

## **ORGANIC CARBON RECOVERY AS BIOPOLYMERS FROM RESIDUALS AND WASTEWATER TREATMENT: STEPS FROM TECHNOLOGY DEVELOPMENT TO DEMONSTRATION**

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### **Abstract**

Valorization of the organic carbon from wastes/wastewater can be achieved in waste bio-refineries, which can transform raw organic and inorganic residuals into renewable products. Polyhydroxyalkanoates (PHAs) are biodegradable polyesters that can be produced as a novel adjunct to biological wastewater treatment in mixed-microbial activated-sludge systems. Process technologies have been developed and investigated for the production of: (i) volatile fatty acids (VFAs) by acidogenic fermentation, (ii) functional biomass with PHA-storage capacity from biological wastewater treatment, and (iii) PHAs from the further biological treatment of different waste and residual organic streams. Experience over 30 years from laboratory-scale and more recent pilot-scale studies has demonstrated the feasibility of treating different wastewaters while producing PHAs. Overall PHA yields of 10-20% (g PHA/g chemical oxygen demand removed) and biomass PHA contents of 40-60% (g PHA/g volatile suspended solids) have been consistently achieved. Steps are underway to establish business models of viable value-added chains for commercial demonstration of mixed-culture PHA production as an integral part of residuals management, as a spin-off from the company-stakeholder-network project BioTRIP. Environmental performance assessments of mixed-culture PHA process technologies are being critically considered. Establishing a stakeholder network in which residual carbon and microbial biomass sourcing is combined with essential services of pollution control while facilitating PHA-product-market combinations is identified as the next pivotal step for achieving the first viable examples of PHA production within a framework of residuals management in a bio-based economy.

### **Key words**

BioTRIP, demonstration, polyhydroxyalkanoates (PHAs), PHA production, residuals, wastewater, waste treatment

### **Introduction**

In the last decades, a family of polyoxoesters of hydroxyalkanoic acids, i.e., polyhydroxyalkanoates (PHAs), has attracted much interest in biotechnology and material science (Braunegg *et al.* 1998; Dias *et al.* 2006; Philip *et al.* 2007; Laycock *et al.* 2013). This interest is motivated by the thermoplastic properties of PHAs combined with their biodegradability and bio-based production feasibility. The possibility of producing

PHAs from renewable resources, especially, from industrial, agricultural and municipal residuals, has spurred advancements in pure and mixed microbial culture technologies for PHA production (Serafim *et al.* 2008a; Nikodinovic-Runic *et al.* 2013). As the opportunities, but also challenges, of PHA production and applications continue to be defined (Gumel *et al.* 2013), PHAs are recognised as key platform chemical raw materials within bio-refinery frameworks of bio-based schemes (Kleerebezem and van Loosdrecht 2007; Koller *et al.* 2011). In particular, in the management of wastewaters and solid wastes, PHA production via open, mixed microbial cultures represents an opportunity for recovering raw wastewater organic carbon by means of biological treatment (de Vegt *et al.* 2012), thus converting a traditional end-of pipe waste or process water treatment plant into a residuals bio-refinery.

Recently, work was undertaken to explore the technical, organizational and economic principles behind potential routes for the viable commercialization of PHA production from process water and residuals management, as part of the company-stakeholder-network project BioTRIP (acronym of the Dutch equivalent of biological transformation of residuals into market-demanded biopolymers) (de Vegt *et al.* 2012). Also, first-of-their-kind prototype pilot facilities for producing 1-2 kg/week of PHA-storing biomass while treating food industry (Eslöv, Sweden) and municipal (Brussels, Belgium) wastewaters have been in operation (AnoxKaldnes /Veolia Water & Solutions; Lund, Sweden) in order to complement laboratory studies of PHA production using a suite of available fermentable residual feedstocks. After more than 30 years of progress documented in numerous scientific publications and patent literature on PHAs, these first piloting prototypes in tandem with social and economic contextual BioTRIP activities represent first steps towards gaining full appreciation of the factors promoting the full-scale demonstration of mixed-culture PHA production as an integral working part of residual management. With this as a basis, the current minor fraction of the biopolymer market occupied by PHAs (Shen *et al.* 2009) may be envisioned to grow from grassroots to niche circular economies in the near future.

