Università
Ca'Foscari
Venezia

Doctoral programme
in Environmental Sciences
XXXIII Cycle

Research Thesis

Urban organic waste valorization through an integrated pilot scale biorefinery for polyhydroxyalkanoates and biogas production

ING-IND/25

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Ringraziamenti

Il Dottorato è stato un percorso lungo e complesso, dal quale ho tratto un insegnamento ed un arricchimento personale che vanno ben oltre il titolo di studio e le conoscenze acquisite. Lavorare in ambiente reale, gestire in autonomia la ricerca, organizzare il lavoro e poter partecipare ad un progetto Europeo sono state alcune tra le sfide che mi hanno permesso di raggiungere una crescita professionale e personale che hanno reso il mio percorso accademico anche un percorso di vita.

Ci tengo a ringraziare innanzitutto il mio supervisor, il Professor Paolo Pavan, per aver creduto in me ed avermi dato l'opportunità di entrare a contatto con il mondo della ricerca e per avermi trasmesso preziosi insegnamenti e consigli durante tutto il mio percorso.

Un ringraziamento molto speciale va al Dottor Francesco Valentino, il quale è sempre stato al mio fianco in questo percorso, dal lavoro sul campo, al lavoro al computer, ai diversi viaggi per i meeting di progetto fino alla revisione e correzione della presente tesi. La sua figura è stata per me una vera guida, un conforto nei momenti di difficoltà ed un incoraggiamento nei momenti di stress dovuti alle innumerevoli scadenze. Il suo affiancamento mi ha aiutato a crescere professionalmente, ma soprattutto personalmente, insegnandomi a gestire anche le collaborazioni con altri gruppi di ricerca. Gli articoli pubblicati sono solo una delle testimonianze di quanto la nostra collaborazione sia stata basata su un sincero rispetto ed una grande fiducia, grazie ai quali sono riuscita a tirare fuori il meglio da questo percorso.

Ci tengo anche a ringraziare tutto lo staff del Campus Scientifico e in particolare della segreteria di Dottorato, che mi ha sempre aiutata nei momenti di dubbio e per aver gestito innumerevoli e-mail e telefonate.

Un pensiero va anche al Dr. Alan Werker, per avermi accolta momentaneamente in un altro gruppo di ricerca ed aver continuato la nostra collaborazione da remoto nonostante il periodo particolare.

Non potrei chiudere i ringraziamenti senza pensare ai miei genitori, Bianca e Sergio, i quali sono sempre stati al mio fianco per aiutarmi nei momenti no e per festeggiare anche il raggiungimento del più piccolo traguardo. Devo anche a loro il fatto di aver continuato fino a questo punto il percorso accademico e di non aver mai smesso di credere in me stessa.

Infine, ultimo ma non per ordine di importanza, un grande ringraziamento va a Vincenzo, con il quale ho condiviso tutto di questo percorso e non solo, nozioni, gioie, dolori, successi ed insuccessi. Grazie per essere sempre al mio fianco in questo meraviglioso momento ed in quelli futuri.

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1. Chapter 1. Introduction

In recent years, the society exponential evolution led to many progresses along with the gradual depletion of non-renewable resources. The uncontrolled and unsustainable natural resources exploitation caused many side-effects, such as greenhouse gases (GHGs) emission, climate change, waste generation, air and water pollution and health risks (Zabaniotou, Kamaterou, 2019). The economic model of linear consumption around which the society has developed since the industrial revolution is no longer sustainable, due to the inevitable resources' depletion and pollution (Russo et al., 2019). The global attention towards a more sustainable economic model grew in particular during the last decades, when politics, especially in the European Union (EU), started to promote new business plans centered around the circular economy concept. The European Commission (EC) promoted a first circular economy action plan in 2015, in order to foster its implementation and to lay the foundation for future challenges and goals. Reducing the pressure on natural resources and ecosystems, limiting the leakage of resources and shifting the economic model from a "make-take-consume-throw away" pattern to an economy based on sharing, leasing, reuse, repair, refurbish and recycling are the key elements of the EC proposal (European Commission, 2015). Waste production reduction and prevention is another target of the circular economy, given that products and materials acquire a higher value and should be consequently maintained as long as possible in the economic cycle in a "closed loop" perspective, through proper recycle and reuse (EEA, 2019). In 2020, the EC published the new circular economy action plan, with a deeper focus directly on the design phase of products, that need to match improved durability, increased recycled content and restricted single use to further prevent waste from the first stages (European Commission, 2020). Indeed, waste generation is one of the major issues of the modern society, since their production exponentially incremented side to side with population growth and industrial development. It was estimated that only in Europe up to 2.5 billion tonnes of waste are generated each year (EPRS, 2016). In urban areas, where most of the population is located, produced waste are generally included in the category of municipal solid waste (MSW). MSW is commonly defined as waste generated by households and commercial activities, or other waste with similar composition to household waste. Up to 46% of MSW is constituted by the biodegradable organic fraction (organic fraction of municipal solid waste, OFMSW), with large quantitative of food waste, garden waste, kitchen waste and leftovers from residences, restaurants and markets (Maina et al., 2017). Another problematic waste falling under the organic waste category, whose production is strictly connected to the urban context, are sludges coming from the wastewater treatment. Sludge is defined as any material produced during primary, secondary or advanced wastewater treatment that has not undergone any process to reduce pathogens or vector attraction. Large quantities of macronutrients, such as carbon, nitrogen and phosphorous as well as pathogens are always present in wastewaters and are usually transferred to the sludge, even if in lower concentrations due to the wastewater treatment process (Tchobanoglous et al., 2014). Waste management, especially for organic waste from urban origin, orientated towards technologies such as composting and anaerobic digestion, that allowed to produce quality compost or biogas in order to recover resources (Demichelis et al., 2018). Nevertheless, these technologies are often applied as isolated systems, without taking into account the huge potential that the integration between them and other production activities could generate (Satchatippavarn et al., 2016). In the past, MSW and stabilized sludge from wastewater treatment were managed through landfill disposal, that represented the cheapest and easiest option (European Commission, 2019). However, the immediate economic advantage was rapidly overcome by detrimental environmental problems, such as GHGs production, soil and groundwater contamination due to leachate formation, odor production and pathogens spreading (Matsakas et al., 2017). The fundament for waste prevention and for basic waste management principles were settled in 2008 with the EU Waste Framework Directive 2008/98/EC, that stated the necessity to avoid endangering human health and harming the environment, promoting also domestic separation of waste fractions (European Commission, 2008). Perfectly in line with the circular economy principle and to answer the need of waste reduction and resource recovery, the biorefinery concept started to develop and to gain more and more attention. This technology chain is similar to the conventional petrochemical refinery and consists in a series of chemical refining processes starting from renewable resources of carbon. Innovative technologies are integrating part of the biorefinery, targeting the production of valuable virgin materials before bioenergy (Battista et al., 2019). The high availability of organic waste in urban scenarios constitutes a perfect linking element that opens the field to the integrated treatment of different kind of waste, overcoming the disadvantages of the isolated treatment. By implementing the biorefinery technology chain, industrial competitiveness and wealth are improved, since biobased product are provided through economically, socially and environmentally sustainable processes (Demichelis et al., 2018). Biorefineries proved to be feasible technology chains for biofuels and chemicals production from oil palm biomass

(Ahmad et al., 2019), food waste (Carmona-Cabello et al., 2018) and biomass from agriculture, forestry and aquacultures (Cherubini, 2010). Recent researches investigated multi-stage processes as biorefineries for polyhydroxyalkanoates (PHAs) production, in order to target carbon recovery maximization from organic waste. PHAs are a class of natural and biodegradable polyesters synthesized as energy and redox storage by different microorganisms in presence of an excess carbon source, typically coupled with other nutrients limiting conditions (Sagong et al., 2018). These polymers are stored in the cellular cytoplasm in the form of granules, up to a concentration that can reach 90% of the cell dry weight (Reddy et al., 2003). The stored polymers can be characterized by a high molecular weight (MW), in the order of 200 – 3,000 kDa, depending on the microorganism type, the available carbon source and the growth conditions (Sudesh et al., 2000; Zinn, 2003). Two of the most common from the hydroxyalkanoates (HAs) class are short chain length homopolymers such as poly(3-hydroxybutyrate), PHB, and medium chain length co-polymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV (Xu et al., 2020). The length and structure of the monomeric units incorporated into the polymeric chains strongly influence the PHAs physical characteristics, with thermoplastic properties typical of the short chain length polymers (3-5 C atoms) and elastomer properties predominant in the medium and long chain length polymers (6-14 C atoms and more than 14 C atoms, respectively) (Keshavarz and Roy, 2010; Lu et al., 2009). PHB is characterized by a glass transition (Tg) equal to 15°C, higher than the Tg of polypropylene (PP), but its melting temperature (Tm, 175°C) near to the degradation temperature (Td, 250°C) implies many difficulties in the manufacturing process with the traditional techniques applied to conventional thermoplastics (Arcos-Hernández et al., 2013). To improve the manufacturing process, the co-polymer PHBV with HV content near to 20% is preferred, since the HV content reduces the crystalline content and enhances the polymer flexibility. While the Tg of the 20% HV co-polymer is almost the same as the one of PHB, the Tm decreases broadening the gap between Tm and Td (Chen et al., 2018). PHAs biodegradability and properties made them eligible substitutes of petroleum-based commercial plastics (Pakalapati et al., 2018). For several years now, PHAs have been produced at industrial scale by means of pure cultures, such as *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, recombinant *Alcaligenes eutrophus* and recombinant *Escherichia coli* fed with synthetic substrates (Salehizadeh, Van Loosdrecht, 2004). However, pure or recombinant cultures require sterile conditions and specific carbon sources followed by a targeted recovery process, resulting in four to nine times higher production costs compared to conventional plastics (Montiel-Jarrillo et al., 2017). Another important cost in the PHAs production and commercialization chain, up to 60% of the total costs, is represented by the polymer extraction from the microbial biomass and subsequent purification, which are also called downstream processing (DSP) (Wang et al., 2014). The applicable methods depend on the biomass composition and matrix, intracellular content, the polymer type, its characteristics, MW and thermal stability. Moreover, the polymer characteristics and quality are influenced by the chosen recovery method (Chan et al., 2017). The two main strategies of DSP can be divided into digestion methods and solvent extraction. The digestion methods target the dissolution of the non-polymer cell mass (NPCM) while keeping the polymer granules as intact as possible, usually chemicals (acids/alkalis) or enzymatic digestion are the preferred protocols. The solvent extraction targets directly the polymer granules inside the biomass using a compatible and selective solvent. Excellent solvents are the halogen-containing ones, such as chloroform and methylene chloride, despite their high environmental burden and associated health risks (Kosseva and Rusbandi, 2018). The research field is currently open to find new alternatives to substitute harmful solvents while achieving similar extraction performances (Pavan et al., 2019). With the purpose of lowering production costs and environmental impacts, mixed microbial cultures (MMCs) represent an effective strategy that allows to combine successful production with resource recovery, since activated sludge from wastewater treatment plants (WWTPs) is considered a good substrate for PHA-storing microorganisms growth (Wang et al., 2019). Typically, PHAs production from MMCs in open biological systems is achieved with the enrichment of the abundance of PHA-storing microorganisms in the waste activated sludge produced by wastewater treatment in WWTPs, by undergoing an aerobic dynamic feeding regime (ADF) that alternates feast phases (carbon abundance) and famine phases (carbon deficiency) (Valentino et al., 2017). The growth of PHA-storing microorganisms is enhanced and almost exclusive due to the selective pressure applied, since the stored PHA is used as carbon source during the famine phase to survive the external carbon deficiency (Silva et al., 2017). These conditions can be tuned based on the type and availability of the carbon source and on the desired process that needs to be applied. The most common configuration successfully applied for PHAs production from MMCs at lab and pilot scale is the three-stage process, that consists in the fermentation of the organic feedstock, mixed culture selection of PHA-storing microorganisms and PHAs accumulation (Valentino et al., 2017). The organic feedstock fermentation covers another key role in the whole PHA production chain, since the polymer quality and composition are strictly dependent also on the available volatile fatty acids (VFAs), main carbon source and direct substrate for PHA synthesis (Valentino et al., 2014). The use of pure and synthetic substrates can represent up to 50% of the total polymer production costs, while the use of waste-derived substrates has the advantages of lowering production costs, using renewable carbon and fostering waste valorization and reduction (Koller et al., 2017). It has been widely reported that the 3-HB component in the polymer is larger when VFAs with even number of carbon atoms (i.e. acetate and butyrate) are predominant in the fermented mixture, while the 3-HV component in the polymer is synthesized when odd number of carbon atoms (i.e. propionate and valerate) are present in various amount (Kiran et al., 2015). The fermentation process is an important step in the anaerobic digestion process of organic substrates into CH_4 and CO_2 (Bengtsson et al., 2008). The process, divided into hydrolysis and acidogenesis, is carried out by a diverse group of facultative and obligate anaerobic bacteria, the most common being *Clostridium* spp., *Peptococcus anaerobus*, *Bifidobacterium* spp., *Desulphovibrio* spp., *Corynebacterium* spp., *Lactobacillus*, *Actinomyces*, *Staphylococcus* and *Escherichia coli* (Tchobanoglous et al., 2014). Operating conditions such as pH, temperature, organic loading rate (OLR), retention time and substrate ratio have a major impact on the VFA production yields (Atasoy et al., 2018). Many VFAs production studies have been carried out with different substrates and operating conditions, e.g. with microalgae biomass, alkaline pH and mesophilic temperature (Jankowska et al., 2015); municipal solid waste (MSW), alkaline pH and mesophilic temperature (Garcia-Aguirre et al., 2017); food waste, neutral pH and mesophilic temperature (Yin et al., 2016); fish waste, alkaline pH and mesophilic temperature (Bermúdez-Penabad et al., 2017) and dewatered sludge, neutral pH and thermophilic temperature (Hao and Wang, 2015). Among the acidogenic fermentation process tailored for the subsequent PHAs selection and production stages, a wide variety of organic substrates were used as carbon source, such as cheese whey (Duque et al., 2014), olive oil mill wastewater (Campanari et al., 2017), lignocellulose feedstock (Dietrich et al., 2019), cellulose primary sludge (Conca et al., 2020) and sewage sludge and OFMSW (Basset et al., 2016). To foster the transition towards a bio-based economy, existing structures and waste treatment facilities should be taken into account for the implementation of a biorefinery technology chain. Indeed, structures such as biogas plants, from which the only products are heat and energy, can be the ideal candidates for the development of a whole technology chain that generates added-value products besides energy (Andersen et al., 2018). However, the implementation of new technologies can be sometimes hard or slow due to different factors. In order to help the public and private sector, the EU developed the

Research and Innovation programme Horizon 2020, financing research projects in many critical areas, i.e. biotechnology, bio-based industries, plastics, etc. (European Commission, 2013). Many projects developed due to the financial help of the EU, two of the most interesting ones in the field of resource recovery and bio-based economy transition are SMART-Plant and RES URBIS. SMART-Plant (Scale-up of low carbon footprint material recovery techniques in existing wastewater treatment plants) ended in May 2020 and demonstrated the feasibility of renovating existing WWTPs to recover resources and added-value products such as biopolymers, cellulose and fertilizers. RES URBIS (Resources from urban bio-waste) ended in December 2019 and demonstrated the feasibility of converting different kind of organic waste produced in urban areas, e.g. OFMSW, garden and park waste and sewage sludge, into PHAs, related PHAs bioplastics, biosolvents and fibers in an integrated biorefinery technology chain (CORDIS, 2020: https://cordis.europa.eu/project/id/690323/it; https://cordis.europa.eu/project/id/730349/it). The valorization of organic waste in a biorefinery perspective can allow also to couple the production of a virgin material, PHAs, with bioenergy production in form of biogas. The biodegradability, chemical and mechanical properties of PHAs, similar to the conventional thermoplastics, are competitive advantages that highlight the potentialities of implementing such a biorefinery technology chain in an urban scenario for the integrated treatment of organic waste of different origin.

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2. Chapter 2. Research Objectives

The projects within the H2020 framework allowed to take important steps ahead for the implementation in real environment of new technology chains. The present thesis developed under the RES URBIS H2020 project; the aim of the research was to implement and demonstrate the feasibility of a biorefinery technology chain for the valorization of urban organic waste into PHAs and biogas. The biorefinery was built up at pilot scale within the Treviso full-scale municipal WWTP in the University experimental platform. The general approach of the study was to implement each process unit in a resource recovery perspective, in order to target added-value products while avoiding the production of secondary waste streams. After the first studies were carried out in each pilot unit, the whole biorefinery was optimized stepwise stage by stage. The key element of the whole thesis consists in the aerobic line for the PHAs selection from MMCs and the PHA accumulation, which is a process widely applied, but the combination of this kind of organic waste in a biorefinery perspective at pilot scale constitutes the innovation of the present work. The promising results obtained paved the way to the transferability of the technology chain for future full-scale installation. The schematic representation of the current thesis is illustrated in Figure 1, in order to give a better comprehension of the topics covered at a glance. The main research activities are reported from chapter 3 to chapter 7, after the main introduction and before the main conclusions. A synthetic chapter description follows the thesis structure scheme.

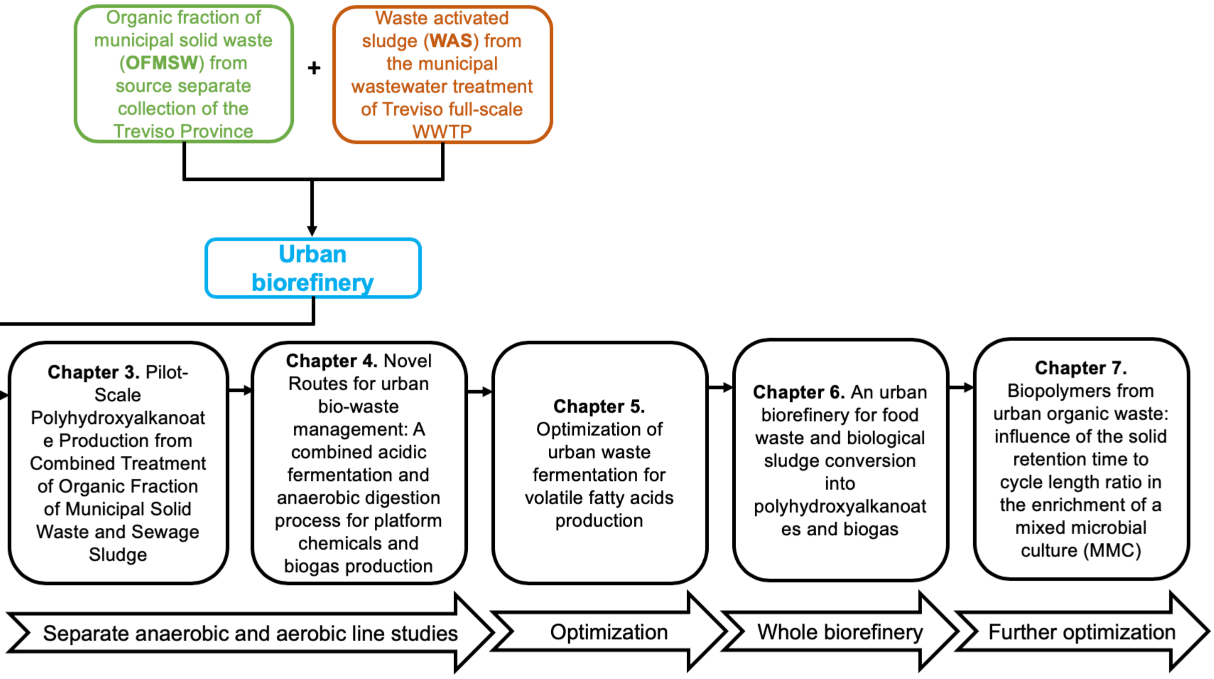

Figure 2–1. Thesis structure and topics covered throughout the whole research.

CHAPTER 3. Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge. This chapter is focused on the aerobic line of the biorefinery, i.e. from the fermentation of the organic waste to the PHAs selection and accumulation from MMCs. Different fermentation conditions were investigated and some preliminary solid/liquid separation stages for the refining of the fermented stream before the aerobic line were implemented.

CHAPTER 4. Novel Routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production. This chapter is focused on the anaerobic line of the biorefinery, i.e. the fermentation reactor and the anaerobic co-digestion process. The fermenter was operated both under mesophilic (37°C) and mesophilic (55°C) conditions and the anaerobic co-digestion section was implemented in order to reuse all secondary waste streams produced in the biorefinery. Secondary waste streams were co-digested with the WAS from the full-scale plant and different operating conditions were tested. The mass and energy balance of the anaerobic line was assessed and the most convenient configuration in terms of energy and economic savings was evaluated.

CHAPTER 5. Optimization of urban waste fermentation for volatile fatty acids production. In this chapter, some lab scale studies were performed in order to test many different fermentation conditions and to fully optimize the pilot scale process. The same starting organic substrate mixture used in the pilot scale reactor was used in each lab scale test (30-36% v/v OFMSW and 70-75% v/v WAS). The aim of this chapter is to demonstrate that the optimization of the fermentation process, that leads to a targeted carbon source for the PHA line, has a direct impact on the overall yields of the aerobic stages.

CHAPTER 6. An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas. In this chapter, the whole biorefinery technology chain is presented, from the fermentation of the organic waste to the anaerobic co-digestion line for “closing the loop” in industrial production systems. The best operating conditions for each pilot unit are presented and a complete mass and energy balance was assessed. A preliminary economic balance was performed by comparing a more traditional organic waste management approach (single anaerobic co-digestion) to the biorefinery technology line.

CHAPTER 7. Biopolymers from urban organic waste: influence of the solid retention time to cycle length ratio in the enrichment of a mixed microbial culture (MMC). This chapter is focused on the optimization of the aerobic line, specifically the PHA-storing microorganisms selection from MMCs. Different operating conditions were tested in the selection reactor and the best performances in terms of specific rates and polymer storage yields. The aim of this chapter was to present the solid retention time (SRT) to cycle length (CL) ratio as another parameter that can give useful further insights on the selective pressure applied to the system, consequently leading to a better process understanding.

CHAPTER 8. Conclusions. This chapter reports the general conclusions drawn from the whole research activity that was carried out and outlines the overall perspectives.

3. Chapter 3. Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge

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PhD Candidate contribution: Formal analysis, scientific investigation and data processing

Valentino, F., Moretto, G., Lorini, L., Bolzonella, D., Pavan, P., and Majone, M., 2019. Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge.
Postprint version of the article published in: *Industrial & Engineering Chemistry Research* 58, 12149-12158. https://doi.org/10.1021/acs.iecr.9b01831.

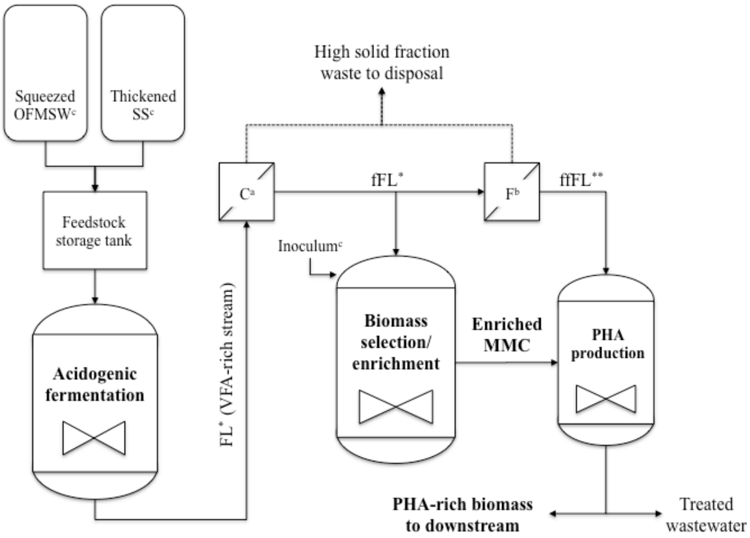
3.1. Introduction

In the frame of applied microbiology and bioprocess engineering, the use of mixed microbial culture (MMC) is attracting remarkable attention for the development of novel bioprocesses that respond to changing environmental frameworks. A well-established example of MMC application consists in the production of a particular family of polyester, namely polyhydroxyalkanoates (PHA). These biopolymers are synthetized 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 (Morgan-Sagastume, 2016). Since they are completely biodegradable and they have thermoplastic properties, the interest on 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 (Kourmentza et al., 2017; Rodriguez-Perez et al., 2018). However, more investments are necessary to promote feasibility studies, possibly at 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 (Valentino et al., 2017). Nowadays, industrial scale PHA production regards pure culture systems, which are based on refine feedstocks and sterile cultivation conditions. Both features entail high-energy requirement that strongly increases the PHA production cost (Gholami et al., 2016). This is the reason why the PHA production from microorganisms' cultivation accounts only for small percentage of global plastic production so far. The possibility to integrate MMC-PHA production into infrastructures typically adopted for bio-waste residuals and wastewater treatment could make this technology economically and environmentally sustainable. In the last decade, this basic concept has led to the process scaling-up from laboratory to pilot-scale facilities with demonstrative plants (Kourmentza et al., 2017). The examples that have been reported in the literature show the integration of MMC-PHA production with municipal wastewater treatment (Bengtsson et al., 2017; Morgan-Sagastume et al., 2015), excess sludge fermentation liquid (Jia et al., 2014), dairy and food-processing wastewater (Chakravarty et al., 2010; Tamis et al., 2014). Independently from the possible configurations, consistently explained in recent reviews (Kourmentza et al., 2017; Valentino et al., 2017), MMC technology provides for the use of activated sludge as inoculum, which microbial composition needs to be enriched in bacteria with high polymer accumulation capacity. This approach is known as "feast-famine" selection strategy and it is realized by applying dynamic feeding to microorganisms in sequencing reactors (Reis et al., 2011). Under this 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 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 recognised as being inefficient, producing large quantities of waste (Pfaltzgraff et al., 2013), 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, bio-energy production). In addition, the interest in 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, Valentino et al., 2018), and related sources such as leachate (Korkakaki et al., 2016), percolate (Colombo et al., 2017), mixture of OFMSW and primary sludge (PS, Basset et al., 2016). 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 pre-treated 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 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 valorised in full-scale anaerobic digestion plant. If compared to the conventional anaerobic digestion technology, PHA bio-based 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 bio-waste are located in Treviso municipal WWTP.

3.2. Materials and Methods

3.2.1. Pilot plant experimental platform

A process schematic of the units installed at the Treviso WWTP is presented in Figure 1. The fermentation reactor produced a hydrolysed 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 (Reis et al., 2011). 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 for 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 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.



*fFL = Centrifuged-filtered Fermentation Liquid; **ffFL = Refined-filtered Fermentation Liquid
[a] Centrifuge unit for first solid/liquid separation
[b] Filter press for further FL refining
[c] Carbon source and biomass inoculum provided by Treviso WWTP (Italy)

Figure 3–1. Process scheme developed at pilot scale for OFMSW and SS valorisation towards PHA production.

3.2.1.1. Sewage sludge (SS) and Organic Fraction of Municipal Solid Waste (OFMSW) collection

The feedstock was weekly collected inside the Treviso municipal WWTP facility. The source-sorted collection of OFMSW was made in the whole Treviso municipality, and transferred in 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, mixed in two different ratios as 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}), VFA. Main features of both mixtures are summarized in Table 1.

Table 3–1. Physical-chemical features of the two influent mixtures of urban origin.

Parameter	Unfermented Feedstock	
	OFMSW 40-45%* SS 60-55%*	**OFMSW 30-35%* SS 70-65%***
Total Solid (TS, g/kg)	92 ± 5	56 ± 3
Total Volatile Solid (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
Total Kjeldahl Nitrogen (TKN; g N/kgTS)	24 ± 2	25 ± 3
Phosphorus (P; g P/kg TS)	2.0 ± 0.1	2.3 ± 0.1
Ammonium ($N\text{-}NH_4^+$; mg/L)	327 ± 35	342 ± 28
Phosphate ($P\text{-}PO_4^{3-}$; mg/L)	163 ± 6	113 ± 7

* Each percentage refers to a volumetric fraction.

