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Review

Scaling-up microbial community-based polyhydroxyalkanoate production: status and challenges

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HIGHLIGHTS

- Microbial community-based PHA production outcomes at pilot-scale are reviewed.
- Challenges to scale-up relate to linking context between process and application.
- Niche applications that can meet process and commercial demands are presented.
- Research needs and practical steps forward are identified and discussed.

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ABSTRACT

Conversion of organic waste and wastewater to polyhydroxyalkanoates (PHAs) offers a potential to recover valuable resources from organic waste. Microbial community-based PHA production systems have been successfully applied in the last decade at lab- and pilot-scales, with a total of 19 pilot installations reported in the scientific literature. In this review, research at pilot-scale on microbial community-based PHA production is categorized and subsequently analyzed with focus on feedstocks, enrichment strategies, yields of PHA on substrate, biomass PHA content and polymer characterization. From this assessment, the challenges for further scaling-up of microbial community-based PHA production are identified.

1. Introduction

Nowadays, as society motivates goals of developing circular economies, converting waste into valuable raw materials is increasingly drawing attention (Kehrein et al., 2020). For example, in 2018 every person in The Netherlands produced an average of 87 kg of organic fraction of municipal solid waste (OFMSW), 104 m^3 of wastewater and 18 kg of dry sewage sludge waste (CBS, 2020a; CBS, 2020b). Most of the OFMSW is currently either used for the production of methane containing biogas or compost. The wastewater from households is commonly treated in municipal wastewater treatment plants (WWTP) which discharge treated water but also produce a significant mass of waste activated sludge (WAS). The WAS is typically incinerated (with or without pre-treatment by anaerobic digestion) and remaining ashes are landfilled (CBS, 2020a). In keeping with the new circular economy package proposed by the EU for 2030, there should be well-defined steps undertaken to further develop the end of waste criteria for different waste streams (Commission, 2020). Therefore, from both legislative and environmental sustainability perspectives, the current waste treatment schemes are challenged to become further expanded into a wider repertoire of products and services from the resources that can be recovered from these waste streams. One promising waste valorization route is to produce biopolymers such as Kaumera gum (extracted from aerobic granular sludge) and/or polyhydroxyalkanoates (PHAs) from waste/wastewater organic matter (Feng et al., 2021; Rodriguez Perez et al., 2018). For instance, in 2020 the first full-scale Kaumera gum installation was launched in Zutphen (The Netherlands). In parallel, microbial community-based PHA production is also moving forward in developments motivating investments for scaling-up.

PHAs are a family of biodegradable polyesters that are naturally

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synthesized by a wide variety of microorganisms as energy and carbon reserves (Dawes and Senior, 1973). Due to an ecological role as storage polymers, PHAs are usually produced under growth limiting conditions and/or in dynamic environments characterized by the alternating presence and absence of carbon source and/or electron acceptor (Van Loosdrecht et al., 1997; Majone et al., 1999; Reis et al., 2003). PHAs are not soluble in water, therefore they are accumulated by bacteria as cytoplasmic intracellular granules forming inclusion bodies (Jendrossek and Pfeiffer, 2014). As polymers, PHAs offer promise in a wide variety of applications e.g. packaging, disposable items and/or biodegradable carriers (Raza et al., 2018).

Currently, PHAs are industrially produced by defined bacterial strains, so-called pure cultures, that can intracellularly accumulate PHAs up to 90% of their cell dry weight (Koller, 2018). Using pure culture fermentations methods, it is estimated that 25.3 kt PHA are produced yearly with an estimated market value of 7.0 US$/kg (Bioplastics, 2020; Vandi et al., 2018). In a pure culture process, high purity substrates and sterile conditions are required to avoid contamination by non-PHA accumulating microorganisms. The combination of refined substrates and white-biotechnology methods for commercial production results in reported high production costs for PHA compared to petroleum-based polymers used within the plastics industry (Raza et al., 2018). Unlike pure cultures, environmental biotechnology aims at designing and engineering process environments rather than working with specific microorganisms and axenic white biotechnology methods (Kleerebezem and van Loosdrecht, 2007). With this in mind, PHA-accumulating bacteria can be enriched by applying selective process conditions that favour PHA-producing microorganisms over non-PHA accumulating microorganisms (Kourmentza et al., 2017). By applying an alternating presence and absence of the carbon source, microorganisms that are able to accumulate PHAs when carbon is present (feast) increase in relative proportions due to a survival advantage during the longer periods without substrate (famine). In such a microbial community-based process, feedstock sterilization for limiting the growth of the non-PHA-storing phenotype is not important and therefore common waste streams can be used as feedstock without onerous steps of pre-treatment. Waste streams are normally fermented first to produce volatile fatty acids (VFAs) that are subsequently metabolized by the suitably enriched cultures to produce PHAs. Microbial community-based PHA production at laboratory scale can achieve a comparable cellular PHA content, as those that have been obtained with pure culture methods (up to 90% cell dry weight) (Johnson et al., 2009).

After the success of microbial community-based PHA production at lab-scale, research efforts continued with focus on the study of different selective conditions for enrichment of PHA-accumulating microorganisms and with the objective to maximize the cellular PHA content (Dias et al., 2006; Verlinden et al., 2007; Chanprateep, 2010; Valentino et al., 2017a; Kourmentza et al., 2017). In parallel, over the past 10 years efforts to scaling-up the technology have resulted in a total of 19 publications describing successes with pilot-scale experiences in which various waste streams have been used as feedstocks for microbial community-based PHA production and recovery. Pilot-scale installations enable the production of large amounts of PHAs making it possible to start to address quality in the context of commercial applications (see Table 1). Additionally, a pedigree of life cycle assessment studies have repeatedly concluded that there is a clear beneficial impact of microbial community-based produced PHA over petroleum-based polymers (Harding et al., 2007; Fernández-Dacosta et al., 2015a; Yadav et al., 2020; Bengtsson et al., 2017; Morgan-Sagastume et al., 2016; Heimersson et al., 2014; Gurieff and Lant, 2007). Despite these successful pilot plant operations to date and positive indications for environmental performance, breakthrough with a demonstration of commercial full-scale PHA production is still not yet realized.

This work aims to review the current status of microbial community-based PHA production, with emphasis on the collective experience gained from work at pilot-scale and, from those experiences, identify bottlenecks that present challenges to come on the road to realize full-scale production of commercial quality PHAs from waste and wastewater organic matter. For more information about PHA production in general, by pure culture methods and/or in the use of different selection pressures to enrich for a PHA-accumulating biomass, other recently published review articles are available (Kourmentza et al., 2017; Rodriguez Perez et al., 2018; Dietrich et al., 2017; Sabapathy et al., 2020; Li and Wilkins, 2020; Nikodinovic-Runic et al., 2013; Valentino et al., 2017b).

2. Pilot-scale PHA production

Nineteen published pilot-scale studies on microbial community-based PHA production can be broadly categorized into two different approaches based on biomass source: *enrichment accumulation* and *direct accumulation* approaches, as illustrated in Fig. 1.

The *enrichment accumulation* approach primarily focuses on the maximization of the PHA production through applied optimum selective pressures. This approach consists of an enrichment/selective step to produce as highly functional biomass as possible for PHA accumulation. This enrichment/selective step has been typically performed in a sequential batch reactor by feeding a pre-fermented VFA-rich stream under the aerobic feast-famine regime (Chakravarty et al., 2010; Tamis et al., 2014, 2018; Valentino et al., 2018, 2019a,b; Moretto et al., 2020b; Mulders et al., 2020a). After the functional biomass is produced, the same pre-fermented VFA-rich stream is most often used with appropriate modulation of nutrient concentrations and under fully aerobic conditions to maximize the biomass PHA content in the PHA accumulation process.

In contrast to *enrichment accumulation*, the *direct accumulation* approach primarily focuses on the use of the PHA-storage capacity of the WAS for PHA production. This approach is based on the subsequent exploitation of WAS produced as a by-product from treating wastewater in a WWTP. A WAS with significant PHA accumulation potential can be found in both industrial or municipal biological WWTP, even though the biological processes were primarily designed for organic carbon and nutrients removal. Municipal or industrial wastewater may not always have significant VFA content, but bioprocess conditions of the WWTP may nevertheless tend to enrich over time for the PHA-storing phenotype (Anterrieu et al., 2014; Morgan-Sagastume et al., 2014, 2015; Bengtsson et al., 2017,; Larriba et al., 2020; Conca et al., 2020). Conditions of dynamic substrate supply in these installations, are mainly due to the configuration/flow pattern, creating an inherent selection pressure (Van Loosdrecht et al., 1997). The use of WAS gives the opportunity to integrate PHA production processes into the municipal wastewater treatment as an extension to present day sludge management goals (Bengtsson et al., 2017). However, other supplies of (waste) organic feedstock are required in order to be able to exploit the polymer storing potential of the WAS exported from the WWTP.

