REVIEW

A review on microbes mediated resource recovery and bioplastic (polyhydroxyalkanoates) production from wastewater

Vishal Ahuja[1], Pankaj Kumar Singh[1], Chandan Mahata[2], Jong-Min Jeon[3], Gopalakrishnan Kumar[4,5], Yung-Hun Yang[6,7] and Shashi Kant Bhatia[6,7*]

Abstract

Background Plastic is widely utilized in packaging, frameworks, and as coverings material. Its overconsumption and slow degradation, pose threats to ecosystems due to its toxic effects. While polyhydroxyalkanoates (PHA) offer a sustainable alternative to petroleum-based plastics, their production costs present significant obstacles to global adoption. On the other side, a multitude of household and industrial activities generate substantial volumes of wastewater containing both organic and inorganic contaminants. This not only poses a threat to ecosystems but also presents opportunities to get benefits from the circular economy.

Main body of abstract Production of bioplastics may be improved by using the nutrients and minerals in wastewater as a feedstock for microbial fermentation. Strategies like feast-famine culture, mixed-consortia culture, and integrated processes have been developed for PHA production from highly polluted wastewater with high organic loads. Various process parameters like organic loading rate, organic content (volatile fatty acids), dissolved oxygen, operating pH, and temperature also have critical roles in PHA accumulation in microbial biomass. Research advances are also going on in downstream and recovery of PHA utilizing a combination of physical and chemical (halogenated solvents, surfactants, green solvents) methods. This review highlights recent developments in upcycling wastewater resources into PHA, encompassing various production strategies, downstream processing methodologies, and techno-economic analyses.
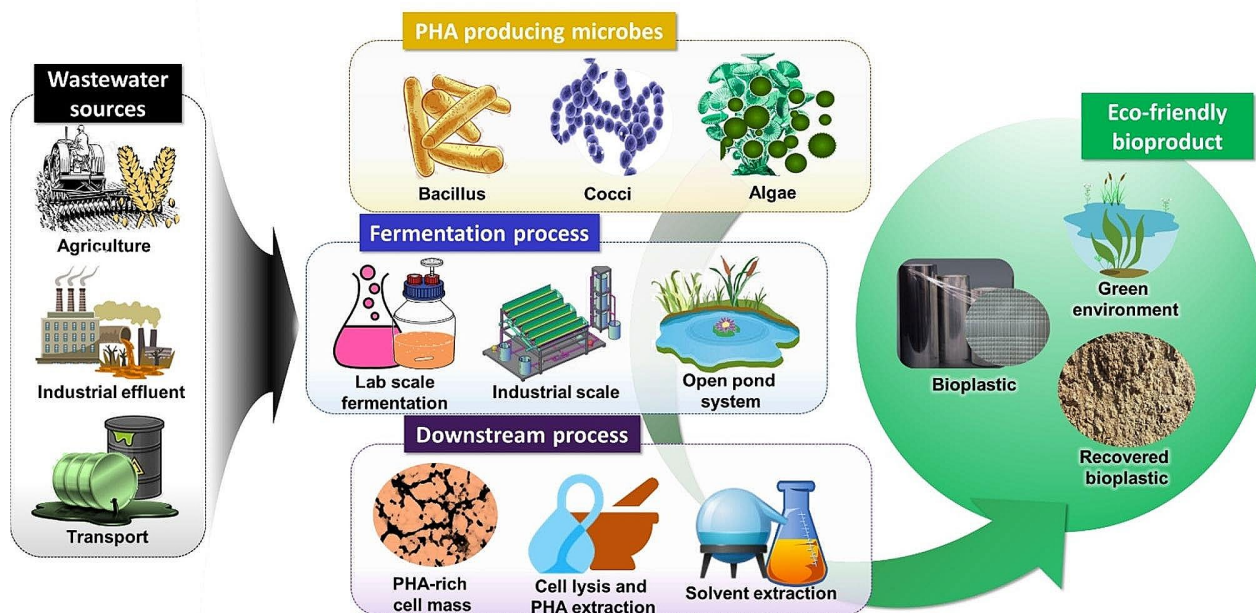
Short conclusion Organic carbon and nitrogen present in wastewater offer a promising, cost-effective source for producing bioplastic. Previous attempts have focused on enhancing productivity through optimizing culture systems and growth conditions. However, despite technological progress, significant challenges persist, such as low productivity, intricate downstream processing, scalability issues, and the properties of resulting PHA.