3.2.1.2. 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 in 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 solid/liquid separation after fermentation: a coaxial centrifuge equipped with 5.0 µm porosity nylon filter bag for solids removal. This step was crucial to obtain a cleaner filtered fermented stream (*fFL*), with a suspended solid level below 10.0 g/L, that was 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 temperature (T); more specifically, different fermentation liquids (FL) were produced according to the following parameters:

FL-I) OFMSW 40-45% v/v and SS 55-60% v/v mixture; T 55°C;
FL-II) OFMSW 30-35% v/v and SS 65-70% v/v mixture; T 55°C;
FL-III) OFMSW 30-35% v/v and 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 at first (from I to II), and then by changing the T from thermophilic (II) to mesophilic (III) conditions. The first trial had been tested up to day 120; condition II from day 121 to day 185; last condition was from day 185 to the end (day 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 d (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 Treviso full-scale WWTP. The aerobic run was conducted for approximately seven months, at 1.0 d of HRT (equal to SRT) and 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) was continuously measured but not controlled. The applied OLR was set at 4.0 g COD_{SOL}/L d. In order to maintain such OLR, the *f*FLs were 6-10 fold diluted with tap water. Feedstock shifts were made at day 93 (from *fFL*-I to *fFL*-II) and 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* (*ffFL*; specifically *ffFL*-II and *ffFL*-III). The *fFL*s 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 *ffFL*/X ratio was chosen to be ≤ 2.0 COD basis, in order to prevent any possible substrate or pH inhibition phenomena (Valentino et al., 2015).

3.2.2. Sampling and Analytical methods

Feedstock mixture was characterized after each collection (weekly basis). The analyses 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 (Valentino et al., 2014). 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 analysed 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 (APHA, AWWA, WEF, 1998). 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 - 0.53 mm - 0.5 mm film thickness) and with a flame ionization detector (200°C). During analysis, the temperature (T) increased from 80°C 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 NaClO solution (5% active Cl_2) and then stored at -4°C for following analysis. In accordance to Braunegg et al. (1978), 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.

3.2.3. Calculations

All the parameters characterising reactor performances were calculated after steady states or pseudo-steady 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). Solubilisation 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 (Valentino et al., 2014). 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 O_2 concentration. The non-polymer biomass (or active biomass, X_A) was calculated as the difference between VSS and PHA concentrations: X_A = VSS-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 (Valentino et al., 2013). 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.

3.3. Results and Discussion

3.3.1. 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, Wang (2015). 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 (2017) also observed lower VFA production by decreasing food waste load in batch acidogenic fermentation test. 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 solubilisation, 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). 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 (Morgan-Sagastume et al., 2015; Valentino et al., 2014). The temperature of 42°C led to lower COD solubilisation (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 (Bolzonella et al., 2005). This difference could be reasonably attributed to the presence of sewage sludge in the mixture.

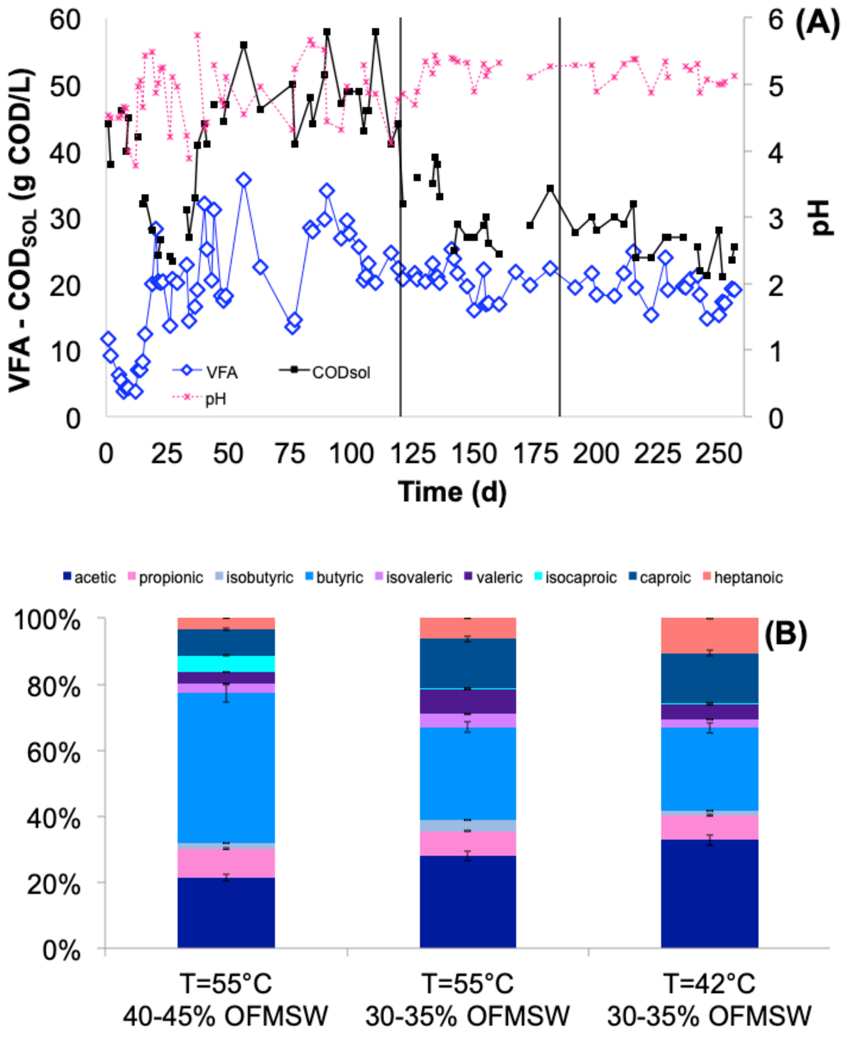


Figure 3–2. VFA, COD_{SOL} and pH evolution in the fermented feedstock (A); VFA distribution in the three different conditions tested (B).

In terms of composition, butyric acid was predominant in FL-I (46% of total VFA, COD basis), followed by acetic (22%) and propionic (9%). 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). In 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 $[C_3/(C_3+C_2)_{VFA}]$ 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 (Bengtsson et al., 2010; Morgan-Sagastume et al., 2015). 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 40-45% 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 (Pittman, Steinmetz, 2017). By decreasing the 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, also important, 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 5-8 g/L in the filtrate. Soluble COD and VFA were not affected by the filtration at all as well as for soluble nutrient level.

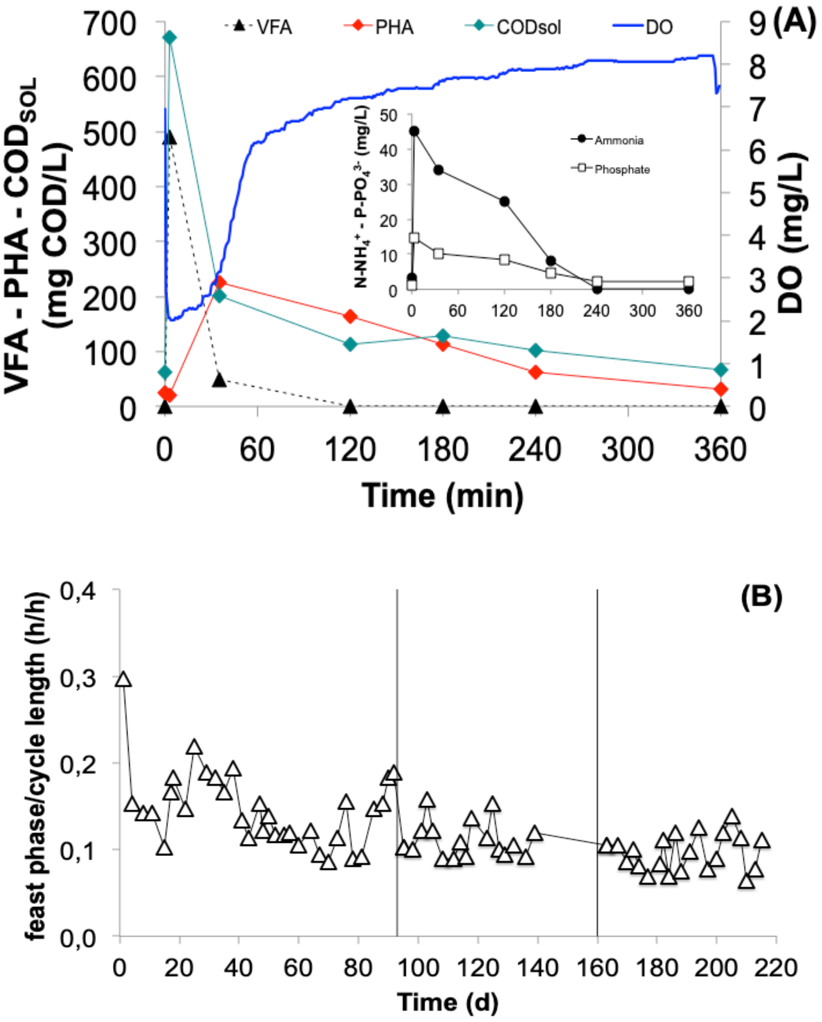
Table 3–2. Fermented feedstock characteristics and related fermentation performances.

Parameter	Fermented feedstock characteristics		
	FL-I	**FL-II**	**FL-III**
Total Solid (TS, g/kg)	82 ± 6	56 ± 4	57 ± 3
Total Volatile Solid (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
Total Kjeldahl Nitrogen (TKN; g N/kgTS)	28 ± 4	23 ± 4	20 ± 2
Phosphorus (P; g P/kg TS)	2.0 ± 0.3	2.2 ± 0.4	2.1 ± 0.3
Ammonium ($N\text{-}NH_4^+$; mg/L)	698 ± 55	724 ± 138	562 ± 44
Phosphate ($P\text{-}PO_4^{3-}$; 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

Parameter		Fermentation performances		
		FL-I	**FL-II**	**FL-III**
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

3.3.2. 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 (Campanari et al., 2014). After VFA (or most of COD_{SOL}) depletion, DO concentration started to increase and it stabilized approximately at 6.0 mg/L and it slowly resumed 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: 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 one week of operation (~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 behaviour 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 run 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 (Reis et al., 2011; Valentino et al., 2017). 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).



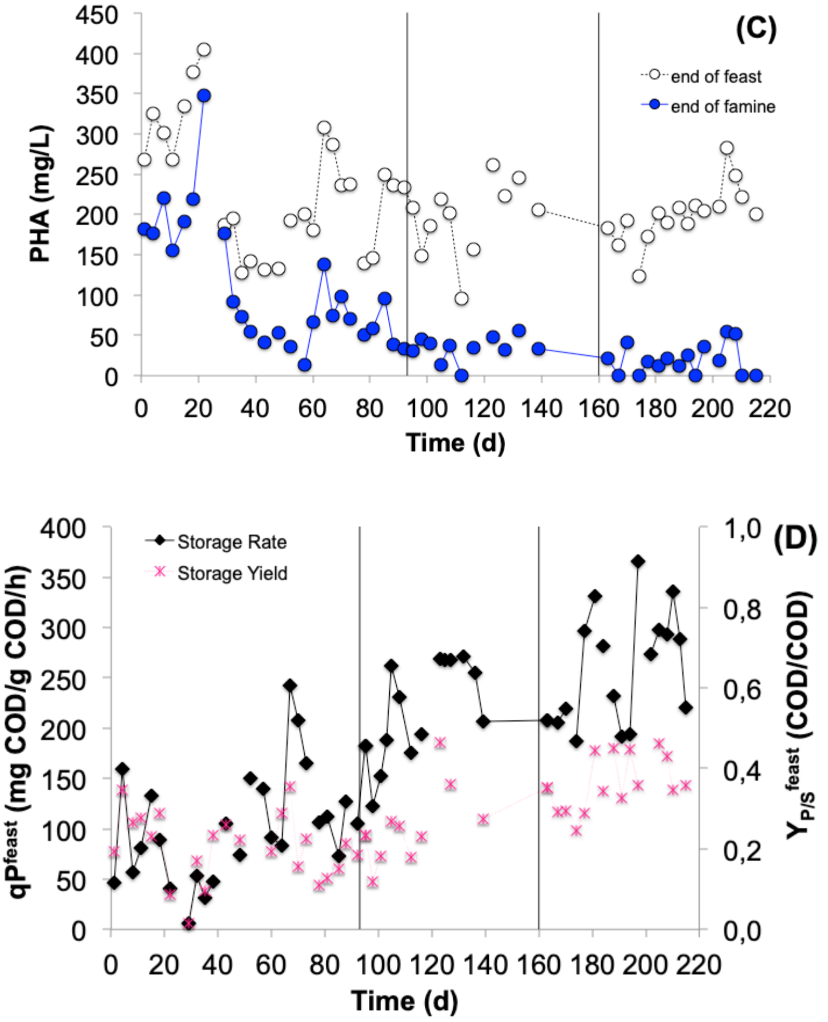


Figure 3–3. Main parameters monitored in SBR: VFA, COD_{SOL}, PHA, ammonium and phosphate in a typical SBR FL-III cycle (A); feast phase/cycle length ratio (B); maximum and minimum PHA concentration (C); storage rate and yield (D) in the three different SBR runs.

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, Dionisi et al., 2004). It is reasonable to suppose that the higher stability of biomass storage response was primarily favoured 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 recorded cycles profiles that non-VFA COD_{SOL} contributed only in minor part to PHA storage, as also reported in literature (Albuquerque et al., 2010; Basset et al., 2016). 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 favour the growth of non-storing bacteria during famine phase, causing a less efficient culture selection (Majone et al., 1996). 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.

Table 3–3. 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 (qP^{feast})	$mgCOD_{PHA}/gCOD_{Xa}$ h	101 ± 52	212 ± 35	258 ± 44
Specific VFA removal rate ($-qVFA^{feast}$)	$mgCOD_{VFA}/gCOD_{Xa}$ h	332 ± 62	619 ± 54	634 ± 53
Specific COD removal rate ($-qS^{feast}$)	$mgCOD_{SOL}/gCOD_{Xa}$ h	509 ± 84	840 ± 77	725 ± 62
Storage yield ($Y_{P/S}^{feast}$)	COD_{PHA}/COD_{SOL}	0.22 ± 0.05	0.26 ± 0.06	0.36 ± 0.04
Storage yield ($Y_{P/VFA}^{feast}$)	COD_{PHA}/COD_{VFA}	0.30 ± 0.06	0.34 ± 0.05	0.40 ± 0.04
Observed yield (Y_{OBS}^{SBR})	COD_{VSS}/COD_{SOL}	0.49 ± 0.05	0.42 ± 0.06	0.54 ± 0.08
Effluent Ammonium ($N\text{-}NH_4^+$)	mg $N\text{-}NH_4^+$/L	2.6 ± 1.2	0.8 ± 0.5	3.5 ± 0.9
Effluent Phospahte (PO_4^{3-})	mg $P\text{-}PO_4^{3-}$/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

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.

3.3.3. PHA accumulation

The PHA accumulation capacity of selected PHA-storing biomass was evaluated in fed-batch accumulation tests by means of multi-spike feeding strategy (Campanari et al., 2014). To do so, *fFL*s 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 (*ffFL*s) characterized by no content of suspended solids. Multiple pulsed addition of *ffFL*-II and *ffFL*-III was used to overcome any potential substrate inhibition phenomena (Albuquerque et al., 2010; Campanari et al., 2014; Korkakaki et al., 2016). 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 hours, 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. Afterwards, VFA started to be consistently accumulated in the reactor and PHA was produced with 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.

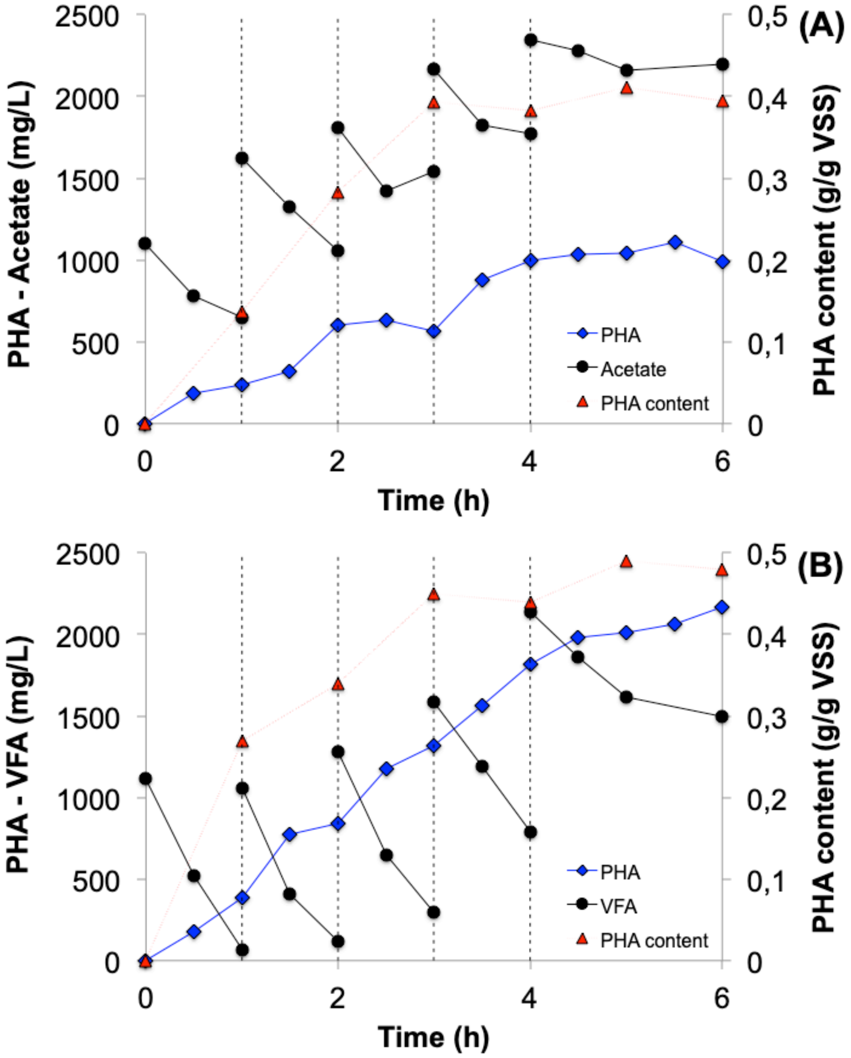


Figure 3–4. Fed-batch accumulations conducted with biomass enriched in SBR FL-III run: synthetic acetate (A) and *ffFL*-III (B) as feeding solutions.

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 (Morgan-Sagastume et al., 2019; Valentino et al., 2015), especially if biomass response needs to be exploited to a large extent (Morgan-Sagastume et al., 2015). In the examined examples, PHA storage had been favoured 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 *ffFL*s than with acetate can be also due to that the SBR biomass has been selected with 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 identical process configuration highlighted the promising results obtained in this study (Valentino et al., 2014). Similar PHA content and yield were achieved with even higher polymer productivity (up to 0.36 g PHA/L h), favoured by the presence of nutrients and, as a consequence, by the growth of new active PHA-storing biomass during accumulation. Other investigations utilising OFMSW sources have been mostly conducted at laboratory scale, at least for the aerobic process steps (Amulya et al., 2015; Basset et al., 2016; Colombo et al., 2017; Korkakaki et al., 2016). Amulya and co-workers (2015) proposed an integrated multi-stage approach for food waste valorisation. 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, one order of magnitude lower than 279-301 mg COD_{PHA}/g COD_{Xa}/h of this study. 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. (2016) used the OFMSW FL, alternatively mixed with PS. 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 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 (Colombo et al., 2017; Korkakaki et al., 2016). 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 by acetate (75-90% v/v). The storage performances obtained with percolate were comparable with those obtained with *ffFL*-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 *ffFL*-III (90% HB and 10% HV weight basis). More recently, Valentino et al. (2018) described a multi-steps pilot scale platform for the conversion of pre-treated OFMSW (not mixed with sludge) into PHA and biogas. Regarding the aerobic stages 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 (Valentino et al., 2018), 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 an efficient and integrated urban waste management.

Table 3–4. 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)**	**Polymer composition (% HB/HV, g/g)**	**$Y_{P/S}^{batch}$ (COD/COD)**	**$Y_{P/VFA}^{batch}$ (COD/COD)**	**$Y_{X/S}^{batch}$ (COD/COD)**	**$Y_{X/VFA}^{batch}$ (COD/COD)**	**qP^{batch} ($mgCOD_{PHA}/gCOD_{Xa}/h$)**	**PHA productivity (gPHA/L h)**	***References***
Acetate (*synth.*)[a]	0.40 ± 0.02	100/0		0.67 ± 0.05		0.08 ± 0.01	202 ± 33	0.20 ± 0.04	*This study*
OFMSW-SS mixture (f**FL-II**)*	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 (f**FL-III)***	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*
Pre-treated 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	*Valentino et al., 2018*
Leachate	0.78[b,c]			0.33[b]-0.40[c]					*Korkakaki et al., 2016*
Percolate*	0.41-0.48	54-57/46-43	0.44-0.52	0.66-1.13				0.18-0.29	*Colombo et al., 2017*
OFMSW*	0.11		0.08	0.22	0.77	0.36	20		*Basset et al., 2016*
OFMSW-PS[d] mixture*	0.09		0.003	0.011	0.79	0.47	11		
VFA mixture (*synth.*)[a]	0.48 ± 0.03	88/12		0.53 ± 0.03			196 ± 13	0.17 ± 0.02	*Valentino et al., 2014*
Food waste (FW)*	0.24	60/40	0.09-0.17				11-42		*Amulya et al., 2015*

[a] No nutrients addition (COD:N:P=100:0:0)
[b] biomass enriched with synthetic VFA mixture (90% v/v) and leachate (10% v/v)
[c] biomass enriched with synthetic VFA mixture (75% v/v) and leachate (25% v/v)
[d] Primary Sludge
*fermented feedstock

3.3.4. 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 PHA, which corresponds to 1.70 kg COD_{PHA}, based on quantified HB-HV wt% monomers composition. The relative mass flow diagram of PHA production is depicted in Figure 5. The backwards calculation started from the final PHA biomass content of 0.46 g PHA/g VSS, obtained with *fFL*-III as feed solution (Table 3). This PHA content means a production of 1.17 kg (or 1.66 kg COD) of active biomass X_A. For both produced X_A and PHA, it has been estimated a total consumption of 6.24 kg COD_{VFA}, of which 3.38 kg 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 ($Y_{P/VFA}^{batch}$) of 0.50 COD_{PHA}/COD_{VFA} (Table 3), and the X_A observed yield (Y_{Xa}^{SBR}) of 0.58 COD_{Xa}/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 COD_{VFA}. This led to 6.42 kg COD_{VFA} in the centrifuged FL, as quantified VFA consumption in both aerobic stages for each kg 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 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 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 (2017) 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. In a more recent study, a complete pilot system was developed for the valorisation of the OFMSW as the only carbon source (Valentino et al., 2018). 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.

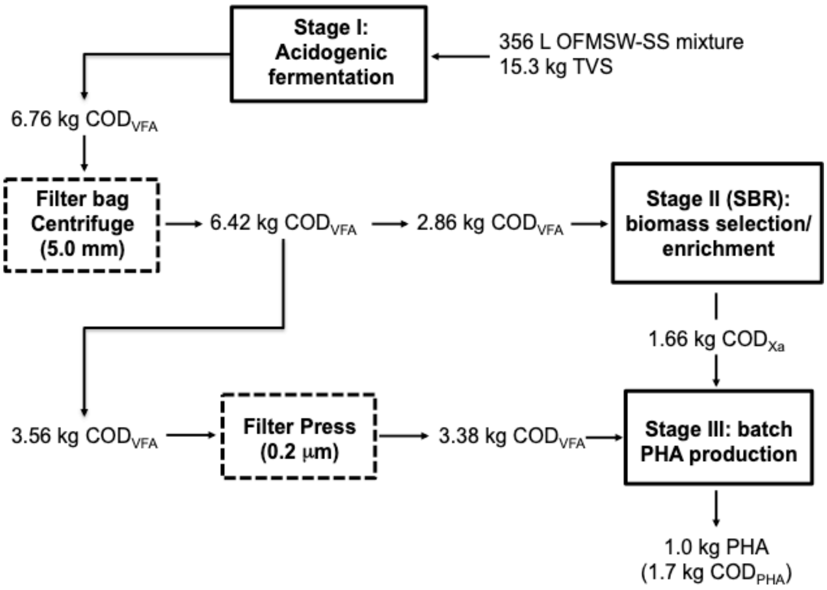


Figure 3–5. Mass flow diagram of PHA production from OFMSW-SS mixture.

In the perspective of a future market scenario for PHA synthetized by MMC, the maximisation of the yield could have a remarkable impact on the process sustainability. Moreover, in a multi-steps 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 (2019) quantified a maximum economic income of 245,000 €/year from the electrical energy produced in the Treviso full-scale anaerobic digestion (AD) plant. The 2,000 m^3 AD reactor was designed for the treatment of OFMSW-SS mixture (roughly 1,150 ton TVS/y), produced in an urban scenario of 70,000 Person Equivalent (PE). With the introduction of a designed PHA line, it can be estimated a request of the 60% of the organic solids (690 ton TVS/y) for the fermentation step and the production of a liquid VFA-rich stream amenable for PHA synthesis (Valentino et al., 2019). Based on the calculated yield (65 g PHA/kg TVS), a production of 45 ton PHA/y can be counted. Following the approach described in Valentino et al. (2019), the residual TVS amount (overflows) is represented by the sum of the 40% unfermented solids (460 ton TVS/y) and the solid-rich pre-fermented fraction (“cake”), which is discharged from the solid/liquid separation unit (80% of the fermentation TVS influent; 552 ton TVS/y). From the specific gas production (SGP) quantified in the mesophilic AD treatment of the overflows (0.40 m^3 biogas/kg TVS), the estimated biogas production is 1,124 m^3/d. The technical feasibility of this approach has been recently demonstrated; a revenue of 134,591 €/y is achievable from the sale of generated electricity (13.03 MWh/d). In theory, taking into account an average PHA value of 4,000 €/ton (Gholami et al., 2016), an additional revenue of 180,000 €/y has to be consider, potentially making the biorefinery platform a more profitable solution if compared to the traditional AD process.

3.4. 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 valorisation of the solid-rich overflow, mainly produced by the filter bag centrifuge and basically composed by fermented OFMSW-SS, through anaerobic digestion (AD, Moretto et al., 2019). This solution allows using one main technology for the conversion of urban bio-wastes 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 bio-based products.

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4. Chapter 4. Novel Routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production

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Valentino, F., Moretto, G., Gottardo, M., Pavan, P., Bolzonella, D., Majone, M., 2019. Novel Routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production.
Postprint version of the article published in: *Journal of Cleaner Production* 220, 368-375. https://doi.org/10.1016/j.jclepro.2019.02.102.