2.1. Feedstocks

The cost of refined feedstocks for a PHA production process can represent up to 48% of the total costs (Rodriguez Perez et al., 2018). Waste streams as feedstock alongside microbial community-based bioprocess methods is an anticipated means to improve the overall PHA production economy at commercial scale. Moreover, the use of waste streams for PHA production fulfills goals in waste valorization objectives. However, waste streams may bring other complexities for production and product quality control, and especially if the accumulating biomass cannot be acclimated to specific production conditions or feedstock variabilities are too large. Potential challenges of PHA production from waste streams include potential for batch or seasonal compositional variations, too low relative VFA contents (0.35–1.00 gVFA-COD/gsCOD), presence of nutrients (100–800 mgNH_4^+/L), high salinity (>5 gNaCl/L), high solids contents (up to 1.5 gTS/L) and other

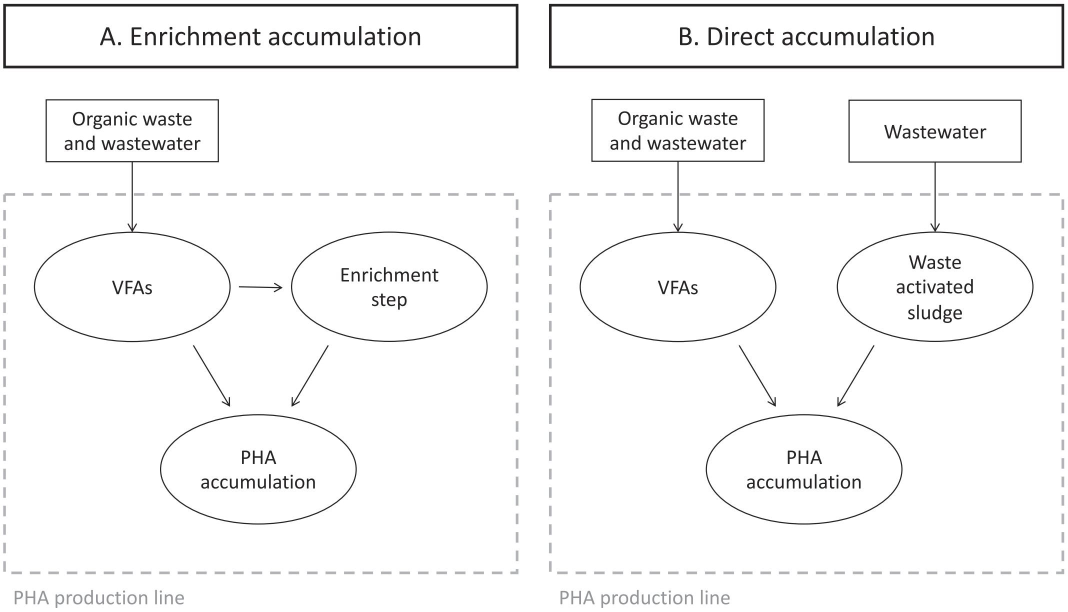

Fig. 1. Critical defining differences between the *enrichment accumulation* and the *direct accumulation* approaches. Adapted from Bengtsson et al. (2017).

Table 1
Definitions of the process properties used in this work.

Property	Description	Units
Feedstock	Substrate used for PHA production	–
Yield on substrate	Fraction of substrate used for PHA production	gPHA-COD/ gVFA-COD
Biomass PHA content	Ratio between PHA and volatile suspended solids	gPHA/gVSS
Polymer properties		
HB content	Fraction of *hydroxybutyrate* (HB) in the PHA co-polymer	gHB/gPHA (%)
HV content	Fraction of *hydroxyvalerate* (HV) in the PHA co-polymer	gHV/gPHA (%)
Molecular weight	Molecular weight (Mw) of the PHA co-polymer	kDa
Impurities	Non PHA fraction in the extracted PHA co-polymer	–

unknown compounds (Table 2). Nutrients and high salinity have been found to negatively affect PHA production (Johnson et al., 2009; Palmeiro-Sánchez et al., 2016). The non-VFA fraction can promote the growth of non-PHA accumulating biomass and together with high solids contents may act to effectively reduce/dilute the final biomass PHA content (Korkakaki et al., 2016). Unknown compounds could be carried over with recovery and this may negatively affect the polymer physical–chemical quality or its application under selected regulatory frameworks (Laycock et al., 2013).

First experiences at pilot-scale for both *enrichment accumulation* and *direct accumulation* approaches were performed with relatively simple wastewater streams characterized by high VFA contents and low nutrients, solids and salts concentrations i.e. fermented dairy wastewater (Chakravarty et al., 2010), fermented beet process wastewater (Anterrieu et al., 2014), fermented candy factory wastewater (Tamis et al., 2014; Bengtsson et al., 2017), fermented paper mill wastewater (Tamis et al., 2018) and starch-rich wastewater (Morgan-Sagastume et al., 2020). In more recent years, more complex waste streams have also been successfully evaluated. These streams were characterized by high non-VFA COD composition, excess nutrients, high suspended solids and unknown compounds concentrations i.e. fermented leachate from the OFMSW (Valentino et al., 2018, 2019a,b; Moretto et al., 2020b; Mulders et al., 2020a; Valentino et al., 2020; Moretto et al., 2020a), fermented tomato waste (Bengtsson et al., 2017) and fermented primary sludge (Morgan-Sagastume et al., 2015; Bengtsson et al., 2017; Conca et al., 2020). To cope with suspended solids, a solid–liquid separation stage for the feedstock prior entering the PHA production line has been evaluated (Valentino et al., 2018, 2019b; Moretto et al., 2020b; Valentino et al., 2020; Moretto et al., 2020a). Suspended solids for PHA accumulation feedstocks after fermentation were reduced to levels in the order of 0.02 gTSS/gsCOD by drum filtration (Bengtsson et al., 2017). To deal with the excess of growth nutrients that may promote the growth of non PHA-accumulating microorganisms, phosphorus levels were reduced to a COD:P of 100:0.1 (mass basis) by iron chloride precipitation before entering the PHA line (Bengtsson et al., 2017). Alternatively, a settling step, in the middle of the enrichment cycle, was used to limit the growth of non PHA-accumulating microorganisms by removing the non-VFA COD fraction, right after the VFA-COD fraction was consumed (Mulders et al., 2020a).

One can conclude that it is technically feasible to produce PHAs from different waste streams, either with biomass produced through specialized enrichment processes or produced as a by-product of mainstream biological wastewater treatment. Further up-scaling of the microbial community-based PHA production requires that at least one potential feedstock specifically for the polymer production is selected. The selected feedstock must be such that the volume of biomass and feedstock supplies are relevant towards meeting the demand in amount and quality of polymers supply in suitable economically supporting commercial application(s). Even if technical feasibility has been established for diverse feedstocks at pilot scale, evaluation and understanding of the relevance of the feedstocks in the context of a commercial production scale is still lacking in the literature.

2.2. *Yields of PHA on substrate*

Yields on substrates are defined as the amount of PHA produced per gram of consumed VFA (gPHA-COD/gVFA-COD) or per gram of consumed waste (gPHA/gWaste). However, how yields on substrate are reported in current literature can be misleading if applied with direct comparison. Many authors have reported the average yields when the PHA content reached saturation levels, while others reported the average yield when 95% of the maximum PHA content was reached (Rodriguez Perez et al., 2018). Similarly, some authors referred to the PHA yield on VFA while others have evaluated the yield on soluble COD. A common basis for description of the process yield is necessary towards

Table 2
Feedstocks used for the PHA accumulation reactors.

