

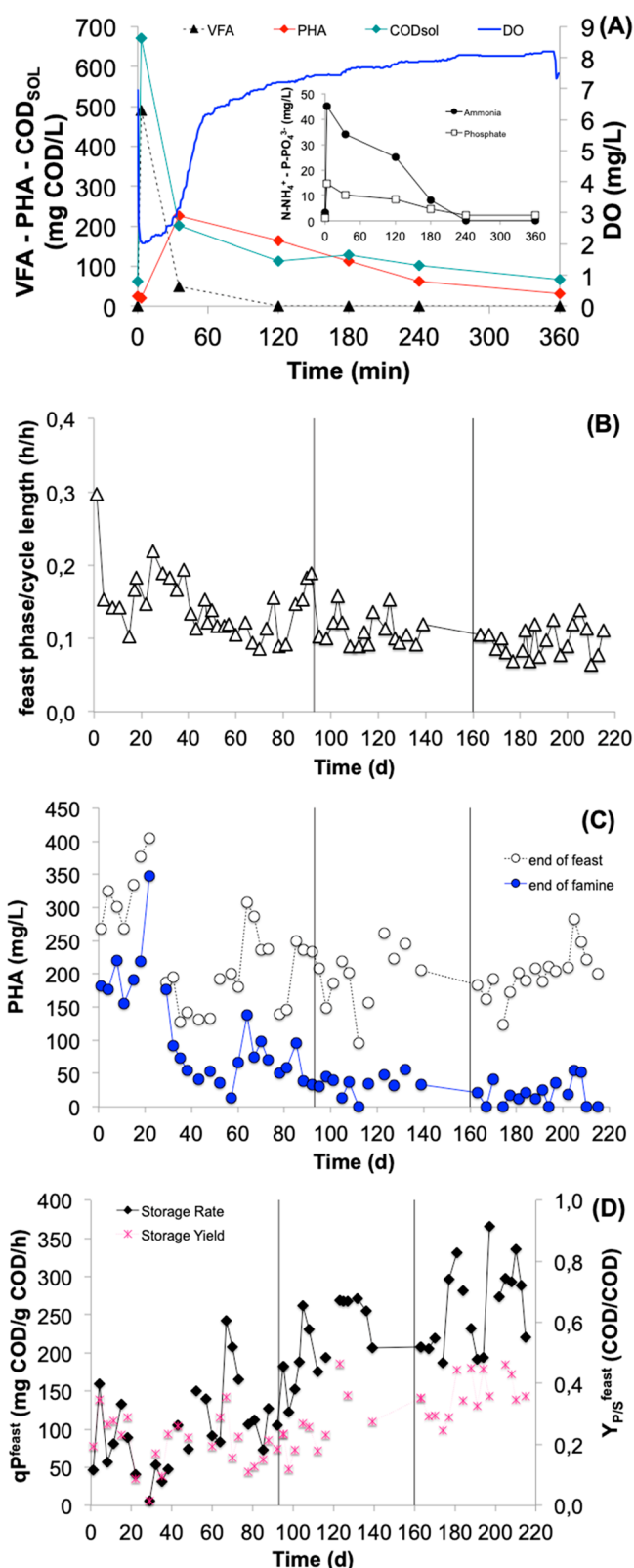


Figure 3. Main parameters monitored in SBR: VFA, COD_{SOL}, PHA, ammonium, and phosphate in a typical SBR FL-III cycle (A). Feast phase/cycle length ratio (B), maximum and minimum PHA concentration (C), and storage rate and yield (D) in the three different SBR runs.

recorded cycles profiles that non-VFA COD_{SOL} contributed only in minor part to PHA storage, as also reported in

literature.^{16,29} This observation was confirmed by the quite similar average storage yields quantified in run FL-III: $Y_{P/S}^{feast}$ and $Y_{P/VFA}^{feast}$ were equal to 0.36 ± 0.04 and 0.40 ± 0.04 , respectively. This non-VFA COD_{SOL} can favor the growth of nonstoring bacteria during the famine phase, causing a less efficient culture selection.³⁰ Therefore, the progressive increase of VFA/COD_{SOL} ratio along the SBR operation had a significant impact on selective pressure and, as a consequence, on biomass storage properties.