4.1. Introduction

Anaerobic digestion (AD) is currently considered the most feasible technology for biogas production from the treatment of high solid content streams, such as manure, organic fraction of municipal solid waste (OFMSW), energy crops, agro-industrial wastewater etc.. (Tyagi et al., 2018). The oldest and more commonly adopted application of AD is the treatment of biological sludge (Mata-Alvarez et al., 2014), even if minimized with membrane bioreactor (MBR) technology (Sepehri and Sarrafzadeh, 2018). In the two last decades, a particular attention has been devoted to the co-digestion processes, which have been designed for a simultaneous treatment of biological sludge and OFMSW (Mata-Alvarez et al., 2014). With regard to the specific case of bio-waste produced in urban scenario, the increasing of global population has led to new concerns for public authorities dealing with the treatment and disposal of biological sludge produced in municipal wastewater treatment plants (WWTPs) and food waste (Ma et al., 2017). Through the conventional AD processes, the degradation of organic matter contained in food and sludge waste streams is not even complete, being in the wide range of 35-60% in most cases (Yin et al., 2016a). This performance is strongly affected by the low efficiency of hydrolysis, which is the rate-limiting step for the urban bio-waste anaerobic degradation process (Ma et al., 2018). Many pre-treatment methods have been explored for enhancing bio-solid hydrolysis and improving chemical oxygen demand (COD) solubilisation in order to increase bio-methane yield; among others, thermal hydrolysis (Li et al., 2017) and aerobic micro-aeration (Montalvo et al., 2018) pre-treatment. Archea have been recognised as main responsible for methane production from volatile fatty acids (VFAs), as well as for ethanol and lactate (Stoyanova et al., 2017) in a typical AD process. However, since different microorganisms with specific biochemical pathways are involved, many other valuable products can be obtained via acidogenic fermentation. These products can have a wide spectrum of possible valorisation. As an example, the VFAs and lactic acid were described as the direct precursors for a particular family of short and medium chain length biopolymers, namely polyhydroxyalkanoates (PHAs) (Kourmentza et al., 2017). Alcohols have been also sometimes reported as possible substrate for PHA synthesis (Beccari et al., 2009). This route allows converting platform chemicals in bio-products at higher added value than methane (Pagliano et al., 2017). In fact, PHAs are one of the most promising classes of biopolymers, which can replace part of the synthetic marketed materials, due to their biodegradability and thermoplastic properties. In the frame of mixed microbial culture (MMC) technology (Valentino et al., 2017), the use of complex and low-cost substrates is a strategy to accomplish both bio-products production and bio-waste treatment following their acidic fermentation. For this reason, acidogenic fermentation can be considered an option to exploit the organic matter contained in sludge and food waste as renewable feedstock, decreasing the amount of bio-waste to be disposed of (Koller, 2017). The VFAs composition and fermentation yield can vary according to the process parameters and settings. A desirable feature for PHA production is the ratio between odd and even carbons in the VFAs, which determine the composition of PHA co-polymers to a large extent (Bengtsson et al., 2010). Several parameters affected the fermentation process outputs such as inoculum (Yin et al., 2016b), pH (Wu et al., 2017), temperature (Dahiya et al., 2015), hydrogen partial pressure (Agler et al., 2011). A previous study showed that a pH between 5.0 and 6.0 was preferable to reach the highest fermentation process yield (Gottardo et al., 2017). However, the accumulation of VFAs produced in the fermentative metabolism generally causes a pH decreasing below the optimal values. Apart from chemicals additions for pH control, some authors recently proposed the recirculation of the anaerobic digestion effluent, as buffering agent, to control the pH in the fermentative stage. This strategy was applied in the combined two-stage fermentation and anaerobic digestion process (Gottardo et al., 2017). The method was particularly efficient when applied to a high solid content waste such as the pre-treated OFMSW. The high content of putrescible solids facilitates their fermentation even at relatively low hydraulic retention time (HRT < 4.0 days); as a consequence, the high level of VFAs (easily achieved) brings the pH below 5.0, inhibiting a further fermentation activity and making the pH-control strategy strictly necessary. In the particular case illustrated in this paper, both VFAs and biogas production has been assessed from the anaerobic treatment of the specific cocktail of urban bio-wastes produced in the Treviso (north-east Italy) municipality. The pre-treated OFMSW was mixed with thickened waste activated sludge (WAS) from Treviso WWTP. The produced VFAs from acidogenic fermentation process were separated from the slurry for other potential uses (such as the PHAs synthesis). The solid-rich waste overflow was originated from a solid/liquid separation unit. This fraction was opportunely diluted with the same thickened WAS (collected in Treviso WWTP), and it has been used as secondary waste feedstock for biogas production through AD process. This approach differs from the traditional WAS-OFMSW co-digestion, developed at the Treviso full scale WWTP (Bolzonella et al., 2006), which allows recovering biogas only. In a frame of urban bio-refinery concept, the renewable carbon source may be driven in different routes that include also VFA production followed by biopolymer synthesis in a high load aerobic process (Valentino et al., 2015). The present pilot scale investigation has the scope to verify the process technical feasibility aimed at maximizing the production of VFA and at recovering biogas from the overflows.

4.2. Materials and Methods

4.2.1. Rationale of the approach

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

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

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

4.2.3. Anaerobic pilot units

The acidogenic fermentation unit consisted of a continuous stirred tank reactor (CSTR) of 0.38 m^3 of operative volume, mechanically stirred and under temperature control by using a thermostatic jacket. The hydrolysis and fermentation of the organic content of WAS-OFMSW mixture into VFAs were carried out under both mesophilic (37°C) and thermophilic (55°C) conditions, in two separate trials. All the other operating conditions are listed as follows: hydraulic retention time (HRT) of 6 days; organic loading rate (OLR) 6.5-7.0 kg VS/m^3 d approximately; uncontrolled pH in the range 5.0-5.5. The CSTR fermenter was connected to a solid/liquid separation unit consisting of a coaxial centrifuge, equipped with 5.0 µm porosity nylon filter bag for solid (TS) removal. The solid-rich stream overflow was separated and then diluted with the excess WAS (collected from the full-scale plant) as feeding for anaerobic digester. Stainless steel CSTR (AISI-304) of 0.23 m^3 operating volume was used as anaerobic digester. Mechanical anchor stirrer ensured mixing and homogenization. Two separated trials were carried-out at 37°C and 55°C, according to the temperature of the previous fermentation stage. The temperature was maintained by hot water recirculation through external jacket. Anaerobic digested sludge inoculum was collected in the same WWTP, from the 2300 m^3 CSTR anaerobic digester, treating thickened WAS and squeezed OFMSW mixture under mesophilic condition at an OLR of 1.8 – 2.0 kg VS/m^3 d. For both AD trials, the digested sludge was acclimatized for 35-40 days (more than 2 HRTs). Operational parameters in both mesophilic and thermophilic trials were similar: HRT of 15 d and OLR between 2.2 – 2.5 kg VS/m^3 d. The WAS/cake ratio in the feedstock was calculated on the basis of the WAS and OFMSW specific production: 0.3 kg OFMSW/PE day (based on wet weight) and 0.06 kg TS/PE day for WAS (Metcalf & Eddy, 2003).

4.2.4. Sampling and analytical methods

Feedstock characteristics were determined once per week on fresh sample, immediately after its collection. The reactor effluents were monitored three times per week for total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and phosphorous (P). For the TS determination, a drying temperature of 105°C was adopted (Peces et al., 2014). The process stability parameters, i.e., the pH, volatile fatty acid content and distribution, total and partial alkalinity, ammonia, were measured two/three times per week. All analyses were performed according to the standard methods (APHA, AWWA, WEF, 1998). The analyses of VFA were conducted using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (T 100°C), a fused silica capillary DB-FFAP (15 m length, 0.53 mm x 0.5 mm film thickness) column, with hydrogen as carrier. The analysis was conducted by increasing the temperature from 80°C to 200°C (10°C/min). Samples were centrifuged at 4000 rpm for five minutes; the supernatant was filtered by using a 0.20 μm cellulose acetate filter before the analysis. The biogas production was monitored using a flow metre (Ritter CompanyTM); methane, carbon dioxide and oxygen in the biogas were continuously determined using a portable infrared gas analyser GA2000TM (Geotechnical InstrumentsTM), and once a day using a gas chromatograph 6890N (Agilent TechnologyTM). The analyses were conducted with an HP-PLOT MOLESIEVE column (30 m length, 0.53 mm ID x 25 mm film thickness), using a thermal conductivity detector and argon as carrier (79 ml/min). H_2, CH_4, O_2 and N_2 were analysed using a thermal conductivity detector (TCD) at a temperature of 250°C. The inlet temperature was 120°C, with a constant pressure in the injection port (70 kPa). Samples were taken using a gas-type syringe (200 mL biogas). Once the entire sample was vaporized, separation of the peaks occurred within the column at a constant temperature of 40°C (8 min).

4.2.5. Calculation

All the parameters characterising reactor performances were calculated after steady states were achieved. Descriptive statistics and exploratory data analysis were performed using the open source program, R (The R Foundation for Statistical Computing, version 3.5.0). For the fermentation stage, the process was considered sufficiently stable and under steady state after consistent VFA production was detected. Solubilisation and fermentation yield

(Y_{VFA}) were determined with respect to the characteristics of initial unfermented feedstock, in particular total COD ($COD_{TOT(0)}$) $TS_{(0)}$ and $VS_{(0)}$. For the anaerobic digestion step, steady state was considered achieved when the biogas production rate (GPR) was constant (below 10% of deviation from average values). The mass and energy balance, as well as the energy yield, were performed by considering both stages in two combination of temperature: a) fermentation and digester reactors under mesophilic condition; b) fermentation reactor under mesophilic condition and digester reactor under thermophilic condition. The aim of this comparison was to evaluate which configuration is more profitable in terms of surplus thermal energy and electricity production. All the parameters were adapted to a scaled-up version of both reactors, in an urban municipality context of average size of 170,000 Person Equivalent (PE); reference parameters and boundary conditions are given in Table 1. In the energy balance analyses, the price of electrical energy has been assumed to be 130 €/MWh (no incentive; Valentino et al., 2018).

Table 4–1. Reference parameters and boundary conditions for energy balance.

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

4.3. Results and Discussion

4.3.1. Bio-waste composition and characteristics

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

Table 4–2. Physical-chemical features of unfermented and fermented feedstock (OFMSW-WAS mixture).

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

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

4.3.2. Acidogenic Fermentation

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

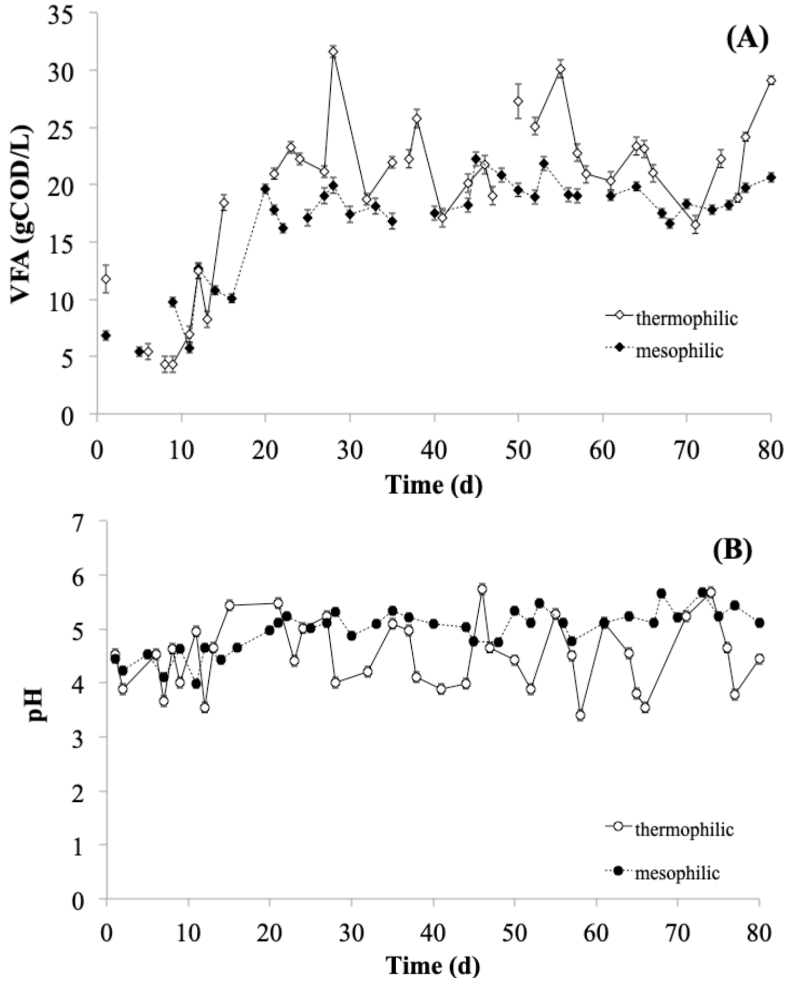


Figure 4–1. VFA (A) and pH (B) trends during mesophilic and thermophilic acidogenic fermentation stage.

Thermophilic fermentation was characterized by a shorted acclimation period (2.5 HRT) compared to mesophilic process. The VFA level in this fermented stream was generally higher than those measured in mesophilic condition, but frequent fluctuations in VFA concentration trend were observed. In addition, the VFA concentrations widely changed over the course of process operation, in a range from 16.5 to 31.6 g COD_{VFA}/L. This profile reflected the equally frequent pH decrease, due to a fast hydrolyzation and fermentation activity (supported by the higher T), not enough buffered by the alkalinity of the feedstock itself. In practice, WAS-OFMSW mixture fermentation at 55 °C appeared more difficult in terms of process control and VFA production stability. Fermentation performances as well as physical-chemical characteristics were affected by this process instability as indicated by the standard deviations of average values (sometimes one order of magnitude higher than those under mesophilic temperature) reported in Table 2. Higher temperatures solubilised more solids and increased the COD_{SOL} and nutrient release in the mixed liquor. The VFA concentration also increased above 20 g COD_{VFA}/L; however, a not negligible fraction of COD_{SOL} remained unconverted into VFA, being the average COD_{VFA}/COD_{SOL} ratio equal to 0.59. Feedstock fermentability at 37°C was found to be less performing for solids and COD solubilisation (0.41 ± 0.02 and 0.26 ± 0.01 on initial VS and COD_{TOT} respectively) even though VFA yields were comparable to those measured at 55 °C. The acidic WAS-OFMSW fermentation was found to be more performing to that WAS only, independently from the chosen process temperature. The VFA yields obtained in this work (0.41-0.44 g COD_{VFA}/g $VS_{(0)}$, 0.26-0.28 g COD_{VFA}/g $COD_{TOT(0)}$; Table 2) are higher than yields reported for WAS at 37°C (0.01-0.06 g COD_{VFA}/g $VS_{(0)}$, Ucisik and Henze, 2008; 0.06–0.14 g COD_{VFA}/g $COD_{TOT(0)}$, Yuan et al., 2009), and at 42°C (0.27 g COD_{VFA}/g $VS_{(0)}$, Morgan-Sagastume et al., 2014). The presence of the OFMSW in the feedstock may have provided a more fermentable bio-waste and explained differences to the literature values. As a consequence, the level of VFA obtained here was higher (roughly double) if compared to those reported in the literature for WAS (6.0-9.4 g COD_{VFA}/L, Morgan-Sagastume et al., 2015) or primary sludge-WAS mixture (7.5-13.5 g COD_{VFA}/L, Pittmann and Steinmetz, 2017), and similar to those obtained with thermally pre-treated sludge (18.5-19.9 g COD_{VFA}/L, Morgan-Sagastume et al., 2010). The process stability is also an important aspect affecting the technical process feasibility: mesophilic fermentation appeared more technically attractive since the VFA-rich stream features were more stable over time and the COD_{VFA}/COD_{SOL} ratio was higher (t – test, alpha value = 0.01) than value obtained in thermophilic fermentation (Figure 2).

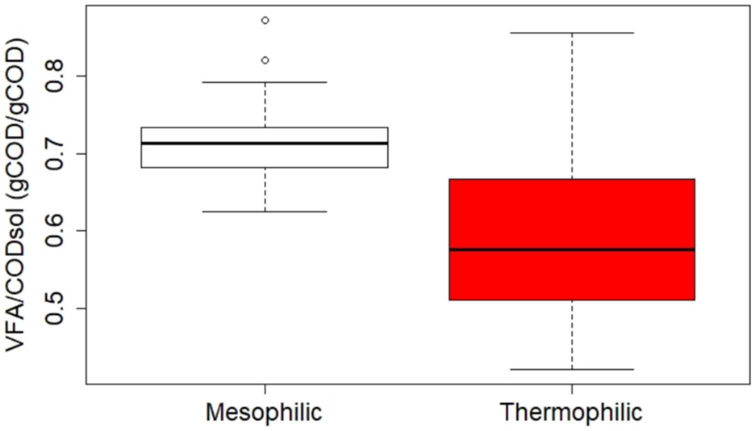


Figure 4–2. Boxplot of VFA/COD$_{SOL}$ for mesophilic and thermophilic fermentation trials.

A VFA-rich stream with consistent composition is also a desirable feature for PHA production since the balance of odd and even carbons in the VFA determines the polymer composition (Bengtsson et al., 2010). Fermentation products in mesophilic and thermophilic conditions were similar and dominated by butyric (41-42%, COD basis), acetic (22-23%, COD basis), propionic (11-10%), valeric (12-11%), caproic (9-8%), eptanoic (4-6%), isovaleric (2-3%) and isobutyric (1-0.8%) acids. The predominance of butyric was probably related to the presence of OFMSW in the feedstock, as reported elsewhere (Girotto et al., 2017), since acetic acid was usually the most abundant in fermentation process performed with sludge only (Morgan-Sagastume et al., 2010, 2011). The molar fraction of VFA with odd number of C-atoms if compared to the total $[C_3/(C_3+C_2)]_{VFA}$ was also similar (Table 2), most likely dependent on feedstock characteristics and not affected by fermentation temperature. Regarding the solid cake (SC), the Table 3 summarizes the main characteristics of both SC overflows, obtained from mesophilic and thermophilic fermentation process. In spite of the observed differences for the features of mesophilic and thermophilic VFA-rich streams, the working temperature did not show any relevant effect on the main characteristics of the two SC (t – test, alpha value = 0.01).

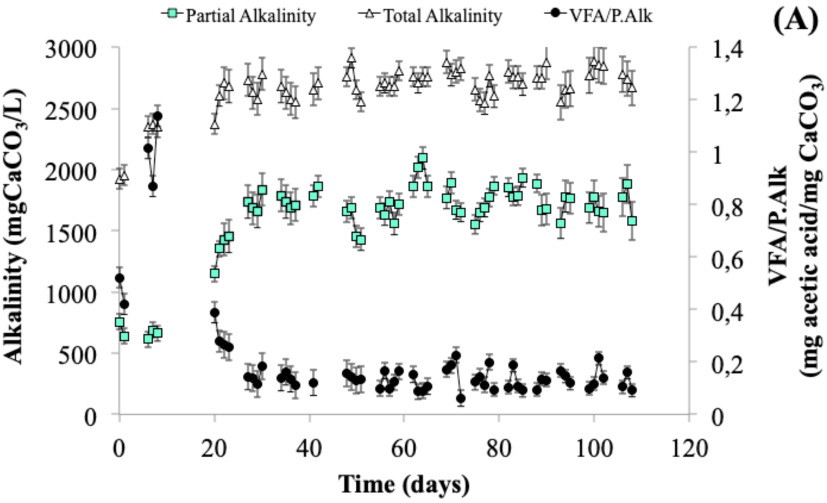
Table 4–3. Physical-chemical features of the solid-rich cake overflows.

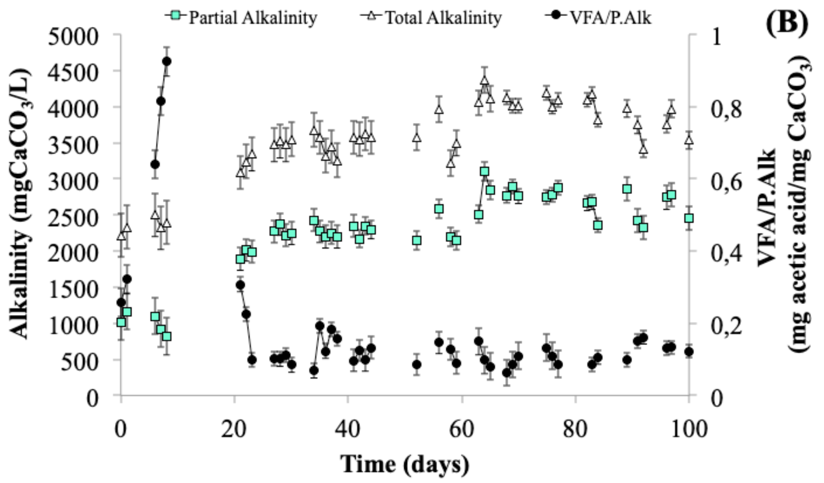
Parameter	Solid-rich cake overflow*	
	Mesophilic	**Thermophilic**
Total Solid (TS, g/kg)	235 ± 21	220 ± 21
Volatile Solid (VS, g/kg)	189 ± 10	177 ± 8
VS/TS (%)	80 ± 2	81 ± 1
COD (g O_2/kg TS)	740 ± 61	755 ± 43
Total Kjeldahl Nitrogen (TKN; g N/kg TS)	29 ± 1	33 ± 1
Phosphorus (P; g P/kg TS)	5.0 ± 0.6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

* Solid-rich cake produced after fermentation process

4.3.3. Anaerobic Digestion

Mesophilic and termophilic processes were started at relatively low OLR (0.7-0.8 kg VS/m^3 d) by using a feed solution mainly composed by thickened WAS. The SC content was gradually increased in order to achieve an OLR of 2.3 - 2.5 kg VS/m^3 d approximately. The SC content strongly affected the operative OLR since it was characterized by TS of one order of magnitude higher than the thickened WAS. In mesophilic condition, steady state was achieved in 40 days (roughly 2 HRTs). In the start-up period, no relevant pH drop was observed even at the highest VFA concentration detected (close to 1.0 g COD/L at day 10th after inoculum). Most of the readily fermentable COD was already converted into VFA in the previous hydrolysis/fermentation step; as a consequence, the process was easily controlled even in the transient conditions when the risk of methanogenic bacteria inhibition is usually high. Average pH value of steady state period was 7.6 ± 0.2, advantageous for the growth of methanogens (Gottardo et al., 2017). The pH remained constant because of the high buffering capacity of the system as indicated by the partial alkalinity (1,738 ± 138 mg $CaCO_3$/L) and by the VFA/Partial Alkalinity (P.Alk) ratio (0.13 ± 0.04 mg acetic acid/mg $CaCO_3$) (Figure 3A). In particular VFA/P.Alk ratio showed a maximum peak (1.14) in the first week after inoculum and then it decreased over the course of operation. In the steady state period, VFA/P.Alk ratio was constantly below 0.20 indicating high process stability and the negligible VFA accumulation. In fact, it is widely recognized that VFA/P.AlK value greater than 0.30 may indicate a possible process upset or instability usually caused by organic matter overload and a consequently VFA concentration increase (Bolzonella et al., 2003). Ammonia release from proteins degradation led to an increase of total ammonium in the liquid phase from 0.46 ± 0.07 to 0.79 ± 0.06 g N-NH_4^+/L. However, this value was abundantly below 3.0-3.5 g N-NH_4^+/L, which is the typical critical value for the inhibition of methanogenic bacteria (Chen et al., 2008). The AD step under thermophilic conditions reached the steady state in 30 days after inoculum, approximately 0.5 HRT less than mesophilic process. Also in this case, the process was easily controlled since no relevant VFA accumulation was detected (maximum value 1.01 g COD/L, day 13) and pH was easily maintained above 7.5. Starting from day 22, VFA/P.Alk ratio was permanently below 0.20 mg acetic acid/mg $CaCO_3$. Figure 3B shows the trends of total-partial alkalinity and VFA/P.Alk ratio along the experimentation: when the steady state was achieved, partial and total alkalinity had average concentrations of 2,524 ± 279 and 3,788 ± 314 mg $CaCO_3$/L; VFA/P.Alk ratio was equal to 0.12 ± 0.03. Figure 3C shows the biogas production rate (GPR) and methane percentage in the produced biogas during AD mesophilic trial. Despite the fast increase of methane content (above 60% v/v after two weeks of operation or 1.0 HRT approximately), the GPR trend showed a slower increase in the course of process stabilization and in parallel to the progressive increase of the applied OLR (roughly up to 1.5 kg VS/m^3 d) during the start-up phase. At the steady state, GPR was 1.38 ± 0.08 m^3 biogas/m^3 r d; the percentage of methane detected in the biogas was 63 ± 3%; most of remaining part was CO_2 (32 ± 3%). The specific gas production (SGP) was found to be equal to 0.40 ± 0.02 m^3 biogas/kg VS. Similarly to the mesophilic condition, in the AD thermophilic trial the OLR was increased from 0.8 to 1.5-1.8 kg VS/m^3 d during the start up, and then it was maintained close to 2.5 kg VS/m^3 d over the course of the run. The progressive increase of the OLR reflected the trend of GPR, which was 1.80 ± 0.09 m^3 biogas/m^3 r d in the steady state, while methane biogas content exceeded 60% in 0.5 HRT approximately (Figure 3D).





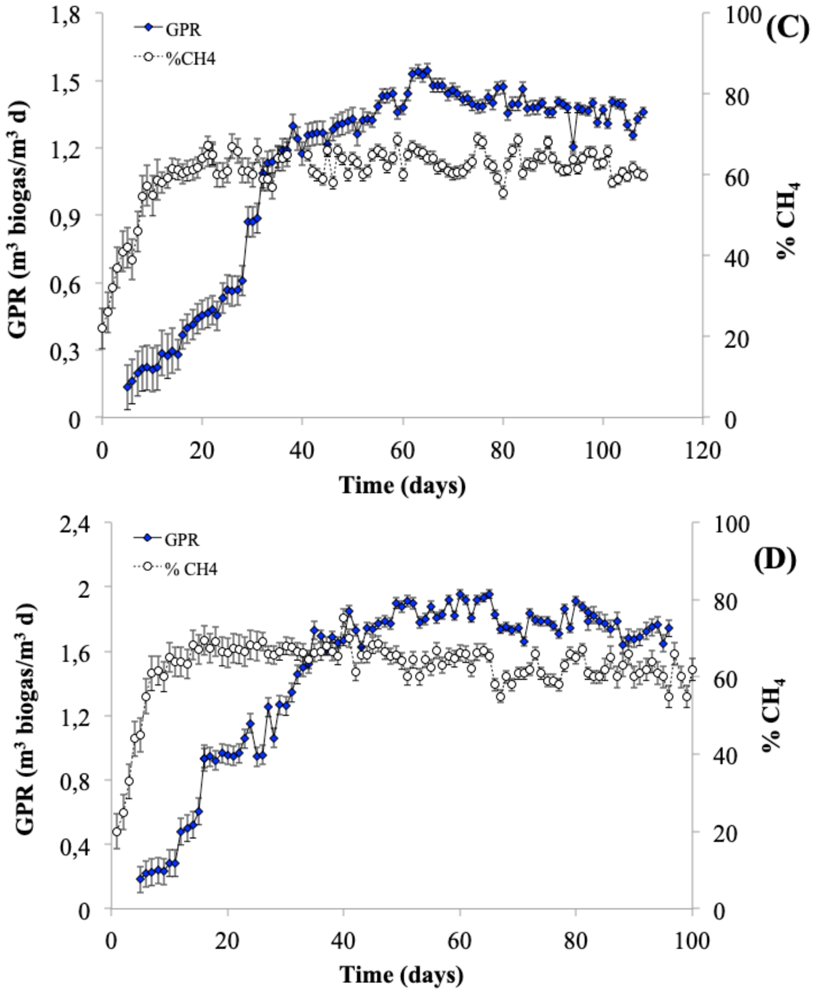


Figure 4–3. Alkalinity and VFA/P.Alk ratio during mesophilic (A) and thermophilic (B) anaerobic digestion stage; GPR and CH_4 percentage in the biogas during mesophilic (C) and thermophilic (D) anaerobic digestion stage.

As for the GPR, also the SGP at 55 °C was higher (Figure 4; t – test, alpha value = 0.01) than SGP quantified at 37 °C, and equal to 0.45 ± 0.06 m^3 biogas/kg VS. This was due to the higher solid abatement, which accounted for 71 ± 5% and 74 ± 4% for TS and VS at 55 °C, and 43 ± 4% and 45 ± 4% for TS and VS at 37 °C.

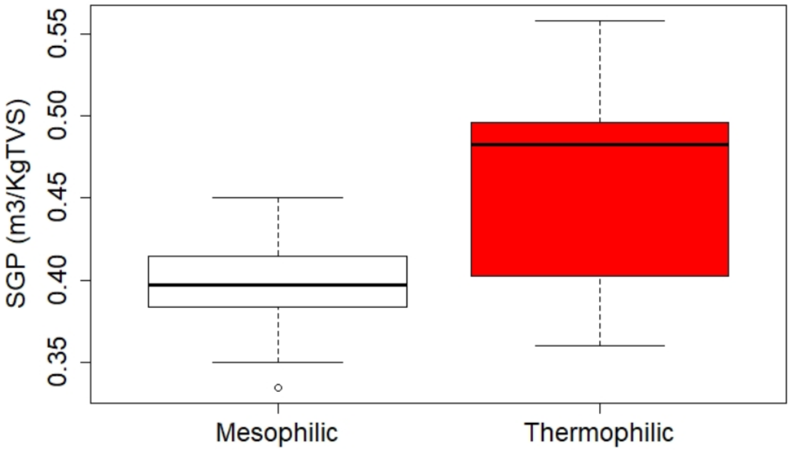


Figure 4–4. Boxplot of SGP for mesophilic and thermophilic Anaerobic Digestion trials.