Feedstock	TS	VS	sCOD	VFA/sCOD	C2	C3	C4	C5	NH_4^+	PO_4^{3-}	Alkalinity	pH	References
	gTS/L	gVS/L	gCOD/L	–	%	%	%	%	mgN/L	mgP/L	mgCaCO$_3$/L	–	
Acetic acid	n.a.	n.a.	172.0	100	100	0	0	0	0	1541	–	–	Patel et al. (2009)
Acetic/Propionic acid	n.a.	n.a.	86.0	100	–	–	–	–	0	1541	–	–	
Fermented dairy wastewater	–	–	2.9–3.2	–	–	–	–	–	–	–	–	6 ± 0.3	Chakravarty et al. (2010)
Fermented beet process water	–	–	9.9 ± 1.3	35–90	28	33	39	–	147 ± 4	23 ± 5	–	–	Anterrieu et al. (2014)
Acetic acid	n.a.	n.a.	–	100	100	0	0	0	0	0	–	–	Morgan-Sagastume et al. (2014)
Fermented candy factory wastewater	–	–	7.8 ± 4.1	64 ± 15	32	14	33	5	Residual	Residual	–	4.5 ± 0.1	Tamis et al. (2014)
Primary sludge centrate	–	–	9.0 ± 1.0	90 ± 9	76	24	0	0	900 ± 100	480 ± 100	–	5.6 –6.4	Morgan-Sagastume et al. (2015)
Acetic acid	n.a.	n.a.	–	100	100	0	0	0	Growth-limiting and excess		–	–	
Tomate waste centrate	–	–	9.7–12.4	80–86	34	23	17	16	174–223	58–74	–	5.6	Bengtsson et al. (2017)
Acetic acid	n.a.	n.a.	80	100	100	0	0	0	800	40	–	4.5	
Synthetic mix	n.a.	n.a.	10	100	85–99	1–15	0	0	100	5	–	5	Bengtsson et al. (2017)
Fermented candy factory wastewater	0.2	–	16	–	C2+C4: 60–95		C3+C5: 5–40		80	16	–	5.5–6.0	
Primary sludge centrate	–	–	7	–	C2+C4: 50–75		C3+C5: 25–50		350	7	–	4.8–5.5	
Fermented paper mill wastewater	–	–	≈6.2	72	37	21	29	16	Residual	Residual	–	5.0	Tamis et al. (2018)
Acetic acid	n.a.	n.a.	–	100	100	0	0	0	0	0	–	–	Valentino et al. (2018)
Fermented OFMSW	n.d.	n.d.	16.0 ± 0.7	91 ± 9	21	13	38	12	400–480	80–112	894 ± 104	5.0 ± 0.2	
Fermented OFMSW and SAS mix	n.d.	n.d.	16.2 ± 0.5	90 ± 2	23	13	37	11	–	–	2800 ± 200	5.0–5.5	Valentino et al. (2019a)
Acetic acid	n.a.	n.a.	–	100	100	0	0	0	0	0	–	–	Valentino et al. (2019b)
Fermented OFMSW and SAS mix I	n.d.	n.d.	32 ± 5	64 ± 7	28	–	28	–	724 ± 138	127 ± 22	4811 ± 741	5.0–5.5	
Fermented OFMSW and SAS mix II	n.d.	n.d.	26 ± 3	75 ± 9	33	–	23	–	562 ± 44	110 ± 9	4451 ± 498	5.0–5.5	
Fermented OFMSW and SAS mix	n.d.	n.d.	34 ± 3	86 ± 5	C2+C4: 75		C3+C5: 25		570–873	130–152	–	5.0–5.5	Moretto et al. (2020b)
Municipal wastewater	–	–	0.14 ± 0.04	–	–	–	–	–	40± 11	4.2 ± 1.1	–	–	Larriba et al. (2020)
Acetic acid	n.a.	n.a.	–	100	100	0	0	0	0	0	–	–	Conca et al. (2020)
Fermented cellulosic primary sludge liquid	–	–	8.8 ± 1.6	94	24	50	12	9	326 ± 23	70 ± 12	–	4.8 ± 0.1	
Fermented OFMSW	1.5 ± 0.8	0.9 ± 0.5	5.8 ± 1.1	50 ± 13	C2+C4: 56		C3+C5: 44		622 ± 159	20.9 ± 10.8	70 ± 10*	7.5 ± 0.4	Mulders et al. (2020a)
Fermented OFMSW	n.d.	n.d.	20 ± 3	73 ± 8	–	–	–	–	615 ± 37	140 ± 13	–	–	Valentino et al. (2020)
Fermented OFMSW and SAS mix	n.d.	n.d.	27 ± 4	85 ± 9	–	–	–	–	673 ± 72	119 ± 11	–	–	
Fermented OFMSW and SAS mix	n.d.	n.d.	36 ± 2	86 ± 5	C2+C4: 54–74		C3+C5: 35–55		689 ± 15	220 ± 6	–	5.0–5.5	Moretto et al. (2020a)
Acetic acid	n.a.	n.a.	9.0	100	100	–	–	–	90	4.5	–	–	Morgan-Sagastume et al. (2020)
Acetic/propionic acid	n.a.	n.a.	9–100	100	C2+C4: 60–100		C3+C5: 0–40		1000	50	–	5.6	
					94	–	–	–	n.d.		–	4.8–6.5	

(continued on next page)

Table 2 (*continued*)

Feedstock	TS	VS	sCOD	VFA/ sCOD	C2	C3	C4	C5	NH_4^+	PO_4^{3-}	Alkalinity	pH	References
	gTS/L	gVS/L	gCOD/L	–	%	%	%	%	mgN/L	mgP/L	mgCaCO$_3$/L	–	
Potato-starch factory effluent	0.3 ± 0.1	0.2 ± 0.1	9.5 ± 1.6	73 ± 10						18 ± 56			

*meq/L; n.a.: not applicable; n.d. not detectable.

making meaningful insights of process or feedstock related differences. As Rodriguez Perez et al. (2018) proposed, it is recommended at least to report both PHA yield related to the added VFA and PHA yield related to the added waste (on a COD basis). Yield should be calculated for the same relative time points in the PHA accumulation process. It is considered that this time point should be relative to degree of saturation, rather than an absolute point in time since kinetics of accumulation may differ even for the same biomass source (Morgan-Sagastume et al., 2020).

Ideally, the yields on substrates should be as close to the maximum theoretical values and as consistent as possible from production batch to batch. The PHA yields on substrate are considered to be affected by the presence or absence of nutrients (N and P) and the VFA composition (Shi et al., 1997). PHA production is usually seen as an overflow mechanism in which PHA is produced when the specific substrate uptake rate exceeds the substrate flux that is used for growth (Tamis et al., 2014). Therefore, if nutrients are limiting in the feedstock, growth can be minimized and PHA production would be maximized (Johnson et al., 2009). Moreover, the maximum theoretical yield is only determined by the feedstock composition. Butyrate and valerate have slightly higher COD-based product yields compared to acetate and propionate. This difference can be explained by the fact that these polymer precursors (butyrate and valerate) require less ATP per unit of COD to be converted into PHA than the others (acetate and propionate). Consequently, the theoretical PHB yield on butyrate is 0.84 gPHB-COD/gButyrate-COD compared to 0.75 gPHB-COD/gAcetate-COD on acetate (Marang et al., 2013). Differences in reported yield between pilot installations may come from the feedstock composition and not only from how the system was operated. Therefore, it is recommended to always report a full description of the VFAs present in the feedstock for meaningful research contribution. Additionally, future work on the fermentation of waste streams prior to the PHA production line can focus on methods for selectively producing more butyrate and valerate rather than acetate and propionate.

Regarding the *enrichment accumulation* approach, reported average yields in the accumulation reactor are found to be similar and close to the theoretical maximum. Yields of 0.61–0.68 gPHA-COD/gVFA-COD were observed when synthetic or nutrient-poor streams were used as feedstocks (Tamis et al., 2014, 2018; Valentino et al., 2019b, 2018; Conca et al., 2020). However, when nutrients-rich streams were applied, a wider range with lower average yields have been reported, 0.33–0.61 gPHA-COD/gVFA-COD (Valentino et al., 2018, 2019b; Moretto et al., 2020b; Valentino et al., 2020; Moretto et al., 2020a). This was not the case for Mulders et al. (2020a) that reported the highest PHA accumulation average yield on substrate so far at pilot-scale, 0.73 gPHA-COD/gVFA-COD while working with leachate from OFMSW, a nutrients-rich and acetate-rich waste stream (0.44 ± 0.11 gAcetate-COD/gVFA-COD). In the *enrichment accumulation* approach, 25–50% of the feedstock is directed to biomass production in the enrichment reactor. Effectively, the overall average PHA yield on substrate is lower, ranging between 0.17–0.55 gPHA-COD/gVFA-COD. Consistent yields are reported within the same work (<10% error), however large differences between different pilot scale works seem to exist. The reason for such case to case variability is unclear and this suggests need for deepened fundamental insight concerning regulating factors of process or context.