In this paper, an overview of the accrued experience on mixed culture PHA production integrated into residuals management is presented. Fundamental and practical developments are highlighted, with references to collaborative technical advances and practical applications. Furthermore, potential opportunities and challenges for achieving the demonstration of this process technology are shared, based on lessons learned in the BioTRIP project.

## **Process technology for PHA production, wastewater treatment and residuals management**

### *Mixed-microbial PHA production and wastewater treatment*

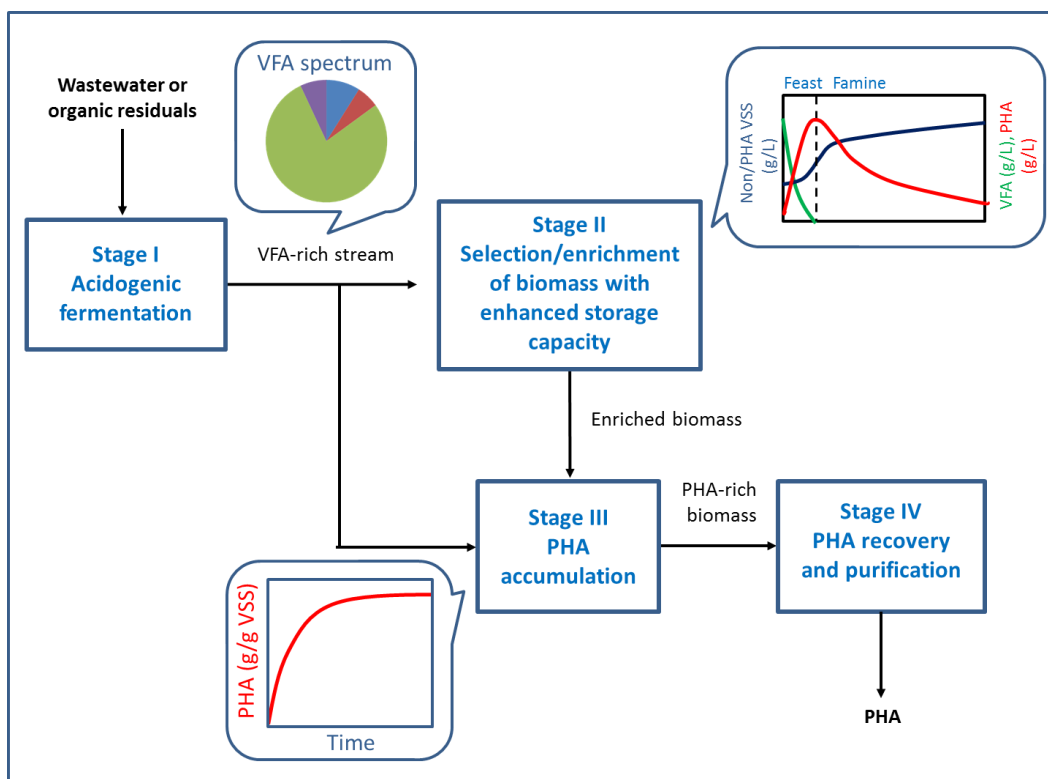
PHAs are naturally produced as intracellular carbon and energy reserves by many ubiquitous species of bacteria, and even archaea, under conditions of available organic substrate but often with some form of other growth limitation (Sudesh *et al.* 2000). Growth-limiting conditions are generally induced by limiting levels of other essential

nutrients (nitrogen or phosphorus) or electron acceptors (oxygen or nitrate). Therefore, PHA production with open, mixed cultures relies on the manipulation of environmental conditions that impose a selective pressure for the enrichment of PHA-storing bacteria in a given biomass and the accumulation of PHA to economically viable levels in this selected biomass.

In general, PHA production integrated into biological wastewater treatment involves selectively growing/enriching PHA-storing bacteria in activated sludge based on engineered environments to subject the biomass to dynamic conditions with respect to substrate feeding, i.e., alternating conditions of carbon availability and unavailability or feast-famine (Dionisi *et al.* 2004), or electron acceptor availability (alternating aerobic and anaerobic/anoxic conditions) (Bengtsson *et al.* 2008b).

