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Review

Carbon Recovery from Wastewater Feedstocks: Synthesis of Polyhydroxyalkanoates for Target Applications

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Abstract

Polyhydroxyalkanoate (PHA) bioplastics are produced from wastewater as a carbon recovery strategy. However, the tuneable characteristics of PHAs and wastewater biorefinery potential have not been comprehensively reviewed. The aim of this study is to review the main challenges and strategies for carbon recovery from wastewater feedstocks via PHA production, assessing potential target biopolymer applications. Diverse PHA-accumulating prokaryotes metabolize organic pollutants present in wastewater through different metabolic pathways, determining the biopolymer characteristics. The synthesis of PHAs using mixed microbial cultures with wastewater feedstocks derived from municipal, agro-industrial, food processing, lignocellulosic biomass processing and biofuel production activities are described. Acidogenic fermentation of wastewater feedstocks and mixed microbial culture enrichment are key steps in order to enhance PHA productivity and determine biopolymer properties towards customized bioplastics for specific applications. Biorefinery of PHA copolymers and extracellular polysaccharides (EPSs), including alginate-like polysaccharides, are alternatives to enhance the value-chain of carbon recovery from wastewater. PHAs and EPSs exhibit a wide repertoire of applications with distinct safety control requirements; hence, coupling biopolymer production demonstrations with target applications is crucial to move towards full-scale applications. This study discusses the relationship between the metabolic basis of PHA synthesis and composition, wastewater type, and target applications, describing the potential to maximize carbon resource valorisation.

Keywords: wastewater; carbon recovery; polyhydroxyalkanoate; extracellular polysaccharide; mixed microbial culture; circular economy

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1. Introduction

Wastewater derived from domestic and different industrial activities is characterized by its organic load. Safe wastewater treatment accounts for 58 and 27% of the total domestic and industrial production, respectively [1,2]. Current wastewater treatment plants (WWTPs) oxidize organic pollutants into carbon dioxide (CO_2) in an energy-intensive,

aerated bioprocess using activated sludge (AS) as a biocatalyst, generating waste AS (WAS), representing up to 60% of the influent chemical oxygen demand (COD) mass [3]. Traditional WAS reduction and valorisation technologies, including anaerobic digestion, pyrolysis, and soil amendment, have been implemented. However, these strategies are challenged with high-cost infrastructure, greenhouse gas (GHG) emissions and the dissemination of heavy metals, emergent contaminants, and antibiotic resistance determinants (e.g., antibiotic resistance genes and resistant bacteria). These issues related to stabilized biosolids valorisation limit sustainable and safe WAS reduction within circular economy principles [4,5]. Novel WAS reduction technologies encompassing carbon recovery strategies through the production of added-value bioproducts, such as biodegradable bioplastics, engage WWTP carbon and energy neutrality [6,7].

Polyhydroxyalkanoate (PHA) production is a promising alternative for carbon recovery from wastewater, achieving WAS reduction and valorisation [8,9]. PHAs are biobased, biodegradable polyesters, which accumulate in diverse prokaryotes. Many of these prokaryotes are part of the microbial community of AS. PHA polymers display versatile thermomechanical properties, resembling those of fossil-based non-biodegradable plastics. The current PHA production cost (USD 1.7–7 kg^{-1}) and selling prices (USD 3–7 kg^{-1}) are higher than those of fossil-based non-biodegradable plastics, whose price is USD 0.8–1 kg^{-1} [10]. Nowadays, industrial pure bacterial cultures are employed for full-scale PHA commercialization, reaching 1000-ton per year approximately, with focus mainly on high-value applications, for example, in the medical field [11,12]. Carbon resources within wastewater feedstocks are useful for PHA production. WWTP technology and infrastructure, such as non-sterile mixed microbial cultures (MMCs) and biomass separation units are suitable for PHA production. Moreover, PHA production costs using wastewater carbon resources reach USD 1.2–2.3 kg^{-1} [13,14]. A lower PHA production cost enables broader bioplastic applications. However, the diluted nature of organic pollution in wastewater, along with high variability across seasons and the wastewater type, represent major challenges for PHA recovery [15]. These issues have been addressed using bioprocess engineering approaches varying the oxygenation regime, culture modalities, and biocatalysts [16–18]. However, the intracellular nature of PHAs and the complexity of MMC biomass surrounding this bioplastic hinders the full-scale commercialization of PHAs recovered from wastewater [19]. The PHA recovery value chain has been improved through the biorefinery concept which has been implemented in pure bacterial cultures to obtain added-value co-products such as extracellular polysaccharides (EPSs), osmoprotectants, and antioxidants [8,20–22].

PHA and added-value polymers, including EPSs, show a wide variety of applications due to their diverse chemical structures, placing them as key molecules for WWTPs' upgrade towards water resource recovery facilities (WRRFs) [8]. PHA properties beyond biodegradability (e.g., customizing monomer and properties profiles) expand bioplastic applications, encompassing circular economic objectives for the cradle-to-cradle management of carbon resources [23]. The aim of this study is to review the main challenges and strategies for carbon recovery from wastewater feedstocks via PHA production, assessing potential target biopolymer applications.

2. Basis of PHA and EPS Synthesis in Prokaryotes

2.1. Types of PHA

PHAs are accumulated by prokaryotes as intracellular granules in response to adverse environmental variations such as nutrient imbalance or extreme osmolarity and temperatures [24,25]. Figure 1A shows the PHAs' synthetic pathways from different carbon sources. PHAs are synthesized from multiple carbon sources, with different polymer yields according to the metabolic characteristics of each biocatalyst

and its culture conditions [26]. More than 70 types of PHA monomers have been described in diverse prokaryotes [27]. Short-chain-length PHA (sclPHA) monomers are C3–C5 in length, while medium-chain-length PHA (mclPHA) monomers are C6–C14 in length [28]. The most studied PHA is poly(3-hydroxybutyrate) homopolymer, which is composed solely of 3-hydroxybutyrate (3HB) monomers [29]. Copolymers of sclPHA, such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)), harbours 3HB and 3-hydroxyvalerate (3HV) monomers with varying compositions [30]. Copolymers of scl-mclPHA, such as the poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)), share properties with elastomers, widening PHA applications. The monomer composition determines the final thermomechanical properties of the PHA copolymer. For instance, incrementing the 3HV monomer fraction of the P(3HB-*co*-3HV) copolymer enhances the polymer flexibility, elasticity, and melting point, boosting its processability and performance for medical applications [31].