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

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

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

Compared to literature values, the performances obtained herein were similar to those reported for mixed sludge digestion rather than sludge-OFMSW mixture co-digestion. For the mesophilic treatment of mixed sludge, Mattioli and co-workers (2017) quantified 0.45 Nm^3 biogas/kg VS (SGP), similar to the average value of thermophilic AD in this study. The authors reported a higher SGP, 0.50 Nm^3 biogas/kg VS, for sludge-OFMSW co-digestion.

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

4.3.4. Mass and energy balance

The mass balance discussed in this paragraph is illustrated in detail in Figure 5.

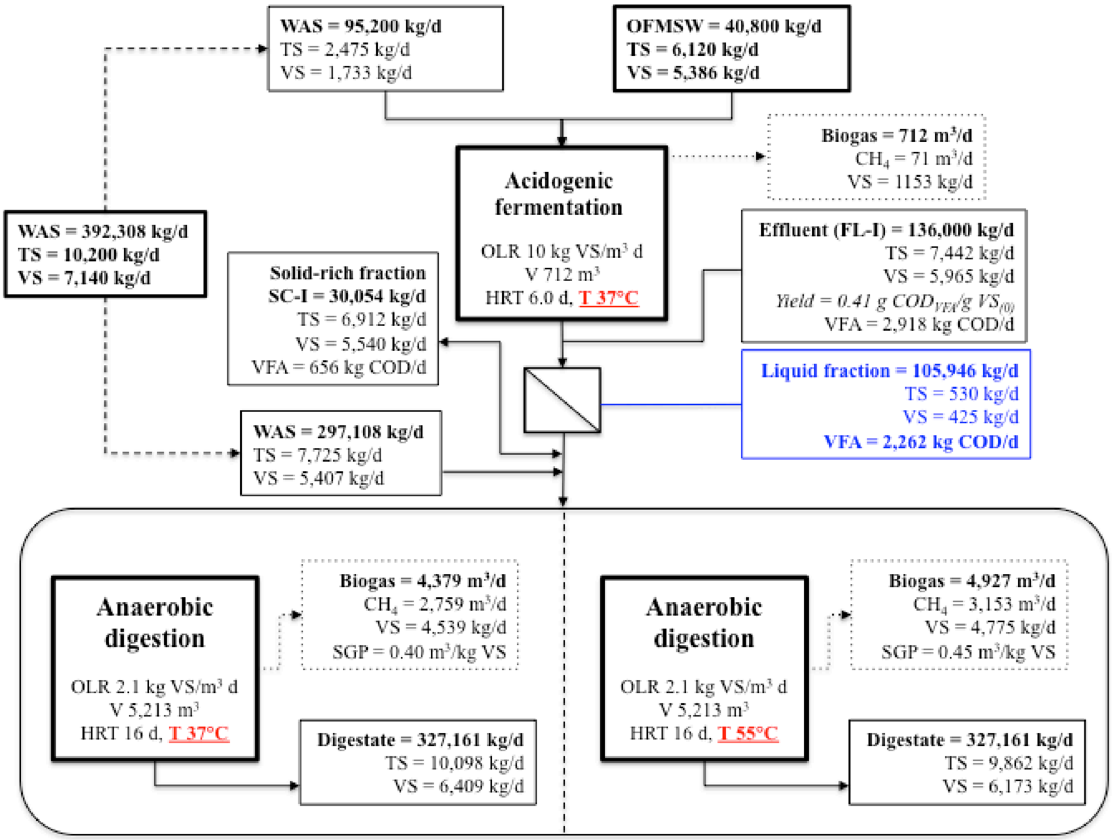


Figure 4–5. Flow rates and mass balance of the scaled up anaerobic process: mesophilic acidogenic fermentation followed by mesophilic or thermophilic anaerobic digestion.

The data analysis of each separate pilot reactor was transferred to an industrial plant of average size, identified as 170,000 PE. Based on the specific production reported in paragraph 4.2.3., the inlet amount of WAS corresponds to 10,200 kg TS/d. A smaller amount is driven to the fermentation reactor (around 24%) and the rest to the AD step (76%) in order to dilute the SC overflow. The inlet amount of the pre-treated OFMSW is 6,120 kg TS/d, based on a dry matter of 15% and a recovery of 80% of TS from the OFMSW screw-press pre-treatment (Majone et al., 2017). For the fermentation process, only the mesophilic condition was considered technically feasible and the relative yield of 0.41 g COD_{VFA}/g $VS_{(0)}$ was taken into account. The bio-waste mixture is conveyed to the mesophilic fermenter with a TS and VS level of 6.3% and 5.2% w/w. As a consequence, the applied OLR was approximately 10 kg VS/m^3 d. The gaseous effluent flow rate out of the acidogenic fermenter is 712 Nm^3/d, corresponding to 1,153 kg VS/d, roughly equal to the 16% of the VS influent amount. The solid/liquid separation unit allows obtaining two streams: the liquid fraction and the SC overflow. The liquid stream has a volumetric flow rate of 105,946 kg/d (almost 80% of the total), corresponding to 2,262 kg COD_{VFA}/d, to be used for the synthesis of bio-products with higher added value than methane (Tamis and Joosse, 2015). In fact, a production of 0.54 tons of marketable biopolymer (PHA) per year is potentially feasible, based on average yield of 0.40 COD_{PHA}/COD_{VFA}, typically quantified in the aerobic MMC-PHA production technology (Valentino et al., 2017). The SC overflow has a flow rate of 30,054 kg/d, which is mixed with 297,108 kg/d of excess thickened WAS. This dilution is necessary to decrease the solid content of the SC overflow, approximately from 23% w/w to 4.5% w/w (TS basin), before being fed into the final AD step. The first scenario provides for the AD step in mesophilic condition (experimental SGP of 0.40 Nm^3/kg VS); the mesophilic AD step produces 4,379 Nm^3/d of biogas. The heat requirements and energy balance analyses were assessed for a working volume of 712 m^3 and 5,213 m^3 for fermentation and methanogenic reactors respectively. Considering the thermal yield of the Combined Heat & Power unit (CHP) of 0.5 (Valentino et al., 2018), the thermal energy produced is 58,577 MJ/d, if AD is performed at 37°C. Indeed, the thermal balance is closed positively because the estimated produced thermal energy overcomes the consumed thermal energy (for heating and heat dispersion balance) of about 36% (Figure 6). In thermophilic conditions (SGP of 0.45 Nm^3/kg VS), the biogas production was higher and equal to 4,927 Nm^3/d; however, the thermal energy consumed for process operation (65,108 MJ/d) is similar to the produced thermal energy (64,875 MJ/d) (Figure 6). In practise, the similarity does not guarantee the process self-sustainability, making this configuration not scalable at full scale. Besides the complete energetic sustainability of the two steps performed at 37°C, the revenues from sale of generated electricity (13.03 MWh/d, from electrical CHP yield of 0.4; Valentino et al., 2018) has been quantified to 609,605 €/year. This potential economic income makes this approach more attractive than the traditional or the two-phase AD process treating excess sludge only (Leite et al., 2016).

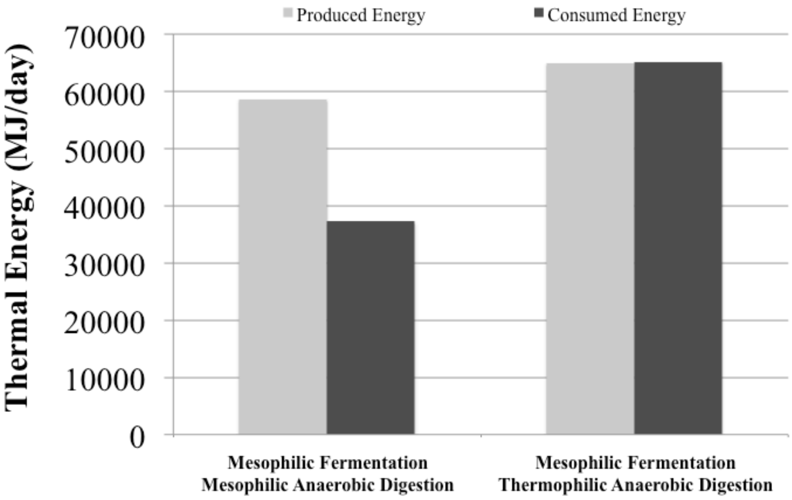


Figure 4–6. Consumed thermal energy and produced thermal energy for the two investigated process configurations.

4.4. Conclusions

This work demonstrated that biogas production is not the only option for anaerobic treatment of WAS-OFMSW mixture produced in an urban scenario. If separated from AD process, the fermentation step allows recovering a consistent amount of solubilized COD, in form of VFA, that can be used as platform chemicals for the synthesis of other bio-products with higher market value than biogas. The mass and energy balances of the anaerobic fermentation-digestion reactors revealed that the overall process was thermally self-sustaining if both stages were performed at mesophilic temperature (37°C). Under this adopted condition, the scaled-up system (170,000 PE) had the 36% surplus of thermal energy (21,360 MJ/d), and a potential economic income from the produced electricity (13.03 MWh/d) of 609,605 €/year. The production of 2,262 kg COD_{VFA}/d makes this approach even more attractive since the VFA-rich valuable stream can be used in other exploitable routes. In fact, this approach represents the application of circular economy concept, focused on the secondary carbon streams treatment process, designed for bioenergy recovery and bio-products creation. Indeed, future works need to be addressed to the development of a new multi-utility bio-refinery, possibly integrated in the existing facilities of an urban context, where each process unit is separately optimized for a specific target, leading to relevant economic and environmental advantages.

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5. Chapter 5. Optimization of urban waste fermentation for volatile fatty acids production

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PhD Candidate contribution: Formal Analysis, Scientific investigation, Original draft writing and review

Moretto, G., Valentino, F., Pavan, P., Majone, M., Bolzonella, D., 2019. Optimization of urban waste fermentation for volatile fatty acids production.
Postprint version of the article published in: *Waste Management* 92, 21-29. https://doi.org/10.1016/j.wasman.2019.05.010.

5.1. Introduction

Waste management and disposal is one of the most pressing issues mainly due to the exponential population increase in the last decades, especially in urban areas where the majority of the population is located (Pfaltzgraff et al., 2013). The main organic refuses produced in this context are food waste, also known as the organic fraction of municipal solid waste (OFMSW), and biological sludge coming from the wastewater treatment. In Europe, the average organic matter production expressed as total solid (TS) per day (d) *pro capita* (person equivalent, PE) is approximately 55 g TS/(PE) d of OFMSW and 39 g TS/(PE) d of biological sludge (Colombo et al., 2017; IPCC, 2006). It is quite clear that these kinds of organic waste are highly available especially in urban areas and at present time they are handled and treated separately. The sludge is typically digested, dewatered and then sent to final disposal, and the OFMSW is sent to composting. The separate treatment of biological sludge and OFMSW is generally not convenient from both energetic and environmental point of view. As a matter of fact, anaerobic digesters treating sludge in wastewater treatment plants (WWTPs), especially waste activated sludge (WAS), are often low loaded and underperforming (Bolzonella et al., 2005), while aerobic composting is a highly energy consuming process. The advantages of a combined treatment of OFMSW and WAS have already been exploited by the anaerobic co-digestion approach, with the experiences reported in previous studies (Mata-Alvarez et al., 2014). This technology directs all the organic matter into a single step, in which the conversion into biogas and energy is realized (Scarlat et al., 2018). Digestate is also produced together with biogas and energy, with environmental concerns regarding its further stabilization and disposal and the possible presence of heavy metals, pharmaceuticals and/or other pathogens (Mata-Alvarez et al., 2014). OFMSW mixing with WAS generates several benefits, such as dilution of potential toxic compounds and improved nutrient balance (Zahedi et al., 2016). A more effective application of this approach can be found in urban contexts where the OFMSW is coming from a source separate collection or a door-to-door collection, since the biodegradability of the collected waste improves substantially due to the increase of organic matter content and the decrease of inert materials (Bernstad et al., 2013). Novarino and Zanetti (2012) reported the application of a mechanical pretreatment method to further separate the inert material and homogenize the organic matter, enhancing the anaerobic co-digestion process. The integration of OFMSW and WAS treatment can be easily realized in existing WWTPs, where anaerobic digesters are in most cases already present, in order to improve the WWTP energy balance. Authors reported some co-digestion experiences in full-scale plants, such as the Rovereto WWTP, located in the Trento province in northern Italy, in which the OFMSW and mixed sludge co-digestion was implemented in 2014 (Mattioli et al., 2017). In the Treviso province (northeast Italy) the co-digestion approach of OFMSW and WAS in the full-scale WWTP was proposed and implemented since 1999 (Bolzonella et al., 2006; Pavan et al., 2000). In this specific urban scenario, the waste separate collection is very efficient and reaches 87.9% on the total wastes (ISPRA, 2017) making the organic waste treatment integration an eligible way for valorization and recovery of the organic matter. A possible and innovative approach for the realization of this treatment integration can be found in an urban biorefinery. The urban biorefinery represent a technology chain in which the organic material of urban waste can be converted into new added-value bio-based products (Valentino et al., 2018). Bio-based products obtainable in an urban biorefinery through the exploitation of organic waste are biofuels (Stephen and Periyasamy, 2018), platform chemicals (Kiran et al., 2014), and bioplastics (Valentino et al., 2017). Some of the most important intermediates that allow the conversion of organic waste into these valuable bio-based products are volatile fatty acids (VFA), which in most cases are the direct precursors for biopolymer synthesis, such as polyhydroxyalkanoates (PHA) obtained from pure and mixed microbial culture (MMC) (Valentino et al., 2014). VFA are produced during the anaerobic fermentation process of almost all kinds of biodegradable organic waste (Strazzera et al., 2018). Previous studies reported experiences of anaerobic fermentation on dewatered sludge (Hao & Wang, 2015), cellulosic substrates (Keating et al., 2013), cheese whey (Colombo et al., 2017; Valentino et al., 2015) and OFMSW (Chen et al., 2017;

Girotto et al., 2017; Korkakaki et al., 2016). In a platform where anaerobic and aerobic processes are combined, VFA obtained from waste fermentation are intermediate chemicals for the conversion of organic matter into the aforementioned biodegradable added-value products (Koller et al., 2017). Indeed, in a scenario where the OFMSW is coming from a highly efficient source separate collection and the anaerobic co-digestion of OFMSW and WAS is already implemented, the urban biorefinery concept finds its perfect integration. The municipality of Treviso (northeast Italy) is a representative example where the OFMSW-WAS mixture is currently sent to anaerobic co-digestion. Indeed, the hypothesis of driving part of this organic source into an anaerobic fermentation step for the VFA production could be an eligible way for an efficient urban organic waste management. Within this route, the combined VFA and methane production from OFMSW-WAS mixture has been recently demonstrated (Valentino et al., 2019) in the same urban context. However, in order to enhance the PHA synthesis and productivity, the fermentation of OFMSW-WAS mixture needs to be optimized, maximizing the VFA production and, as a consequence, the PHA potentially obtainable. In this study, different fermentation conditions were tested by means of batch tests on a mixture of OFMSW and WAS. These batch trials were conducted in order to find the best working conditions for the fermentation process, namely optimum pH value and temperature. Once the best condition was found, a continuous lab scale trial in a continuous stirred tank reactor (CSTR) was set-up to better represent an acidogenic fermentation process under different applied HRT and OLR.

5.2. Materials and Methods

5.2.1. Substrate characterization

The substrates used in this study were thickened WAS and pre-treated OFMSW both available inside the Treviso WWTP. The WAS has been collected from the static thickener of the full-scale plant; the OFMSW came from the source sorted collection in 50 districts of the Treviso Province and was transferred to the full-scale WWTP after its pre-treatment (squeezing and homogenization) in a dedicated plant. The mixture used for all trials was composed by volumetric fractions of 65-70% thickened WAS and of 30-35% squeezed OFMSW. Thickened WAS and squeezed OFMSW had TS contents of 30 ± 1 g TS/kg and 139 ± 7 g TS/kg respectively, with a volatile solid (VS) fraction of 70% VS/TS for WAS and 90% VS/TS for OFMSW. The high VS/TS ratio of the OFMSW was favoured by the quality of the source-sorted collection, which effectively increased the readily biodegradable organic content. On the other hand, the relatively low nutrients (nitrogen and phosphorus) level in the squeezed OFMSW was counterbalanced by the high volumetric fraction of WAS, which contributed to the final COD:N:P ratio of 100:2.5:0.7 in the mixture. Table 1 shows the average values obtained from the chemical-physical characterization of both separated streams, of the final urban waste mixture and of the waste mixture after thermal pre-treatment.

Table 5–1. Chemical-physical characteristics of the thickened WAS, pre-treated OFMSW, urban waste mixture and thermally pre-treated substrate used for batch tests and continuous trial.

Parameter	Unit	Thickened WAS	Pre-treated OFMSW	Urban waste mixture	Thermally pre-treated urban waste mixture
Total Solid (TS)	g/kg	30 ± 1	139 ± 19	62 ± 7	58 ± 6
Volatile Solid (VS)	g/kg	21 ± 1	125 ± 14	50 ± 2	46.4 ± 0.9
VS/TS	%	70 ± 4	90 ± 2	81 ± 2	83 ± 1
COD_{SOL}	g COD/L	0.15 ± 0.06	81 ± 7	32 ± 3	38 ± 3
VFA	g COD/L	-	8.3 ± 0.6	2.4 ± 0.3	2.2 ± 0.2
pH	-	6.6 ± 0.2	4.3 ± 0.3	5.1 ± 0.3	5.0 ± 0.2
COD_{VFA}/COD_{SOL}	-	-	0.11 ± 0.02	0.08 ± 0.02	0.06 ± 0.01
Total Kjeldahl Nitrogen (TKN)	g N/kg TS	42 ± 3	27 ± 1	35 ± 2	36.0 ± 0.8
Phosphorus (P)	g P/kg TS	15 ± 1	7.0 ± 0.4	10 ± 1	10 ± 2

5.2.2. Batch fermentation tests

Batch tests were performed in order to investigate the effects of different combinations of temperature and initial pH on urban waste fermentation. Mesophilic (37°C) and thermophilic (55°C) conditions were tested. In parallel, another mesophilic trial was performed with the same urban waste mixture after a thermal pre-treatment (72°C for 76 h). For each of the three conditions, three different initial pH values (5.0, 7.0 and 9.0) were investigated. Sodium hydroxide (NaOH) was added to reach both initial pH values of 7.0 and 9.0; no chemicals addition was necessary for the tests at initial pH value of 5.0 since it was the typical pH of the urban waste mixture. Each test was performed in duplicate, in 1.0 L glass bottles sealed with a silicon plug and an aluminium seal (working volume 0.75 L). No inoculum was added since more than a sufficient amount of fermentative microorganisms were already present in the initial waste mixture. The investigated conditions are displayed in Table 2. Batch fermentation tests were monitored up to ten (trials A-B) and five (trial C) days. Liquid samples (15.0 mL) were collected each day for VFA analysis and pH measurements as well as for ammonium ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$) and soluble COD (COD_{SOL}). The slurry was manually mixed twice a day, one hour before and 6 hours after each sampling. The bottles were opened for the sampling and then maintained under N_2 flux for 20 min to reestablish anaerobic conditions.

Table 5–2. Summary of the operating conditions investigated in the nine batch tests.

Temperature (°C)	Thermal pre-treatment	Initial pH	Test name
55	-	5.0	A1
55	-	7.0	A2
55	-	9.0	A3
37	-	5.0	B1
37	-	7.0	B2
37	-	9.0	B3
37	72°C, 76 h	5.0	C1
37	72°C, 76 h	7.0	C2
37	72°C, 76 h	9.0	C3

5.2.3. Urban waste fermentation process in a CSTR

The best performing condition obtained in batch tests was further investigated by means of a fermentation process in a 6.0 L CSTR that was maintained at a fixed temperature (37°C) by an external thermostatic jacket and stirred with a mechanical impeller at 80 rpm. No inoculum was added. The reactor was fed once per day, in a semi-continuous manner. Acidogenic fermentation was started up and initially conducted with an OLR of 7.7 kg VS/(m^3 d), which was then increased up to 9.3 and 11.3 kg VS/(m^3 d). Each condition was characterized under steady state and compared in terms of VFA yields and composition. The increasing OLR was combined with a corresponding decrease of HRT, from 6.0 to 5.0 and 4.1 days. The reactor was not equipped with pH control; however, alkaline fermentation was performed by adjusting the pH of the feedstock at pH 9.0. The effluent was collected before the feeding for pH measurements and VFA analysis (up to five times a week) as well as TS, VS, ammonium, phosphate and COD_{SOL} analysis (twice per week).

5.2.4. Analytical methods

Analyses were conducted according to Standard Methods (APHA, AWWA, WEF, 1998). Volatile fatty acids were determined using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) (T = 250°C). Samples were analysed through an Agilent J&W DB-FFAP fused silica capillary column (15 m length, 0.53 mm i.D. x 0.5 mm film) using hydrogen as carrier. The inlet was working in split mode, with a split ratio of 20:1. The instrument was programmed with a ramp temperature from 80°C to 100°C (10°C/min). Before GC analyses, samples were centrifuged at 4,500 rpm for five minutes and the supernatant was filtered at 0.2 µm using acetate cellulose syringe filters (Whatman).

5.2.5. Data analysis

According to the sampling described above, data were collected daily and analysed at the end of each test batch. In the CSTR experiment, data were processed according to the adopted frequency of sampling (paragraph 5.3.2.). In order to better represent VFA distribution, the molar ratio between odd numbered acids and the total concentration was determined. The nutrient concentration, related to the release of ammonium and phosphate in the medium, was also quantified by means of the COD:N:P ratio expressed in grams. The substrate solubilisation was calculated by the ratio between the final soluble COD (as net concentration subtracted to the initial COD_{SOL}; $COD_{SOL(0)}$) and the initial VS of the feedstock ($VS_{(0)}$), as it follows:

$$Solubilisation = \frac{(COD_{SOL} - COD_{SOL(0)})}{VS_{(0)}} \qquad (1)$$

The VFA yield was determined by the ratio between the produced VFA (as net concentration subtracted to the initial VFA; $COD_{VFA(0)}$) and the initial VS of the feedstock ($VS_{(0)}$), as it follows:

$$Yield = \frac{(COD_{VFA} - COD_{VFA(0)})}{VS_{(0)}} \qquad (2)$$

Substrate solubilisation and VFA yield were calculated for each batch test and for the CSTR process. In batch tests with the thermal pre-treated substrate (C1, C2 and C3) and in the CSTR process both substrate solubilization and VFA yield were calculated considering the substrate after thermal pre-treatment as starting point.

5.3. Results and Discussion

5.3.1. Batch fermentation tests

All batch tests were started with the same urban waste mixture; indeed, the initial solids as well as the macronutrients (nitrogen and phosphorus) content were similar in all trials. The urban waste mixture was extremely homogeneous since the OFMSW was squeezed in the pre-treatment and resulted as liquid slurry after mixing with thickened WAS. VFA concentrations were monitored daily for each batch test. The main results are summarized in Table 3.

5.3.1.1. Effect of temperature on the urban waste fermentation in batch tests

Figure 1 shows the VFA concentrations in the thermophilic (A), mesophilic (B) and pre-treated mesophilic (C) batch tests, which have been performed at initial pH value of 5.0 (1), 7.0 (2) and 9.0 (3). In terms of VFA production rates and yields, the mesophilic condition was better performing. Some literature studies report that mesophilic temperature ranges are preferable to thermophilic ones, not only for OFMSW (Garcia-Aguirre et al., 2017) but also for sewage sludge (primary or WAS) fermentation process (Morgan-Sagastume et al., 2015). Under thermophilic condition, VFA production lagged in the first two days and started noticeably only from the third day, especially in those trials started under alkaline pH value (9.0). This observation was in line with previous studies (Peces et al., 2016; Jiang et al., 2013), where the initial lower acidification yields observed at 55°C was due to the slower adaptation of the thermophilic culture. In the tests performed under mesophilic condition, with or without thermal pre-treatment, the fermentation activity was immediately observed both at pH 7.0 and 9.0. Overall, at the same initial pH investigated, VFA concentrations obtained under condition B (18.6-30.0 g COD_{VFA}/L) and C (22.5-41.0 g COD_{VFA}/L) are higher than those obtained under condition A (4.7-27.5 g COD_{VFA}/L), as most of literature studies suggest (Jiang et al., 2013; Morgan-Sagastume et al., 2015; Wan et al., 2016). Thermophilic temperatures favoured the organic matter solubilisation (0.22-0.36 g COD_{SOL}/g $VS_{(0)}$) but a substantial part of the solubilized COD was not efficiently converted into VFA (0.06-0.52 g COD_{VFA}/g $VS_{(0)}$). Indeed, thermophilic tests A1-A2-A3 were characterized by higher COD_{SOL} (39-46 g COD_{SOL}/L) than mesophilic tests B1-B2-B3 (34-37 g COD_{SOL}/L), but lower final VFA content independently from the chosen initial pH. On the other hand, thermal pre-treatment (tests C1-C2-C3) increased the solubilisation yield (0.07-0.15 g COD_{SOL}/g $VS_{(0)}$) in the mesophilic fermentation process compared to tests B1-B2-B3 performed without thermal pre-treatment (0.06-0.09 g COD_{SOL}/g $VS_{(0)}$). The mesophilic fermentation process applied to an already solubilized substrate allowed increasing the VFA content (22.5-41.0 g COD_{VFA}/L), much more than tests B1-B2-B3 where the thermal pre-treatment was not applied (19.2-30.0 g COD_{VFA}/L), being VFA yields comparable in the fermentation series B (0.29-0.49 g COD_{VFA}/g $VS_{(0)}$) and C (0.26-0.57 g COD_{VFA}/g $VS_{(0)}$). Moreover, VFA production rate was substantially improved after the organic matter pre-treatment at 72°C: approximately 35 g COD_{VFA}/L were achieved in less than 5 days (at least under initial neutral and alkaline pH), which reasonably meant a doubled production rate compared to the thermophilic alkaline test A3 and mesophilic neutral-alkaline tests B2-B3. Usually, thermophilic temperatures enhance the hydrolysis process in which complex molecules are solubilized and become more available for the microorganisms' metabolism (Dahiya et al., 2015; Morgan-Sagastume et al., 2015). Indeed, process temperature strongly affected the nutrients release from the substrate. Ammonium and phosphate levels quantified at the end of tests C (0.94-1.22 g N-NH_4^+/L; 0.56-0.67 g P-PO_4^{3-}), where the organic waste was thermally pre-treated, were higher than those achieved in thermophilic series A (0.41-1.04 g N-NH_4^+/L; 0.28-0.43 g P-PO_4^{3-}) and mesophilic series B (0.43-0.54 g N-NH_4^+/L; 0.25-0.33 g P-PO_4^{3-}).