The main substrates used for the selective growth of PHA-storing organisms and PHA production have been volatile fatty acids (VFAs) since VFAs are efficiently converted into PHAs (Luengo *et al.* 2003), whereas carbohydrates are preferably stored as polysaccharides (Dircks *et al.* 2001).

Until now, PHA production together with wastewater treatment has been preferentially investigated in a four-stage process (Fig. 1) (Serafim *et al.* 2008a): (i) acidogenic fermentation of wastewater for transforming organic carbon into VFAs and producing a VFA-rich stream, (ii) selection, enrichment and production of PHA-storing biomass based on ADF, (iii) PHA accumulation or production with excess biomass using the VFA-rich stream, and (iv) downstream processing of PHA-rich biomass and PHA recovery and purification.



**Figure 1:** Schematic representation of a typical 4-stage process for PHA production with open, mixed microbial cultures. Typical process assessments are illustrated.

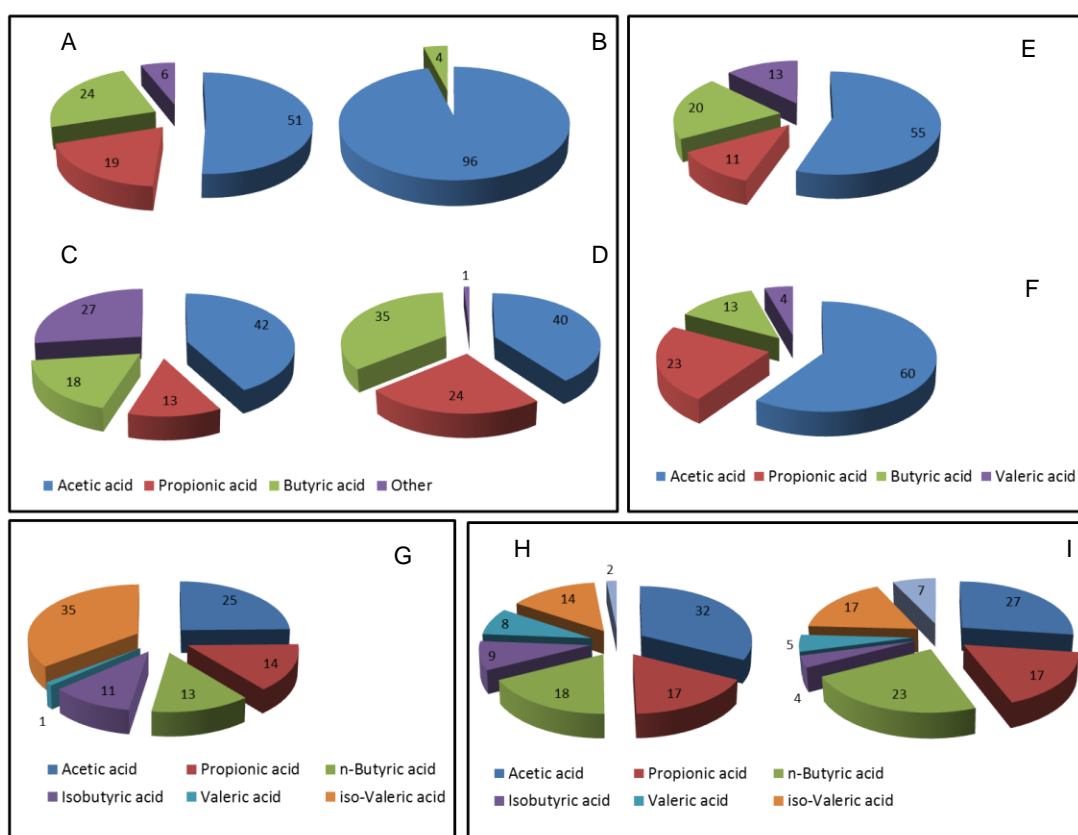
#### *Volatile fatty acids(VFAs) as platform chemicals for PHA production*