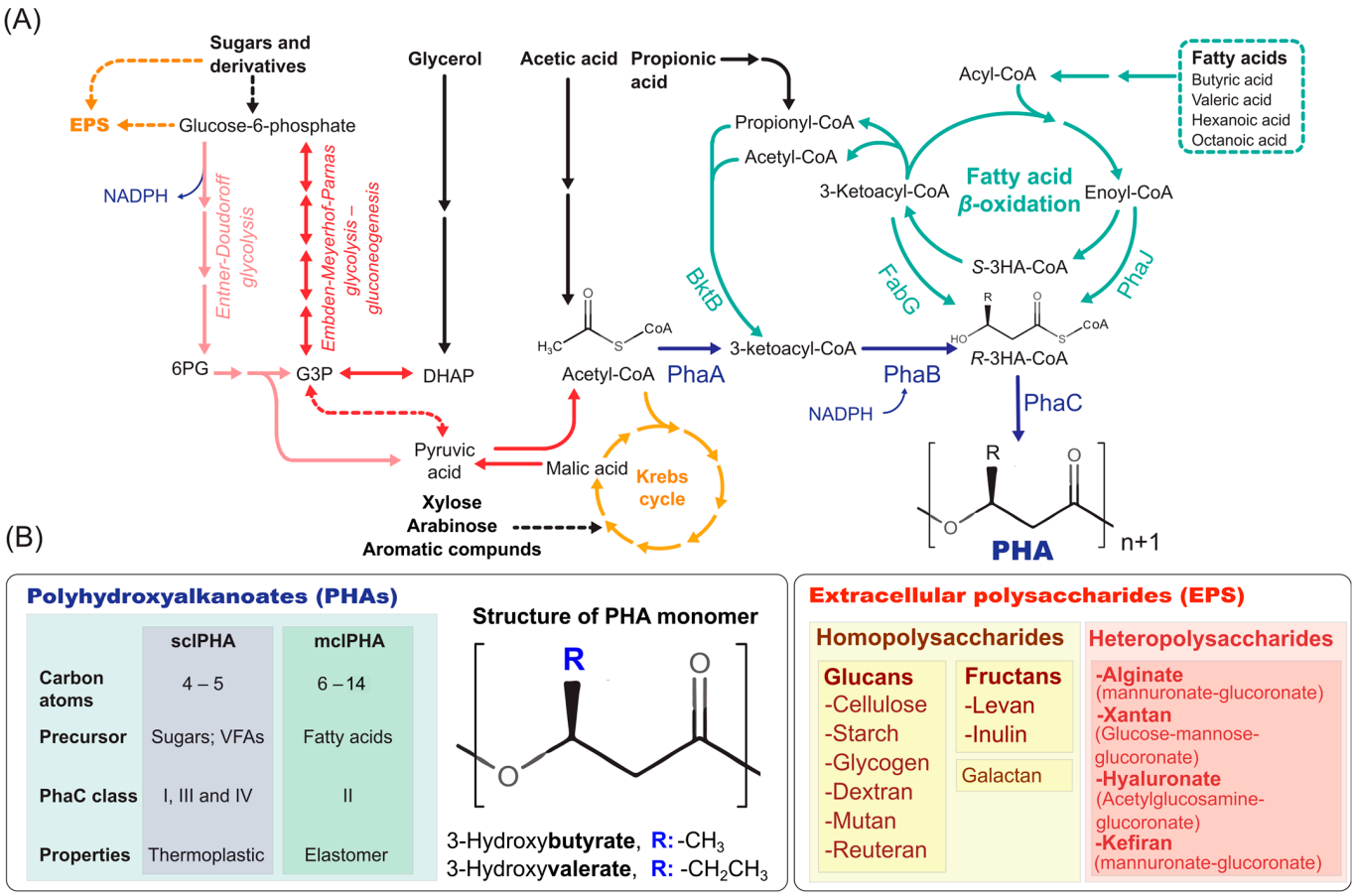

Figure 1. Metabolic basis of biopolymer production in prokaryotes. (**A**) Metabolic pathways involved in polyhydroxyalkanoate (PHA) and extracellular polysaccharide (EPS) synthesis. Each arrow represents an enzymatic reaction while dashed arrows depict several enzymatic reactions. Abbreviations: 6PG, 6-phosphogluconate; G3P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetonephosphate; *R*/*S*-3HA-CoA, (*R*/*S*)-3-hydroxyacyl-CoA; PhaA, 3-ketothiolase; BktB, 3-ketovaleryl-CoA producing 3-ketothiolase; PhaB, acetoacetyl-CoA reductase; PhaC, PHA synthase; FabG, 3-ketoacyl-ACP reductase; PhaJ, R-specific enoyl-CoA hydratase. (**B**) Classification of PHA and EPS biopolymers according to chemical structure. Roman numbers indicate PhaC classes I, II, III and IV. sclPHA, short-chain-length PHA; mclPHA, medium-chain-length PHA. Homopolymers and heteropolymers of sclPHAs, mclPHAs, and scl-mclPHAs have been described.

2.2. Metabolic Pathways of PHA Synthesis

The monomer composition of PHAs depends on the substrate and the metabolism of the prokaryote organisms used as the biocatalyst [26]. The synthesis of the P(3HB) precursor (*R*)-3-hydroxybutyryl-CoA (*R*-3HB-CoA) consists in the condensation of two acetyl-CoA molecules, followed by a NADPH-dependent reduction through the enzymes 3-ketothiolase (PhaA) and acetoacetyl-CoA reductase (PhaB), respectively. Acetyl-CoA is usually obtained from sugar metabolism through different glycolytic pathways, or the β-oxidation of fatty acids (Figure 1). The synthesis of 3HV monomer is obtained from odd-chain fatty acid β-oxidation intermediates, including propionyl-CoA or 2-*trans*-pentenyl-CoA. The 3-ketothiolase (BktB) and *R*-specific enoyl-CoA hydratase (PhaJ, MaoC) enzymes are important in 3HV synthesis. Synthesis of mclPHA monomers is achieved by intermediates of medium-chain-length fatty acid β-oxidation (e.g., hexanoic acid, heptanoic acid, and octanoic acid). The enzymes PhaJ, MaoC, and 3-ketoacyl-ACP reductase (FabG) are relevant catalysts for the synthesis of mclPHA monomer and precursors (Figure 1). Intermediates of *de novo* fatty acid synthesis can be channelled toward mclPHA monomer precursor synthesis via the 3-hydroxyacyl-ACP transacylase (PhaG) enzyme [32]. Finally, polymerization is performed by the PHA synthase (PhaC). Four PhaC classes have been described according to their phylogenetic and structural characteristics and substrate specificity [33,34]. Class I, III, and IV PhaCs polymerize specifically sclPHA monomers and are distributed in a wide diversity of prokaryotes, including Burkholderiales and Bacillales [34,35]. Class II PhaCs polymerize specifically mclPHA monomers and are mostly associated to Pseudomonadales bacteria and some *Paraburkholderia* species [32]. A wide repertoire of PHA depolymerases (PhaZs) mobilize PHA chains into their monomeric precursors or fatty acids through thiolytic and hydrolytic mechanisms [25]. PHA accumulation is subjected to synthesis and degradation rates. Advances in metagenomic analyses in an enriched AS-treating wastewater revealed the relevance of PhaJ, PhaC, and PhaZ enzymes in enhancing PHA accumulation, determining mclPHA monomer compositions [36].

Advances in PHA metabolic and regulatory networks in pure bacterial cultures have improved yields, allowed for tailored bioplastic synthesis, and enabled the biorefinery of other valuable products [12]. Otherwise, in MMC-based systems, the microbial community of the MMC is engineered to enhance PHA productivity. For example, an adding an additional settling phase or modulating fatty acid profiles in the influent increased the abundance of the *Paracoccus* genus, enhancing 3HV monomer incorporation [36,37]. Additionally, the co-production of PHA, EPS, and high-value lipids has been modulated by adjusting the dissolved oxygen, feeding modality, or pH to achieve biorefinery synergies [38]. Bioaugmentation of MMCs with specific taxa is an attractive alternative to engineer microbial communities. The bioaugmentation with microalgae enabled the biorefinery of specific EPSs from a municipal wastewater treatment system [39]. These examples highlight the potential of microbiology knowledge on bioaugmentation to overcome the productivity and process reliability issues of wastewater biorefinery using MMCs.