*Correspondence:
Shashi Kant Bhatia
shashibiotechhpu@gmail.com

Full list of author information is available at the end of the article

Graphical abstract

Keywords Bioplastic, Polyhydroxyalkanoates, Wastewater, Downstream processing

Background

Plastic waste and environmental pollution pose significant global challenges, stemming from a dramatic increase in production and usage over recent decades [1]. Primary plastic production globally surged from 9,200 million metric tons (1950–2017) to a projected 34 billion metric tons by 2050 [2]. A critical concern lies in plastic's enduring presence in landfills, water bodies, and ecosystems [3]. Of the total 7 billion tons of plastic waste generated, less than 10% undergoes recycling, while 14% is incinerated, leaving 76% to either accumulate in landfills or infiltrate natural environments [2]. Annually, approximately 14 million metric tons of plastic find their way into the ocean, resulting in an estimated 170 trillion plastic particles dispersed throughout the world's oceans. Experts predict this figure to triple within the next two decades [4].

Plastic waste undergoes degradation through physical, chemical, and biological processes. Physical and mechanical forces work to reduce particle size, while energy transfer, like heat weakens structural integrity and functionality [5–7]. Chemical methods, including exposure to UV radiation, ozonolysis, or catalytic conversion, induce chemical changes that diminish mechanical strength, cause embrittlement, and release residues [5, 7]. The biological degradation (biodegradation) of plastics, considered a safer process occurs under the influence of microbial (bacteria, fungi, and algae) metabolism and enzymatic action. This phenomenon can be seen distinctly in various environments including landfills, oceans, and soil [8]. First microbes colonize on particles and secrete enzymes to depolymerize polymers and then use them as a carbon source. Microorganisms employed various enzymes including PETase, esterase, lacasses, cutinases, etc., for plastic degradation by acting on carbon back bone, side chains, and hetero atoms [9]. From the mangrove sediments, Auta et al. (2018) isolated *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, reporting 6.4% and 4.0% polypropylene (PP) degradation in 27 and 40 days, respectively [10]. Many other microbes have been reported to degrade various plastics including *Pseudomonas putida* IRN22, *Acinetobacter pittii* IRN19, *Micrococcus luteus* IRN20 [11] *Pseudomonas citronellolis* [12]. Besides microorganisms, *Tenebrio molitor* larvae have also been reported to depolymerise the polyvinyl chloride [13]. However, these processes are slower and result in the generation of plastic traces, including microplastics and nanoplastics, which can easily infiltrate marine and terrestrial environments, posing risks to both humans and animals [8, 14]. Ingestion or inhalation of microplastics may lead to oxidative stress, inflammatory reactions, and metabolic disorders [14, 15].

Various biobased materials, including starch, chitin, chitosan, and cellulose, have been explored for their potential in packaging applications [16–18]. Polyhydroxyalkanoates (PHA) emerge as a promising alternative to fossil-based plastics, offering comparable strength and environmental friendliness [19]. However, the cost-effectiveness of PHA production is predominantly influenced by feedstock expenses and product recovery, with nearly 40% of the cost attributed to feedstock and facing issues with low yield [20, 21]. In this context, waste resources such as wastewater from various sources including municipal, industrial, and agricultural practices present a viable and cost-effective alternative for bioplastic production, while also enabling resource reclamation [22–24]. According to United Nations reports from 2023 [25] a total of 320 billion m^3 of wastewater is generated globally. More than 70% of freshwater is utilized for agricultural purposes, while industries consume 22% [26]. Only 11% of wastewater from domestic and industrial sources is earmarked for reuse, leaving over 42% of household wastewater partially treated. The wastewater generated alone accounts for approximately 1.57% of greenhouse gas (GHGs) emissions [25]. Wastewater from various sources contains high organic content, such as sugars and fatty acids, which can be harnessed by microorganisms for the production of PHA [27]. Numerous studies have explored PHA production using diverse substrates, including municipal wastewater [28], cheese whey by anaerobic mixed culture [29], olive oil mill wastewater by acidogens [30], secondary wastewater sludge, and waste sludge [31], hardwood sulfite spent liquor [32], paper industry effluent [33], raw sludge by methanotrophs [34], phototrophic mixed culture [35] and by anaerobes [36]. These studies highlight the versatility of wastewater as a substrate for PHA production, showcasing its potential to revolutionize the bioplastic industry by utilizing diverse waste streams.