VFAs alongside sugars and oils are being increasingly considered as platform chemicals from which fuels as well as chemicals can be produced in a bio-based economy (Chang *et al.* 2010). VFAs are short-chain carboxylic acids containing 3 to 6 carbons that can be produced by microbial fermentation. Acidogenic fermentation of organic residuals has been extensively applied for converting waste organics into substrates for biogas production, as in anaerobic digestion, and for nutrient removal in biological wastewater treatment. VFA production via acidogenic fermentation helps to convert more complex organic streams, containing carbohydrates, lipids and proteins, into a more chemically uniform influent for valorising these organic residuals in wastewater and waste solids (e.g., sludge) and produce PHAs with open, mixed microbial cultures.

The feasibility of producing VFAs as feedstocks for PHA production from diverse industrial and waste streams has been demonstrated in batch and continuous acidogenic fermentations (Fig 2), e.g., olive oil mill wastewater (Dionisi *et al.* 2005a), molasses (Albuquerque *et al.* 2007), dairy wastewater and pulp and paper mill effluents (Bengtsson *et al.* 2008a), primary sludge (Coats *et al.* 2011), thermally hydrolysed waste activated sludge (WAS) (Morgan-Sagastume *et al.* 2011) and raw WAS (Xiong *et al.* 2012). The obtained VFA-rich streams have been used as substrates for ADF biomass enrichment and/or for direct PHA accumulation. The original wastewater composition

and fermentation process conditions greatly influence the VFA composition of the fermented stream, which in turn determines to a great extent the types of monomers in the final PHA product. Therefore, evaluating the acidogenic potential from different organic residual sources in relation to PHA production is important.

Although acidogenic fermentation has been generally applied as a *sine-qua-non* process step for PHA production with mixed microbial cultures, other forms of readily biodegradable organic carbon may be used to produce enrichment cultures or to accumulate PHA in a biomass. For example, methanol in pulp and paper mill foul condensate (Coats *et al.* 2007a), a mixture of ethanol, glycerol, esters and fatty acids in biodiesel wastewater (Coats *et al.* 2007a), and glycerol (Moralejo-Gárate *et al.* 2011) have been demonstrated to lead to biomass enrichment and different levels of PHA production.



**Figure 2:** Examples of spectra of VFAs obtained (% g COD<sub>VFA</sub>/L basis) from the acidogenic fermentation of different effluents for PHA production: (A) dairy, (B) pulp and paper mill, (C) paper mill white water, (D) pulp mill (Bengtsson *et al.* 2008a), molasses at a (E) C:N = 100:6 and (F) C:N = 100:3 (Albuquerque *et al.* 2007), (G) WAS (Xiong *et al.* 2012), and (H & I) thermally hydrolysed WAS from two full-scale plants (Morgan-Sagastume *et al.* 2010, 2011). COD = chemical oxygen demand

*Production of biomass with PHA-storage capacity*

Activated sludge biomass with enhanced PHA-storage capacity has been successfully produced by selectively growing and enriching PHA-storing bacteria under ADF or feast-famine, in which a growth selective pressure is applied to the biomass based on sequential periods of excess and exhaustion of VFA carbon under aerobic conditions (Salehizadeh and van Loosdrecht 2004; Dionisi *et al.* 2005b). Under ADF, biomass is subjected to an internal growth limitation and physiological adaptation, arising from alternate VFA-carbon substrate availability (feast) and starvation (famine). The extended famine period causes bacteria to decrease their internal amounts of cell-growth enzymes, and to store carbon as PHA under a following feast period, in which cells cannot reach maximum growth rates due to limited enzymatic amounts. The stored PHA can thus be used as internal carbon source during famine by PHA-storing bacteria, which provides them with a competitive advantage over others.