2.3. Microbial Synthesis of EPS

EPSs are produced by bacteria and archaea and accumulated in the extracellular medium. These polysaccharides are synthesized from sugars or through glycolytic or gluconeogenic intermediates (Figure 1) under nutrient imbalance, such as carbohydrate excess, nitrogen or phosphorus scarcity, low pH, or extreme temperatures [40]. The biological function of EPS is described mainly in biofilm formation, cell interaction and aggregation, and surface adhesion [41]. Microbial EPSs are composed of different carbohydrates (e.g., glucose, fructose, mannose, galactose, and arabinose), some of them with modifications to their functional groups (e.g., acetylation, methylation, and sulfonation) [42]. These

carbohydrates are polymerized linearly, branched, or in a combination of both, resulting in polymers of varying molecular masses [43]. The monomeric composition, structure, and molecular mass of EPSs determine their thermomechanical and active properties, providing different biological functions and potential biotechnological applications [41]. Polysaccharide accumulation during PHA production (e.g., glycogen) by MMC has been associated with decreased bioplastic yields [17]. However, a wide variety of polysaccharides produced by diverse bacteria have shown high-value applications, representing a potential biorefinery synergy [42–44]. Moreover, EPS recovered from wastewater systems has shown suitability for a wide spectrum of applications, including medicine, tertiary wastewater treatment, and construction.

3. Wastewater Feedstocks

The usage of pure carbon sources represents up to 60% of overall PHA production costs [45]. Low-cost substrates may decrease the overall PHA production cost by 93% [46]. Table 1 shows studies on PHA production from wastewater feedstocks derived from diverse sources, such as municipal wastewater treatment plants, agro-industrial activities, the food processing industry, paper mill and kraft mill effluents, and the biodiesel production industry. Wastewater streams present distinct compositions, with pH values varying from 2–6 [47] and COD values ranging from 3.7–360 g COD L^{-1}, consisting of carbohydrates, VFAs, and proteins. The relative abundance of these components varies according to each wastewater type [48].

PHA production from different wastewater feedstock sources has been addressed through different bioprocess strategies (Figure 2). Strategy I consists in the direct usage of wastewater carbon towards PHA without feedstock pretreatment, using non-enriched MMC [14,49]. Strategy II consists of the pretreatment of the wastewater feedstock through acidogenic fermentation (AF), transforming organic components into VFAs. Moreover, thermic hydrolysis of wastewater or WAS prior to AF further enhances organic matter bioavailability [50]. In strategy II, the AF effluent is used as feedstock for PHA accumulation using non-enriched MMC (Figure 2). Finally, strategy III uses VFAs from AF as a carbon source to produce PHA with enriched MMC. MMC enrichment uses mostly bioprocess engineering in an independent unit to shape a microbial community towards a higher abundance of PHA-accumulating prokaryotes [51]. In general, MMC enrichment consists in subjecting the microbial community to a feast phase, where carbon is stored within PHA-producing microorganisms. After nutrient depletion, a famine phase takes place. In the famine phase, the intracellular accumulated polymers, such as PHA, are consumed. Storage polymer consumption enables polymer-accumulating prokaryotes to outcompete non-producers, enhancing the relative abundance of PHA producers [51]. Several enrichment strategies have been studied, highlighting important factors, such as high organic loading rates, long hydraulic retention times, and additional settling phases [37]. These strategies increase MMC contact with readily biodegradable COD [37,49,52]. MMC enrichment changes the microbial community profile, influencing the metabolic capabilities and thus determining monomer PHA composition [37]. However, the addition of specific taxa (i.e., bioaugmentation) could be an attractive strategy to enhance the PHA accumulation capability within MMCs. For example, bioaugmentation of AS or aerobic granular sludge (AGS) with specific species to improve recalcitrant pollutant removal, resistance under harsh conditions, and AGS settleability [53–61]. Full-scale PHA production from wastewater represents specific challenges according to the wastewater feedstock composition, such as the organic load, nutrient availability, presence of toxic compounds, or high salinity.

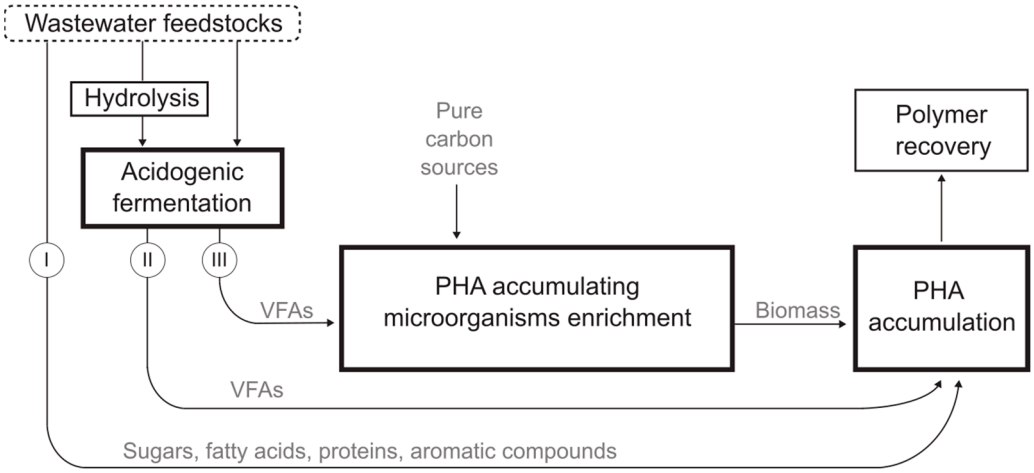

Figure 2. Strategies for carbon recovery from wastewater feedstocks through polyhydroxyalkanoate (PHA) production. I, strategy for PHA accumulation directly from wastewater feedstocks. II, strategy for PHA accumulation from volatile fatty acids (VFAs) obtained through anaerobic fermentation of wastewater feedstocks. III, strategy for PHA accumulation from VFAs using enriched mixed microbial cultures.

Table 1. Carbon (C) recovery from wastewater feedstocks through polyhydroxyalkanoate (PHA) synthesis.