Quantitatively, both specific storage rate and storage yield were strongly correlated with the increasing VFA/COD_{SOL} ratio of used fFL, as easily deducible from Table 3, where the main parameters measured in the three periods of SBR run are reported.

Ammonium, phosphate, and COD_{SOL} concentrations were systematically measured in SBR effluent (Table 3). Despite different characteristics of the three fFL, the selection stage provided stable performance for carbon and nutrients removal. The percentage of removal from the influent was in a narrow range between the three runs: 85–97%, 69–82%, and 86–92% for ammonium, phosphate, and COD_{SOL} respectively.

3.3. PHA Accumulation. The PHA accumulation capacity of selected PHA-storing biomass was evaluated in fed-batch accumulation tests by means of multispike feeding strategy.²⁷ To do so, fFLs effluents were further refined by means of filtration on the filter press above-described (for fFL-II and fFL-III only) in order to obtain a refined filtered fermented liquids (ffFLs) characterized by no content of suspended solids.

Multiple pulsed additions of ffFL-II and ffFL-III was used to overcome any potential substrate inhibition phenomena.^{14,27,31} As an example, Figure 4 shows the profile of PHA biomass content, VFA and PHA concentration during a typical accumulation test performed with run FL-III biomass, fed with either acetate as benchmark (Figure 4A) or ffFL-III (Figure 4B). In both cases, PHA production occurred linearly for at least 3 h, although at different rate. In the case of acetate, the PHA biomass content increased linearly up to 0.40 g PHA/g VSS; then, PHA production was no longer sustained and acetate uptake rate was remarkably reduced after the last pulse addition (4 h). On the other hand, a faster VFA depletion was observed with ffFL-III, at least within the four initial hours. This relatively high VFA uptake rate was associated with a considerable polymer production, since PHA concentration increased almost linearly in the first 4 h of accumulation and its biomass content reached 0.45 g PHA/g VSS. Afterward, VFA started to be consistently accumulated in the reactor and PHA was produced with a lower rate, achieving a final concentration above 2.0 g/L and PHA biomass content of 0.48 g PHA/g VSS. Quantitatively, PHA concentration was almost doubled, and PHA biomass content was roughly 20% higher than those obtained with acetate accumulation. This was likely due to the presence of nutrients in the carbon source, which probably did not limit the microbial activity in terms of PHA storage and new PHA-storing biomass growth. On the contrary, the acetate synthetic solution did not allow the growth of new biomass, and as a consequence, the whole PHA production potential of the consortium was limited.

These results highlighted the importance of the nutrients in the expression of storage potential of biomass appropriately selected. Lack of nutrients is not always better than excess and/or partial limitation of nutrients,^{32,33} especially if biomass response needs to be exploited to a large extent.⁶ In the

Table 3. Main Parameters (Average Values and Standard Deviations) Monitored in the SBR Runs