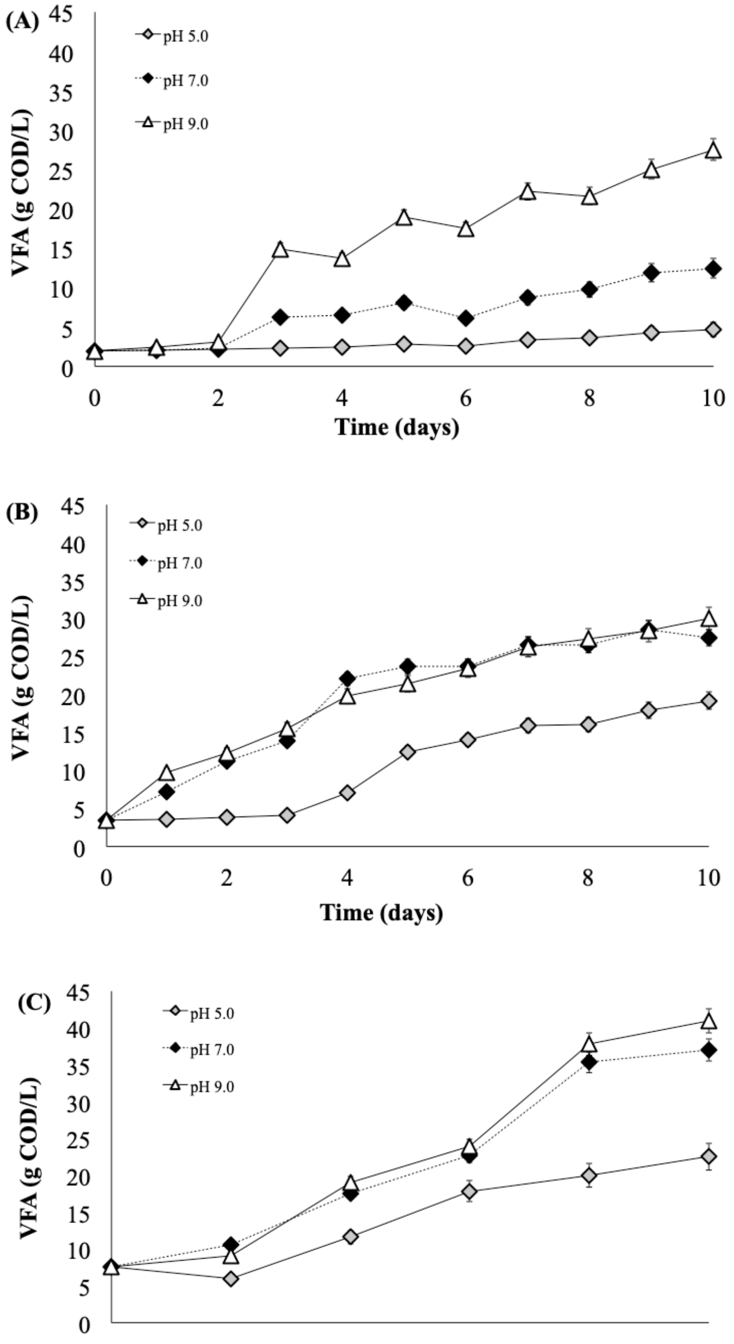

Figure 5–1. VFA concentration trends in the thermophilic batch tests A1-A2-A3 (a) under initial pH 5, 7 and 9; mesophilic batch tests B1-B2-B3 (b) under initial pH 5, 7 and 9; mesophilic batch tests with thermal pre-treatment C1-C2-C3 (c) under initial pH 5, 7 and 9.

Table 5–3. Final concentrations and fermentation performances obtained in all batch tests.

Parameter	Unit	Test name								
		A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Total VFA	g COD/L	4.7 ± 1	12.5 ± 0.7	27.5 ± 2	18.6 ± 0.9	27.5 ± 2	30 ± 2	22.5 ± 0.7	37 ± 2	41 ± 2
Acetic acid	g COD/L	3.4 ± 0.5	5.4 ± 0.3	7.1 ± 0.5	4.8 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	6.4 ± 0.3	7.3 ± 0.6	10.0 ± 0.8
Propionic acid	g COD/L	1.2 ± 0.4	2.5 ± 0.2	6.8 ± 0.2	0.8 ± 0.1	3.8 ± 0.3	6.9 ± 0.4	3.2 ± 0.1	9.6 ± 0.1	13.0 ± 0.3
Isobutyric acid	g COD/L	0	0	0.6 ± 0.1	0	0	0	0	0	0
Butyric acid	g COD/L	0	3.1 ± 0.1	6.9 ± 0.4	3.9 ± 0.1	3.8 ± 0.2	4.0 ± 0.3	6.3 ± 0.1	7.6 ± 0.3	8.2 ± 0.4
Isovaleric acid	g COD/L	0	0	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.4	1.2 ± 0.1	0	0	0
Valeric acid	g COD/L	0	0	4.2 ± 0.4	1.4 ± 0.1	5.0 ± 0.5	4.2 ± 0.5	1.8 ± 0.1	5.0 ± 0.5	5.8 ± 0.3
Caproic acid	g COD/L	0	1.5 ± 0.1	0.7 ± 0.3	3.5 ± 0.4	2.9 ± 0.1	2.8 ± 0.3	3.3 ± 0.2	3.0 ± 0.2	1.4 ± 0.1
Heptanoic acid	g COD/L	0	0	0	2.9 ± 0.1	3.9 ± 0.4	3.9 ± 0.1	1.6 ± 0.1	4.6 ± 0.3	2.4 ± 0.1
Soluble COD	g COD/L	39 ± 2	40 ± 3	46 ± 1	34 ± 2	35 ± 2	37 ± 3	41 ± 3	44 ± 4	45 ± 3
Ammonium	g $N\text{-}NH_4^+$/L	0.41 ± 0.05	0.88 ± 0.06	1.04 ± 0.05	0.43 ± 0.04	0.47 ± 0.06	0.54 ± 0.08	0.94 ± 0.07	1.07 ± 0.09	1.22 ± 0.09
Phosphate	g $P\text{-}PO_4^{3-}$/L	0.28 ± 0.04	0.37 ± 0.05	0.43 ± 0.07	0.25 ± 0.05	0.28 ± 0.05	0.33 ± 0.04	0.56 ± 0.05	0.62 ± 0.08	0.67 ± 0.07
Solubilisation	$gCOD_{SOL}/gVS_{(0)}$	0.22 ± 0.02	0.24 ± 0.04	0.36 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.13 ± 0.02	0.15 ± 0.02
Yield	$gCOD_{VFA}/gVS_{(0)}$	0.06 ± 0.03	0.22 ± 0.03	0.52 ± 0.03	0.29 ± 0.04	0.44 ± 0.05	0.49 ± 0.05	0.26 ± 0.03	0.50 ± 0.04	0.57 ± 0.03
COD:N:P*	g	100/1.1/0.7	100/2.2/0.9	100/2.3/0.9	100/1.3/0.7	100/1.3/0.8	100/1.5/0.9	100/2.2/1.4	100/2.4/1.4	100/2.7/1.5

*COD refers to COD_{SOL}; N and P refer to ammonium and phosphate

5.3.1.2. Effect of initial pH on the urban waste fermentation in batch tests

Another crucial parameter investigated in this work is pH. It is well known from literature that in anaerobic processes the pH affects the hydrolysis and fermentation rate, substrate solubilisation and methanogenic activity (Chen et al., 2007; Li et al., 2018; Maspolim et al., 2015). Generally, at relatively low reaction time methanogenic bacteria do not show remarkable activity and VFA can be accumulated without being consumed. However, hydrolytic and fermentative bacteria can be strongly inhibited if pH drops below 4.5-5.0, limiting the organic matter solubilisation and VFA accumulation (Zou et al., 2018). VFA productions caused a constant drop in pH during the tests, especially in those starting from alkaline conditions. In test A1 and B1, performed under initial pH 5, pH dropped from 5 (day 0) to 4.2 (day 10) and from 5 (day 0) to 4.13 (day 10) respectively. Test C1 showed a quicker drop from 5 (day 0) to 4.25 (day 5) in less time. Tests A2 and B2, performed under initial pH 7, were affected by a pH drop from 7 (day 0) to 5.88 (day 10) and from 7 (day 0) to 5.24 (day 10) respectively. In test C2, pH dropped from 7 (day 0) to 5.18 (day 5). In tests A3 and B3, both performed under initial alkaline conditions, pH dropped from 9 (day 0) to 6.51 (day 10) and from 9 (day 0) to 5.86 (day 10) respectively. In test C3, pH dropped from 9 (day 0) to 6.18 (day 5) in five days. Tests performed with the thermal pre-treated substrate showed an overall quicker pH drop, reaching almost the same final pH value in half of the time respect to other tests, in accordance with quicker VFA productions. Independently from the temperature range or pre-treatment adopted, the final VFA concentration reached its maximum value under initial alkaline pH. In condition A, B and C the maximum VFA concentrations achieved under alkaline pH were 27.5, 30.0 and 41.0 g COD_{VFA}/L respectively. At neutral pH, the maximum VFA concentrations achieved were 12.5, 27.5 and 37.0 g COD_{VFA}/L in condition A, B and C respectively. Apart from condition A, where the process temperature negatively affected the VFA production yield (in particular at initial pH 5.0 and 7.0), conditions B and C showed similar values both under initial neutral and alkaline pH. The drop in pH seemed to affect adversely VFA production only in tests A1, B1 and C1 starting from pH 5, since lower pH values were reached (around 4.2) due to the initial lower pH value. Hence, the fermentation process appeared to be more sensitive to the initial pH value at which tests were performed. These results revealed an interesting outcome about the optimum initial pH value for urban waste fermentation, also in agreement with previous literature studies. Considering the two separated sources, literature reports an optimum pH around 5.5 and 6.0 for the OFMSW fermentation (Jiang et al., 2013; Lim et al., 2008; Valentino et al., 2018), and an optimum pH ranging between 10-11 for the WAS fermentation

(Wu et al., 2016). Dogan and Demirer (2009) found that an increase in pH value from 5.5 to 6.5 decreased hydrolysis performance and VFA production in a CSTR fed with OFMSW at HRT 2 days and OLR 15 g VS/(L d). Since the substrate used for these fermentation batches is a mixture of OFMSW and WAS, both initial neutral and alkaline pH could be eligible working conditions in order to reach high VFA concentrations or high acidification yields, especially related to the solubilized COD (COD_{SOL}). In fact, one of the most relevant factors in the utilization of fermented organic feedstock for biopolymers synthesis (e.g. PHA) is the COD_{VFA} content in relation to COD_{SOL}. High VFA production associated with high COD_{SOL} could not be suitable for PHA production since the non-VFA COD_{SOL} stimulates the growth of non-PHA storing bacteria (Valentino et al., 2017). In order to ensure an appropriate PHA storing bacteria selection process, especially with nutrient rich carbon sources (e.g. the one studied in the present research) high COD_{VFA}/COD_{SOL} ratio is the key element to obtain a good PHA storing biomass selection process (Albuquerque et al., 2010). Therefore, the COD_{VFA}/COD_{SOL} ratio is a useful parameter to understand the fermentation performances. At constant temperature, the initial alkaline fermentation process increased the substrate solubilisation compared to initial acidic and neutral condition. The higher COD_{SOL} achieved (46, 37 and 45.0 g COD_{SOL}/L in test A3, B3 and C3 respectively) was also associated to the higher VFA production. VFA increased from 4.7 to 27.5 g COD_{VFA}/L in series A, from 18.6 to 30 g COD_{VFA}/L in series B and from 22.5 to 41 g COD_{VFA}/L in series C as initial pH increased from 5 to 9. In general, all tests under acidic pH showed lower COD_{SOL}/COD_{VFA} ratio. The lowest COD_{VFA}/COD_{SOL} ratio of 0.12 was obtained in test A1, where the activity of fermentative bacteria was strongly inhibited. In the other thermophilic trials, the final COD_{VFA}/COD_{SOL} ratio was equal to 0.31 (A2) and 0.60 (A3). In condition B, the COD_{SOL} concentrations were lower than those obtained in other conditions, but the remarkable VFA production increased the COD_{VFA}/COD_{SOL} ratio up to 0.55, 0.79 and 0.81 with initial acidic (B1), neutral (B2) and alkaline (B3) initial pH, respectively. The combined effect of thermal pre-treatment and initial alkaline condition in test C3 allowed achieving the highest value of 0.91 COD_{VFA}/COD_{SOL}, due to the enhanced substrate solubilisation combined with a subsequent mesophilic temperature range. Fermentation at initial neutral pH (test C2) also produced a VFA-rich stream with high COD_{VFA}/COD_{SOL} ratio (0.84). In terms of nutrients, ammonium and phosphate were higher in the tests conducted under alkaline fermentation. In the three series, independently from the applied temperature, nutrients concentration increased at increasing initial pH from 5.0 to 9.0. As observed for VFA production, in test C3 the ratio of nutrients related to the COD_{SOL} reached their maximum values (COD:N:P =

100:2.7:1.5).

5.3.1.3. VFA distribution

The distribution of VFA in the fermented feedstock is an important aspect of the process, especially if the production needs to be addressed to a specific group or single VFA. The effect of pH on the VFA composition and production has been widely investigated on different kind of waste (Cavinato et al., 2017; Girotto et al., 2017; Lee et al., 2014; Morgan-Sagastume et al., 2015). In general, the optimum pH level is strictly dependent on the type of waste. In addition, in the context of MMC-PHA production, the content of even numbered C-atoms VFA (3-hydroxybutyrate precursors; 3HBp) and the odd numbered C-atoms VFA (3-hydroxyvalerate precursors; 3HVp) affects monomers synthesis and consequently the polymer composition (Valentino et al., 2017). In the tests conducted in the present study, the qualitative VFA spectrum appeared to be regulated by the feedstock characteristics more than from the fermentation conditions. A dominance of acetic, butyric and propionic acid was observed, with a not negligible amount of valeric, caproic and heptanoic acid. Similarly, acetic, butyric and propionic acid were the most abundantly observed (respectively 27-32%, 18-23% and 15-17% COD basis) in the acidogenic fermentation of thermally pre-treated sludge (Morgan-Sagastume et al., 2011), or WAS fermentation (Feng et al., 2009). Valeric and caproic acid were in some cases also reported at lower concentrations in WAS fermentation (Morgan-Sagastume et al., 2011). Butyric acid was usually predominant (40-60%) in food waste fermentation followed by acetic acid (25-40%) (Girotto et al., 2017; Valentino et al., 2018). The relative VFA percentage seemed to be affected also by the initial pH, more than temperature. Series A showed a net dominance of acetic acid at pH 5.0 (72%) and 7.0 (43%); the initial alkaline condition increased the production of propionic, butyric and valeric acid, whose percentages were 25%, 25% and 15% of total VFA respectively. Acetic acid still remained relatively high at 26% but the molar ratio between [3HVp/(3HB+3HV)p] was more than doubled (0.36 mol/mol), if compared to the molar ratio quantified at acidic (0.17 mol/mol) and neutral (0.16 mol/mol) condition. In both mesophilic fermentation tests (series B and C), the [3HVp/(3HB+3HV)p] ratio similarly changed as pH increased from 5.0 (0.20 mol/mol) to 7.0 (0.40 mol/mol) and 9.0 (0.44 mol/mol) (Figure 2). These results were mainly related to the progressive increase of propionic (up to 13.0 g COD/L, 31% COD basis) and valeric acid (up to 5.8 g COD/L, 14% COD basis). Under initial neutral and alkaline conditions, the percentage of butyric acid was constant at 25% in the tests A2-A3, at 14-13% in the tests B2-B3, and at 21-20% in the tests C2-C3. The acidic fermentation seemed to enhance its production up to 3.9 g COD/L (21%) and 6.3 g COD/L (28%) in the tests B1 and C1 respectively, with the only exception of test A1, where the fermentative bacteria were inhibited and the fermentation process was only partially observed. Caproic acid was also abundant under the same conditions: 19% (3.5 g COD/L) and 10% (3.3 g COD/L) in tests B1 and C1 respectively. Some variability was observed in the percentage of acetic acid, even though it remained one of the most predominant VFA in all tests performed. Its percentage ranged between 20-28% with two maximum peaks of 43% (5.4 g COD/L, test A2) and 72% (3.4 g COD/L, test A1). Heptanoic acid was particularly affected by the temperature more than pH: it was detected only in the mesophilic tests with relative higher content in those trials performed without thermal pre-treatment: 16% (2.9 g COD/L, test B1), 14% (3.9 g COD/L, test B2) and 13% (3.9 g COD/L, test B3). Initial alkaline fermentation of squeezed OFMSW and thickened WAS mixture enhanced the production of some acids, propionic in particular, while lowering the production of butyric and caproic acid, which achieved higher level at acidic pH, as confirmed by previous studies (Horiuchi et al., 2002).

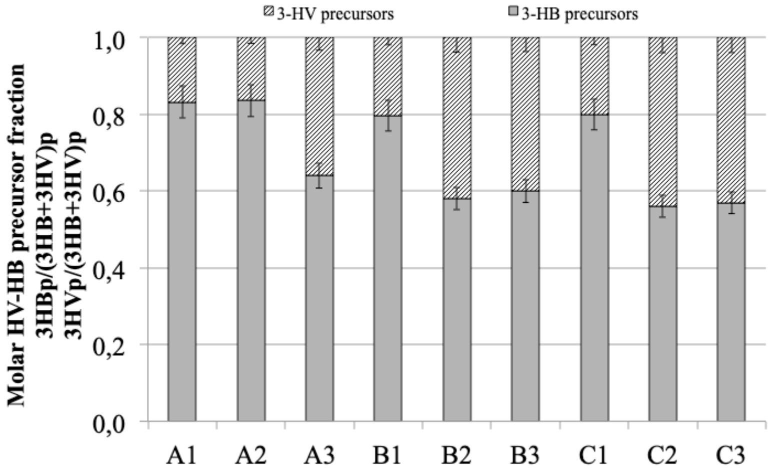

Figure 5–2. VFA distribution expressed as 3HB (even numbered C-atoms VFA) and 3HV (odd numbered C-atoms VFA) precursors obtained in batch tests.

5.3.2. Effect of different HRTs and OLRs on urban waste alkaline fermentation at mesophilic temperature in a CSTR

The best performing condition C3 (thermal pre-treatment followed by alkaline mesophilic fermentation) was reproduced in a CSTR for further investigations on the effects of different HRTs and OLRs on VFA production. The reactor was started with Run I; then, the OLR was increased by decreasing the HRT. The narrow OLR range [7.7 - 11.3 kg VS/(m^3 d)] was chosen in order to find a threshold or a limit OLR value, above which the process may be considered not feasible in terms of stable VFA production. Table 4 resumes all the parameters and the process yields analysed in the three conditions. The COD_{SOL} in the effluent reached similar values in the range 41 - 43 g COD/L in all the three trials and it was not correlated to the progressive increase of the OLR. Probably, the increase of OLR from 7.7 to 9.3 kg VS/m^3 d may not have been so high to justify an increase of the COD_{SOL}. At the highest OLR (11.3 kg VS/m^3 d, Run III) the expected increase of COD_{SOL} was not observed most likely due to the lower VS solubilisation (0.07 g COD_{SOL}/g $VS_{(0)}$), reduced by almost 50% if compared to Run I and II (0.12 g COD_{SOL}/g $VS_{(0)}$). The mixture acidification was remarkable from the 5th day (less than 1.0 HRT) and it was steadily maintained at high level, with VFA concentration above 35 g COD_{VFA}/L, for the whole Run I and II. In the last Run III, the reactor seemed to be overloaded, especially in the first part (immediately after the OLR increase), where the VFA dropped from 40 to 24 g COD_{VFA}/L. Over the course of the process, the VFA concentration was affected by frequent fluctuations (Figure 3) and in general the process suffered the imposed high rate condition. Other authors found out that VFA production was similarly affected by high substrate concentration and high applied OLR in a CSTR fed with OFMSW, while high HRT favoured substrate solubilization and VFA production (Argelier et al., 1998). The average VFA concentrations achieved were 39 ± 3, 38 ± 2 and 31 ± 4 g COD_{VFA}/L in Run I, II and III respectively. Run I exhibited the highest COD_{VFA}/COD_{SOL} ratio of 0.91. This high value makes this VFA-rich stream suitable for those applications that strictly required this feature (e.g. PHA from MMC; Valentino et al., 2017); Run II was also promising, providing a fermented stream with a ratio of 0.88. Both runs exhibited the highest VFA yields of 0.74 g (Run I) and 0.71 (Run II) g COD_{VFA}/ g $VS_{(0)}$; while Run III showed the lowest yields equal to 0.60 g COD_{VFA}/g $VS_{(0)}$. In terms of VFA composition, acetic and propionic acid were the most predominant in all three conditions (22-25% acetic and 22-28% propionic acid, COD basis), representing together the 50% of all species, in line with the observation of the previous C3 batch test. Butyric (20-22%) and valeric acid (10-13%) were the other predominant species in all three runs. The highest molar [3HVp/(3HB+3HV)p] ratio was obtained in Run II with 0.39 mol/mol, followed by 0.38 mol/mol in Run I, and 0.35 mol/mol in Run III. It seems that no significant correlation exists between those ratios and the applied OLR (or HRT). Nutrient concentrations were investigated in terms of ammonium and phosphate quantification. The highest concentrations of both nutrients (1.02 g $N\text{-}NH_4^+$/L; 0.28 g $P\text{-}PO_4^{3-}$/L) were found at higher HRT and lower OLR (Run I), with a slight difference respect to Run II, probably due to the sufficiently high process stability and the available prolonged time for the substrate solubilisation and degradation (Lee et al., 2014). The combined effect of higher HRT and lower OLR in an alkaline system (Run I) resulted as the best operating condition, in terms of VFA production and process stability (39 g COD_{VFA}/L; 0.91 COD_{VFA}/COD_{SOL}). The slight decrease in HRT and increase in OLR (Run II) resulted also as eligible process condition for the soluble COD acidification and further VFA utilisation (38 g COD_{VFA}/L; 0.88 COD_{VFA}/COD_{SOL}). Indeed, every process configuration can have different optimum condition for VFA production. In previous studies focused on OFMSW fermentation, where a different process configuration was applied (leach-bed reactor), an increase in leachate recycle rate favoured VFA production (Yesil et al., 2014). Depending on their composition and characteristics, each feedstock has its optimum fermentation conditions that can be tuned on the scope of future applications (Atasoy et al., 2018). Since the feedstock used in this study was composed by a mixture of OFMSW and WAS, the optimum fermentation conditions in terms of VFA level maximization can be intermediate between the one for OFMSW and WAS. Authors found out that increasing OLR lead to increasing VFA productions in OFMSW fermentation; however, OLR higher than 13 kg VS/m^3 d made the process unstable (Lim et al., 2008). Generally, high rate processes for OFMSW fermentation are considered unsuitable since the easy putrescible matter may lead to a quick VFA production, not properly buffered unless an efficient pH control system is applied (Valentino et al., 2018). On the other hand, prolonged HRTs (up to a maximum of 12 days) can enhance VFA production and process stability with complex substrates such as OFMSW and WAS (Lim et al., 2008; Zhou et al., 2018). Hong and Haiyun (2010) investigated the effects of the interactions of different parameters (food waste composition as co-substrate, HRT, OLR and pH) on food waste and dewatered excess sludge fermentation process through the application of experimental design methods. They observed significant correlations between pH and OLR, pH and HRT, pH and food waste composition, showing that with increasing OLR, VFA production decreased above 7.9 g VSS/(L d). VFA production also decreased with too long HRT, above 8.92 days, in accordance to the findings of the present study.

Other authors reported VFA concentrations up to 60 g/L (Girotto et al., 2017) with food waste fermentation. However, such high value was obtained in batch test and presumably a continuous process implementation could require effective strategies to maintain the pH at the desired value. Indeed, this high rate VFA production could probably cause a drastic drop in the pH with the risk of system imbalance. On the contrary, WAS fermentation generates less VFA than OFMSW (no more than 6 g COD/L) in the same temperature range (Chen et al., 2007; Girotto et al, 2017; Hao, Wang, 2015; Horiuchi et al., 2002; Strazzera et al., 2018). A total VFA concentration close to 40 g COD_{VFA}/L with limited amount of non-VFA soluble COD, as herein obtained (Run I and II) is quite promising since it would be a valuable carbon source for those applications that require high VFA productions in high organic loading rate processes (e.g. MMC-PHA production). In fact, higher VFA production would translate in higher volumetric PHA productivity [kg PHA/(L d)], which is defined on the overall PHA storage yield over the utilised VFA (Valentino et al., 2018). Economic viability of this approach needs to be addressed for the evaluation of associated costs of heating requirements and chemical supply, in a specific biorefinery scenario where the MMC-PHA production technology is intended to be integrated in existing WWTP facilities.

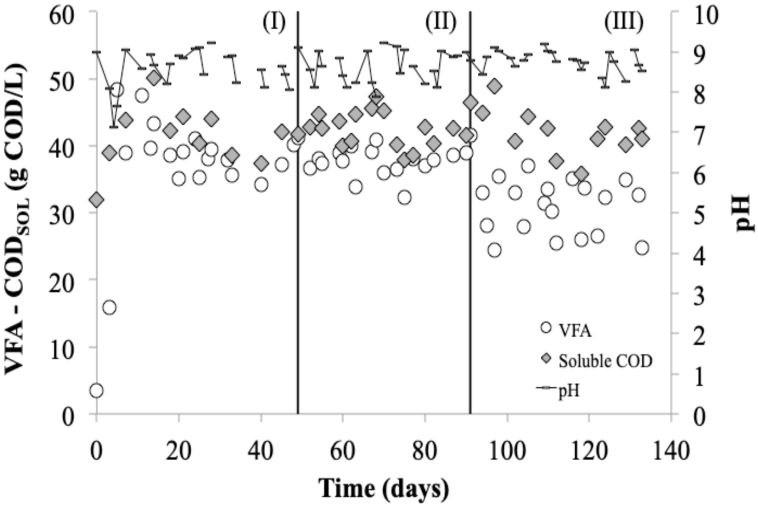

Figure 5–3. Soluble COD, VFA concentrations and pH obtained during the continuous lab-scale trial in the three conditions tested.

Table 5–4. Final concentrations and fermentation performances obtained in the three CSTR trials.

Operating condition	**Unit**	**CSTR trial**		
		Run I	**Run II**	**Run III**
HRT	d	6	5	4.1
OLR	kg VS/(m^3 d)	7.7	9.3	11.3
Parameter	**Unit**	**Run I**	**Run II**	**Run III**
TS	g/kg	54 ± 4	52 ± 4	57 ± 5
VS	g/kg	40 ± 3	41 ± 3	42 ± 4
VFA	g COD/L	39 ± 2	38 ± 2	31 ± 4
COD_{SOL}	g COD/L	43 ± 4	43 ± 3	41 ± 3
COD_{VFA}/COD_{SOL}	-	0.91 ± 0.04	0.88 ± 0.06	0.76 ± 0.07
[3HVp/(3HB+3HV)p]	mol/mol	0.38 ± 0.03	0.39 ± 0.02	0.35 ± 0.04
Solubilisation	g COD_{SOL}/g $VS_{(0)}$	0.12 ± 0.07	0.12 ± 0.06	0.07 ± 0.06
Yield	g COD_{VFA}/g $VS_{(0)}$	0.74 ± 0.05	0.71 ± 0.03	0.60 ± 0.06
Ammonium	g $N\text{-}NH_4^+$/L	1.02 ± 0.04	0.87 ± 0.05	0.82 ± 0.05
Phosphate	g $P\text{-}PO_4^{3-}$/L	0.28 ± 0.08	0.27 ± 0.02	0.17 ± 0.02

5.4. Conclusions

This study assessed how the fermentation process of a specific waste mixture of urban origin could be optimized in terms of organic matter solubilisation and VFA production. For this work, a mixture composed by thickened WAS (65-70% v/v) and squeezed OFMSW (30-35% v/v) was used as renewable feedstock. A first screening of batch trials revealed that alkaline pH (9.0) and mesophilic temperature (37°C), coupled with thermal pre-treatment (72°C, 76 h) gave the best performances in terms of maximum VFA concentration and COD_{VFA}/COD_{SOL} ratio. The same working conditions were proposed in a CSTR. The effluent VFA-rich stream was characterized by a stable production close to 40 g COD_{VFA}/L with consistent composition and limited amount of non-VFA soluble COD (0.91 COD_{SOL}/COD_{VFA}), at HRT of 6 days and OLR of 7.7 kg VS/m^3 d. The outcomes of this work could be used as a beneficial tool for the development of a biorefinery platform dedicated to the valorisation of urban organic waste and recovery of added-value bio-products (such as biopolymers) through a combined anaerobic/aerobic multi-steps process.

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6. Chapter 6. An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas

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Moretto, G., Russo, I., Bolzonella, D., Pavan, P., Majone, M., Valentino, F., 2020. An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas.
Postprint version of the article published in: *Water Research* 170, 115371. https://doi.org/10.1016/j.watres.2019.115371.

6.1. 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 feedstock 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 minimization (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 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.

6.2. Materials and Methods

6.2.1. Biorefinery process scheme

The biorefinery process scheme is represented in Figure 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).