Recovering PHA from fermentation broth poses another challenge in the production process. Traditionally, recovery methods involve either cell lysis or solvent-based extraction. The conventional approach favors chloroform ($CHCl_3$) as a standard solvent [37]. However, alternative methods, such as antisolvent systems [38] using nonhalogenated solvents, have been explored to extract PHA. Concerns over environmental and user toxicity have steered the search for greener and non-toxic solvents. Advanced techniques have introduced biobased solvents like 3-hydroxybutyrate-*co*-3-hydroxyvalerate and methyltetrahydroxyfuran (2-MTHF) [39], as well as dimethyl carbonate, ethanol, ethyl acetate, ethyl lactate, and methanol [40], which have proven to be equally effective in extraction without toxic effects compared to the standard process. The primary considerations for downstream processing remain environmental impact and overall cost which can be achieved by use of greener solvent-based extraction, solvent recycling, and revalorization of residues. The exploration of bioplastics has garnered significant attention in both research and industrial sectors. Previous literature has examined various facets of bioplastics, including their environmental benefits, production from different waste sources such as food, vegetables, downstream processing, and stability in different environments. For instance, Lavagnolo et al. [41] delved into the stability of bioplastics in aquatic environments, while Gong et al. [42] and Ali et al. [43] summarized bioplastic production from fruit-vegetable residues and organic waste, respectively. Additionally, Bhat et al. [44] addressed greener approaches for bioplastic recovery. However, there is limited information available on utilizing wastewater as a feedstock. The current article comprehensively covers various aspects from feed conversion to bioplastic production, including techno-economic analysis and cost considerations.

Microbial PHA synthesis pathways

The microbial synthesis of PHA stands as a remarkable example of nature's innovation, offering a sustainable avenue for producing biodegradable bioplastics [45, 46]. Microorganisms produce PHA as intracellular carbon reserves in response to nutrient imbalances, particularly an excess of carbon and limited nitrogen or phosphorus, storing them as granules [18, 47]. The biosynthetic operon for PHA consists of a cluster of genes, including PHA synthase, β-ketothiolase, and NADPH-acetoacetyl-CoA reductase, organized in close proximity [48, 49]. Carbon sources are converted into acetyl-CoA, a precursor for PHA synthesis, through pathways such as glycolysis or beta-oxidation of fatty acids [50]. The synthesis of polyhydroxybutyrate (PHB) involves the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, catalyzed by the enzyme β-ketothiolase. Subsequently, acetoacetyl-CoA is reduced to (R)-3-hydroxybutyryl-CoA by (R)-specific acetoacetyl-CoA reductase. Finally, (R)-3-hydroxybutyryl-CoA is polymerized into PHB by the enzyme PHA synthase [51, 52]. This pathway utilizes the organic components available in wastewater, such as oil, volatile fatty acids (VFAs), glucose, fructose, xylose, and amino acids, transforming them into PHA and intermediates (Fig. 1).