parameter	unit	average value		
		run FL-I	run FL-II	run FL-III
TSS	mg TSS/L	2717 ± 132	1912 ± 64	1930 ± 101
VSS	mg VSS/L	2136 ± 95	1510 ± 61	1718 ± 78
VSS/TSS		0.79 ± 0.02	0.80 ± 0.01	0.88 ± 0.01
feast phase/cycle length ratio	h/h	0.14 ± 0.05	0.11 ± 0.02	0.10 ± 0.02
PHA (end of feast)	mg PHA/L	228 ± 58	196 ± 30	200 ± 16
PHA (end of famine)	mg PHA/L	104 ± 24	34 ± 12	20 ± 9
HV fraction	g HV/g PHA (%)	13.7 ± 0.4	16.1 ± 0.8	14 ± 1
specific PHA storage rate (q_{P}^{feast})	mgCOD _{PHA} /gCOD _{Xa} /h	101 ± 52	212 ± 35	258 ± 44
specific VFA removal rate ($-q_{\text{VFA}}^{\text{feast}}$)	mgCOD _{VFA} /gCOD _{Xa} /h	332 ± 62	619 ± 54	634 ± 53
specific COD removal rate ($-q_{S}^{\text{feast}}$)	mgCOD _{SOL} /gCOD _{Xa} /h	509 ± 84	840 ± 77	725 ± 62
storage yield ($Y_{P/S}^{\text{feast}}$)	COD _{PHA} /COD _{SOL}	0.22 ± 0.05	0.26 ± 0.06	0.36 ± 0.04
storage yield ($Y_{P/VFA}^{\text{feast}}$)	COD _{PHA} /COD _{VFA}	0.30 ± 0.06	0.34 ± 0.05	0.40 ± 0.04
observed yield ($Y_{\text{OBS}}^{\text{SBR}}$)	COD _{VSS} /COD _{SOL}	0.49 ± 0.05	0.42 ± 0.06	0.54 ± 0.08
effluent ammonia (N-NH_4^+)	mg N-NH ₄ ⁺ /L	2.6 ± 1.2	0.8 ± 0.5	3.5 ± 0.9
effluent phosphate (PO_4^{3-})	mg P-PO ₄ ³⁻ /L	1.8 ± 0.5	1.9 ± 0.4	2.1 ± 0.3
effluent COD _{SOL}	mg COD _{SOL} /L	204 ± 37	102 ± 24	116 ± 8

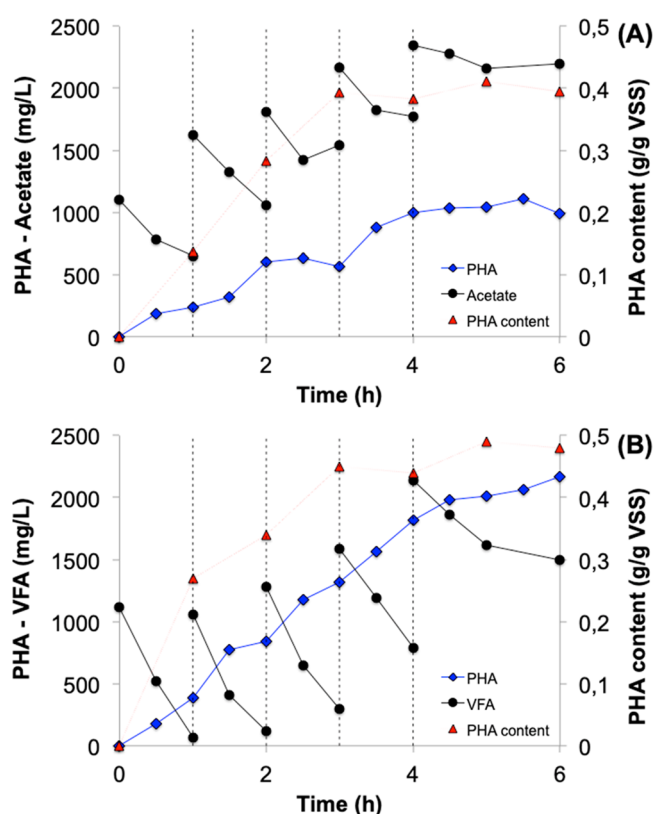


Figure 4. Fed-batch accumulations conducted with biomass enriched in SBR FL-III run: synthetic acetate (A) and fFL-III (B) as feeding solutions.

examined examples, PHA storage had been favored by the presence of nutrients (partially limiting respect to COD_{SOL}) and consequently by the simultaneous growth of PHA-storing organisms. However, better performance with fFLs than that with acetate can also be due to the SBR biomass having been selected with a very similar fFL mixture instead of with pure acetate.