ADF or feast-famine selective growth and enrichment of PHA-storing bacteria in activated sludge has been demonstrated in integration with the treatment of different industrial effluents, solid waste and other residual streams, e.g., fermented olive mill wastewater (Dionisi *et al.* 2005a), effluent from a cellulose-acetate fibre manufacturing facility (Punrattanasin *et al.* 2006), palm oil mill effluent (Md Din *et al.* 2006), fermented molasses (Albuquerque *et al.* 2007), fermented alkaline WAS (Mengmeng *et al.* 2009), and thermally hydrolysed WAS (Morgan-Sagastume *et al.* 2010). Sequencing batch reactors (SBRs) have been commonly used in this process due to their easiness of control and process adaptability; nevertheless, continuous activated sludge processes are also applicable (Bengtsson *et al.* 2008b; Albuquerque *et al.* 2010a) as long as the feast-famine regime is sustained. The feast-famine regime can thus be applied in systems with selectors, return biomass or biomass recirculation and plug flow configurations among others.

Another approach for achieving selective growth of PHA-storing organisms in tandem with wastewater treatment is based on applying anaerobic-aerobic or anoxic-aerobic cycling or dynamic conditions, more specifically anaerobic/anoxic feast and aerobic famine. Anaerobic-aerobic cycling in connection with PHA production has been applied to the treatment of VFA-rich synthetic wastewaters (Sato *et al.* 1998; Takabatake *et al.* 2000; Dai *et al.* 2007; Bengtsson 2009), municipal wastewater and fermented primary sludge (Coats *et al.* 2007b), phosphorus-poor wastewaters, such as fermented food waste centrate (Rhu *et al.* 2003), and fermented sugar molasses (Bengtsson *et al.* 2010b). This approach selects for mixed microbial cultures rich in glycogen-accumulating organisms (GAOs) that are able to accumulate PHA from excess VFAs under anaerobic conditions, using internally stored glycogen as reducing power and energy source. Under aerobic conditions, glycogen is regenerated from PHAs. The characteristic channelling of carbon through glycogen by GAOs leads to differences in PHA production with respect to that of the ADF approach, such as different fractions of PHA monomers in the final product, possibility to operate the PHA accumulation either under anaerobic or aerobic conditions (Dai *et al.* 2007; Bengtsson 2009; Bengtsson *et al.* 2010b). Recently, anoxic-aerobic cycling has also been applied to

the treatment of nutrient-rich process waters from a sugar-beet factory (Anterrieu *et al.* 2013), in which case nitrification, denitrification and phosphorus removal were successfully achieved alongside feast-famine for the selection and growth of PHA-storing organisms.

Not only VFA-rich streams but also VFA-poor streams can be used for the selection, enrichment and production of PHA-storing biomass. The possibility of using directly wastewaters rich in readily degradable carbohydrates in combination with VFAs for the ADF enrichment of PHA-accumulating organisms was first indicated by Gurieff (2007) and Voltolini (2009). Recently, enrichment of PHA-producing organism has been achieved based on the readily biodegradable organic content of municipal wastewater (Morgan-Sagastume *et al.* 2013), which avoids using VFAs for this step of the PHA production process and thus most likely increases PHA production yields from incoming organic carbon into the treatment plant.

#### *PHA accumulation and production*

VFAs are the preferred substrates for the production of PHAs in biomass presenting PHA-storage capacity. Although preliminary studies on mixed-microbial PHA production used VFA-rich streams obtained from the fermentation of wastes/residuals for achieving PHA saturation in the biomass, the biomass enrichment/production was conducted with pure VFAs only (e.g., Dionisi *et al.* 2005a; Albuquerque *et al.* 2007). However, later on, fermented residual streams were also applied for both biomass enrichment and PHA accumulation, e.g., primary sludge (Coats *et al.* 2007b), sugar cane molasses (Albuquerque *et al.* 2007; Albuquerque *et al.* 2010a, b), paper mill wastewater (Bengtsson *et al.* 2008b), olive oil mill effluent (Beccari *et al.* 2009), and thermally hydrolysed WAS (Morgan-Sagastume *et al.* 2010), and the number of such studies with different substrates keeps increasing (e.g., Jiang *et al.* 2012).