In the *direct accumulation* approach, the reported average accumulation yields have generally been lower and more variable in nature, independently of the nutrients levels and composition of the feedstock, 0.20–0.61 gPHA-COD/gVFA-COD (Anterrieu et al., 2014; Morgan-Sagastume et al., 2015; Bengtsson et al., 2017,; Conca et al., 2020; Morgan-Sagastume et al., 2020). An acclimation to the feedstock has been reported to improve the performance of direct accumulation (Morgan-Sagastume et al., 2017). It is important to emphasize that in the *direct accumulation* approach the feedstock is used only for PHA synthesis in the accumulation reactor, in contrast to the *enrichment accumulation* approach. Thus, the overall performance of PHA production yield is similar (neglecting biomass production as a by-product of services in waste water treatment).

Independently of the approach used, it was observed that the PHA average yield on substrate decreased over the time of accumulation, when feedstocks with growth nutrients are used. It was, therefore, suggested to keep the accumulation periods as short as needed to reach PHA saturation levels (Bengtsson et al., 2017; Tamis et al., 2018). In order to keep high average PHA yields in longer accumulation runs, Valentino et al. (2015) suggested to incorporate simultaneous growth of the PHA-accumulating microorganisms in the PHA accumulation run. Daughter cells have been observed to contain half of the cellular PHA content of the mother cells (Pfeiffer and Jendrossek, 2012). As new cells (with more available space for PHA) are produced, PHA production rates can be maintained high for longer periods of time even if more substrate is directed away from conversion to PHA. Mechanisms to favour the growth of PHA-accumulating microorganisms over non PHA-accumulating microorganisms do not appear to be well-established in the research literature and these methods remain to be further developed. A combination of nutrient levels and a feed-on-demand feeding strategy were shown to promote the growth of only PHA-accumulating microorganisms during the PHA accumulation without loss of overall biomass PHA content (Valentino et al., 2015; Mulders et al., 2020b). Sustained selective growth under conditions of partial phosphorus limitation has been applied as a biomass enrichment method starting with municipal activated sludge (Cavaillé et al., 2013).

The feedstock complexity, the presence of nutrients and different salinity levels can be factors limiting the potential level of PHA yields on substrate. However, recent research still suggests a potential to achieve consistent PHA yields from batch to batch, and close to the theoretical maximum, when either the *enrichment accumulation* or the *direct accumulation* approaches were used. Overall it can be concluded that specific feedstock dependent pre-treatment methods enable high PHA yields. As the PHA yield on soluble COD determines how much substrate eventually ends up in PHA, these results facilitate improvements in performance and economy for the further scaling-up of microbial community-based PHA production.

2.3. *Operational conditions and dominant species*

The feedstock composition and the operational conditions of the bioprocess selecting for growth of the PHA-storing phenotype determine the microbial community structure in the process and therefore the maximum PHA content that can be achieved per unit of total biomass. It was already shown that the alternating presence of carbon source (feast-famine regime) effectively enriches for PHA-accumulating bacteria and this has been the adopted approach for the biomass production explicitly (*enrichment accumulation*) or implicitly (*direct accumulation*) in all the reported research work performed at pilot-scale. However, modifications of the operational conditions in the selection reactor have resulted in very different outcomes (Table 3). In most of the pilots, pH was only monitored, but not controlled and pH ranged from 6.5 to 9.2. Only in a couple of studies has pH been explicitly controlled between 6.5 and 7.5 (Tamis et al., 2014, 2018). Hydraulic and solids retention times during biomass enrichment were lower than 2 d, excluding those from the *direct accumulation* approach, in which nutrients removal and sludge production were coupled, and were in the range 5–20 d (Bengtsson et al., 2017; Conca et al., 2020; Morgan-Sagastume et al., 2020). Temperature was generally not controlled and in the range 15 to 35 °C, but when temperature was controlled, the values were 22–25 °C (Valentino et al., 2019b), 25–28 °C (Moretto et al., 2020b,a) and 30 ± 2 °C (Tamis et al., 2014, 2018; Mulders et al., 2020a).

Notwithstanding, selection has been suggested to be sensitive to temperature and pH based on studies where the resultant dominant microorganisms were assessed under the applied selection conditions. For the *enrichment accumulation* approach, under well-defined conditions of temperature and pH (pH 7 and 30 °C), *Plasticicumulans acidivorans* was found to be the dominant microorganism (Tamis et al., 2014, 2018). When temperature remained at 30 °C, but pH was not controlled, and resulted in pH ranging between 8.5 and 9.2, an uncultured *Rhodocyclaceae* bacterium clone JT01 was found to be the dominant microorganism (Mulders et al., 2020a). Under similar pH conditions, but lower temperature (15–29 °C) a member of the *Hydrogenophaga* spp. were enriched (Crognale et al., 2019). These outcomes emphasize that changes in operational conditions, such as pH or T, may result in different dominant microorganisms (Crognale et al., 2019; Mulders et al., 2020a), which may ultimately be an important factor that influences the maximum PHA content that can be achieved in an accumulation process. Temperature was found to be optimum at 30 °C in lab-scale systems for the enrichment of high PHA-accumulating microorganisms (Stouten et al., 2019). However, reasons why different dominant species are enriched under apparently similar conditions remains an open question begging deepened understanding.

In the *direct accumulation* strategy, there has been already a lot studied about the microbial structure of WAS (Cydzik-Kwiatkowska and Zielińska, 2016). However only a limited focus has been given in different studies on the abundance of the PHA production phenotype in WAS samples (Morgan-Sagastume, 2016). It has been observed that the PHA accumulation potential of biomass produced in enhanced biological phosphorus removal processes (bio-P sludge) was lower than those from biomass enriched under aerobic and/or anoxic conditions (Bengtsson et al., 2017). Fundamental understanding about why bio-P activated sludge has shown less PHA accumulation potential compared to non bio-P activated sludge is still unknown and also remains a point of misunderstanding especially in the municipal sector given the common popular association of bio-P metabolism with PHA storage.

One of the main differences between the *enrichment accumulation* and the *direct accumulation* approach is the process operational conditions. Differences in operational conditions have also resulted in different dominant species with different characteristics. The fundamental reason of why PHA-accumulating microorganisms with high maximal PHA contents are enriched instead of PHA-accumulating microorganisms with low maximal PHA contents with similar feedstocks, feast-famine cycle lengths, and temperature requires further investigation.

2.4. Biomass PHA content

Biomass PHA content, in most cases, refers to a relative amount of PHA and with respect to the biomass volatile and/or total suspended solids (gPHA/gVSS or gPHA/gTSS). The reported maximum attainable amounts of biomass PHA content is one of the major differences in expectation between *enrichment accumulation* and *direct accumulation* approaches as shown in Table 4. Processes of *enrichment accumulation* are typically tuned to be able to produce a more specialized biomass for PHA production, and as such, with expected higher PHA accumulation potential. Biomass PHA content is important because it can influence on the costs of the downstream processing (DSP) and the recovered polymer quality. To recover the same mass of recovered PHA, greater amounts of biomass need to be processed the lower the biomass PHA contents.

With the *enrichment accumulation* approach, high PHA contents, 0.7–0.8 gPHA/gVSS, were attained in pilot accumulations with pre-fermented industrial wastewater streams from candy and paper mill factories (Tamis et al., 2014, 2018). With similar process configuration but without pH control (Mulders et al. (2020a)) reported 0.77 gPHA/gVSS by using fermented OFMSW as feedstock. Using fermented OFMSW or a filtered fermented mixture of 30% OFMSW and 70% biological sludge (v/v), a series of accumulation batches PHA contents in the PHA-rich biomass ranged between 0.33 gPHA/gVSS and 0.59 gPHA/gVSS (Valentino et al., 2018, 2019a,b; Moretto et al., 2020b; Valentino et al., 2020; Moretto et al., 2020a).