Table 4 summarizes the main outcomes of conducted accumulations. A first comparison with previous lab-scale investigation adopting an identical process configuration

highlighted the promising results obtained in this study.¹⁸ Similar PHA content and yield were achieved with even higher polymer productivity (up to 0.36 g PHA/L h), favored by the presence of nutrients and, as a consequence, by the growth of new active PHA-storing biomass during accumulation. Other investigations utilizing OFMSW sources have been mostly conducted at laboratory scale, at least for the aerobic process steps.^{14–16,34} Amulya and co-workers proposed an integrated multistage approach for food waste valorization.³⁴ In that study, PHA production was strongly limited by low VFA level in the fermented feedstock (approximately 6.0 g COD_{VFA}/L), which made it difficult to operate at relatively high OLR. Hence, enriched biomass produced PHA with a specific rate of 11–42 mg COD_{PHA}/g COD_{Xa}/h, 1 order of magnitude lower than this study, 279–301 mg COD_{PHA}/g COD_{Xa}/h. Maximal PHA content achieved was 0.24 g PHA/g VSS, negatively affected by the abundant amount of COD_{SOL} other than VFA. Basset et al. used OFMSW FL alternatively mixed with PS.¹⁶ The approach proposed by the authors was different from this pilot-scale demonstration, since it consisted in an integration of MMC selection with nitrogen removal (through aerobic–anoxic feast–famine regime) in a municipal WW treatment line. As a consequence, the storage performances quantified in fed-batch tests were still far, even though not properly comparable, from those exhibited by the fully aerobically selected biomass.

Better performance was obtained with leachate and percolate.^{14,15} In the first case, the biomass was able to accumulate high level of polymer (0.78 g PHA/g VSS), but it has to be considered that its enrichment in SBR had been performed with a synthetic mixture mainly composed of acetate (75–90% v/v). The storage performances obtained with percolate were comparable with those obtained with fFL-III with the exception for the composition of produced PHA. Percolate was characterized by higher level of HV precursor (mainly propionic acid, 29–46% w/w); HB and HV monomer fractions were consequently closer (54–57% and 46–43% g/g for HB and HV respectively) if compared to those fractions derived from fFL-III (90% HB and 10% HV weight basis).

More recently, Valentino et al.¹³ described a multistep pilot-scale platform for the conversion of pretreated OFMSW (not mixed with sludge) into PHA and biogas. Regarding the

Table 4. Summary of Main Results Obtained in Fed-Batch Accumulations and Comparison with Previous Studies Focused on Food Waste Valorization