The accumulation of PHA in enriched biomass has been generally conducted in batch or fed-batch mode with single or multiple substrate feeding pulses, and a wide range of biomass PHA contents from approximately 20 to 75 % (g PHA/g VSS or TSS) have been reported based on the different VFA-rich streams used as substrates and enriched biomasses (Table 1). Overall, a PHA content of about 45-55% (g PHA/g TSS) appears consistently reported using VFA-rich waste/residual streams with open, mixed microbial cultures. A biomass PHA content above about 40% is believed to be a rough threshold towards commercially viable PHA recovery. The quality of PHA produced in the biomass and the importance of producing and recovering thermally stable PHA of high molecular weight has been also recently reported (Werker *et al.* 2012). Other key productivity-related performance parameters are also frequently reported, such as, PHA storage yields (g PHA/g COD substrate consumed), PHA production rates (g PHA/g active biomass/h) and PHA productivities (g PHA/L/d or g PHA/g VSS/d). When considering both biomass enrichment/production and PHA accumulation from the same VFA-rich stream, overall PHA production yields of 0.10-0.2 kg PHA per kg COD treated could be expected (Bengtsson *et al.*, 2008b), but such assessments remain to be more widely and consistently reported.



**Table 1:** Performance overview of PHA accumulation processes with ADF open, mixed microbial biomass using fermented waste feedstocks as substrate

Fermented feedstock	Process	PHA composition (3HB:3HV, % molar basis)	Biomass PHA content (% g PHA/g TSS)	PHA storage yield (g COD <sub>PHA</sub> /g COD)	PHA production rate (mg COD <sub>PHA</sub> /g COD <sub>x</sub> /h)	PHA productivity (g PHA/L/h)	Source
Primary sludge	Batch	50:50 (mass)	53	n.a.	n.a.	n.a.	Coats <i>et al.</i> 2007b
	Batch	79-83:21-17	n.a.	0.44-59 (Cmol/Cmol VFA)	0.12-0.14 (Cmol/Cmol/h)	0.35 <sup>a</sup>	Albuquerque <i>et al.</i> 2007
Sugar cane molasses	Sequential batches with biomass retention	82-87:18-13	44-61 (g PHA/g VSS)	0.68-0.71 (Cmol/Cmol VFA)	n.a.	n.a.	Albuquerque <i>et al.</i> 2010a
Paper mill wastewater	Batch	39-47:61-53	43-48	0.55-67 (Cmol/Cmol VFA)	0.05-0.06 (Cmol/Cmol/h)	n.a.	Bengtsson <i>et al.</i> 2008
Olive oil mill effluent	Batch	n.a.	20 (g COD/g COD)	0.35	100-160	0.6 (g COD <sub>PHA</sub> /L/h)	Beccari <i>et al.</i> 2009
Alkaline WAS	Fed-batch	88:18	57	0.33-0.39 (g PHA/g VFA)	310 (mg TOC/g TSS/h)	0.38 <sup>b</sup>	Menmeng <i>et al.</i> 2009
Thermally hydrolysed WAS	Fed-batch	74:26	20	0.28 (Cmol/Cmol)	0.10 Cmol/Cmol/h	0.23 <sup>c</sup>	Morgan-Sagastume <i>et al.</i> 2010
Kraft mill effluent	Batch	n.a.	26-30****	0.10-0.14 (g PHA/g COD)	n.a.	n.a.	Pozo <i>et al.</i> 2011
Paper mill wastewater	Fed-batch	88:12	56-77 (g PHA/g VSS)	0.70-0.80	112-384 (mg PHA/g VSS/h)	n.a.	Jiang <i>et al.</i> 2012
WAS	Batch	90-91:10-9 (mass)	17-24	n.a.	n.a.	n.a.	Xiong <i>et al.</i> 2012
WAS	Fed-batch	66:34 (mass)	52 (g PHA/g VSS)	0.48	130 (mg PHA/g VSS/h)	n.a.	Morgan-Sagastume <i>et al.</i> 2013

<sup>a</sup>Estimated from calculated 16.4 Cmmol/L/h

<sup>b</sup>Estimated assuming 57% (g PHA/g VSS) with reported 2.473 g VSS/L at 3.67 h