Source	Feedstock	Pre-Treatment	Main C Sources [a]	Bioprocess [b]	MMC	Products	Ref.
Municipal WWTPs	WAS	AF	Acetic acid: 0.9 g L^{-1}; propionic acid: 0.2 g L^{-1}; butyric acid: 0.1 g L^{-1}	Pilot SBR	Enriched AS	P(3HB-*co*-15 mol%-3HV)	[62]
	WAS	AF	Acetic acid: 0.8 g L^{-1}; propionic acid: 0.1 g L^{-1}; butyric acid: 0.2 g L^{-1}	Pilot SBR	Enriched AS	P(3HB-*co*-3 mol%-3HV)	[63]
	WAS	TH and AF	Acetic acid: 3.9 g L^{-1}; propionic acid: 1.4 g L^{-1}; butyric acid: 2.6 g L^{-1}; valeric acid: 0.5 g L^{-1}	Pilot fed-batch	Enriched AS	P(3HB-*co*-10 mol%-3HV)	[64]
	Primary sludge	AF	Acetic acid: 1.5 g L^{-1}; propionic acid: 2.4 g L^{-1}; butyric acid: 1.2 g L^{-1}; valeric acid: 0.4 g L^{-1}	Pilot fed-batch	Non-enriched AS	P(3HB-*co*-30–40 mol%-3HV)	[49]
	WAS or primary sludge	AF	Acetic acid: 2.3–2.7 g L^{-1}; propionic acid: 1.0–1.2 g L^{-1}; butyric acid: 1.3–1.4 g L^{-1}; valeric acid: 0.5–0.8 g L^{-1}	Pilot fed-batch	Enriched AS	P(3HB-*co*-25–30 mol%-3HV)	[65,66]
Agro-industry	Olive mill effluent	AF	Acetic acid: 3.6–7.1 g L^{-1}; propionic acid: 1.0–1.3 g L^{-1}; butyric acid: 2.1–6.0 g L^{-1}; valeric acid: 0.6–1.5 g L^{-1}; oleanolic acid	Pilot batch, pilot SBR	Enriched and non-enriched AS	P(3HB-*co*-11 mol%-3HV)	[47,67]

Table 1. *Cont.*

Source	Feedstock	Pre-Treatment	Main C Sources [a]	Bioprocess [b]	MMC	Products	Ref.
Food processing industry	Fruit processing effluent	AF	Acetic acid: 1 g L^{-1}; butyric acid: 1.6 g L^{-1}; valeric acid: 0.02 g L^{-1}; hexanoic acid: 11.8 g L^{-1}	Pilot SBR	Enriched AS	P(3HB-*co*-3HV); P(3HB-*co*-1 mol%-3HV-*co*-41 mol%-3HHx)	[68,69]
	Tomato processing effluent	AF	Acetic acid: 3.3 g L^{-1}; propionic acid: 2.1 g L^{-1}; butyric acid: 2.4 g L^{-1}; valeric acid: 1.2 g L^{-1}	Pilot SBR	Enriched AS	P(3HB-*co*-45 mol%-3HV)	[52]
	Whey permeate	AF	Acetic acid: 0.69 g L^{-1}; propionic acid: 0.03 g L^{-1}; butyric acid: 0.8 g L^{-1}; valeric acid: 0.02 g L^{-1}	Pilot SBR	Non-enriched MMC	P(3HB-*co*-1 mol%-3HV)	[70]
	Ice cream factory effluent	AF	VFAs: 3.1 g L^{-1}	Pilot aerobic continuous	Non-enriched AS	PHA	[71]
	Citrus processing effluent	Non	Acetic acid, sucrose, fructose, glucose	Lab fed-batch	Enriched AS	P(3HB) and EPS	[72]
	Potato processing effluent	Non	Acetic acid: 6.3 g L^{-1}	Pilot SBR	Enriched AS	P(3HB)	[73]
	Mussel processing effluent	AF	Acetic acid: 0.6–1.2 g L^{-1}; propionic acid: 0.2–0.4 g L^{-1}; butyric acid: 0.10–0.15 g L^{-1}; valeric acid: 0.08–0.1 g L^{-1}; carbohydrates: 0.17–0.41 g L^{-1}; Protein: 0.1–0.2 g L^{-1}	Lab fed-batch	Non-enriched AS	P(3HB-*co*-30 mol%-3HV)	[37]
	Candy industry effluent	AF	VFAs	Pilot fed-batch	Enriched AS	P(3HB-*co*-3HV)	[74]
Paper mill and Kraft mill effluents	Paper mill effluent	AF	Acetic acid: 1.8 g L^{-1}; propionic acid: 1 g L^{-1}; butyric acid: 1.5 g L^{-1}; valeric acid: 0.8 g L^{-1}	Pilot SBR	Enriched AS		[75]
	Kraft mill effluent	Non	Total phenolic compounds: 0.25 g L^{-1}; lignin derivates	Lab batch MBBR	Non-enriched AS from kraft-mill WWTP	PHAs	[76]
	Kraft mill effluent	Non	Total phenolic compounds: 0.25 g L^{-1}; lignin derivates	Lab aerobic batch	Non-enriched AS from (i) kraft mill; (ii) paper mill; (iii) municipal WWTPs	PHAs	[14]
	Paper mill effluent	Non	acetic acid: 0.3 g L^{-1}; propionic acid: 0.4 g L^{-1}; butyric acid: 0.2 g L^{-1}	Lab MBBR	Non-enriched AS	PHAs	[77]

Table 1. *Cont.*

Source	Feedstock	Pre-Treatment	Main C Sources [a]	Bioprocess [b]	MMC	Products	Ref.
	Paper mill effluent	AF	Acetic acid: 1.4–2.1 g L^{-1}; propionic acid: 1.6–2.4 g L^{-1}; butyric acid: 0.8–1.2 g L^{-1}; valeric acid: 0.2–0.3 g L^{-1}	Lab batch	Enriched AS	P(3HB-*co*-53–69 mol%-3HV)	[78]
Biodiesel production	Crude glycerol	Non	0.8w w^{-1} glycerol	Lab SBR	Enriched AS	P(3HB)	[79]

[a], Concentration of volatile fatty acids (VFAs) are expressed on a chemical oxygen demand basis. [b], PHA accumulation bioprocess. WAS, waste-activated sludge (secondary sludge); AF, acidogenic fermentation; TH, thermic hydrolysis; SBR, sequence batch bioreactor; MMC, mixed microbial culture; AS, activated sludge; WWTP, urban wastewater treatment plant. Lab-scale and pilot-scale refer to laboratory experiments in flasks or bench-scale reactors or pilot-scale bioreactors, respectively. Ref., references.

3.1. Municipal Wastewater

Municipal wastewater harbours a complex mixture of organic compounds with diluted and variable compositions across seasons, challenging PHA product quality control [80]. VFAs derived from AF (strategies II and III) are the main carbon source for PHA production using municipal WWTP streams (Table 1). Up to 0.38 g PHA $gVSS^{-1}$ of P(3HB-*co*-3HV) copolymer with a 3HV content of 30 mol% using VFAs was obtained from fermented sludge [65]. Similarly, 0.4 g PHA $gVSS^{-1}$ was reported from fermented primary sludge using a non-enriched MMC from a WWTP [49]. High 3HV content (40 mol%) can be explained by propionic acid being the most abundant VFA in primary sludge digestate [49]. Propionic acid and acetic acid can be metabolized through the BktB enzyme into 3-ketovaleryl-CoA, the precursor of the 3HV monomer (Figure 1). However, not all microorganisms possess this BktB enzyme [32]. Notably, the genus *Paracoccus* has been associated with a wide range of substrate uptake capability, enabling increased 3HV content in the final polymer in the presence of propionic acid [81]. Preliminary WAS thermal hydrolysis (TH) enhances the release of VFAs for further PHA accumulation [64]. TH represents a bioprocess strategy to modulate VFA profiles and P(3HB-*co*-3HV) monomer composition. For example, hydrolysed secondary sludge was anaerobically fermented and used to produce P(3HB-*co*-3HV), reaching 0.53 g PHA $gVSS^{-1}$ with a low 3HV monomer content (9.5 mol%) [64]. This is attributed to the higher abundances of acetic acid (3.9 g COD L^{-1}) and butyric acid (2.6 g COD L^{-1}) compared to that of propionic (1.4 g COD L^{-1}) and valeric acid (0.5 g COD L^{-1}) [64]. Similarly, P(3HB-*co*-3HV) produced from WAS as carbon feedstock showed low 3HV content (3 mol%), as propionic acid was 87 and 50% less abundant than acetic and butyric acids. Meanwhile, higher propionic acid (0.2 g COD L^{-1}) than butyric acid (0.1 g COD L^{-1}) resulted in a higher 3HV monomer composition (15 mol%) in a similar set up [62].