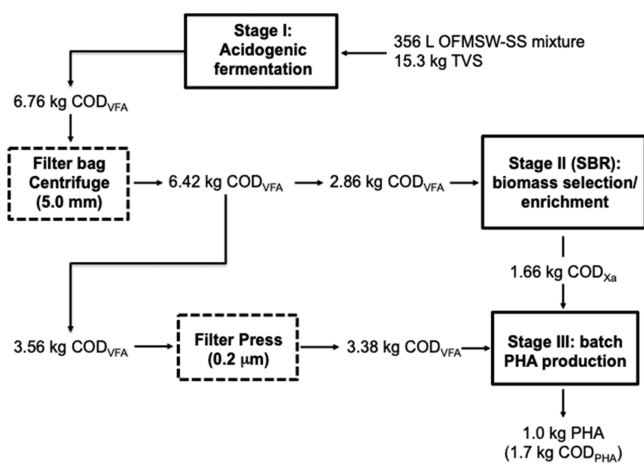
VFA-rich stream	PHA content (gPHA/gVSS)	PHA composition (wt % HB/HV)	Yields				q_P^{batch} (mgCOD _{PHA} /gCOD _{X_A} /h)	PHA productivity (gPHA/L h)	refs
			$Y_{P/S}^{\text{batch}^a}$	$Y_{P/VFA}^{\text{batch}^a}$	$Y_{X/S}^{\text{batch}^a}$	$Y_{X/VFA}^{\text{batch}^a}$			
acetate (synth.) ^b	0.40 ± 0.02	100/0		0.67 ± 0.05			202 ± 33	0.20 ± 0.04	this study
OFMSW-SS mixture (fFL-II) ^f	0.43 ± 0.01	87/13	0.37 ± 0.06	0.44 ± 0.03	0.22 ± 0.05	0.24 ± 0.07	301 ± 81	0.29 ± 0.04	this study
OFMSW-SS mixture (fFL-III) ^f	0.46 ± 0.05	90/10	0.41 ± 0.05	0.50 ± 0.04	0.21 ± 0.01	0.26 ± 0.08	279 ± 41	0.36 ± 0.05	this study
pretreated OFMSW	0.39–0.52	7–13/93–87	0.39–0.47	0.43–0.57	0.18–0.24	0.19–0.28	255–436	0.28–0.49	13
leachate	0.78 ^{c,d}			0.33 ^c –0.40 ^d					14
percolate ^f	0.41–0.48	54–57/46–43	0.44–0.52	0.66–1.13				0.18–0.29	15
OFMSW ^f	0.11		0.08	0.22	0.77	0.36	20		16
OFMSW-PS ^c mixture ^f	0.09		0.003	0.011	0.79	0.47	11		16
VFA mixture (synth.) ^b	0.48 ± 0.03	88/12		0.53 ± 0.03			196 ± 13	0.17 ± 0.02	18
food waste (FW) ^f	0.24	60/40	0.09–0.17				11–42		34

^aCOD basin. ^bNo nutrients addition (COD/N/P = 100:0:0). ^cBiomass enriched with synthetic VFA mixture (90% v/v) and leachate (10% v/v).

^dBiomass enriched with synthetic VFA mixture (75% v/v) and leachate (25% v/v). ^ePrimary sludge. ^fFermented feedstock.

aerobic stage performances, the obtained results were comparable in terms of PHA production rate and yield, volumetric productivity, and polymer composition. However, compared to the previous approach,¹³ the process scheme herein proposed offers the possibility for a simultaneous treatment of two different streams collected in the same urban area. In this way, a single technology can be used for efficient and integrated urban waste management.

3.4. Overall PHA Yield and Possible Future Perspective. Data analysis of each pilot unit, including the two solid/liquid separation units (“C” and “F”), have been related to the quantification of the overall PHA yield. This number indicates the amount of TVS necessary for the production of 1.0 kg of PHA, which corresponds to 1.70 kg of COD_{PHA}, based on quantified HB–HV wt % monomers composition. The relative mass flow diagram of PHA production is depicted in Figure 5. The backward calculation started from the final PHA biomass content of 0.46 g PHA/g VSS, obtained with fFL-III as feed