<sup>c</sup>Estimated from reported 11 Cmmol/L/h

<sup>d</sup>Ratio of PHA-containing cells versus total cells

The types of PHAs produced during the accumulation process strongly depend on the composition of VFAs in the feedstock used as substrate, and on the composition of the microbial biomass enriched and produced under ADF or anaerobic-aerobic cycling. The composition of VFAs depend on the content of the stream as well as on the operating conditions during acidogenic fermentation (e.g., hydraulic and solids retention times, pH and temperature) (e.g., Bengtsson *et al.* 2008a). When using ADF-enriched biomass, these VFAs generally lead to the accumulation of PHAs containing 3-hydroxybutyrate and 3-hydroxyvalerate monomers (Table 1). In contrast, biomass enriched in GAOs by anaerobic-aerobic cycling can produce a wider range of monomers when fed with VFA-rich streams. Production of PHA containing 2-methyl-3-hydroxybutyrate and 2-methyl-3-hydroxyvalerate has been repeatedly reported, and recently also the production of significant levels (up to 23 mol-%) of the medium-chain-length (MCL) monomer 3-hydroxyhexanoate has been observed in GAOs treating fermented sugar cane molasses (Bengtsson *et al.* 2010a,b). Incorporation of MCL type monomers in the PHA leads to lower crystallinity and higher elongation to break. In addition, the lower melting temperature obtained leads to a broader thermal processing window (Bengtsson *et al.* 2010b).

#### Downstream processing, PHA recovery and final product

PHA recovery is necessary from the PHA-rich biomass produced in the accumulation process in order to separate the PHA resin from non-PHA cellular mass. This last, fourth stage in the PHA production process (Fig. 1) involves a downstream series of physico-chemical unit operations that can be grouped into three main steps: (i) biomass pre-treatment, (ii) PHA recovery generally by extraction, and (iii) PHA post-treatment or purification. For example, a downstream processing train employed in pure-culture PHA production may consist of homogenisation, centrifugation, solvent rinsing, centrifugation with buffer washing, and drying (Gurieff and Lant, 2007). Nevertheless, for mixed cultures, these unit operations need to be tailored towards a centralized process that can support recovery of PHA from PHA-rich-biomass sources containing a range of PHA contents (40



to 70%) and a range of co-polymer compositions. Such downstream processing should not only provide a final PHA with appropriate quality and purity for the envisioned application, but also consist of environmentally friendly operations with competitive costs, since all these aspects are crucial for the commercialization of the PHA production process. Ideally, the change from conventional "sludge disposal" to a route of PHA-rich-biomass exploitation should enable parallel benefits in the utilization/recovery of nutrients, energy content, and other chemicals from the non-PHA biomass.

The physico-chemical properties of the produced PHAs determine to a great extent the scope of application of these polymeric products; however, the PHA physico-chemical characteristics can also be customised based on the manipulation of the accumulation process. PHA characterization has been an important aspect of studies on PHA production with mixed microbial cultures. Although early studies focused mainly on determining PHA composition, molecular weight, and thermal stability (e.g., Serafim *et al.* 2008b; Albuquerque *et al.* 2011), more recent studies have also considered the interplay between physico-chemical properties, microstructure, crystallisation kinetics, molecular weight, rheological and mechanical properties (Arcos-Hernandez *et al.* 2013; Laycock *et al.* 2013). Physico-chemical properties of the biopolymers have been evaluated based on standard techniques including gas chromatography/mass spectrometry (GC/MS), Fourier transform infrared spectroscopy (FTIR) (Arcos-Hernandez *et al.* 2010), size exclusion chromatography (SEC), differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA). Polymer melt rheology and mechanical testing are currently under assessment (unpublished results).

Although PHAs are recognised as polymers with a wide range of applications (Philip *et al.*, 2007; Gumel *et al.*, 2013), Laycock *et al.* (2013) underlined the importance of demonstrating that the quality of mixed-culture PHAs can consistently meet the standards required for use in commercially interesting plastic applications. The path in this direction is being advanced by pilot-scale studies (e.g., Morgan-Sagastume *et al.*, 2013, Arcos-Hernandez *et al.* 2013), in which greater amounts of PHAs can be produced for assessing the polymer thermoplastic workability and compounding potential into bioplastics. Furthermore, the process performance in producing a defined PHA quality can be evaluated with respect to defined, real-life demands rather than speculated niche applications.