Using the *direct accumulation* approach, accumulation up to 0.52 gPHA/gVSS were reported when feeding fermented waste VFA-rich streams to WAS from selected municipal and/or industrial WWTPs (Bengtsson et al., 2017; Conca et al., 2020; Morgan-Sagastume et al., 2020). These results were obtained without any modification to the wastewater treatment line, even if simple adjustment or process modifications were foreseen to introduce improvements due to imposed periodic feast stimulation to the process biomass. It was interpreted that the quality of the “feast” environment established in the full-scale process was influential to the WAS capacity for PHA storage. To demonstrate a potential to engineer selective pressure for the PHA storing phenotype while treating the municipal wastewater, biomass was produced based on a pilot-scale anoxic-feast and aerobic- famine selection pressure (Bengtsson et al., 2017). The WAS from the pilot system was found to accumulate up to 0.49 gPHA/gVSS compared to the WAS coming from the full-scale installation that could only accumulate up to 0.15 gPHA/gVSS. An essential difference in process was the quality of the feast environments between pilot and full-scale systems given the same wastewater.

Pilot-scale PHA contents achieved have been reported in the range of 0.4–0.8 gPHA/gVSS for the *enrichment accumulation* approach and in the range of 0.4–0.6 gPHA/gVSS for the *direct accumulation* approach. Results within individual studies have been relatively robust even if outcomes in accumulation potential are varied between the respective piloting experiences. Reasons why outcomes of PHA accumulation potentials are varied between the piloting experiences provided in the literature may be related to:

1. growth of non-storing organisms, diluting the PHA-rich biomass;
2. degree of enrichment with a reduced fraction of PHA producing biomass;
3. production of other storage products;
4. differences in the individual species respective maximum possible PHA content;
5. differences in the physiological state of the PHA-accumulating biomass at the time of accumulation.

All these interpretations are plausible in the context of the studies that have been made. However, as mentioned above, the measurement of biomass PHA content to date are ambiguous to understand if and/or when these five factors may apply, more or less. At the same time, these five points also motivate that other kinds of measurements than just PHA content, coupled with continued fundamental investigations are warranted towards improved productivity and control from a greater command in applied methods of environmental biotechnology.

Overall, both enrichment accumulation and direct accumulation strategies have repeatedly achieved biomass PHA content in excess of

Table 3
Operational conditions of the selection reactor at pilot-scale level.

Feedstock	Enrichment	OLR gCOD/(L d)	HRT d	SRT d	Cycle length d	Temperature °C	pH	Dominant Microorganism	Reference
Acetic acid	Aerobic feast Aerobic famine	0.6	1	10	0.5	35	8.3–8.7	–	Patel et al. (2009)
Acetic acid/propionate		0.6	1	5	0.5	35	8.3–8.7	–	
Fermented dairy wastewater	Aerobic feast Aerobic famine	–	0.72	2.96 ± 2	–	–	8.2 ± 0.2	–	Chakravarty et al. (2010)
Fermented beet process wastewater	Aerobic feast Aerobic famine	–	5	–	–	–	–	–	Anterrieu et al. (2014)
Municipal wastewater	Aerobic feast Aerobic famine	3	0.21	1–2	0.08	–	–	–	Morgan-Sagastume et al. (2014)
Fermented candy factory wastewater	Aerobic feast Aerobic famine	≈5	1	1	0.5	30 ± 2	6.5–7.5	P. acidivorans	Tamis et al. (2014)
Municipal wastewater	Anoxic feast Aerobic famine	3.0 ± 0.8	0.125		1.7 ± 1.2	8.4–22.8	–	–	Morgan-Sagastume et al. (2015)
Tomate waste centrate	Anoxic feast Aerobic famine	1.8 ± 0.7	0.125–0.25	5.9	0.08	20	–	–	Bengtsson et al. (2017)
Municipal wastewater	Anoxic feast Aerobic famine	–	–	17	–	10–23	–	–	Bengtsson et al. (2017)
Fermented paper mill wastewater	Aerobic feast Aerobic famine	≈5	1	1	0.5	30 ± 2	6.6–7.2	P. acidivorans	Tamis et al. (2018)
Fermented OFMSW	Aerobic feast Aerobic famine	2.5–3.0	1	1	0.25	14–29	8.0–8.5	–	Valentino et al. (2018)
Fermented OFMSW	Aerobic feast Aerobic famine	2.0–3.4	1	1	0.25	16–28	8.0–8.7	Hydrogenophaga spp	Valentino et al. (2019a)
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	4.0	1	1	0.25	22–25	8.0–9.0	Hydrogenophaga spp	Valentino et al. (2019b)
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	3.1–5.9	1	1	0.25	25–28	–	Hydrogenophaga spp	Moretto et al. (2020b)
Municipal wastewater	Anaerobic feast Anoxic famine	–	1.0–1.5	10–15	0.3–0.5	–	–	–	Larriba et al. (2020)
Fermented cellulosic primary sludge liquid	Aerobic feast Anoxic famine	0.89–1.58	1.7–2.3	6–7	≈0.5	18.8–26.8	–	–	Conca et al. (2020)
Fermented OFMSW	Aerobic feast Aerobic famine	≈8	0.71	1	0.5	30±3	8.5–9.2	Uncultured Rhodocyclaceae	Mulders et al. (2020a)
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	2.2–4.4	1	1	0.25	15–34	8.0–9.0	–	Valentino et al. (2020)
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	4.0	1–2	1–2	0.25–0.5	25–28	–	–	Moretto et al. (2020a)
Potato-starch factory effluent	Aerobic feast Aerobic famine	2.2 ± 0.4	4.5 ± 0.6	7.1 ± 1.1	0.33	27 ± 3	8.5 ± 0.4	–	Morgan-Sagastume et al. (2020)

Table 4
Operational conditions of the accumulation reactor and PHA quality at pilot-scale level.

Feedstock	Enrichment	Biomass	$Y_{PHA/VFA}$	PHA Content	HV	Mw	Effluent	Reference
			gPHA-COD/ gVFA-COD	gPHA/gVSS	%	kDa		
Acetic acid	Aerobic feast Aerobic famine	Same as enrichment	–	0.21 ±0.02	0	2200	Yes	Patel et al. (2009)
Acetic/Propionic acid				0.25 ±0.03	5.6	2300		
Fermented dairy wastewater	Aerobic feast Aerobic famine	Same as enrichment	0.21–0.26	0.39–0.43	–	–	–	Chakravarty et al. (2010)
Fermented beet process wastewater	Aerobic feast Aerobic famine	Same as enrichment	–	0.60	–	–	No	Anterrieu et al. (2014)
Municipal wastewater	Aerobic feast Aerobic famine	Municipal activated sludge	0.20–0.38	0.19–0.34	15	980	–	Morgan-Sagastume et al. (2014)
Fermented candy factory wastewater	Aerobic feast Aerobic famine	Same as enrichment	0.30 ±0.04*	0.70–0.80	16	–	N,P	Tamis et al. (2014)
Primary sludge centrate	Anoxic feast Aerobic famine	Municipal activated sludge	0.25–0.37	0.27–0.38	0–30	500	Yes	Morgan-Sagastume et al. (2015)
Acetic acid				0.33–0.39	–	–		
Tomato waste centrate	Anoxic feast Aerobic famine	Municipal activated sludge	0.30–0.39	0.34–0.45	42–49	–	Yes	Bengtsson et al. (2017)
Acetic acid			0.34–0.53	0.19–0.49	0			
Synthetic mixture	Anoxic feast Aerobic famine	Municipal activated sludge	0.35–0.48	0.37–0.43	0–44	700–1500	–	Bengtsson et al. (2017)
Fermented candy factory wastewater			0.28–0.52	0.37–0.43				
Primary sludge concentrate			0.28–0.55	0.28–0.42				
Fermented paper mill wastewater	Aerobic feast Aerobic famine	Same as enrichment	0.68	0.65–0.76	25	–	No	Tamis et al. (2018)
Fermented OFMSW	Aerobic feast Aerobic famine	Same as enrichment	0.43–0.57	0.39–0.52	7–13	–	Yes	Valentino et al. (2018)
Acetic acid			0.61–0.64	0.37–0.42	0			
Fermented OFMSW	Aerobic feast Aerobic famine	Same as enrichment	0.33–0.44	0.38–0.49	11–13	–	–	Valentino et al. (2019a)
Fermented OFMSW and SAS mix I	Aerobic feast Aerobic famine	Same as enrichment	0.50 ± 0.04	0.43±0.01	10	–	–	Valentino et al. (2019b)
Fermented OFMSW and SAS mix II			0.44±0.03	0.46±0.05	13			
Acetic acid			0.67±0.05	0.40±0.02	0			
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	Same as enrichment	0.59±0.04	0.52±0.04	–	–	–	Moretto et al. (2020b)
Municipal wastewater	Anaerobic feast Aerobic (Anoxic) famine	Municipal activated sludge	–	0.03–0.07	–	–	–	Larriba et al. (2020)
Fermented cellulosic primary sludge liquid	Aerobic feast Anoxic famine	Same as enrichment	0.61±0.07	0.44±0.06	21–41	–	Yes	Conca et al. (2020)
Acetic acid			0.60±0.06	0.47±0.05	0			
Fermented OFMSW	Aerobic feast Aerobic famine	Same as enrichment	0.44**	0.77±0.18	50	–	–	Mulders et al. (2020a)
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	Same as enrichment	0.33–0.47	0.36–0.48	–	–	–	Valentino et al., 2020
Fermented OFMSW and SAS mix	Aerobic feast Aerobic famine	Same as enrichment	0.47–0.59	0.40–0.59	–	–	–	Moretto et al., 2020a
Acetic acid	Aerobic feast Aerobic famine	Same as enrichment	0.67±0.15	0.52±0.05	0.1±0.1	548±66		Morgan-Sagastume et al., 2020
Acetic/Propionic acid			–	0.52–0.61	24/63	181/602		
Potato-starch factory effluent			0.69±0.15	0.45±0.06	1.9±0.8	547±78	–	