**Figure 5.** Mass flow diagram of PHA production from OFMSW-SS mixture.

solution (Table 3). This PHA content means a production of 1.17 kg (or 1.66 kg of COD) of active biomass X_A . For both produced X_A and PHA, it has been estimated a total consumption of 6.24 kg of COD_{VFA}, of which 3.38 kg of COD_{VFA} for polymer production in batch accumulation reactor and 2.86 for X_A production in SBR enrichment reactor. These separated contributions have been quantified based on the process yields the storage yield ($Y_{P/VFA}^{\text{batch}}$) of 0.50 COD_{PHA}/COD_{VFA} (Table 3), and the X_A observed yield ($Y_{X_A}^{\text{SBR}}$) of 0.58 COD_{X_A}/COD_{VFA}, for PHA and X_A production, respectively. Since the use of filter press reduced the volumetric load of fFL of 5% v/v with respect to influent FL, the equivalent amount of VFA has to be increased up to 3.56 kg of COD_{VFA}. This led to 6.42 kg of COD_{VFA} in the centrifuged FL, as quantified VFA consumption in both aerobic stages for each kilogram of produced PHA. In addition, taking into account the slight retention of FL in the filter bag centrifuge (roughly 5%), it has been overall estimated a total consumption of 6.76 kg of COD_{VFA}, which corresponds to 356 L of FL-III at a VFA level of 19.0 g COD/L. By considering the TVS concentration of 43 g/kg in the initial feedstock (before acidogenic fermentation), the amount of TVS needed for the production of 1.0 kg PHA is 15.3 kg of TVS, which corresponds to an overall yield of 65 g PHA/kg TVS. Such deep analysis is rarely reported in the literature, and the recent practical advancements of the MMC technology on the pilot-scale platforms showed yields in the same order of magnitude. Colombo and co-workers reported a global yield in the range 62.9–114 g PHA/kg TS by using the percolate fraction of fermented OFMSW; however, only the first fermentation step was performed at pilot scale.¹⁵ In a more recent study, a complete pilot system was developed for the valorization of the OFMSW as the only carbon source.¹³ From the mass balance assessment, the authors quantified an overall yield of 37 g PHA/kg TVS. Hence, the approach described in this study increased the yield of 40% approximately.}

In the perspective of a future market scenario for PHA synthesized by MMC, the maximization of the yield could have

a remarkable impact on the process sustainability. Moreover, in a multisteps biorefinery approach, the simultaneous bio-products and biogas production from the same organic feedstock could be more beneficial compared to a single product recovery. With reference to the same urban scenario described in this study, Moretto and co-workers quantified a maximum economic income of 245 000 €/year from the electrical energy produced in the Treviso full-scale anaerobic digestion (AD) plant.³⁵ The 2000 m³ AD reactor was designed for the treatment of OFMSW-SS mixture (roughly 1150 ton TVS/year), produced in an urban scenario of 70 000 Person Equivalent (PE).³⁵

With the introduction of a designed PHA line, it can be estimated a request of the 60% of the organic solids (690 ton TVS/year) for the fermentation step and the production of a liquid VFA-rich stream amenable for PHA synthesis.³⁶ Based on the calculated yield (65 g PHA/kg TVS), a production of 45 ton PHA/year can be counted. Following the approach described in Valentino et al., the residual TVS amount (overflows) is represented by the sum of the 40% unfermented solids (460 ton TVS/year) and the solid-rich prefermented fraction ("cake"), which is discharged from the solid/liquid separation unit (80% of the fermentation TVS influent; 552 ton TVS/year).³⁶ From the specific gas production (SGP) quantified in the mesophilic AD treatment of the overflows (0.40 m³ biogas/kg TVS),³⁶ the estimated biogas production is 1124 m³/day. The technical feasibility of this approach has been recently demonstrated,³⁶ a revenue of 134 591 €/year is achievable from the sale of generated electricity (13.03 MWh/day).³⁶ In theory, taking into account an average PHA value of 4000 €/ton,⁵ an additional revenue of 180 000 €/year has to be considered, potentially making the biorefinery platform a more profitable solution if compared to the traditional AD process.

4. CONCLUSIONS

The present pilot-scale plant is an example of novel "biorefinery" for urban waste valorisation, providing an option to implement a synergic treatment of relevant organic waste streams produced and collected in the same urban area. The overall process yield of PHA production from selected organic waste has been estimated to 6.5 wt % with respect to the TVS on untreated waste stream, composed by the source sorted OFMSW and sewage sludge. In addition, a possible integration of the proposed approach could be the valorization of the solid-rich overflow, mainly produced by the filter bag centrifuge and basically composed by fermented OFMSW-SS through anaerobic digestion (AD).³⁵ This solution allows using one main technology for the conversion of urban biowastes into PHA, while also minimizing any residual or consequent waste to be disposed.

This work demonstrated how the innovation in urban waste management could contribute to better waste collection and processing for the production of higher value biobased products.

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Notes

The authors declare no competing financial interest.

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