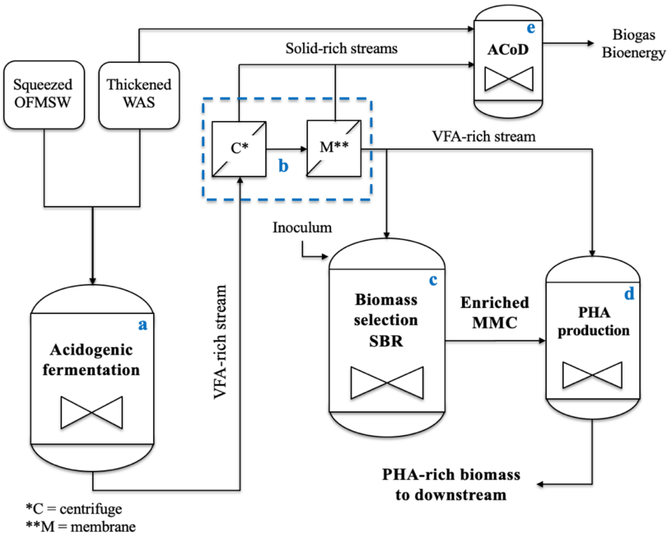


Figure 6–1. Biorefinery process scheme. (a): Acidogenic fermenter reactor; (b): Solid-liquid separation stage; (c): Aerobic PHA selection SBR; (d): Aerobic PHA accumulation reactor; (e): Anaerobic digester.

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.

6.2.2. 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 ($N\text{-}NH_4^+$), phosphorus as orthophosphate ($P\text{-}PO_4^{3-}$), Total Kjeldahl Nitrogen (TKN) and organic phosphorus (P). Substrates characterization is reported in Table 1.

Table 6–1. 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

6.2.3. 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 ultrafiltration 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 2,000 m^3 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) 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.

Table 6–2. Operating conditions of all pilot units and different runs operated.

Anaerobic Fermentation (AnF)					
Run	**HRT (d)**	**SRT (d)**	**OLR (kg VS/m^3 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/m^3 d)**	**Temperature (°C)**	
ACoD1	15	15	3.0 - 3.5	37	
ACoD2	15	15	3.0 - 3.5	55	

6.2.4. 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 6890N 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 4,500 rpm for five minutes, 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.

6.2.5. 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:

$$Solubilization = \frac{\left(COD_{SOL} - COD_{SOL(0)}\right)}{VS_{(0)}} \tag{1}$$

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:

$$Y_{VFA} = \frac{\left(COD_{VFA} - COD_{VFA(0)}\right)}{VS_{(0)}} \tag{2}$$

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:

$$X_A = VSS - PHA \tag{3}$$

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:

$$-qS^{feast} = {}^{\Delta COD_{SOL}}/_{t \cdot X_A} \tag{4}$$

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:

$$qP^{feast} = {}^{\Delta PHA}/_{t \cdot X_A} \qquad (5)$$

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

$$Y_{P/_S}^{feast} = {}^{\Delta PHA}/_{\Delta COD_{SOL}} \qquad (6)$$

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:

$$Y_{OBS}^{SBR} = {}^{X_A}/_{(OLR \cdot HRT)} \qquad (7)$$

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 3. Thermal and electrical yields of the Combined Heat & Power unit (CHP) are also listed.

Table 6–3. Reference parameters and boundary conditions applied for the energy balance.

Parameter	Unit	Value
BIOGAS[1]		
Low Heat Value Biogas	kJ/Nm3	23,012
COMBINED HEAT AND POWER (CHP)[1]		
Termical Energy yield	-	0.5
Electrical Energy yield	-	0.4
Total Energy yield		0.9
BOUNDARY CONDITIONS[1]		
Operative Temperature Anaerobic Processes	°C	37-55-72
Operative Temperature Aerobic Processes	°C	25
Water Temperature	°C	15
Air Temperature	°C	20
Ground Temperature	°C	25
HEAT TRANSFER COEFFICIENT[2]		
Outer Concrete Reactor Wall	W/(m^2°C)	0.7
Inner Concrete Reactor Wall	W/(m^2°C)	1.2
Floor	W/(m^2°C)	2.85
AERATION SYSTEM[3]		
Electric Motor Adsorbed Power	kWh/kgCOD*	0.753

*estimated based on removed COD.

[1] Micolucci, F., Gottardo, M., Pavan, P., Cavinato, C., Bolzonella, D., 2018. Pilot scale comparison of single and double-stage thermophilic anaerobic digestion of food waste. *J. Cleaner Prod*. 171, 1376-1385.

[2] Tchobanoglous, G., Burton F.L., and Stensel, H.D., 2003. Wastewater Engineering Treatment and Reuse, Metcalf & Eddy Inc., 4th ed. McGraw-Hill, New York.

[3] Foladori, P., Vaccari, M., Vitali, F., 2015. Energy audit in small wastewater treatment plants: methodology, energy consumption indicators, and lessons learned. *Water Sci. Technol.* 72, 1007-1015.

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).

6.2.6. 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 bioplastic, 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. 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.

6.3. Results & Discussion

6.3.1. Anaerobic line

6.3.1.1. 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 maintained itself between 5.0 and 5.5, due to the biological sludge buffering capacity (Cabbai et al., 2016). In Figure 2 are reported the VFA production and the COD_{VFA}/COD_{SOL}

ratios obtained at the end of every fermentation batch.

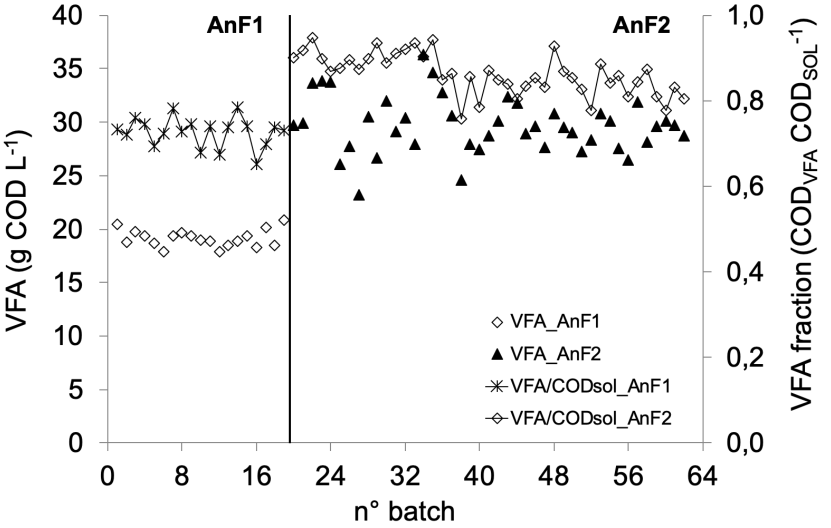


Figure 6–2. VFA concentrations and COD_{VFA}/COD_{SOL} ratio obtained during the two fermentation runs.

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 4 reports the average fermented stream characteristics obtained in both runs.

Table 6–4. 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]*	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

*ratio between odd numbered acids precursors (3HVp) and the sum of even and odd numbered acids precursors (3HB + 3HV)p

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.

6.3.1.2. 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/m^3 d approximately. The fed mixture was composed by 66% of 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/m^3 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 5.

Table 6–5. 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 $CaCO_3$/L	1890 ± 129	1598 ± 119
Total Alkalinity	mg $CaCO_3$/L	2903 ± 201	3192 ± 223
COD_{VFA}	g COD/L	0.30 ± 0.04	0.43 ± 0.07
Ammonium	mgN-NH_4^+/L	670 ± 101	879 ± 192
VFA/P.Alk	mg Acetic acid/mg $CaCO_3$	0.14 ± 0.03	0.16 ± 0.05

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 m^3_{biogas}/m^3 d and an SGP equal to 0.51 ± 0.02 m^3_{biogas}/kg VS. In ACoD1, the GPR and SGP were equal to 1.5 ± 0.1 m^3_{biogas}/m^3 d and 0.44 ± 0.02 m^3_{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 Figure 3 are shown the GPR and CH_4 content trends during the whole monitoring period.

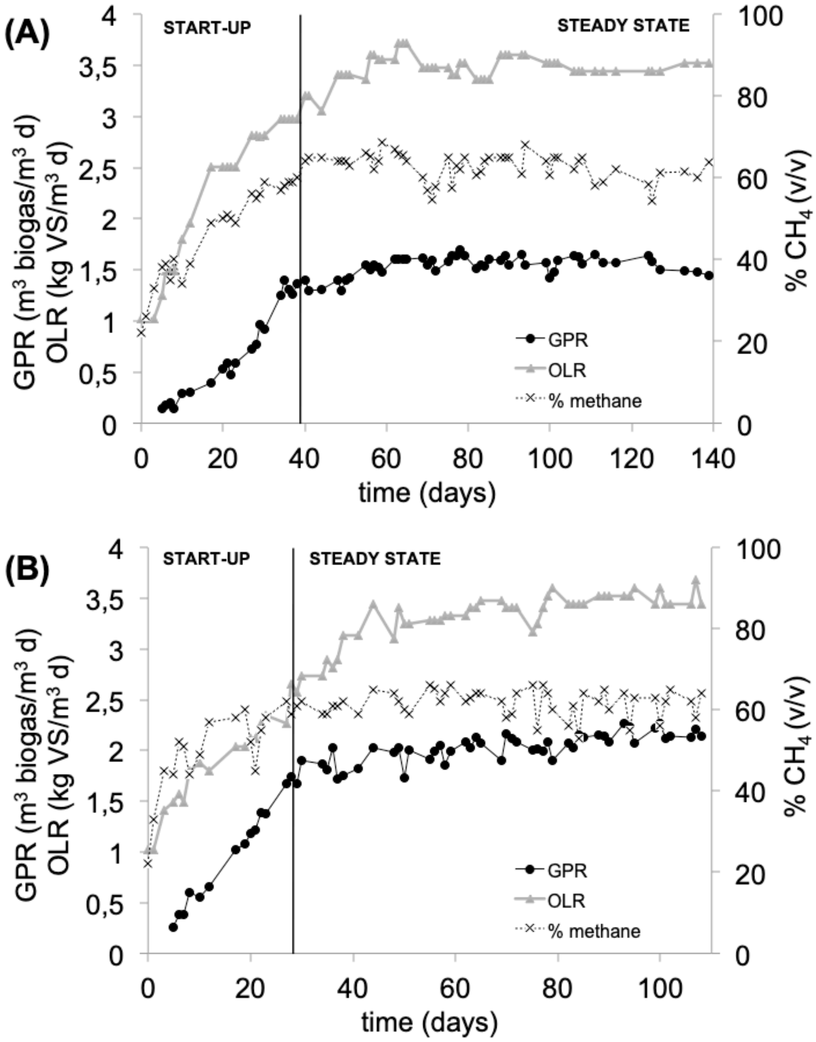


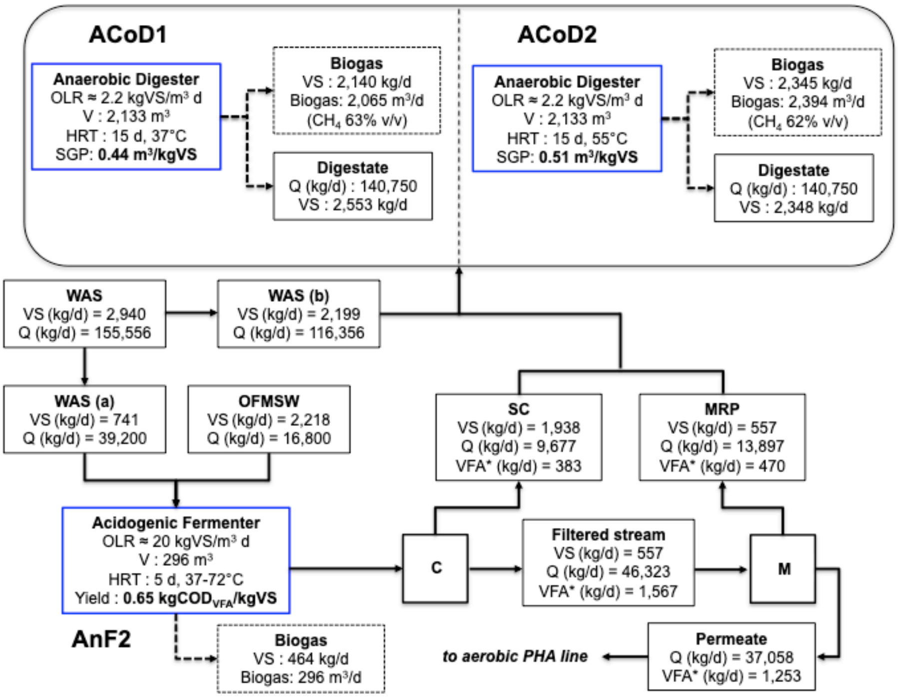
Figure 6–3. OLR, GPR and methane percentage trends of biological sludge, SC and MRP anaerobic co-digestion in mesophilic (A) and thermophilic (B) conditions.

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 m$^3_{biogas}$/m^3 d) to day 32 (1.34 m$^3_{biogas}$/m^3 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 m$^3_{biogas}$/kg VS with applied OLRs equal to 4.3 and 4.5 kg VS/m^3 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 m^3_{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/m^3 d) than the one adopted in this 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.

6.3.1.3. 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 (9,677 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; 1,175 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 (Figure 4).



*COD based.

Figure 6–4. 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.

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 (2,689 m^3/d) was higher than for the AnF2-ACoD1 process (2,361 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 (Figure 5).

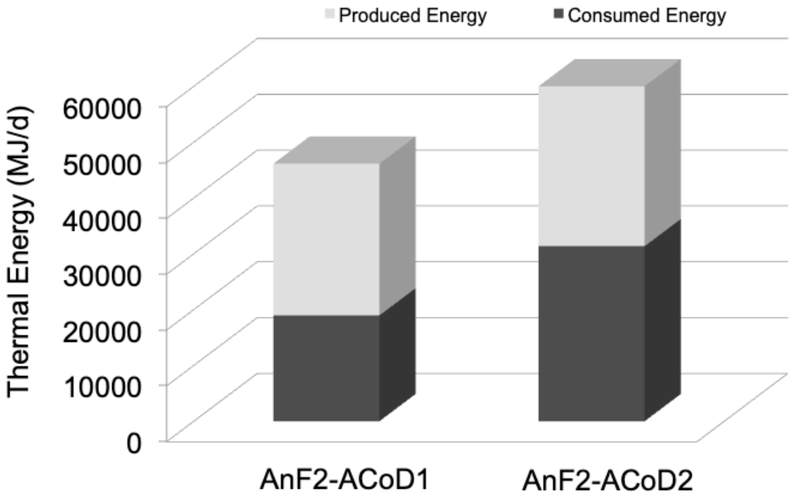


Figure 6–5. Thermal energy balance of the two anaerobic processes configurations AnF2-ACoD1 and AnF2-ACoD2.

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).

6.3.2. Aerobic PHA line

6.3.2.1. 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) (Figure 6).

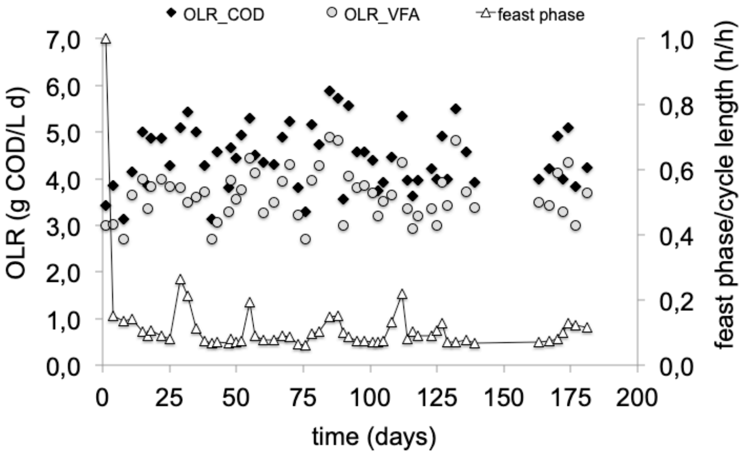


Figure 6–6. Trends of the organic loading rates (COD_{SOL} and VFA based) and feast phase length over the course of the single SBR run.

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 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 Figure 7.

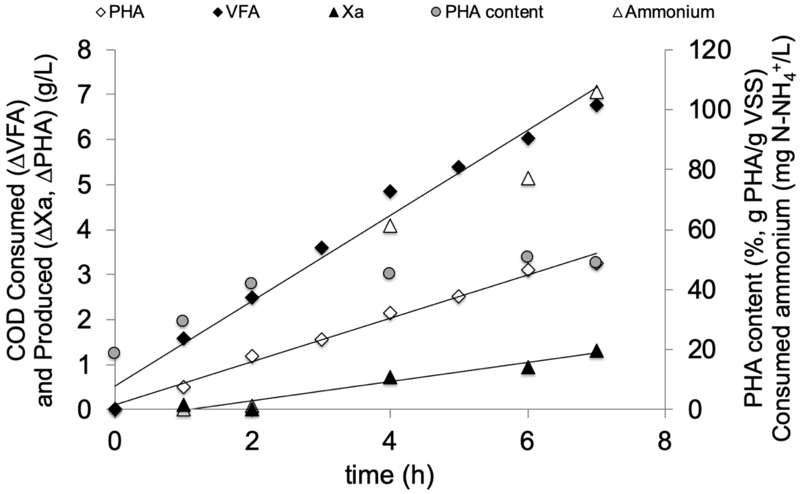


Figure 6–7. Trends of PHA concentration and content, consumed VFA and produced active biomass during a typical accumulation batch.

An intracellular PHA content of 0.51 g PHA/g VSS was obtained after 7 hours 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. 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 hours. 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.

6.3.2.2. *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 Figure 8.

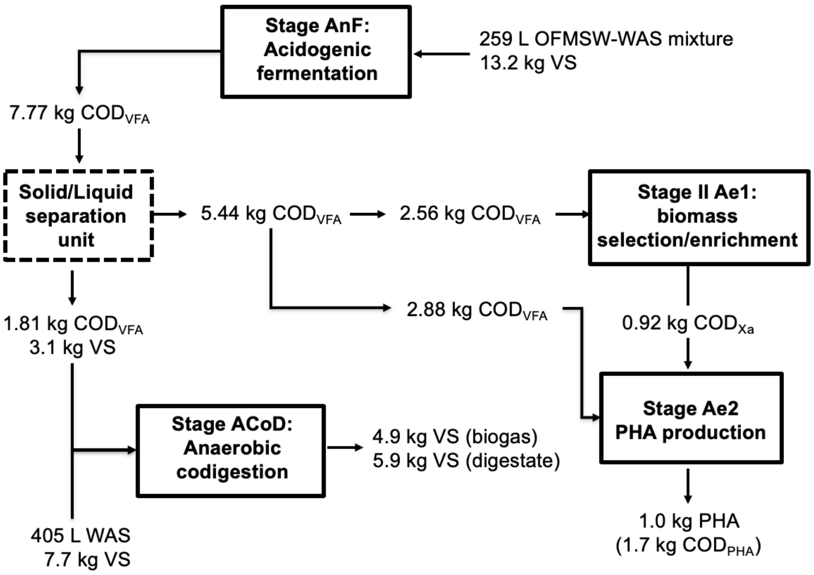


Figure 6–8. Mass balance of the whole biorefinery technology chain.

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 ($Y_{P/VFA}^{batch} = 0.59 \pm 0.04$ COD_{PHA}/COD_{VFA}), and 2.56 kg COD_{VFA} for X_A growth ($Y_{Xa/VFA}^{SBR} = 0.51 \pm 0.02$ 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 Figure 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 (2,000 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 Figure 4 (2,959 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 3). 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 preliminary economic evaluations for each of the compared scenarios can be found in Table 6.

Table 6–6. Preliminary economic evaluation of each process configuration compared.

Parameter	Unit	AnF1-ACoD1	AnF2-ACoD1	AnF1-ACoD2	AnF2-ACoD2
PE	-	70,000	70,000	70,000	70,000
VFA Yield	COD/COD	0.37	0.65	0.37	0.65
VFA	kg COD/d	1095	1923	1095	1923
Biogas	m^3/d	2058	2065	2386	2394
SGP	m^3/kg VS	0.44	0.51	0.44	0.51
PHA produced*	t/y	47	81	47	81
Thermal energy produced**	MJ/d	+ 6,200	+ 3,658	- 2,356	- 4,893
Electric energy produced	MWh/d	5.26	4.86	-	-
Revenue***	€/y	433,312	552,031	-	-

*calculated from global PHA yield related to both aerobic stages (0.30 kg PHA/kg VFA) of the present study

**taking into account the heating cost in the aerobic line as reported in Valentino et al. (2018)

***estimated from both PHA production and electric energy from biogas

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.

6.3.3. 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.

6.3.4. Social acceptance survey of bio-based products

One of the first outcomes from the social acceptance survey was 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 Figure 9.

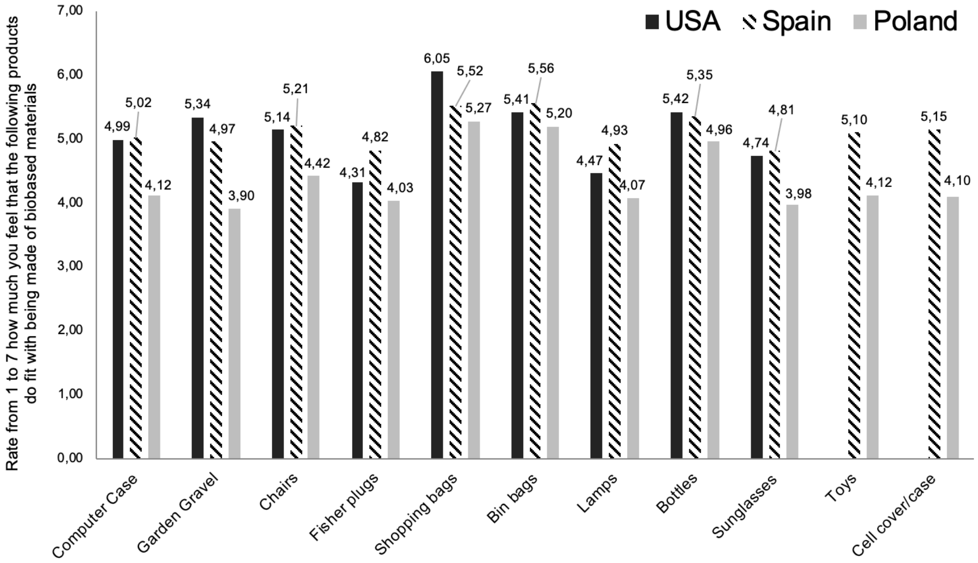


Figure 6–9. Outcomes of the survey regarding the possible most suitable application for bio-based products derived from waste.

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.

6.4. 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).

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7. Chapter 7. Biopolymers from urban organic waste: influence of the solid retention time to cycle length ratio in the enrichment of a mixed microbial culture (MMC)

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Moretto, G., Lorini, L., Pavan, P., Crognale, S., Tonanzi, B., Rossetti, S., Majone, M. and Valentino, F., 2020. Biopolymers from urban organic waste: influence of the solid retention time to cycle length ratio in the enrichment of a mixed microbial culture (MMC).
Postprint version of the article published in: *ACS Sustainable Chemistry & Engineering* https://dx.doi.org/10.1021/acssuschemeng.0c04980.

7.1. Introduction

7.1.1. Background

Switching the primarily fossil resource-based economy into a bio-based economy can contribute to the process of slowing down global warming and natural resources depletion (Andersen et al., 2018). Hence, global research focused on the development of many new functional materials and sustainable products obtainable from renewable resources, such as lignocellulosic biomass (Dutta et al., 2017; Liao et al., 2018; Liao et al., 2020), food waste (Morone et al., 2019), corn and sugarcane biomasses (Parada et al., 2017), etc. Polyhydroxyalkanoates (PHAs) are natural polyesters of hydroxyalkanoates (HAs) synthesized by microorganisms as intracellular granules for carbon and energy storage (Raza et al., 2018). Generally, PHA is stored under nutrient-limited conditions coupled with an excess of carbon source by a wide variety of microorganisms (Sagong et al., 2018). For several years now, PHAs have been produced at industrial scale mostly by means of pure cultures (Sabapathy et al., 2020). However, pure or recombinant cultures require sterile conditions and specific carbon sources followed by a targeted recovery process, resulting in four to nine times higher production costs compared to conventional plastics (Montiel-Jarrillo et al., 2017).

7.1.2. Processes for PHA production from MMCs and types of PHA produced

To enhance the market competitivity, PHAs production from mixed microbial cultures (MMCs) using waste streams or wastewaters as carbon sources has been exponentially investigated (Rodriguez-Perez et al., 2017). Typically, PHAs production from MMCs is achieved with the application of discontinuous feeding regimes (feast and famine regime) and alternating redox conditions to enrich the presence of PHA-storing microorganisms in the activated sludge of wastewater treatment plants (WWTPs, Valentino et al., 2017). These conditions can be tuned based on the type and availability of the carbon source and on the desired process that needs to be applied. More recently, the three-stage process (divided into acidogenic fermentation of the organic feedstock for volatile fatty acids (VFAs) production, PHA-storing microorganisms' selection from the MMC and PHAs accumulation maximization) has been implemented at pilot scale for both wastewater and organic waste valorization in a new biorefinery technology chain perspective (Conca et al., 2020). A wide variety of substrates have been used in previous studies, such as organic fraction of municipal solid waste (OFMSW, Colombo et al., 2017), cheese whey (Duque et al., 2014; Gouveia et al., 2017), olive oil mill wastewater (Campanari et al., 2017), etc. However, this process can be quite challenging, mostly due to the intrinsic variability of non-synthetic organic substrates used as carbon sources, that consequently translates into a variability of the selected microbial culture and of the obtained final polymer. The amount and quality of the produced polymer is tightly bound to many factors, especially the performance of the selection reactor, the carbon source quality, the accumulation strategy and the microorganisms type (Kaur et al., 2017). The most common of the class are poly(3-hydroxybutyrate), P(3HB), poly(3-hydroxyvalerate), P(3HV) and the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate), P(3HB-co-3HV), which exhibit thermoplastic characteristics with mechanical properties comparable to polypropylene and polyethylene (Anjum et al., 2016). Previous studies demonstrated that the polymer composition changed with respect to the substrate used (Duque et al., 2014), feedstock composition and feeding strategies (Arcos-Hernández et al., 2013) and to the strategy adopted for the accumulation performance, including the important role played by environmental factors (temperature, pH, dissolved oxygen, by-products inhibition and nutrient concentrations; Kosseva, Rusbandi, 2018).

7.1.3. Assessment of the selection performances

Both PHA stages (selection and accumulation) have a major impact on the overall process yield. However, it has been demonstrated that in MMCs the core element to achieve a high PHA storage performance in the accumulation stage (in terms of high PHA yields, high specific storage rate and maximum intracellular content) consists in the efficiency of the culture enrichment stage to select high-performing PHA-storing microorganisms (Oehmen et al., 2014). The higher the selective pressure applied, the more the culture is enriched exclusively in PHA-storing microorganisms. Many studies report the feast to cycle length ratio (feast/CL, Morgan-Sagastume et al., 2015; Valentino et al., 2019) or the feast to famine ratio (F/F, Colombo et al., 2017; Dionisi et al., 2005a) as the most important indicators for a good and stable selection process. These two parameters are calculated from the duration of the feast phase, usually observed by the dissolved oxygen (DO) profile. Both proved to be useful to assess the enrichment of a MMC and values of F/F lower than 0.3-0.4 are required for the imposition of an effective internal limitation, achieved through a long enough famine phase, to induce the PHA storage (Oliveira et al., 2017). Nevertheless, when dealing with real substrates, non-sterile conditions and open cultures, many other factors can influence the selection performances. Parameters such as the solid retention time (SRT), hydraulic retention time (HRT), cycle length (CL), organic loading rate (OLR), DO, carbon source quality, nutrient concentrations and ratios play a fundamental role, translating into a difficulty to properly assess the selection process (Rodriguez-Perez et al., 2018). Many authors also reported internal nitrogen (N) or phosphorous (P) limitation being triggering factors for the achievement of maximal storage capacity (Korkakaki et al., 2017; Valentino et al., 2015a). Indeed, the complexity of the process promoted the search for other indicators that could give useful information on the selection process. Previous studies focused also on the cycle length (Amulya et al., 2015; Dionisi et al., 2007) and the number of cycles per SRT (Jiang et al., 2011) to further investigate the selection performances. In the present study, the ratio between SRT and CL is discussed and evaluated as main parameter that gives deeper insights on the quality of the selective pressure applied in the selection reactor. The study was carried out at pilot scale inside a full-scale WWTP, with a mixture of organic fraction of municipal solid waste (OFMSW) and waste activated sludge (WAS) as carbon source for the PHA line.