0.4 gPHA/gVSS. Above this level, it has been suggested that DSP becomes increasingly more economically viable (Reis et al., 2003). Even though differences in PHA content are found case-to-case, results from piloting experiences nevertheless suggest a maturity in technological feasibility and readiness level to produce polymers by a microbial community-based approach.

2.5. Polymer quality characterization

PHAs as polymers can be characterized in terms of attributes related to co-polymer composition and its distribution, average molecular weight (Mw) and its distribution, thermal properties, mechanical properties and impurities (Laycock et al., 2013; Bengtsson et al., 2017). The polymer properties are not constant through the PHA production process. Different operational conditions in the upstream will lead microorganisms to accumulate PHAs with different physico-chemical properties. The evolution of polymer properties during the PHA accumulation process it is not well understood. Importantly, the polymer properties generated in the upstream are interlinked with selection of DSP methods, and the DSP also will further modulate polymer properties. The final PHA properties after DSP determines the range of possible applications for the polymers. From these 19 pilot studies, the polymer properties after the accumulation and before the DSP are the most frequently reported.

Currently, for microbial community-based PHA production, the most common polymers are comprised of a blend of HB (3-hydroxybutyrate) and HV (3-hydroxyvalerate) monomers. From different pilot studies, the reported HV fraction has varied between 0 to 50%, as described in Table 4. The main reason for differences obtained for HB/HV ratios is the feedstock VFA composition (Jiang et al., 2011). It has been demonstrated that even for a full-scale activated sludge over 4-seasons of operations, polymer type was predictable as a function of the feedstock composition (Werker et al., 2020). Polymer properties were directly related to the average HV content assuming distributions in blends of random co-polymers. Feedstock variability influencing batch-to-batch polymer composition product quality was shown to be controllable by suitable blending of batches into a master batch as part of the polymer recovery and purification. After blending, PHAs can be extracted from this master batch at a larger scale which favours an improved scale in economy for the cost of the extraction processes (Bengtsson et al., 2017; Werker et al., 2020). More importantly, it was indicated that the HV content of the blended master batch is predictable if the HV contents from the different inputs are known. This predictability gives an opportunity to manipulate the HV content during the DSP for different industrial applications. HV content and its distribution influences crystallization and crystallinity of these co-polymer blends can mean specific requirements for tuning of DSP conditions (Laycock et al., 2013; Koyama and Doi, 1997; Chan et al., 2017; Cal et al., 2019; Werker et al., 2020). It was reported with the same thermal history, the higher the average HV content up to the eutectic point of the co-polymer blend, the lower the maximum crystallinity of the polymer. The maximum crystallinity of the co-polymer blends to be recovered are closely tied to the extraction conditions such as the selection of the type of extraction solvent, the optimum extraction temperature and the duration of the extraction. When it comes to water-based methods of polymer purification, polymer crystallinity influences the survivability of the polymer as a function of time, pH and temperature (Yu et al., 2005; Yu, 2009; Porter and Yu, 2011).

Besides HV content, modulating crystallinity, Mw and thermal stability of recovered PHAs is important with respect to possible methods of formulation and processing alongside targeted properties in the specific context of the intent for the material in application. For example, using PHA for fiber spinning would require higher Mw than using PHA as an additive in other polymers (Bengtsson et al., 2017). Even though the produced polymer with different Mw would have different opportunities in types of possible applications, one still may prefer to produce the polymer with a higher Mw since it will enlarge the window of opportunity for the range of possible applications. However, so far, Mw has been only characterized in few studies and range up to 2300 kDa (Bengtsson et al., 2017; Patel et al., 2009; Morgan-Sagastume et al., 2014, 2015, 2020). The Mw of the polymer could be affected by the feeding strategy, the presence of alcohols in the upstream process and the drying in the DSP (Werker et al., 2020). The effects of the DSP on Mw and thermal stability are shown to be predictable, nonetheless, similar to the HV content the prediction requires the knowledge of Mw and thermal stability of PHA in the biomass after the accumulation and before the DSP.

As discussed before, biomass PHA content has an impact on the cost of DSP, besides that higher levels of co-extracted non-PHA biomass add complexity to the purification. Additionally, some specific impurities, such as cations, can negatively influence the polymer chemical and/or thermal stability (Csomorova et al., 1994). Carry-over of non-polymer impurities including heavy metals and priority organic pollutants will also influence the scope for application of the polymers due to regulatory frameworks under EU directives or similar (Werker et al., 2020; Astolfi et al., 2020; Riccardi et al., 2020). Thus, the type and amount of specific impurities are critical to consider rather than simply polymer purity for a given method of DSP.

A so-called demonstration scale project using feedstocks intended for full-commercial activities will need to address the specifics of opportunities and challenges in the product quality assurance control methods. This is the context where a more detailed polymer characterization can address the most relevant and very case specific knowledge gaps in jumping from current levels of success in findings of technical feasibility to details of process and method for a given waste-to-renewable resource value chain with economic viability including secured supply chains, and well-defined targets of products within a given regulatory framework. A better polymer characterization would be beneficial for developments in both the upstream bioprocesses and downstream purification steps with regards to process and product stability, and this in the end would help towards building of an overall well-functioning value chain.

3. Challenges for the scaling-up from piloting experience

From the published experiences with microbial community-based PHA at pilot-scale, as summarized in Fig. 2, the level of developments from the published experiences decreases progressively from upstream to downstream to application. In general, the upstream bioprocess technology developments support that a PHA-rich biomass can be consistently produced and is adaptable within a wide range of different scenarios. Even though there are continued fundamental research questions yet to be answered, it is already technically feasible to generate mixed cultures highly enriched in PHA producing biomass, either through specialized enrichment processes (*enrichment accumulation* approach) or by means of mainstream biological wastewater treatment (*direct accumulation* approach). *Enrichment accumulation* and *direct accumulation* methods are complimentary to one and another, and as such offer a flexibility to exploit regional catchments of organic waste streams in a way that can maximize productivity while meeting other constraints and requirements depending on context and feedstock. The obtained functional biomass can be used to produce a range of co-polymer blends from a wide mix of possible simple and complex fermented VFA-rich industrial and municipal feedstocks. Independent of the feedstock used for the polymer accumulation bioprocess, PHA yields on substrate can be close to theoretical maximum levels, and significant PHA contents may be robustly achieved even though the maximum biomass PHA content before downstream processing may currently be considered to be the characteristic difference between *enrichment accumulation* and *direct accumulation* approaches. The difference of biomass PHA content might affect the choice of the DSP and the quality of the final extracted polymer. However, the basic outcomes and requirements for scaling-up of the process are equally valid for *enrichment* or *direct accumulation* PHA production methods. One can even expect that any practical differences between so-called *enrichment* and *direct* accumulation processes would become trivial if, for instance, the *direct accumulation* approach was applied with WAS that could produce biomass with up to 0.6 gPHA/gVSS. Even though the production potential of biomass can be further optimized especially for municipal activated sludge, one can conclude the pilot-scale experience in PHA-rich biomass production to date positively motivate an initiative to scaling-up production of the PHA-rich biomass semi-product.