## **Process impacts on waste management and environmental performance**

Due to the attractive advantage of diverting renewable organic carbon from waste biomass (waste sludge) into a value-added product, the synergistic integration of PHA production with biological waste/water treatment has the potential to reduce waste sludge production and its associated management costs (Werker *et al.*, 2008), and change sludge minimization/disposal into an opportunity of utilization of a functional biomass. Currently, the potential of two process configurations coupling municipal wastewater treatment and PHA production for achieving sludge reduction is being evaluated as part of the project Routes (Braguglia *et al.*, 2012). In addition, the integration of PHA production with biological waste/water treatment is increasingly

being evaluated in terms of environmental impacts and sustainability characteristics since the resulting insights can help in optimizing the use of energy and chemicals, and can identify further non-PHA biomass resource recovery within the mixed microbial PHA production process (Koller *et al.* 2011; Heimersson *et al.* 2013). A first life cycle assessment (LCA) indicated that PHA production as a component of industrial wastewater treatment has merit from perspectives of environmental and economic impacts compared to pure-culture PHA production and conventional waste management producing biogas (Gurieff and Lant 2007). Furthermore, specific methodological challenges in LCAs of mixed-microbial PHA production together with essential services of waste treatment and renewable resource utilization are beginning to be more critically assessed by means of suitably selected allocation approaches (Heimersson *et al.* 2013) since currently definitive conclusions from LCAs on biopolymers are difficult to draw (Yates and Barlow 2013).

### **Technology demonstration steps and future perspectives**

In the case of mixed-culture PHA production, no full-scale installation exists up to date; however, a first step towards advancing the mixed-culture process technology for PHA production from fundamental/applied research to a first demonstration facility was pursued in the BioTRIP project (de Vegt *et al.* 2012). BioTRIP explored the technical, organizational (social) and economic needs for commercializing PHA production from waste and residuals in the Netherlands using AnoxKaldnes Veolia's Cella™ technologies for PHA production in synergy with biological wastewater treatment. Specifically, the technical integration of PHA production with residuals and waste management was evaluated based on specific case study, namely the sugar beet industry (Anterrieu *et al.* 2013). The positive outcomes of BioTRIP have motivated initiatives to advance further to demonstration scale the potential of carbon value chains, regional development and new economic networks in the Netherlands with piloting production of VFAs from agro-industry resources and piloting the use of municipal and industrial biomass sources for PHA production.

BioTRIP's experience has provided us with the basis for a philosophy of approach in which understanding how to combine efficiently residual carbon and microbial biomass sources as to generate mutual benefits in bio-based stakeholder networks is critical for establishing healthy value-chain business activities in which the first successful technical demonstration of mixed-culture PHA production could be achieved. This stakeholder chain would include different levels of public and private organizations, individuals and enterprises contributing to the process chain of going from waste/residuals to PHA-based products, encompassing a network that includes VFA production, essential residuals management and treatment, PHA-rich biomass (not any longer "waste sludge") management, residuals recovery and exploitation, PHA-based bioplastic or chemical production, and even final product disposal or recycling. Therefore, PHA production is just one element that, if considered in isolation of this value-chain stakeholder network, may be insufficient to justify its own economical implementation. The integration of PHA-rich-biomass production into the activities of water quality engineering opens a door to tackle an old problem of sludge with a new opportunity for renewable resources. At least for first implementations, a challenge is to achieve a balance in benefits to offset

respective costs associated with this chain for which reliable essential services of waste treatment and residual management are sustained with net benefits across all the stakeholders in the value chain.

## Conclusions

1. Process technology for PHA production as an integral part of wastewater treatment and wastes/residuals management is technically viable using volatile fatty acids as platform chemicals and mixed microbial cultures subjected to substrate or electron-acceptor dynamic conditions.
2. Specific PHA product market combinations, viable technology integration strategies and material flows that are motivated from life cycle assessment considerations underpin efforts and advancements under current investigation.
3. The successful demonstration of mixed-culture PHA production integrated to reliable essential services of waste treatment and residual management are likely to be achieved within stakeholder networks for which a positive balance of benefits and costs can be established along the whole value chain.

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