7.2. Materials & Methods

7.2.1. Pilot plant process scheme and reactors

The proposed pilot plant configuration illustrated in Figure 1 is the same adopted in a previous study (Moretto et al., 2020), as well as the pilot reactors used.

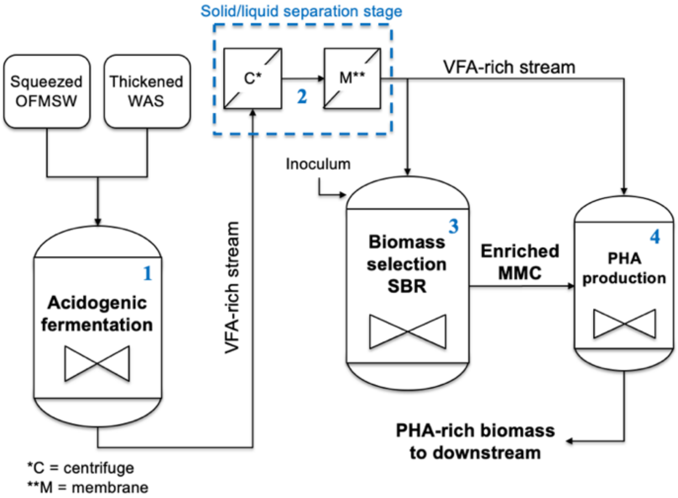

Figure 7–1. Pilot plant process configuration adopted in the present study.

The pilot plant platform was located inside the Treviso municipal WWTP (north-east Italy). The well-known three-stage process was applied for PHAs selection and production. The first stage consisted in the acidogenic fermentation (unit 1) of a mixture composed by 30% v/v WAS and 70% v/v OFMSW for VFAs production, followed by a solid/liquid separation stage for the refining of the fermented stream (unit 2). The refined fermented VFA-rich stream was then fed to the sequencing batch reactor (SBR, unit 3) for PHA selection and also to the PHA accumulation reactor (unit 4). Both reactors for selection and accumulation worked under fully aerobic regime and were automatically operated through a programmable logic controller (PLC, MyRio Labview from National Instrument©), that also acquired real time signals from immersion probes. The acidogenic fermentation was carried out in batch mode in a 400 L reactor equipped with a mechanical stirrer (HRT of 6 days; OLR 12 – 15 kg volatile solids (VS)/m^3 d). Roughly 8-10 batch fermentation run were conducted during each SBR operation. The temperature was controlled by means of a thermostatic jacket and maintained at 72°C for 48 hours for the mixture thermal pre-treatment, then set at 37°C for four days. The thermal pre-treatment was applied in order to foster substrate solubilization, according to the results obtained in previous studies (Moretto et al., 2019; Moretto et al., 2020). The fermenter pH was left uncontrolled and maintained itself around 5.0 – 5.5 for the whole duration of the study. The solid/liquid separation stage was divided first into a coaxial centrifuge equipped with a 5.0 μm porosity nylon filter bag and a subsequent ultrafiltration membrane with 0.2 μm porosity. The PHA selection from MMC was performed in a 100 L working volume reactor equipped with Bibus EL-S-250 linear membrane blowers (ensuring a continuous maximum DO level of 8.0 mg O_2/L and a complete stirring of the mixed liquor) and with immersion probes (DO; pH; temperature). The temperature was maintained between 25°C and 28°C with an immersion heater. Since no settling phase was programmed, the HRT was equal to the SRT in all runs. The SBR was operated and monitored under four different runs with intermediate OLR (4.0 g COD/L d) with a real substrate at pilot scale. Different settings of SRTs and CLs were tested, and process performances were assessed in each run after steady-state conditions were reached. As a consequence, different SRT/CL ratios of 2 (run Ae3), 4 (runs Ae1 and Ae4) and 8 (run Ae2) were investigated. Maintaining the same carbon source and OLR in all four runs allowed to focus only on the effects of SRT and CL. The experiments started from a 0.25 day cycle length with a SRT varying from 1 day (run Ae1) to 2 days (run Ae2), up to a 0.50 day cycle length again with a SRT varying from 1 day (run Ae3) to 2 days (run Ae4). In all four runs, the end of the feast phase was identified by the DO profile acquired by the PLC. The DO trends were analyzed for feast-famine regime evaluation as widely described in the literature (Valentino et al., 2017). In each run performed, a stable feast-famine regime was observed approximately after three SRTs. The feast phase length was recorded in real time by the PLC during the whole duration of the research. Feast phase length to CL ratios were calculated once per day taking into account one representative feast phase duration for each day and for each SBR run: 44 days for run Ae1, 48 days for run Ae2, 45 days for run Ae3 and 76 days for run Ae4. Operating conditions tested in the selection process are reported in Table 1.

Table 7–1. Operating parameters applied in the four runs tested in the selection process.

Run	HRT (d)	SRT (d)	OLR (g COD/L d)	CL (d)	SRT/CL (d/d)	Feeding frequency (d^{-1})	Operation length (d)	Load per cycle (g COD/L)
Ae1	1	1	4	0.25	4	4	44	1.0
Ae2	2	2	4	0.25	8	4	48	1.0
Ae3	1	1	4	0.5	2	2	45	2.0
Ae4	2	2	4	0.5	4	2	76	2.0

PHA accumulation batches were conducted in fed-batch mode with selected biomass (X) collected at the end of the feast phase. The reactor was provided with the same equipment of the selection reactor. The same permeate fed in the selection reactor was also used in the accumulations. The carbon source was progressively dosed in spikes to reach 3 g

COD/L concentration in the accumulation reactor immediately after each addition. When the DO level recorded by the PLC started to increase, indicating that the carbon source was consumed, another spike was added. Each accumulation lasted about 6-8 h on average, after that time the biomass reached its maximum PHA intracellular content and the accumulation was stopped. Accumulation batches started with an initial volume of 70-80 L and ended when the reactor working volume reached approximately 120 L. From 8 to 15 accumulation batches per SBR run were performed, for a total of 50 accumulation batches approximately.

7.2.2. Organic substrates

The characteristics of the mixture used in the acidogenic fermentation, of the fermented stream and of the permeate coming from the S/L separation which was used for the PHA selection and accumulation reactors are listed in Table 2.

Table 7–2. Organic substrates characterization.

Parameter	**Organic waste mixture**	**Fermented stream**	**Permeate (after S/L separation)**
TS (g TS/kg)	64 ± 2	46 ± 2	-
VS (g VS/kg)	51 ± 2	35 ± 2	-
VS/TS (%)	80 ± 1	76 ± 1	-
COD_{SOL} (g COD/L)	22 ± 1	37 ± 3	36 ± 2
VFA (g COD/L)	2.8 ± 0.5	32 ± 3	31 ± 2
VFA/COD_{SOL}	0.13 ± 0.05	0.86 ± 0.05	0.86 ± 0.05
Ammonium (mg $N\text{-}NH_4^+$/L)	380 ± 28	691 ± 15	689 ± 15
Phosphate (mg $P\text{-}PO_4^{3-}$/L)	121 ± 7	221 ± 6	220 ± 6
TKN (g N/kg TS)	29 ± 3	28 ± 3	28 ± 3
P (g P/kg TS)	2.3 ± 0.1	2.9 ± 0.5	2.9 ± 3

All the parameters refer to a monitoring period of two years approximately. The permeate fed to the PHA line showed a predominance of acetic acid (24-34%) and propionic acid (10-20%), along with butyric acid (30-40%) and caproic acid (25-35%) expressed on a COD basis. The values given in brackets represent a range of variation from the minimum to the maximum value over the whole operation period. The OFMSW was collected door-to-door in 50 districts of the Treviso province and sent to a dedicated plant, where a mechanical pre-treatment by means of a screw-press was applied. The solid fraction was sent to composting and the liquid fraction (ca. 15% total solids basis) was sent to the Treviso WWTP for full-scale anaerobic co-digestion with biological sludge. The WAS was collected from the static thickener after the biological nutrient removal (BNR) process applied in the water line of the full-scale WWTP. The same activated sludge was also used as inoculum for the PHA selection.

7.2.3. Analytical methods

The organic substrates were weekly collected and characterized. Samples from the selection SBR were collected daily. TS, VS, total suspended solids (TSS), volatile suspended solids (VSS), soluble chemical oxygen demand (COD_{SOL}), ammonium, phosphate, total Kjeldahl nitrogen (TKN), total phosphorus (P) were determined according to Standard Methods (APHA, AWWA, WEF, 1998). VFAs were analyzed using a gas chromatograph (GC), the sample was centrifuged at 4,500 rpm for 5 mins, the supernatant was then filtered with a 0.2 μm acetate cellulose filter (Whatman$^{©}$) and acidified at pH 2 with ortophosphoric acid. The GC used was an Agilent 6890 N equipped with an inlet set at 220°C in split mode with a split ratio of 20:1, a flame ionization detector (FID) set at 230°C and an Agilent J&D DB-FFAP fused silica capillary column (15 m length, 0.53 mm i.D., 0.55 mm film). The run consisted in a ramp temperature from 80°C to 200°C and the analytes were determined with an internal standard (2-ethyl butyric acid). Regarding PHA analyses, samples were prepared by adding 1 mL of NaClO (5% active Cl_2) to 5 mL of unfiltered fresh sample and the solution was stored at -4°C for subsequent extraction, hydrolyzation and esterification. Then, 3HB methyl ester and 3HV methyl ester monomers were quantified with a GC using P(3HB-co-3HV) standard polymer (Sigma-Aldrich) at 5% wt HV content as reference (Braunegg et al., 1978).

7.2.4. Calculations

Performances of the selection SBR were assessed after reaching steady state conditions, identified with the achievement of a constant feast phase length for at least two SRTs. All parameters were calculated taking into account the actual carbon source (permeate after S/L separation) fed into the selection and accumulation reactors.

The active biomass concentration (X_A, g/L) was calculated as difference between VSS concentration (g/L) and PHA concentration (g/L) at the end of the cycle:

$$X_A = VSS - PHA \qquad 1$$

PHA content in the biomass (g PHA/ g VSS) was determined as ratio between PHA concentration (g/L) and VSS concentration (g/L) both at the end of the feast phase:

$$PHA\ content = \frac{PHA}{VSS} \qquad 2$$

The specific substrate uptake rate ($-qS^{feast}$), expressed in mg COD_{SOL}/g COD_{Xa} h was assessed as ratio between the substrate (soluble COD) consumed during the feast phase and the length of the feast phase per unit of X_A:

$$-qS^{feast} = {\Delta COD_{SOL}}/{t_{feast} \cdot X_A} \qquad 3$$

The specific PHA consumption rate ($-qP^{famine}$, mg COD_{PHA}/g COD_{Xa} h) was calculated as ratio between the PHA consumed during the famine phase and the length of the famine phase per unit of X_A:

$$-qP^{famine} = {\left(mg\ PHA_{feast} - mg\ PHA_{famine}\right)}/{t_{famine} \cdot X_A} \qquad 4$$

The polymer specific storage 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:

$$qP^{feast} = {\Delta PHA}/{t \cdot X_A} \qquad 5$$

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:

$$Y_{OBS}^{SBR} = \frac{\left({X_A}/{(OLR \cdot HRT)}\right)}{0.93} \qquad 6$$

A correction factor of 0.93 was applied to the equation in order to refer the final results to the loaded COD_{SOL} instead of the consumed one, since the consumed COD_{SOL} represents the 93% of the loaded one.

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

$$Y_{P/S}^{feast} = {\Delta PHA}/{\Delta COD_{SOL}} \qquad 7$$

All PHA and X_A concentrations are referred to COD basis. The factor used for biomass conversion to COD is equal to 1.42 g COD/g X_A, while the factor used for PHA monomers conversion to COD is based on the oxidation stoichiometry and is equal to 1.67 g COD/g HB monomer and to 1.92 g COD/g HV monomer for HB and HV monomers respectively.

7.2.5. DNA extraction

Aerobic sludge samples (10 mL) were taken over the SBR operation at four different sampling times during steady state operation: day 21 for run Ae1, day 47 for run Ae2, day 23 for run Ae3 and day 24 for run Ae4. The samples were centrifuged at 15,000 rpm for 15 mins and the resulting pellet was immediately stored at −20°C until further processing. DNA extraction was performed by using PowerSoil® DNA Isolation Kit (MoBio - Carlsbad, CA) following the manufacturer's instructions. Purified DNA from each sample was eluted in 100 µL sterile Milli-Q water and the quality of the extracted DNA ($1.6 < A260/280 < 1.8$ and $A260/230 > 2$) was analysed with a Nanodrop 3300 (Thermo Scientific, Italy). DNA was stored at −20°C in small aliquots.

7.2.6. High-throughput 16S rRNA gene sequencing and bioinformatic processing

Extracted DNA was amplified in a first PCR with the primer pairs 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 534R (5'-ATTACCGCGGCTGCTGG-3') targeting the region V1-V3 of bacterial 16S rRNA gene according to the procedures described in a previous work (Crognale et al., 2019). Reactions were set up in 25 µL volumes containing 15 ng of DNA, 0.5 µM primers and 1X Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, MA USA). PCR settings were as follows: initial denaturation at 98°C for 10 s, 30 cycles of 98°C for 1 s, 60°C for 5 s, 72°C for 15 s and final elongation at 72°C for 1 min. The amplicon libraries were purified using the Agencourt® AMpure XP bead protocol (Beckmann Coulter, USA). Sequencing libraries were prepared from the purified amplicon libraries using a second PCR followed by purification and pooling in equimolar concentration (4 nM). Library concentration was measured with Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA USA). The samples were paired end sequenced (2x301bp) on a MiSeq platform (Illumina) using a MiSeq Reagent kit v3, 600 cycles (Illumina, USA) following the standard guidelines for preparing and loading samples. 10% Phix control library was added to avoid low complexity issues. After checking read quality with fastqc, the sequences were processed and analyzed using QIIME2 v. 2018.2 (Bolyen et al., 2019). The reads were demultiplexed using demux plugin (https://github.com/qiime2/q2-demux) and the primer sequences were removed by using cutadapt plugin (https://github.com/qiime2/q2-cutadapt). The demultiplexed reads were denoised, dereplicated and chimera-filtered using DADA2 algorithm (Callahan et al., 2016). Moreover, DADA2 resolved amplicon sequence variants (ASVs) differing by as little as one nucleotide (Callahan et al., 2017). Taxonomy was assigned to ASVs using a pre-trained naïve-bayes classifier based on the 16S rRNA gene database at 99% similarity of the Silva132 release (Quast et al., 2013). High-throughput sequencing of the region V1-V3 of the bacterial 16S rRNA gene yielded a total of 166˙908 sequence reads after quality control and bioinformatic processing that resolved into 1517 ASVs. Sequencing results were used for the calculation of biodiversity indices for each sample (Dominance, Simpson, Shannon, Evenness) by using PAST software (PALAEONTOLOGICAL STATISTICS, ver. 2.17, Hammer et al., 2001).

7.3. Results and Discussion

7.3.1. Biomass selection performances based on the SRT/CL ratio

In every run tested the feast to CL ratio was abundantly below 20%, widely recognized as the threshold for the establishment of a proper selection process (Dionisi et al., 2005b; Johnson et al., 2009; Reis et al., 2011). Overall, the feast to CL ratio showed more differences in run Ae1 and Ae4, with values equal to 11.8% and 7.1%. In runs Ae2 and Ae3 this parameter was quite similar, 9.6% and 9.4% respectively, even if both SRTs and CLs were different from run Ae2 to run Ae3. From these results, it could be mistakenly interpreted that the most performing selection process in terms of specific storage rates and storage yields was run Ae4, since the duration of the feast phase was shorter than the one of other runs. The PHA concentrations (mg/L) at the end of the feast and famine phase and the feast/CL ratio obtained in each run are showed in Figure 2.

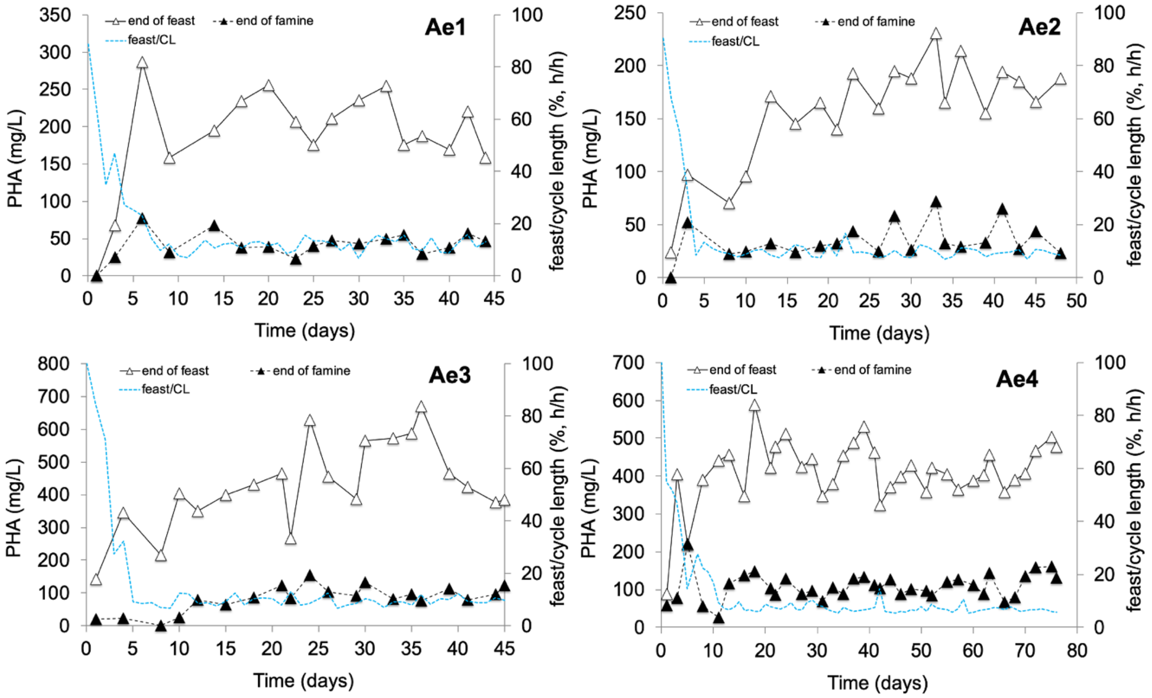

Figure 7–2. Trends of PHA concentrations (mg/L) at the end of the feast and famine phase and feast/CL ratio in all four runs.

The SRT plays an important role in determining the reactor X_A concentration, indeed higher concentrations of biomass were obtained in runs Ae2 and Ae4 with 2 days of SRT (2.36 g/L and 2.54 g/L respectively) rather than in runs Ae1 and Ae3 with SRT equal to 1 day (1.37 g/L and 1.30 g/L respectively). However, the intracellular PHA content at the end of the feast phase did not reflect the increase in X_A concentration observed in run Ae2 and Ae4. Run Ae2 and Ae4 showed a PHA content equal to 0.07 and 0.14 g PHA/g VSS respectively, while in run Ae1 and Ae3 the PHA content was 0.13 and 0.20 g PHA/g VSS respectively. Hence, the storage response was differently affected and a combination of both parameters, SRT and CL, seemed to be more relevant. Run Ae1 and Ae4 were more similar in terms of PHA level accumulated at the end of the feast phase but run Ae3 showed a visible increase with respect to all other runs. If the selective pressure applied was the same in all runs, the PHA content would have been expected to be similar. The observed differences may suggest that in run Ae3, where the CL was the highest (0.50 day) and the SRT the shortest (1 day), the culture is stimulated to store more PHA. A previous study already showed how the SRT may affect the selective pressure (Chua et al., 2003), however, a deeper data analysis revealed that a combined effect of CL and SRT has to be taken into account for a comprehensive process evaluation. Clear differences between the runs were also observed in terms of specific substrate uptake rate ($-qS^{feast}$) and specific storage rate (qP^{feast}). The obtained values for both parameters showed a decreasing trend when correlated with the SRT/CL ratio, as illustrated in Figure 3A. Run Ae3, which had the lowest SRT/CL ratio (2 d/d), was characterized by the highest specific rates according to the highest PHA content at the end of feast phase obtained: $-qS^{feast}$ equal to 816 mg COD_{SOL}/g COD_{Xa} h and qP^{feast} equal to 375 mg COD_{PHA}/g COD_{Xa} h. Even with different CL and SRT, run Ae1 and Ae4 exhibited similar kinetics as a consequence of the same SRT/CL ratio (4 d/d): $-qS^{feast}$ equal to 617 and 553 mg COD_{SOL}/g COD_{Xa} h and a qP^{feast} equal to 253 and 235 mg COD_{PHA}/g COD_{Xa} h respectively. Run Ae2 showed the lowest rates, with a $-qS^{feast}$ equal to 440 mg COD_{SOL}/g COD_{Xa} h and a qP^{feast} equal to 156 mg COD_{PHA}/g COD_{Xa} h, at the highest SRT/CL ratio (8 d/d). From these results, it appears that lower SRT/CL ratios stimulate higher specific rates, both in terms of consumed substrates and of stored polymer. The storage yield ($Y_{P/S}^{feast}$) correlated with the SRT/CL ratio showed a similar trend, with highest values in run Ae3 (0.46 COD_{PHA}/COD_{SOL}) and lowest in run Ae2 (0.35 COD_{PHA}/COD_{SOL}). The $Y_{P/S}^{feast}$ trend of all runs is reported in Figure 3B.

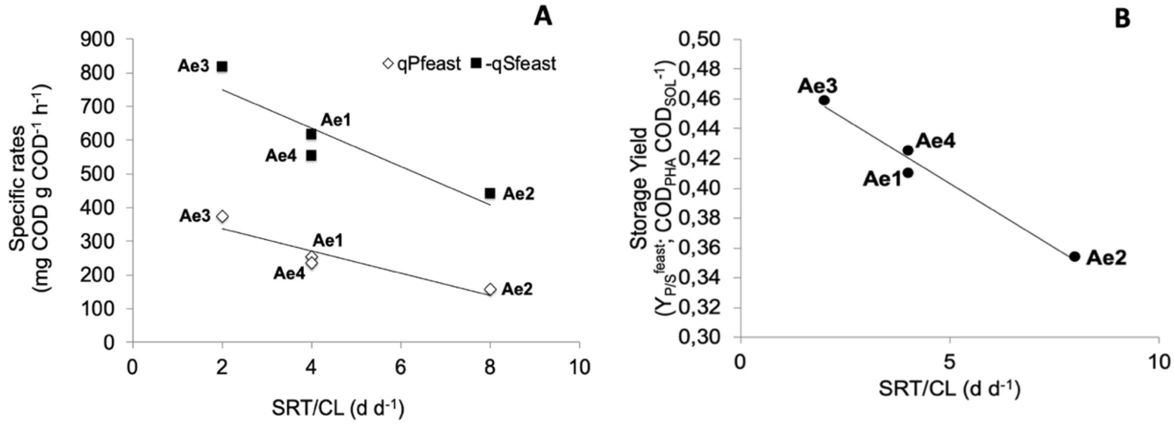

Figure 7–3. (A) Specific substrate uptake rate ($-qS^{feast}$) and specific storage rate (qP^{feast}) obtained in all four runs in relation to the SRT/CL ratio; (B) storage yield ($Y_{P/S}^{feast}$) trend related to the SRT/CL ratio in all four runs.

Clearly, the storage yield is in agreement with the observed specific rates. Unexpectedly, run Ae4, where the lowest feast phase to CL ratio of 7.1% was observed, did not show the best performances in terms of specific rates and polymer storage yield. Indeed, Ae3 turned out to be the most performing in terms of PHA storage stimulation, compared to the other runs. From these results, it seems that the influence of the SRT/CL ratio is crucial for the evaluation of the selective pressure applied to the system. Main parameters and performances obtained in the selection and accumulation processes of all runs are summarized in Table 3. The F/M has also been calculated and was equal to 2.92, 1.69 3.08 and 1.57 g COD/g X_A d respectively for run Ae1, Ae2, Ae3 and Ae4. Apparently, no correlation seemed evident, since the lowest performances (run Ae2) did not correspond to the lowest F/M value. This value can be assumed as a direct consequence of the operating conditions applied in the selection phase, especially the OLR, which was further discussed with respect to the SRT/CL.

Table 7–3. Main parameters and performances obtained in the selection and accumulation processes of the four runs.

Parameter *(Selection/enrichment stage)*	**Unit**	**Value (Avg. ± St. Dev.)**			
		Ae1	**Ae2**	**Ae3**	**Ae4**
Feast/CL	%	11.8 ± 0.2	9.6 ± 0.1	9.4 ± 0.1	7.1 ± 0.2
X_A (end of feast)	g/L	1.37 ± 0.04	2.36 ± 0.05	1.30 ± 0.01	2.54 ± 0.02
PHA content (end of feast)	g PHA/g VSS	0.13 ± 0.05	0.07 ± 0.04	0.20 ± 0.03	0.14 ± 0.02
F/M	g COD/g X_A d	2.92 ± 0.12	1.69 ± 0.13	3.08 ± 0.11	1.57 ± 0.12
Specific storage rate (qP^{feast})	mg COD_P/g COD_{Xa} h	253 ± 29	156 ± 19	375 ± 36	235 ± 38
Specific substrate uptake rate ($-qS^{feast}$)	mg COD_{SOL}/g COD_{Xa} h	617 ± 47	440 ± 32	816 ± 41	553 ± 44
Storage yield ($Y_{P/S}^{feast}$)	COD_P/COD_{SOL}	0.41 ± 0.03	0.35 ± 0.07	0.46 ± 0.02	0.43 ± 0.03
Observed yield (Y_{OBS}^{SBR})	COD_{Xa}/COD_{SOL}	0.56 ± 0.05	0.47 ± 0.04	0.59 ± 0.04	0.54 ± 0.03

Parameter *(Accumulation stage)*	**Unit**	**Value (Avg. ± St. Dev.)**			
		Ae1	**Ae2**	**Ae3**	**Ae4**
PHA content	g PHA/g VSS	0.51 ± 0.07	0.40 ± 0.03	0.59 ± 0.03	0.49 ± 0.04
Storage yield ($Y_{P/VFA}^{batch}$)	COD_P/COD_{VFA}	0.58 ± 0.09	0.47 ± 0.09	0.62 ± 0.05	0.55 ± 0.07

7.3.2. The role of SRT/CL ratio in relationship with the applied OLR and microbial community

Few other authors investigated the performances of the selection process in terms of the CL or SRT/CL impact on PHA production and on microbial composition of the selected culture, and results are quite variable. A previous study (Jiang et al., 2011) investigated the microbial competition in several SBRs fed with pure synthetic acetate (OLR 2.0 g COD/L d) at different temperatures (20°C and 30°C), with different CLs (1 h, 4 h, 12 h, 18 h), and SRT-HRT both fixed at 1 day. The authors found out that the SRT/CL ratio had a strong impact on the PHA production, but not on the variability of the microbial community. This study demonstrated that the lower the SRT/CL ratio is, the higher selective pressure the culture has to deal with, since a higher intracellular polymer content needs to be achieved. The amount of substrate fed per cycle is increased, while biomass concentration at the beginning of the cycle decreased, resulting in a higher substrate to biomass ratio. Temperature instead was found to be the main parameter affecting the different types of microorganisms selected in the mixed culture, with a predominance of *Plasticicumulans acidivorans* at 30°C. A maximum PHB intracellular content of 71.3 wt% was obtained at 30°C with a CL of 18 h (SRT/CL equal to 1.33 d/d). Another study (Dionisi et al., 2007) investigated the impact of the CL on polymer production and on microbial community selection in a SBR operated at different CLs (1 h, 2 h, 4 h, 8 h) with SRT-HRT both equal to 1 day and high OLR (20.0 g COD/L d). The CL was recognized as the main parameter affecting both the storage and the growth response. This was due to the different extent of the famine and feast phase, which turned out to be crucial especially in this high load process. The highest storage yield of 0.46 COD/COD was obtained with a high-intermediate SRT/CL of 12 d/d. A strong influence of CL was assessed also on microbial composition, unlike in the other cited study (Jiang et al., 2011), probably due to the much higher applied OLR. A following work (Valentino et al., 2014) confirmed the statement of Dionisi et al. (2007). The authors tested four different runs with decreasing cycle lengths (8, 8, 6 and 2 h) coupled with different feeding frequency of the carbon source. Results indicated that the lowest SRT/CL ratio values of the selection reactor did not correspond to the most performing runs. The runs with the lowest SRT/CL were indeed characterized by higher concentration of non-polymer biomass and lower storage response. The most performing results in terms of storage response were obtained at the highest SRT/CL ratio, which corresponded to the lowest cycle length investigated of 2 h. Moreover, the microbial community changed during the different runs investigated, showing an influence of the feeding frequency and of the cycle length on the culture selection. An explanation to the different results achieved in the present study might be identified in the applied OLR, which was more than doubled compared to this work (8.5 g COD/L d vs. 4.0 g COD/L d). This theory can be also supported by the study of Dionisi et al. (2007), in which the OLR was even higher (20.0 g COD/L d) and the microbial community was found to be strongly affected. It may be deduced that with higher OLRs, the selective pressure applied on the biomass is more driven by high-intermediate SRT/CL, leading also to changes in the microbial community. On the contrary, in processes with lower or intermediate OLRs, the SRT/CL ratio needs to be maintained at low values to maximize the selective pressure applied to the system. It is still not clear the effect of SRT/CL on microbial community cultivation at low-intermediate OLRs, since the outcomes from the work of Jiang et al. (2011) are quite contradictory with respect to the microbial community differentiation obtained in this study. It has to be taken into account that the studies of all previous cited works were conducted with synthetic substrates, and the applied OLRs were substantially different from the one applied in this study. Comparable results in terms of storage rate and storage yield, equal to 339 mg COD_{PHA}/g COD_{Xa} h and 0.56 COD/COD respectively, were obtained with a similar OLR (4.7 g COD/L d) in a SBR fed with a real substrate (pre-treated olive mill wastewater, Campanari et al., 2014) and SRT/CL of 4 d/d. The authors investigated the effect of the OLR on the selection performances, by maintaining a fixed SRT/CL ratio. The length of the famine phase was recognized as the main selective pressure applied to the system, since when it was not long enough it did not ensure the required internal growth limitation, leading to less PHA accumulation and a higher growth response during the feast phase. Indeed, the feast to CL or to famine phase ratio can give an immediate and direct indication on the effective management of the selection process. However, when evaluating more thoroughly the performances of the selection process and especially with complex and nutrient-rich real substrates, the SRT/CL ratio parameter can give good and better insights on how well the selection process is actually taking place and how much the culture is enriched only in PHA-storing microorganisms.