Notably, some reports of PHA production with municipal wastewater or digested WAS uses low VFA concentrations. Low VFA concentrations (e.g., 0.25 mg COD L^{-1}) in contrast with other studies (e.g., 2–7 g COD L^{-1}) show similar PHA content (0.37 g PHA $gVSS^{-1}$), while minimizing GHG emissions [62–66]. On the other hand, an increasing C:N ratio resulted in a lower PHA accumulation (0.3–0.4 g PHA $gVSS^{-1}$), demonstrating the relevance of the C:N ratio for PHA synthesis reliability [62]. On the contrary, adding non-carbon nutrient excess (i.e., low C:N and C:P ratios) to the PHA accumulation bioreactor promoted MMC growth when using whey permeate digestate, increasing overall PHA productivity due to the higher biocatalyst amount [69]. The trade-off among microbial growth and

PHA accumulation is an important factor since nutrient limitation (e.g., N, P) enhances polyester accumulation. However, supplementing N or P may increase microbial growth, which can be an advantage to obtain other added-value compounds under a biorefinery approach. In order to increase C:N and C:P ratios, additional VFAs can be added to the PHA accumulation process. For example, the P(3HB-*co*-3HV) produced in a municipal WWTP was enhanced by adding a side stream consisting of tomatoes processing wastewater digestate with a high VFA organic load, reaching 0.49 g PHA $gVSS^{-1}$ [52]. Using co-substrates from local industrial activities, such as agro-industrial by-products, may facilitate the decentralization of carbon recovery infrastructure development.

3.2. Agro-Industrial Wastewater

Agro-industrial wastewater presents different compositions due to the diversity of agricultural activities according to each region, making it an interesting source producing different PHAs (Table 1). Olive mills produce 6–30 million m^3 of wastewater annually with a high organic load [47]. Digested olive mill wastewater yielded different VFA profiles according to the wastewater's physical pretreatment, obtaining P(3HB-*co*-3HV) with a maximum 3HV content of 11 mol%, while non-digested wastewater yielded P(3HB) [67]. High PHA content (0.8 g PHA $gVSS^{-1}$) has been reported using fruit juice industry wastewater as feedstock throughout AF, MMC enrichment, and accumulation (strategy III, Figure 2) [68]. A similar three-stage process using fruit wastewater digestate led to poly(3-hydroxybutyrate-3-hydroxyvalerate-3-hydroxyhexanoate) (P(3HB-*co*-3HV-co-3HHx)). The 3HHx content in the terpolymer was 66 mol%, while the 3HV content was 1 mol% due to low abundance of odd-chain VFAs in the digestate (Table 1). Optimized AF of fruit wastewater increased hexanoic acid production up to 11 g COD L^{-1}, making it useful for mclPHA production, including the enrichment of mclPHA synthesizers within the MMC [69]. This study used activated sludge as a biocatalyst (Table 1), likely harbouring Pseudomonadaceae species encoding Class II PhaC enzymes, which enables mclPHA synthesis in the presence of hexanoic acid (Figure 1). A shotgun metagenomic analysis in WAS revealed high abundances of Pseudomonadaceae species and Class II PhaC enzymes, explaining mclPHA synthesis using this biocatalyst [82]. Otherwise, when using whey permeate digestate as carbon feedstock, low 3HV content (1 mol%) was obtained due to decreased odd-chain VFAs (<0.01 g COD L^{-1}) in the substrate [70]. Agro-industrial carbon feedstock composition is highly dissimilar. While some feedstocks show potential for mclPHA synthesis, other feedstocks yield mostly sclPHA. Further research in AF of each feedstock is required to assess the retrieved organic acid profile and the PHA type that is possible to obtain. This gap must be addressed to demonstrate the potential of agro-industrial wastewater to generate suitable digestates for a tailored bioplastic portfolio that is aligned with regional agro-industrial needs.

3.3. Food Processing Wastewater

The food processing industry generates wastewater of variable compositions according to the respective activity. Citrus processing wastewater possesses high VFA and sugar content that enable the simultaneous PHA and EPS accumulation, reaching up to 0.44 g PHA $gVSS^{-1}$ and 0.36 g EPS $gVSS^{-1}$ without feedstock pretreatment [72]. Using a membrane SBR was crucial to achieving high PHA content without AF of the feedstock [72]. A non-digested wastewater feedstock from a potato-starch factory was used to produce P(3HB), reaching 0.4–0.7 g PHA $gVSS^{-1}$. Despite high organic load fluctuations in potato wastewater, with VFA variations of 50–90%, P(3HB) yields varied less than 10%, highlighting the robustness of the process [73]. Candy industry wastewater digestate enriched with C2-C7 fatty acids was used as carbon feedstock in a pilot-scale fed-batch P(3HB-*co*-

3HV) accumulation process, reaching 0.32–0.46 g PHA $gVSS^{-1}$ [74]. Mussel processing wastewater digestate rich in VFAs showed a high NaCl concentration (4.3–12.1 g L^{-1}) [37]. An halotolerant acclimated MMC was able to produce P(3HB-*co*-3HV) from this saline feedstock with an increased 3HV content (18–30 mol%) due to an additional feast settling phase within the SBR operation [37]. Similarly, fish-canning wastewater with salinity of up to 10 g NaCl L^{-1} used as feedstock allowed up to 0.39 g PHA g VSS^{-1} production with the co-production of triacylglycerol, indicating a biorefinery synergy potential [83]. Notably, a gradual exposure of an MMC to salinities of 0.2–2.8% w w^{-1} maintained PHA and EPS accumulation, which correlated with an increment of PHA-producing genera *Thauera*, *Paracoccus*, *Flavobacterium*, and the halotolerant *Vitellibacter* genus [84,85]. Furthermore, these genera maintained PHA synthetic gene expression upon salinity increment, including 3HV synthetic genes, such as BktB. These results place these genera as relevant bacteria for P(3HB-*co*-3HV) production process reliability upon salinity exposure, which is useful for canning wastewater feedstocks.