While production of PHA-containing biomass has been well studied even at pilot-scale, projects reporting on piloting experience with the downstream processes of PHA recovery are lacking. DSP could be done with both solvent-based and water-based methods. A solvent-based DSP typically includes process steps of dewatering, acidification, drying and

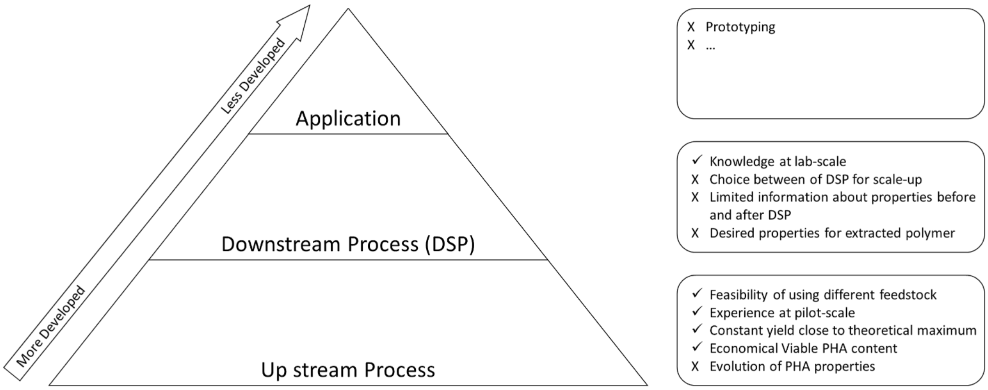

Fig. 2. Summarized current development levels for PHA production process.

solvent extraction (Werker et al., 2020). Heat for drying and non-chlorinated (solvent) extraction are principal recovery costs (Fernández-Dacosta et al., 2015b). Drying costs are linked to the amount of moisture per total mass dried, and extraction costs are limited to the volume of mass that can be processed per batch. A high degree of solvent recovery and its reuse is furthermore important to the environmental and economic performance of solvent-based methods. Spent non-PHA biomass is a retained resource with application for its chemical and heat value. On the other hand, DSP by water-based methods involve steps of selective non-polymer biomass solubilization followed by granule separation and associated washing steps (Lorini et al., 2020; Burniol-Figols et al., 2020; Kosseva and Rusbandi, 2018). It may be expected that water-based methods become more costly due to a greater amount of chemical consumption to remove non-polymer biomass the lower the biomass PHA content. The solubilized solids including digestion chemicals as well as the released solubilized biomass organic, nitrogen and phosphate contents generate a wastewater that must be treated. Consequently, solvent extraction methods might be more applicable at larger scales for processing PHA-rich biomass with moderate polymer content. Water-based methods may be initially more attractive at smaller scales and with PHA-rich biomass having higher biomass PHA content.

To our knowledge, there is only one detailed reported experience on pilot-scale DSP and production quality control for microbial community-based PHA (Werker et al., 2020). The polymers were recovered from dried biomass by using simple alcohols and/or acetone (Werker et al., 2020). Optimal conditions of recovery were influenced by the average co-polymer composition, molecular mass, particle size, and polymer-in-biomass chemical and thermal stability. The degree of polymer decomposition during recovery was predictable, and a pure polymer of commercial quality (98%) could be recovered even with biomass containing 0.4 gPHA/gVSS.

The challenge of developing a specific DSP is an uncertainty of what the feedstock quality, type of polymer, and requisite polymer properties will be, initially, in scaling-up efforts. Up until now the published research is normally linked to the goal to obtain a high purity polymer with moderate to high molecular mass. However, the question and demands of purity and molecular mass are very much linked to the polymer specifications for application in a product. In many cases, the base polymer properties are modulated in formulations that influence, for example, crystallization in processing. These formulations are best developed with supply of the same type of pending commercial grades of the polymer. Currently, the majority of the PHA production development is still on microbial PHA production supposing that a market exists because biopolymers are needed due to a crisis of plastics in the environment. However, no market can exist until a supply is available. The dilemma is without significant amounts of the commercial prototype for the polymer, one cannot test the feasibility of specific types and grades of the prototype PHAs for opportunities within promising and sometimes unforeseen applications. Therefore, the next steps for scaling-up microbial community-based PHA production are challenging because optimal downstream methods depend on the upstream (type of PHA, PHA-in-biomass quality, biomass quality, PHA content), the application intent (scale of production, polymer property quality window, regulatory frameworks) and the overall value chain economic viability starting with the supply of VFAs.

To break this Catch-22, next steps of scaling-up upstream processes of production of PHA-rich biomass, such as a relevant demo-scale installation, can provide for a prototype stream of representative raw material, to more fully establish methods and process for the product recovery, as illustrated in Fig. 3. Even the establishment of a demo-scale installation of PHA-rich biomass production will naturally involve a context with specific potential volumes in supplies of organic waste and/or wastewater conversions to PHA-rich biomass in the scope of methods from *direct accumulation* to *enrichment* culture bioprocesses. Scaling-up embodies a business understanding to reliably supply a mass of polymer for a given price towards commercially viable application(s). The type of fermented streams used for PHA production will also determine upstream engineering details of process volumes etc, as well as the specifics of the polymer type, i.e. co-polymer blend composition and this will, in turn, govern range and scope of application. Range and scope of application may furthermore determine the most appropriate methods for commercial DSP. Thus, the most relevant developments require a context of the specific organic materials being converted for the initial (commercial) scale of production, the regional growth potential in scale of organic material supply, and an application that fits with the potential market and specificities of the supply chain. The involvement and close collaboration of stakeholders all the way from feedstock, upstream process, DSP and application brings extra challenges. However, such cooperation is required to move the microbial community-based PHA production efficiently forward technically as well as economically.

4. Application case studies

In the scientific and popular literature, PHAs are purported to be drop in substitutes for traditional polymers for the plastics industry with properties similar to polypropylene and polystyrene. Biopolymers are implied to replace traditional plastics in many mainstream applications including durable products, as well as short term use applications, like packaging. This market for PHAs would require demanding DSP to produce PHAs of sufficient general quality for the open market. It would also require a scale of supply that would be unrealistic to expect to achieve, at least in the short term. The pilot studies to date give an impression of what the scale of supply may reasonably be.

As a practical example, if the OFMSW produced every year in the European Union (38,802 kt OFMSW; year 2018) was collected and *all* of it was used to produce PHAs, around 394 kt PHA could be produced per year (Appendix). The estimated amount of PHA that can be produced

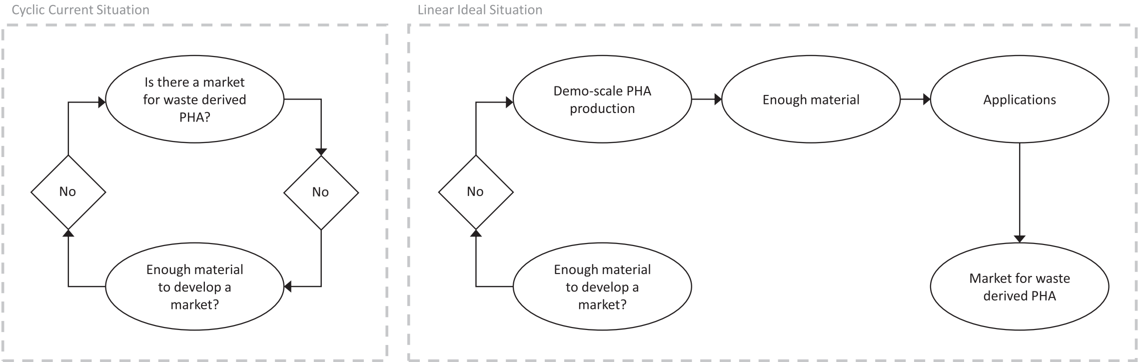

Fig. 3. Depiction of dilemma in current scaling-up efforts of microbial community-based PHA production and how a demo-scale installation may contribute to support potential industrial implementation.

from this supply of organic waste represents only 0.6% of the current production scale and demand of petroleum-based polymers. The difference in production scale suggest that PHA will not be able to readily compete with traditional demands for polymers from petrochemical industries and highlights the importance of developing applications for PHAs that do not pretend to compete with petroleum-based polymers or mislead to unreasonably offer a drop in solution to global plastic pollution problems. Applications should be based on the unique PHA polymer properties, including but not limited to biodegradability. Even from the above very simple mass balance, it is clear that niche applications are required that can match realistically a scale in production volume that reliably secures stakeholder commercial investments in the supply chain. Towards this end, two application case studies are provided below to illustrate and develop the thoughts of strategy and challenges in next steps for scaling-up from published demonstrated pilot-scale technical successes to date at pilot-scale.