7.3.3. Microbiome composition

The majority of the bacterial taxa obtained from the samples taken under steady-state operating conditions was affiliated with phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. *Proteobacteria* was the most abundant phylum in almost all runs representing between 15.3% and 70.5% of total reads. Within this phylum, members of *Alphaproteobacteria* affiliated with *Brevundimonas* and *Mesorhizobium* were mainly found in runs Ae1 and Ae4 with relative abundances up to 15% and 8.4% (Figure 4).

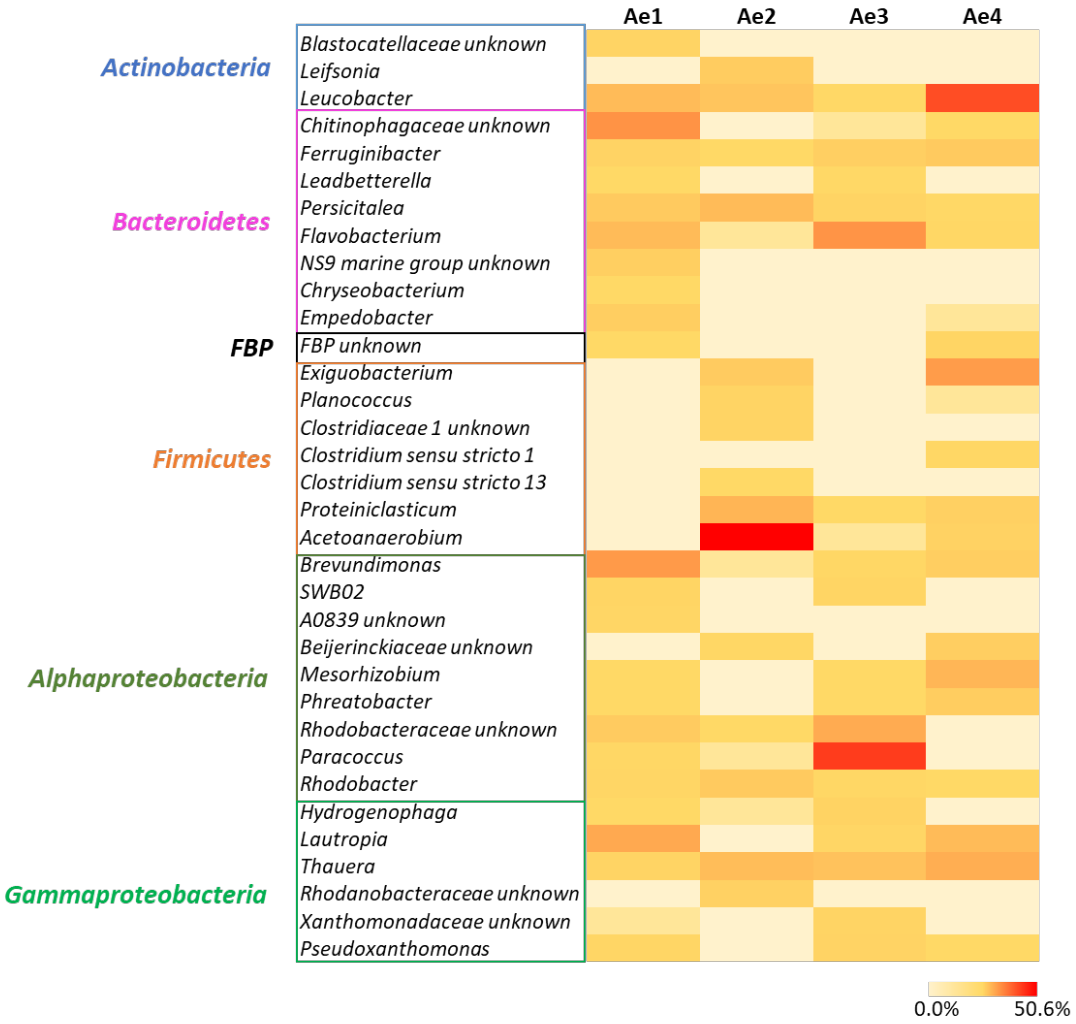

Figure 7–4. Frequency heat-map of bacterial communities at genus level: the colour intensity in each cell shows the relative abundance (relative abundance of total reads ≥1% in at least one sample).

In run Ae3, 48.5% of total sequences were affiliated with family *Rhodobacteraceae* with the predominance of genus *Paracoccus* (36.8%), in contrast to the low abundances observed in all other samples (<1%). Within *Gammaproteobacteria*, sequences affiliated with genera *Hydrogenophaga*, *Thauera* and *Pseudoxanthomonas* were retrieved in all samples. Members of genus *Lautropia* were only found in runs Ae1 and Ae4 (11.5 and 7.4% of total reads, respectively). *Bacteroidetes* represented between 6% and 38.6% of total reads and were affiliated with genera *Ferruginibacter*, *Leadbetterella*, *Persicitalea* and *Empedobacter* in all samples and with *Flavobacterium* mostly in Ae3 run (16.3% of total reads). The occurrence of *Firmicutes* was mainly observed in run Ae2, in which sequences affiliated with *Clostridiaceae* family reached up to 60.8% of total reads with the dominance of genus *Acetoanaerobium* (50.6%). Sequences belonging to *Exiguobacterium* were mainly retrieved in run Ae4 (14.5%). Lastly, *Actinobacteria* counted between 1.2% and 33.2% of total reads in all samples, with a large occurrence of *Leucobacter* in run Ae4 (32.9%). Overall, the analyses revealed distinct bacterial profiles over different runs. In particular, the relative abundance of putative PHA-storing bacteria selected under different runs varied and decreased with the increase of the SRT/CL ratio (Figure 4, Table 4).

The highest relative abundance of sequences affiliated with known putative PHA-storing bacteria was found in Ae3 (up to 64%). In contrast, only 17.9% of PHA-storing bacteria was found in Ae2 and around 28% in Ae1 and Ae4 (Figure 5).

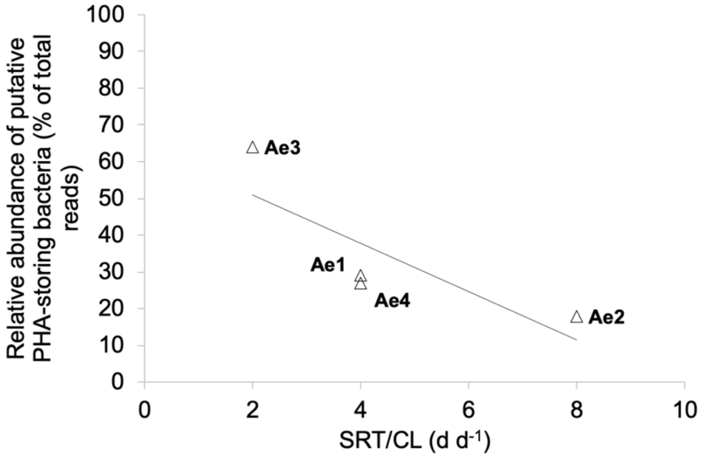

Figure 7–5. Relative abundance of sequences affiliated with putative PHA-storing bacteria out of total sequences in relation to SRT/CL ratio.

Table 7–4. Relative abundance (% of total reads) of bacterial taxa known for their capability to store PHA. For each taxonomic group, a reference reporting the ability to store PHA is also provided.

phylum	class	order	family	genus	Ae1	Ae2	Ae3	Ae4	Reference
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	0.0	3.4	0.0	0.0	Kumar et al., 2018
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Leadbetterella	0.3	0.0	0.6	0.0	Coats et al., 2016
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	7.4	0.1	16.3	0.8	Dionisi et al., 2005b
Firmicutes	Bacilli	Bacillales	Family XII	Exiguobacterium	0.0	3.5	0.0	14.5	Kung et al., 2007
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	15.0	0.1	0.7	2.8	Bhuwal et al., 2013
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0.8	0.1	36.8	0.0	Sawant et al., 2015
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	1.0	3.7	0.7	0.3	Jiang et al., 2009
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Hydrogenophaga	0.2	0.1	1.7	0.0	Valentino et al., 2015b
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Thauera	1.4	6.9	5.6	10.4	Serafim et al., 2006
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.9	0.0	1.6	0.3	Mwamburi et al., 2019

This finding showed that the adoption of different SRT/CL ratios may have different selective pressure on the mixed microbial communities and strongly impact on the PHA storage process. In particular, *Flavobacterium* and *Paracoccus* were enriched in run Ae3 whereas *Brevundimonas*, *Exiguobacterium* and *Thauera* were the main PHA-storing genera in runs Ae1 and Ae4. Lastly, run Ae2, characterized by the lowest PHA-storage yield and rates, showed low abundances of PHA-storing bacteria mainly affiliated with *Leifsonia*, *Exiguobacterium*, *Rhodobacter* and *Thauera* genera. In line with this finding, the highest selective pressure occurring in Ae3 was mirrored by the lowest evenness and biodiversity values (Table 5).

Table 7–5. Biodiversity indexes.

	Ae1	**Ae2**	**Ae3**	**Ae4**
Dominance_D	0.1	0.1	0.2	0.0
Simpson_1-D	0.9	0.9	0.8	1.0
Shannon_H	3.8	3.3	3.0	3.5
Evenness_e^H/S	0.3	0.3	0.2	0.5

7.3.4. Global process yields

The overall yield was calculated for the most performing run (Ae3), in terms of storage yield and maximum PHA content achieved. The mass balance started from the amount of COD necessary for the production of 1 kg of PHA (equal to 1.7 kg COD_{PHA}). The overall mass flow and process diagram is illustrated in Figure 6. Stages and performances adopted from a previous study (Moretto et al., 2020) are indicated in a different color.

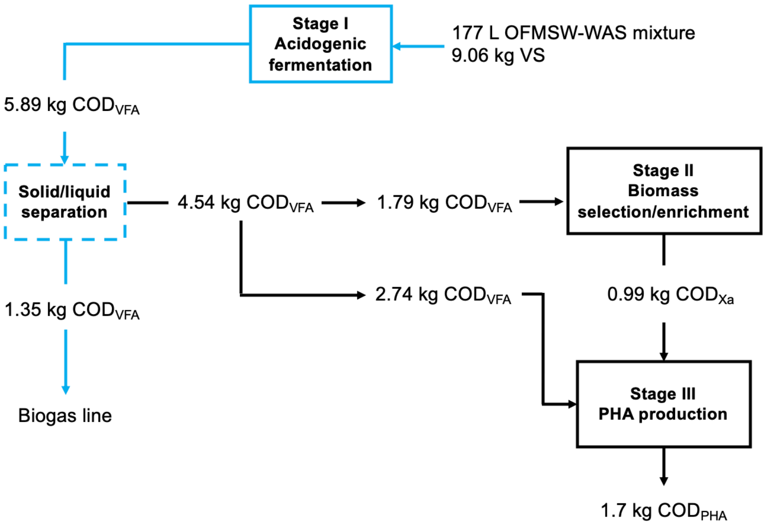

Figure 7–6. Overall mass balance and process diagram of the most performing run (Ae3). Stages and performances adopted from a previous study (Moretto et al., 2020) are indicated in a different color.

Taking into account an obtained storage yield in the accumulation reactor ($Y_{P/VFA}^{batch}$) equal to 0.62 COD_{PHA}/COD_{VFA} and an obtained growth yield in the selection reactor ($Y_{Xa/VFA}^{SBR}$) equal to 0.55 COD_{Xa}/COD_{VFA}, the COD needed is 2.74 kg COD_{VFA} and 1.79 kg COD_{VFA} for the accumulation and selection reactor respectively, with a total of 4.54 kg COD_{VFA} entering the aerobic line. The configuration adopted, reported in Figure 1, is equipped with a solid/liquid separation stage (centrifuge and membrane filtration system) for the clarification of the fermented stream entering the aerobic line. This stage causes a volume loss of the COD_{VFA} stream equal to 23%, meaning that the total COD entering the solid/liquid separation stage is equal to 5.89 kg COD_{VFA}, with a loss of 1.35 kg COD_{VFA} that is sent to the anaerobic co-digestion line for biogas production. Taking into account a fermentation yield (Y_{VFA}) based on the most performing condition proposed in Moretto et al. (2020) of 0.65 kg COD_{VFA}/kg VS_{IN}, 9.06 kg VS_{IN} are needed for the fermentation process in order to produce 5.89 kg COD_{VFA}. The corresponding TS_{IN} needed for the fermentation process are equal to 11.33 kg TS, considering that the OFMSW-WAS mixture is characterized by a VS/TS ratio of 80%. Since the TS concentration of the mixture is 64 kg TS/m^3, the volume of mixture needed for the fermentation process is equal to 177 L. This scenario leads to a calculated overall yield of 110 g PHA/kg VS, which is 45% more compared to the yield calculated in the cited previous work[32] adopting the same process configuration.

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8. Chapter 8. General conclusions and future perspectives

The biorefinery technology chain represents a promising productive system that is perfectly in line with the circular economy concept promoted by recent EU policies. In the field of waste recycling and resource recovery, the production of added-value materials from renewable carbon sources acquires a double importance, since both waste reduction and sustainable industrial productive chain are targeted. Another element that needs to be taken into account is the advantage that is created by the adoption of such a technology chain into an urban context. The potentialities coming from the integration of different technologies and waste treatments are indeed very broad, touching social, environmental and economic aspects by enhancing the overall life quality, lowering general pollution with the avoidance of waste transportation and by creating new job positions. The proposed technology chain is relatively simple to implement, since currently many pre-existing infrastructures, such as existing WWTPs, are equipped with an anaerobic digester which is often under-loaded. More than 36,000 digesters located in Europe are affected by operational problems, caused by the low loading imposed by secondary sludges digestion, which are already partially mineralized and are not able to ensure a good and efficient energy recovery. Apart from self-sustainability issues, less sludge is being degraded, resulting in a significant amount of waste that needs to be disposed of and that currently in some parts of Europe is still disposed through landfill or incineration. On the other hand, OFMSW coming from the separate collection is, in the best scenario, presently managed through the composting process, which has very high energetic and space requirements. The studied technology chain proved its applicability to treat both OFMSW and WAS, that are highly available in urban contexts, through a more sustainable and efficient process compared to the current separate waste management situation. Nowadays, the only full-scale technology that is still not much diffused is anaerobic co-digestion, that directs all waste of different origin to a single stage process for bioenergy production. The additional great value of the proposed biorefinery is the production of a new material apart from compost and energy only, PHA, a bio-based polymer considered and used as bioplastic precursor. With bioplastics, the requirements imposed by the new 2020 EU Circular Economy Action Plan on newly designed materials are targeted, since the problems linked to oil-based plastics are avoided. Even if oil-based plastics are properly recycled, their bio recalcitrant properties are still rising concern about controlled or uncontrolled disposal. The unique features presented by PHAs, such as the full biodegradability under both aerobic and anaerobic conditions, the availability of a family of copolymers with largely tunable properties, the microbial synthesis with mild process conditions and the obtainability from a large range of available waste feedstock enhance the great potentialities for a quite broad market. The biorefinery studied in the present thesis outlined an initial overall yield of 65 g PHA/kg VS, improved up to a final yield 110 g PHA/kg VS, proving the effectiveness of the optimizations integrated in the previous chapters. The consolidated feast-famine regime allowed to produce a selected PHA-storing biomass with good stability and improved performances both in the selection and accumulation stage (selection storage yield $Y_{P/S}^{feast}$ equal to 0.46 COD_P/COD_{SOL} and accumulation storage yield $Y_{P/VFA}^{batch}$ equal to 0.62 COD_P/COD_{VFA}). The obtained yields were confirmed also by the microbiology analysis performed in chapter 7, that highlighted a predominance of *Flavobacterium* and *Paracoccus* in the selected mixed culture. Noticeable improvements were also studied at lab scale and then achieved at pilot scale with respect to the fermentation process, crucial for the polymer final composition and quality. The introduction of the thermal pre-treatment allowed to achieve a fermentation yield (Y_{VFA}) equal to 0.65 g COD_{VFA}/g $VS_{(0)}$ and a ratio between odd numbered acid 3HV precursors (3HVp) and the sum of even and odd numbered 3HB and 3HV precursors (3HB+3HV)p of 0.25 mol/mol. The implementation of the co-digestion process in a “closing the loop” perspective allowed to valorize the secondary waste streams produced within the biorefinery with an SGP of 0.44 m^3_{biogas}/kg VS in the mesophilic configuration, that proved to be more energetically convenient than the thermophilic one due to a 30% energy surplus. As final considerations, the overall mass balance can be resumed as it follows: 6,720 kg TS/d entering the biorefinery, with a biological sludge contribution of 4,200 kg TS/d and an OFMSW contribution of 2,520 kg TS/d. The first outlet as PHA produced is equal to 225 kg/d, while the volumetric flow entering the digester is equal to 6,256 kg TS/d (spilt into 2,419 kg TS/d of solid cake from the centrifuge, 692 kg TS/d of membrane retained phase and 3,142 kg TS/d of biological sludge). As a result, the co-digestion process gives 1,331 m^3/d of CH_4, 1,030 m^3/d of CO_2 and 4,059 kg TS/d of digestate as outputs. The overall mass balance has been calculated for a 70,000 PE scenario, in a scaled-up perspective as reported in the previous chapters, in order to be more representative. Even though the technical feasibility of this technology at pilot scale is so clear that strongly supports its transferability to a larger scale, the possibility to integrate the MMC-PHA production in the context of a full-scale facility, while also meeting standards and demands of product quality for market exploitation, still needs to be confirmed. The PHA market for pure culture production is already established, however, since their price is ranging between 5-8€/kg, their market expansion is also linked to other aspects and assumptions. Their current cost decrease, their properties further improvement in order to adapt to a wide range of final destinations and their environmental impact reduction through less energy and chemicals consumptions are important features that need to be considered. While an important contribution to all these aspects is given by the application of MMCs production processes, as proposed in the urban biorefinery of the present thesis, the extraction process of the biopolymer from the microbial cell is still a bottleneck that is crucial for cost and environmental burden reduction. The most performing extraction process for PHAs is based on the use of chloroform, which is considered as the most excellent solvent for this class of polyesters, or other chlorinated compounds, with all associated environmental and health risks. In the RES URBIS project, the extraction part was also targeted by other work packages dedicated to the downstream processing and to the potential applications, leading to the successful development of an extraction process using bio-based solvents that is currently under patent procedure. Subsequent analyses performed on the extracted polymers of the project revealed that a high recovery with high molecular weight polymers (370 – 640 kDa) and 100% purity was achievable. However, some limitations to the exploitation of potential market applications were represented by the quantity of polymer produced. Even though around 15 kg of PHA were produced in the total project duration, wider PHA characterization and the development of bioplastic formulation require higher amounts of polymer by product-market developers. The polymer composition (7-20% 3HV content) could translate into a good stiffness and improved brittleness and flexibility, but for thermoplastic applications a MW higher of equal to 600 kDa is required, which was not met by all samples. Moreover, an important variable impacting the profit margin is the purity of the extracted PHA, in general purity higher than 95% is preferred. A simpler application, with less requirements, can be represented by groundwater remediation with permeable barriers. A demonstration scale production is required in order to reach an advanced and improved downstream process, consequently allowing a deeper exploitation of potential market applications. PHAs provide a higher added value with respect to biogas, both in terms of economic advantage and range of applications. While one product does not exclude the other, since both are being synthesized in the biorefinery, the majority of the carbon recovered from waste is directed towards PHAs production instead of biogas, guaranteeing the biorefinery energetic self-sustainability and lowering some environmental burden caused by oil-based plastics. The studies conducted in the three years research shed a light on some possible weaknesses and optimizations, that were deeply investigated and led to an overall improvement of the biorefinery. Indeed, the technology proved its robustness to face organic waste heterogeneity and the requirements needed for the end products for their application in targeted market segments. The practical studies also opened the field to manage related regulatory gaps and legislative barriers that can be encountered along the way to convert organic waste into bio-based products, since clearly the present regulations are inadequate to deal with innovative technologies. As targeted by the preliminary “End of Waste” considerations and by other Work Packages of the RES URBIS project, the end products can be considered “clean” enough to avoid falling into the waste category. Moreover, the contaminants migration assessed by another Work Package demonstrated the broad market applicability of the bio-based polymer produced in the biorefinery, whose only restriction is the use for direct food contact packaging. Social aspects and the consumers’ perception of waste derived products proved their willingness to pay for recycled products (“green” products) and confirmed an eligible use of waste derived PHAs into bin bags. This analysis gave an interesting perspective, confirming at least one concrete market sector that could immediately be positively accepted, closing also the loop of the whole system from cradle to end. The successful studies concluded on the pilot scale biorefinery led to its natural continuation, which is a demonstration plant that will be integrated as a part of a waste treatment facility. Indeed, an important factor to take into account when assessing profitability and up-scaling is the plant size. In the RES URBIS project, the scale of 500,000 inhabitants has been considered and evaluated as sufficient but necessary to ensure an amenable profitability range in a full-scale production. The perspectives and potentialities of this technology chain and its derived products are already confirmed by the market and enterprises attention, that will hopefully lead to a full valorization and integrated treatment of organic waste of urban origin in the next years.

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Matricola (se posseduta) 834872 Autore della tesi di dottorato dal titolo:

Urban organic waste valorization through an integrated pilot scale biorefinery for polyhydroxyalkanoates and biogas production

Dottorato di ricerca in Scienze Ambientali

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Student: Giulia Moretto Student ID: 834872
PhD: Environmental Sciences
Cycle: 33°

Thesis title: Urban organic waste valorization through an integrated pilot scale biorefinery for polyhydroxyalkanoates and biogas production (S.S.D. ING/IND-25)

Abstract:

The main three-year doctoral research activity is centered around the pilot scale implementation of a biorefinery aimed at the valorization of organic waste into polyhydroxyalkanoates (PHAs) and biogas. The whole production chain comprises the organic waste fermentation, the aerobic line for PHA production and the anaerobic co-digestion of the secondary waste streams produced within the biorefinery. A mixture of organic fraction of municipal solid waste and waste activated sludge from the municipal wastewater treatment was used as primary carbon source. The initial phases of the study focused on the fermentation process and the aerobic line, the most performing conditions corresponded to a fermentation yield (Y_{VFA}) equal to 0.40 g COD_{VFA}/g VS and a storage yield in the accumulation phase ($Y_{P/VFA}^{batch}$) equal to 0.50 COD_{PHA}/COD_{VFA}. Then, the studies focused on the anaerobic line, i.e. the acidogenic fermentation and anaerobic co-digestion. Following studies aimed at the optimization of the fermentation process, the best obtained yields guaranteed a Y_{VFA} of 0.57 and 0.74 gCOD_{VFA}/g VS in batch and continuous tests respectively. The most performing conditions were then tested at pilot scale, the overall yield was considerably increased, reaching 76 g PHA/kg VS. In addition, legislative barriers and product social acceptance of waste derived bioplastics were preliminarily investigated. As final optimization, different operating conditions were investigated in the MMCs PHA selection process, that allowed to reach further improvement of the biorefinery overall yield, equal to 110 g PHA/kg VS. The results obtained in the present thesis shed a light on promising innovations in the field of organic waste valorization, proving the feasibility of the whole productive chain and its future industrial scale implementation.

Student signature

Giulia Moretto

Studente: Giulia Moretto Matricola: 834872
Dottorato: Scienze Ambientali
Ciclo: 33°

Titolo della tesi: Urban organic waste valorization through an integrated pilot scale biorefinery for polyhydroxyalkanoates and biogas production (S.S.D. ING/IND-25)

Abstract:

La principale attività di ricerca dei tre anni di dottorato è focalizzata sull'implementazione in scala pilota di una bioraffineria per la valorizzazione di rifiuti organici urbani in poliidrossialcanoati (PHA) e biogas. La filiera di processo studiata è divisa in fermentazione del rifiuto organico, linea aerobica per la produzione di PHA e co-digestione degli scarti prodotti nella bioraffineria. Come fonte di carbonio è stata utilizzata una miscela di frazione organica dei rifiuti solidi urbani e fanghi secondari dal trattamento delle acque reflue municipali. Le fasi iniziali si sono focalizzate sulla fermentazione e sulla linea aerobica, sono state ottenute una resa di fermentazione (Y_{VFA}) pari a 0.40 g COD_{VFA}/g VS ed una resa di stoccaggio di PHA in accumulo ($Y_{P/VFA}^{batch}$) pari a 0.50 COD_{PHA}/COD_{VFA}. Gli studi sono poi stati focalizzati sulla linea anaerobica, costituita da fermentazione e da co-digestione. Studi successivi sono stati diretti all'ottimizzazione della fermentazione in scala di laboratorio. Le migliori rese ottenute sono pari a Y_{VFA} di 0.57 e 0.74 gCOD_{VFA}/g VS rispettivamente in batch e in continuo. Queste sono state poi testate in scala pilota con una visione generale, che ha portato ad un notevole incremento della resa globale (76 g PHA/kg VS). Sono inoltre stati preliminarmente studiati barriere legislative ed accettazione sociale del prodotto legati all'implementazione sul mercato di bioplastiche derivanti da rifiuto. Come ottimizzazione finale, sono state indagate diverse condizioni operative nel processo di selezione, che hanno portato ad un netto miglioramento della resa globale dell'intera bioraffineria, pari a 110 g PHA/kg VS. I risultati emersi dalla presente tesi hanno dimostrato la fattibilità della filiera produttiva e la sua implementazione in scala industriale.

Firma dello studente

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