3.4. Lignocellulosic Biomass Processing Wastewater

Lignocellulosic biomass processing wastewater includes paper mill and kraft mill effluents harbouring toxic organic compounds (Table 1). This wastewater type is usually treated with AS, representing potential for carbon recovery through PHA synthesis within decentralized circular economy models, especially in forestry-based industries [76]. Paper mill wastewater digestate enables the accumulation of 0.7–0.8 g PHA g VSS^{-1} with nitrogen (N) or phosphorus (P) supplementation, which increases the total VSS, enhancing overall PHA productivity [75]. However, another report indicates that supplementing N and P nutrients led to a 11–17% decrease in the PHA VSS content when using a paper-mill wastewater digestate as feedstock [78]. On the other hand, a P-limiting condition resulted in the highest PHA accumulation (0.48 g PHA g VSS^{-1}) and 3HV content (69 mol%) compared to N-limiting conditions [78]. Non-carbon supplementation is a bottleneck for carbon recovery from paper-mill and kraft-mill wastewater. A trade-off among increased biomass (e.g., nutrient excess) or increased PHA accumulation (e.g., non-carbon nutrient limitation) is observed when comparing nutrient excess or limitation, respectively [75,78]. Additionally, N or P limitation generates bulking issues in WWTPs; hence, additional bioprocess strategies address this issue to enrich PHA-accumulating microorganisms. A moving-bed biofilm reactor (MBBR) was able to enrich an MMC (i.e., AS from a WWTP) using paper-mill wastewater, avoiding bulking and increasing PHA-producing microorganisms from 30% to 74%, according to flow cytometry estimations [77]. Kraft-mill wastewater harbours a complex mixture of organic compounds; many of them are recalcitrant (BOD_5:COD = 0.46), including aromatics and lignin [76]. An MBBR reactor removed COD, including lignin derivatives from kraft-mill wastewater by 15–21% using a low C:N:P ratio (BOD_5:N:P = 100:1:0.2) that enhanced PHA-accumulating species abundance (95%) in the MMC biofilm [14]. Inoculum origin is highly relevant for resisting the toxic effects of kraft-mill composition, as several bacteria capable of degrading and resisting the toxicity of aromatic compounds may be present in AS from kraft-mill WWTPs [14,76].

3.5. Crude Glycerol

Biofuel production generates wastewater with a crude-glycerol phase with up to 85% w w^{-1} glycerol, which has been used frequently for PHA production using pure microbial cultures, reaching up to 0.8 g PHA g $biomass^{-1}$ [86]. Crude glycerol by-product from the biofuel industry was used as carbon feedstock for P(3HB) production in an SBR reactor [79]. A crude glycerol concentration gradient was used to enrich an MMC culture, reaching 0.27 g PHA $gVSS^{-1}$ of P(3HB) while pure propionic acid was added to

obtain P(3HB-*co*-HV), as glycerol metabolism usually yields only P(3HB) homopolymer (Figure 1) [79]. Crude glycerol feedstocks usually contain methanol and other compounds that inhibit cell growth and decrease P(3HB) production and molecular weight, deteriorating bioplastic properties [86]. Some bacteria, such as *Methylorubrum extorquens* (formerly classified as *Methylomonas extorquens*), metabolize methanol and recalcitrant compounds found in crude glycerol to accumulate P(3HB) [87]. *Paracoccus* species are reported to metabolize methanol and co-produce PHA and carotenoids from crude glycerol [88]. These reports highlight the biotechnological potential of some bacterial strains to overcome growth inhibition issues related to carbon recovery from crude glycerol feedstocks.

3.6. Challenges and Opportunities of PHA Production with Wastewater Feedstocks

Table 1 highlights the AF of wastewater feedstocks (strategies II and III; Figure 2) as a key process to obtain different VFA profiles as carbon feedstocks, affecting the monomeric composition of PHA copolymers. For instance, AF with an increased food-to-biomass ratio (e.g., F/M = 0.2) or lower sludge retention times favours the synthesis of odd VFAs that leads to 3HV monomers [62,65]. Additionally, long biomass acclimatation time (e.g., 53–60 days) in AF and a suitable carbon source choice in the acclimation and accumulation steps (e.g., caproic acid) may yield mclPHA precursors, such as hexanoic acid [69]. Furthermore, AF decreases sugar content in wastewater to avoid undesirable non-PHA biomass that may increase PHA productivity and enhance the economics of downstream processing. However, further research is required in the AF process to determine the range of VFA profiles that can be obtained from each wastewater feedstock in order to produce tailored PHA bioplastics. Hence, each wastewater feedstock, by providing a PHA repertoire, can support specific bioplastic applications aligned with the respective industry needs.

Recent metagenomic analyses provide new insights on the MMC community dynamics during enrichment and PHA accumulation processes. Pseudomonadota is the main PHA-producing phylum in MMC using wastewater feedstocks. The bacterial genera *Thauera*, *Azoarcus*, *Paracoccus*, *Flavobacterium,* and *Methylorubrum* have been highlighted as relevant for PHA accumulation with broad substrate preferences and resistance to harsh conditions (e.g., salinity, presence of toxic compounds) [81,82,84,89,90]. However, MMC diversity is still unknown, as several unclassified bacteria have been reported [82]. Novel PHA synthetic pathways, such as fatty acid de novo synthesis, have been poorly addressed in wastewater feedstocks to obtain mclPHA [32]. Furthermore, enzymes including R-specific enoyl-CoA hydratase (PhaJ) and phylogenetically distant PHA synthases represent potential tools for tailoring bioplastic properties and enhance productivity [32,36,82]. Additionally, determining the MMC metabolic capability for synthesizing added-value bioproducts will reveal the biorefinery synergy potential of wastewater feedstocks. For example, high-salinity wastewater increased ectoine, triacylglycerols, and EPS synthetic genes' richness, revealing novel potential biorefinery synergies [80,83,90].

4. Recovery of EPS from Wastewater Streams

Downstream polymer purification processes represent 40–60% of overall PHA production costs due to the complexity of the surrounding residual biomass [91]. The biorefinery of PHAs and EPSs has been well achieved in pure cultures, increasing the value chain of recovery processes [19]. Table 2 shows several reports of carbon recovery through EPS production from wastewater. A synthetic MMC produced 4.9 g PHA $gVSS^{-1}$ using WAS as carbon feedstock [92]. An alginate-like exopolysaccharide (ALE) was obtained from AS from a full-scale WWTP and from a laboratory-scale AGS system, reaching 200–440 mg ALE g VSS^{-1} [93]. The ALE was composed mainly of glucuronic acid and mannuronic acid blocks, and the

content of this polysaccharide in AGS was 56–67% higher than those of the AS full-scale system. This indicates that AGS formation conditions correlated with an ALE-producing microorganism enrichment. ALE recovery from an AGS bioaugmented with the microalgae *Tetradesmus* sp. reached 70 mg ALE g VSS^{-1} [39]. The biorefinery of P(3HB-*co*-3HV) and ALE was achieved from AS from a laboratory-scale fed-batch bioreactor, obtaining 0.61 g PHA $gVSS^{-1}$ with 3.5 mol% 3HV and 0.12 g ALE g VSS^{-1} [94]. This P(3HB-*co*-3HV) and ALE biorefinery reduced the overall GHG emissions by 3.4 g CO_2 equivalents, WAS mass by 24% w w^{-1}, and overall PHA cost by 58.6% [94]. Carbon recovery through PHAs and ALE biorefinery shows potential for WWTPs' upgrade towards WRRFs. EPS consisting of methyl furaldehyde and levoglucosenone was recovered from AGS from a municipal WWTP, reaching 140 mg EPS g AGS^{-1} [95]. A comprehensive modulation of MMC composition will contribute valuable tools for optimizing biorefineries and for broadening the portfolio of bioproducts, strengthening the value chain of carbon recovery [38].