Self-healing concrete is a type of concrete where the spores of specific haloalkaliphilic limestone-producing bacteria and poly-lactate polymer are mixed with the concrete raw materials in the production process (Jonkers et al., 2010). When cracks are formed, oxygen and moisture enter the concrete, this activates the spores. The growing bacteria consume the poly-lactate and generate limestone. The limestone seals the cracks preventing further crack growth and blocking further intrusion of water, prolonging the service life-time of the concrete. Due to the longer expected material service life-time, an environmental benefit comes also from lower associated concrete production CO_2 emissions (Wiktor and Jonkers, 2016). PHA could be used as an attractive substrate replacing lactate, as it is expected to be cheaper to use than lactate. In a recent publication, the feasibility of using waste-derived PHA for self-healing concrete has been demonstrated (Vermeer et al., 2021).

Controlled (or slow) release fertilizers (CRFs) are fertilizer products with embedded nutrients that are released to the soil in pace with plant growth requirements (Azeem et al., 2014). As a consequence, CRFs provide corollary benefits by mitigating nutrient run-off with associated environmental impacts of ground water contamination, eutrophication of surrounding water bodies, and emissions of greenhouse gases (nitrous oxide) (Boyandin et al., 2017). Commercially available CRFs apply a poorly biodegradable fossil-based polymer skin around a selected fertilizer pellet formulation as a diffusion membrane. The leftover of the polymer skin will remain as a micro-plastic waste in the soil. To exploit the benefits of CRFs without spreading micro-plastic waste and in accordance with the EU Fertilizing Products Regulation (Regulation (EU) 2019/1009), PHAs as bio-based and biodegradable polymers are an alternative to achieve the CRF function. There are several reports on the feasibility of different formulations and methods using high purity commercially available pure culture derived PHAs in CRFs (Boyandin et al., 2016; Volova et al., 2016; Boyandin et al., 2017).

Self-healing concrete and CRFs are emerging technologies which have a modest but significant market and raw material demands. As an example, 5000 t/year of PHA may be estimated to be required if *all* the applied mineral fertilizer in Europe would be substituted by PHA-based CRFs (Appendix). At the same time, waste activated sludge from a 1 million p.e. municipal wastewater treatment plant could have the capacity to produce PHAs in the order of 2500 t/year of PHA (Bengtsson et al. (2017)). This example illustrates context of a niche application where initial supply chains may readily target a valued market within the scope in scale of demand. The application developments would be best achieved when production capacity and market demand can be in balance. The demands from these emerging technologies will be modest to begin, but so will the supply chain too. One might think, for a single emerging technology, the PHA demand seems small. However, it is important to keep in mind that the expected market demand and supply chain infrastructure can be stimulated to blossom within an evolution of emerging applications. This evolution is driven from ongoing discovery from exploiting unique properties of PHAs produced from specific organic waste streams once there is a commercial scale supply.

These two emerging technologies, as examples, were selected to illustrate their common question of relevance in scaled-up supply for the polymers given a particular waste organic feedstock. They also contrast in quite different considerations of up- and downstream methods and process of production. These considerations enable the delivery of polymers with different qualities that are linked to very specific objectives in the applications. For self-healing concrete, it is anticipated that the molecular weight and the HV content of the PHA are of limited importance to the polymer function in the application. However, the concrete quality may be sensitive to other impurities which can potentially harm the strength of the concrete. Therefore, purity requirements for the polymer recovery are directed towards avoiding very specific kinds of biomass components rather than presence of trace contaminants of a polymer sold on the open market. For CRFs, the biodegradation rate of PHAs will affect the delivery of the fertilizer. The biodegradability of the PHAs are affected by the environmental conditions and the polymer properties (Emadian et al., 2017; Kale et al., 2007; Ferreira and Akesson, 2020). Crystalinity, HV content and thermal stability are essential factors for the biodegradation of the polymer (Zaheer and Kuddus, 2018; Ferreira and Akesson, 2020). Crystalinity affects the accessibility of the PHA degradation enzymes (Zaheer and Kuddus, 2018). The higher the crystallinity of the polymer the lower the degradation rate is expected to be. HV content will also affect the crystallinity of the polymer (Yu et al., 2005; Yu, 2009; Porter and Yu, 2011). A lower average HV content correlates to less crystalline polymers with higher melting temperature. Higher melting temperature also contributes to decreasing PHA biodegradation rates (Zaheer and Kuddus, 2018).

As discussed before, piloting experience has shown that these properties can be predicted and modulated during the PHA production process. Tuning the bioprocesses and DSP can become very technically and economically strategic when the principal objectives are linked to specific kinds of impurities (self-healing concrete) and/or crystallinity and Mw (CRFs). The DSP can furthermore be efficiently integrated into conversion steps for applications if the polymers do not need to be recovered and sold first as pure chemicals on the open market. The scaled up production and polymer quality is best to not be considered with respect to references of absolute quality targets, but with respect needs for a specific application.

5. Research and development directions

Based on the review of the published piloting research and developments, the following elements are recommended as necessary to bridge specific context for necessary linking between the upstream and downstream research and development efforts:

- Focus on factors underlying ambiguities in observed differences in the performance of selection and understanding the evolution of polymer properties in the PHA accumulation process;
- A focus on niche applications for microbial community-based PHA based on the unique polymer properties, including but not limited to biodegradability;
- The impact of different downstream processes on PHA product specifications needs to be better investigated for a product oriented DSP;
- A realistic context of feedstock supply for commercial scale production at demo-scale that would provide for production of the representative type of PHA for the downstream processing and application engineering, as well as nurturing necessary stakeholder relations and commercial developments;
- Building sound business cases with help of substantive techno-economic evaluations using regional data from both public and private stakeholders, represented in the supply chain, towards understanding reliable flows in material supply that could support viable business(es) driven by niche applications with market potential suitable in scale to the emerging commercial supply.

6. Conclusions

Microbial community-based PHA production from organic waste and wastewater has been shown at pilot scale to be a ready technology that offers meaningful contribution for resource recovery. Commercial quality polymers can be consistently produced. Main knowledge gaps remain in the bioprocess and downstream processing in linking relationship to the production methods for PHA applications with specific product specifications. Further, commercial production will require a greater depth in fundamental understanding of the polymer characteristics towards process control of the polymer properties over the different stages in the whole PHA production chain from organic waste to value added products and services.

CRediT authorship contribution statement

Ángel Estévez-Alonso: Conceptualization, Writing - original draft. **Ruizhe Pei:** Conceptualization, Writing - original draft. **Mark C.M. van Loosdrecht:** Conceptualization, Writing - review & editing. **Robbert Kleerebezem:** Conceptualization, Writing - review & editing. **Alan Werker:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

From CBS data (CBS, 2020a,b), in 2018 in The Netherlands 1,487,000,000 kg OFMSW were produced (87 kg per person). Based on Moretto et al. (2020b), on average OFMSW has 0.132 kgVS/kgOFMSW and 13 kgVS are required to produce 1 kg PHA. This means that 15,098 tPHA (15 ktPHA) can be potentially produced from organic waste per year in The Netherlands. If the same calculation is done for Europe, assuming a population of 446 million, 393,990 tPHA/year (394 ktPHA) could be produced. These numbers can be compared with the traditional plastic industry. Europe produced in 2018, 62 mt of plastics (62,000 kt) (PlasticsEurope, 2019), which means that the PHA production would represent only 0.6% of the plastic industry at European level. These numbers strongly suggest that PHAs will not be able to compete with traditional petroleum-based polymers and should find other entry markets.

About 10 million tons of mineral fertilizer are applied annually in Europe (European Commision, 2020). At the limit of ideal nutrient delivery, CRFs would reduce fertilizer demand by nominally 50% (Mosier et al., 2013). Then, if all the fertilizer application in Europe was to be based on CRFs, and the mass of PHA in the fertilizer was 0.1% of the total weight, the supply to meet EU market demand for PHAs based CRFs would be in order of 5000 t/year of PHA.

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