Table 2. Extracellular polysaccharide (EPS) recovery from wastewater streams.

Wastewater/Resource	MMC	Bioprocess	EPS Type	Application	Ref.
Municipal wastewater	Enriched AS	Fed-batch	ALE	N.D.	[93]
Municipal wastewater	Non-enriched AGS and AS	SBR	ALE	Wastewater and surplus biosolid treatment	[94]
Municipal wastewater	AGS bioaugmented with microalgae	SBR	ALE	N.D.	[39]
Municipal wastewater	Non-enriched AGS	SBR	N.D.	Flame retardant	[95]
Municipal wastewater	Non-enriched AS	Aerobic continuous reactor		Flame retardant	
Municipal/Slaughterhouse wastewater	Non-enriched AGS	SBR	Methyl furaldehyde and levoglucosenone	Tissue coating enhancing. Hydrophobicity	[96]
WAS [a]	Synthetic MMC	Aerobic batch	N.D.	Flocculation and metal chelator agent	[92]

[a], Thermically hydrolysed waste-activated sludge (WAS). MMC, mixed microbial cultures; EPS, extracellular polysaccharides; ALE, alginate-like exopolysaccharide; SBR, sequence batch reactor; AGS, aerobic granular sludge; AS, activated sludge; N.D., not determined.

5. Applications of Microbial Biopolymers

PHAs and EPS offer a wide diversity of polymer types with varying monomeric compositions, hence versatile and tuneable thermomechanical properties for target applications. Figure 3 shows some PHAs applications in medicine, agriculture, packaging, bioremediation and construction, depicting distinct quality control requirements that must be aligned with feedstock characteristics.

In medicine, the low melting temperature (122–133 °C) and flexibility of P(3HB-*co*-3HV) and P(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) according to the 3HV or 4HB proportion is useful for the manufacture of medical devices [97]. Similarly, the elastomer P(3HB-*co*-3HHx) was used for the manufacture of biodegradable scaffolds with different geometries through additive manufacturing. P(3HB-*co*-3HHx) scaffolds enabled growth of Human Dermal Fibroblast cells, showing applicability in tissue engineering [98]. PHA-based composites and blends are of increasing trend to reach enhanced properties for target applications. P(3HB-*co*-3HV) combined with the bacterial compound violacein provides antibacterial properties to the formulation [99]. P(3HB) combined with natural products quercetin, curcumin, or caffeic acid provide antioxidant activity [100]. P(3HB) combined with gelatine enhances hydrophobicity and mechanical properties for tissue

engineering scaffolds [101]. Biopolymer production from wastewater is of major concern, as hazardous elements such as heavy metals, toxins, and pathogens increase the risk of cytotoxicity, inflammation, irritation, allergic reactions, and acute and chronic systemic toxicity [102]. Some agro-industrial or food processing wastewater are subject to rigorous upstream quality control standards [103], placing them as attractive feedstocks for carbon recovery through PHA production intended for medical applications [104].

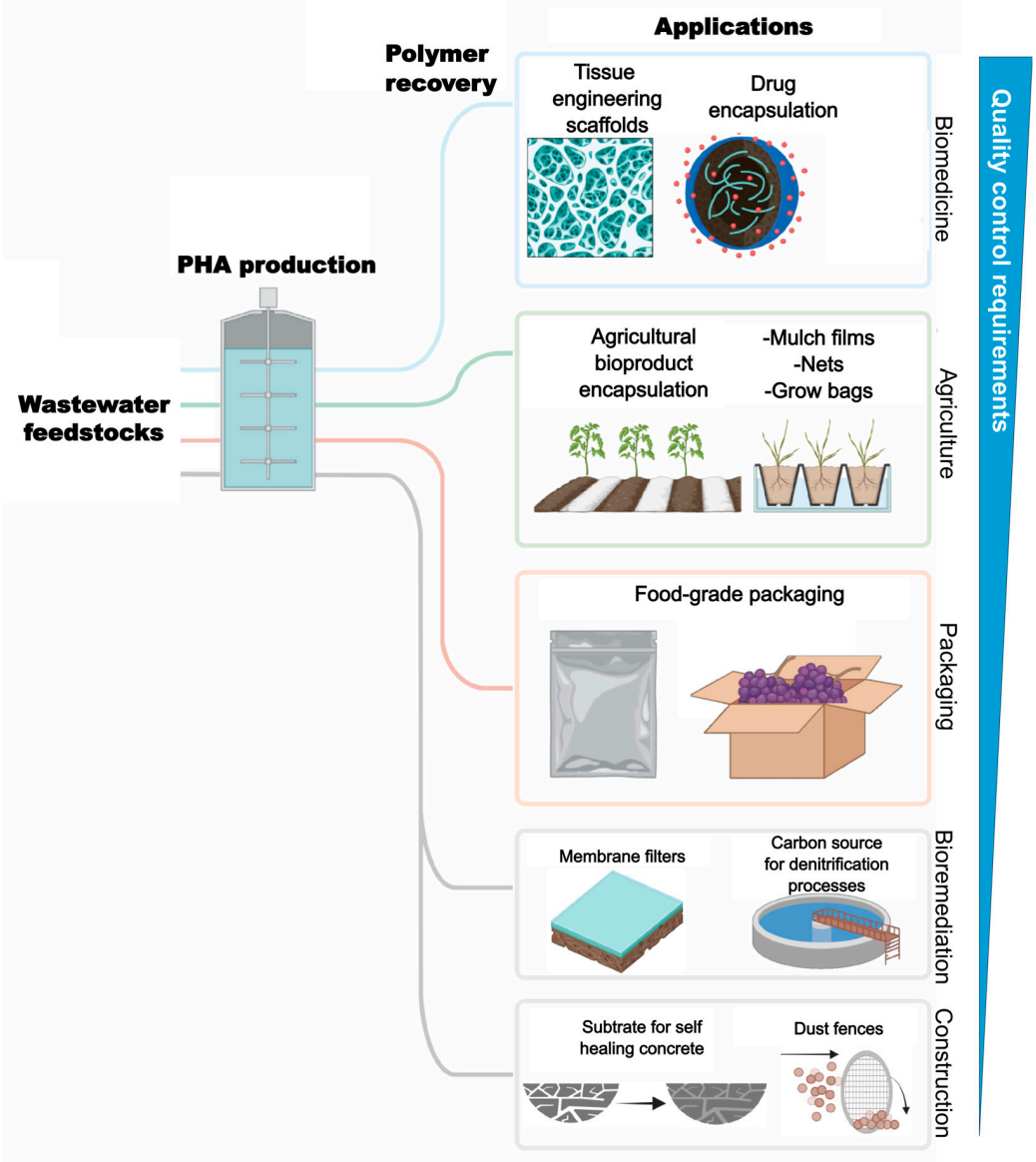

Figure 3. Potential applications of polyhydroxyalkanoates (PHAs) recovered from different wastewater carbon feedstocks. Suitability of each wastewater carbon feedstock is constrained by the respective target application according to quality and safety control requirements, which is represented in arrows of different colours.

In agriculture, PHAs are used for the manufacture of mulch films for weed control, preventing contamination and retaining soil moisture [105]. Mulch films of P(3HB-*co*-3HHx) copolymer exhibited up to 80% biodegradation in soils after 4 months of incubation [106]. Poly(4-hydroxybutyrate) has been used to manufacture agricultural nets and grow bags due to its similar properties compared to those of polyethylene [97]. P(3HB), P(3HB-*co*-3HV), cellulose, or alginate formulations for the controlled release of plant-growth-promoting microorganisms, fertilizers, and other agricultural products increase their viability and efficiency in field applications [107–109]. Agricultural applications forecast an interesting

market opportunity for biopolymers derived from agro-industrial wastewater streams, promoting a decentralized circular scheme to reduce economic and environmental burden associated with transportation [110].

Packing applications are the most acknowledged PHA applications due to the infrastructure and market development of single-use fossil-based plastics as well as the environmental awareness related to their disposal [111]. P(3HB) blended with lignocellulosic biomass or EPS for the manufacture of packing materials with enhanced hydrophobicity or properties enable products with water-resistant properties or their processing with 3D printing manufacture [96,112]. Additionally, P(3HB-*co*-3HV) and P(3HB-*co*-3HHx) maintained their thermal and gas barrier properties after being reprocessed up to six times through thermal extrusion, demonstrating the recycling feasibility [113].

Environmental pollution management is an interesting application field for PHA and EPS biopolymers. PHAs' use as a slow-release carbon source for denitrification improvement has been reported [114]. PHA-based membrane filters show potential for upgrading current non-biodegradable fossil-based membranes enhancing water purification sustainability [115]. Wastewater-derived ALE showed better flocculant and gel-forming properties than commercial additives, removing 82–94% from brewery or municipal wastewater and other specific pollutants [39,92,93]. Biopolymers recovered in a WRRF will reduce the economic and environmental burden associated with transportation and hazardous pollutant dissemination [110].

Construction materials are another promising application for PHAs, such as with self-healing concrete. Self-healing concrete consists of a bioproduct harbouring a microbial inoculum with its respective substrate. Upon microbial activation, respiration leads to CO_2, which is readily precipitated into calcium carbonate due to the alkalinity and high calcium content of concrete. Calcium carbonates fill up concrete cracks, extending the life span of construction and enhancing the sustainability of concrete usage. A recent study used waste-derived PHA as a carbon source for a concrete-healing formulation [116], highlighting the flexible quality control requirements, (e.g., monomer composition, molar mass, and hazardous components).

6. Quality Control

Carbon recovery from wastewater via PHA and EPS is an attractive strategy due to the biodegradability, thermomechanical, and active properties of these biopolymers. However, the presence of persistent organic pollutants and heavy metals in wastewater may be transferred to the final biopolymer product, which leads to stricter regulatory scrutiny [18,117]. While agricultural resource recovery is already embedded in circular economy policies, medical and food contact uses remain under regulatory uncertainty, requiring clear frameworks. In the medical field, ISO 10993 and ISO 7405 guide the biological evaluation of medical devices, covering biocompatibility assessments (i.e., cytotoxicity, sensitization, hemocompatibility, pyrogenicity, implantation issues, genotoxicity, carcinogenicity, reproductive toxicity, degradation, and chemical assessments) [118]. Yet, medical or food contact devices derived from wastewater resources require harmonized monitoring and management of substances of very high concern [21]. On the other hand, in agricultural applications, nutrient circulation through biosolids and soil amendments has long been regulated. These regulations include standards limiting heavy metals, pathogens, and organic pollutants [119]. As an example, stricter thresholds apply to the usage of wastewater-derived struvite and digestate applications as phosphorus biofertilizers [120,121]. In this case, the cadmium threshold is below 20 mg kg^{-1} P [119]. Such policies are valuable examples for PHA and EPS regulation in mulch films and slow-release fertilizers designed to degrade in soils. In packaging applications, food contact materials are subject to strict

safety policies to protect health and consumers [122]. Biopolymer purity, device constituent migration limits, and traceability requirements will be critical for wastewater-derived devices in this field. Safety control of PHA and EPS-based products place polymer recovery strategies as an essential research topic to implement carbon recovery within one health scope. Nevertheless, the fields concerning bioremediation and construction convey interesting PHA and EPS applications due to low quality and safety control requirements.

7. Concluding Remarks

Wastewater from municipal and agro-industrial activities, food processing, lignocellulosic biomass processing, and biofuel production are suitable carbon feedstock to produce PHA and EPS. Wastewater carbon recovery faces challenges such as substrate variability and diluted nature. Acidogenic fermentation and enrichment of MMC with robust PHA-producing microorganisms are pivotal for a reliable production of bioplastics with tailored properties.

The biorefinery of PHAs and alginate-like biopolymers from wastewater is a cross-cutting approach that enhances carbon recovery in WWTPs. This biorefinery approach promotes low-cost production of bio-based biodegradable polymers that replace fossil-based non-biodegradable plastics. A deeper understanding of the MMC community profile and the conditions for customizing and optimizing PHA and EPS synthesis will improve biorefinery synergy to increase the value chain of carbon recovery and upgrade WWTPs towards WRRFs.

Specific target applications, including biomedicine, agriculture, packaging, or construction, define PHA and EPS quality standards and provide a framework for application-oriented production under suitable quality benchmarking for feasible carbon recovery from wastewater feedstocks.

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Abbreviations

The following abbreviations are used in this manuscript:

AF	Acidogenic fermentation
AGS	Activated granular sludge
ALE	Alginate-like exopolysaccharide
AS	Activated sludge
BktB	3-Ketovalerate producing 3-ketothiolase
COD	Chemical oxygen demand
EPS	Extracellular polysaccharide
GHG	Greenhouse gas

MBBR	Moving-bed biofilm bioreactor
MMC	Mixed microbial culture
PHA	Polyhydroxyalkanoate
PhaC	Polyhydroxyalkanoate synthase enzyme
PhaA	3-Ketothiolase
PhaB	Acetoacetyl-CoA reductase
PhaJ	*R*-specific enoyl-CoA hydratase
PhaG	(*R*)-3-Hydroxyacyl-ACP-CoA transacylase
PhaZ	Polyhydroxyalkanoate depolymerase
mclPHA	Medium-chain-length polyhydroxyalkanoate
P(3HB)	Poly(3-hydroxybutyrate)
P(3HB-*co*-3HV)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
P(3HB-*co*-3HHx)	Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)
P(3HB-*co*-4HB)	Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
R-3HB-CoA	(R)-3-hydroxybutyryl-CoA
SBR	Sequence batch bioreactor
sclPHA	Short-chain-length polyhydroxyalkanoate
TH	Thermic hydrolysis
VSS	Volatile suspended solids
WAS	Waste-activated sludge
WWTP	Wastewater treatment plant
WRRF	Wastewater resource recovery facility
3HB	3-Hydroxybutyrate monomer
3HV	3-Hydroxyvalerate monomer
4HB	4-Hydroxybutyrate monomer

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