The acidogenic effluent derived from dairy waste presents itself as a cost-effective, acid-rich wastewater stream suitable for producing PHA [53]. In a study by Pagliano et al. [54], the efficacy of dairy wastewater-derived effluent for PHB production in specific biobased industrial applications was demonstrated, achieving a significant PHB content of 0.31%, twelve times higher than the control group at the same time (0.025%). *Cupriavidus necator* DSM 13,513 was identified as the most efficient strain
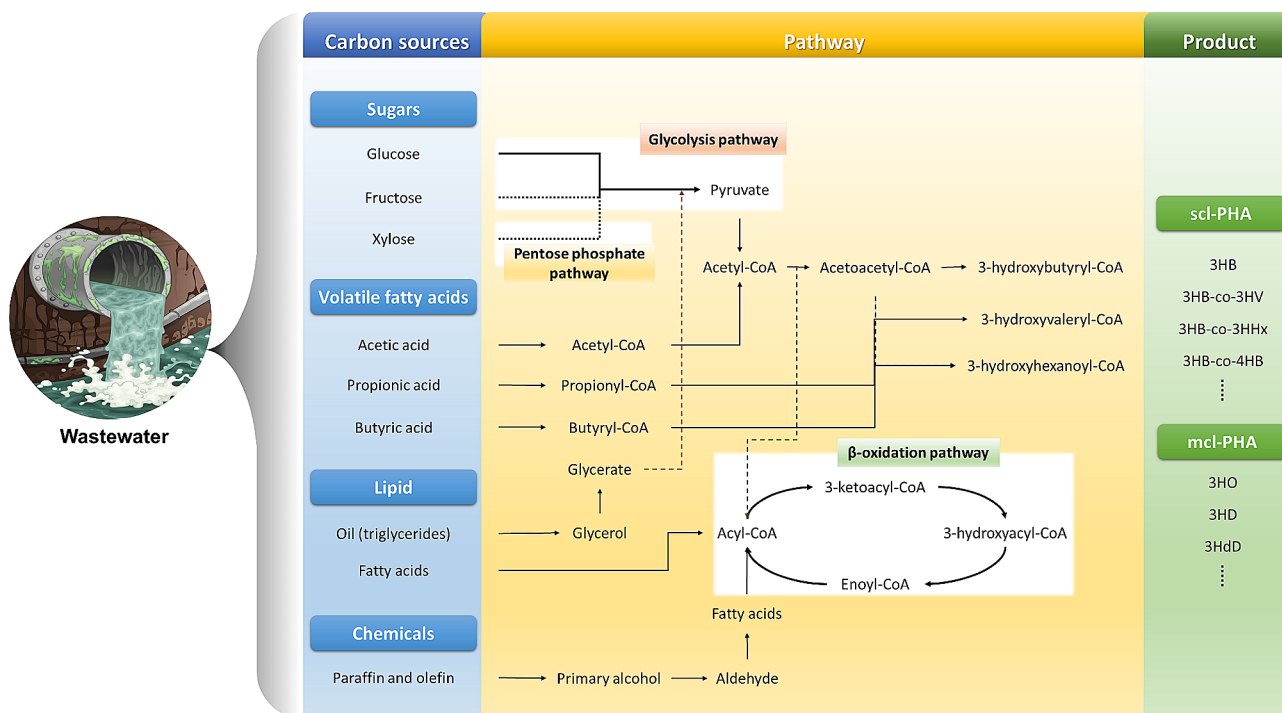
Fig. 1 Different pathways for the production of bioplastic from pollutants in wastewater

for accumulating PHB from the fatty acid-rich effluent of an anaerobic process fed with dairy wastewater. Other research endeavors have successfully produced PHB using effluents from waste sewage and food industry wastewater [55]. Furthermore, studies indicate the potential utilization of food industry wastewaters, such as confectionery wastewater (CWW) and rice parboiling water (RPW), as cost-effective substrate sources for PHB production [56].

Microorganisms possess the capability to utilize oils and produce medium-chain-length polyhydroxyalkanoates (*mcl*-PHA) [57]. (R)-3-hydroxyacyl-CoA, an intermediate of fatty acid metabolism, directs the conversion of fatty acids into PHA. By bypassing the fatty acid β-oxidation pathway, acetyl-CoA and de novo pathway intermediates are directed towards PHA biosynthesis. Transacylase, a critical enzyme in PHA biosynthesis, transfers the (R)-3-hydroxyacyl moiety from the respective acyl carrier protein (ACP) thioester to CoA [58, 59]. Notably, the genes encoding transacylase and enoyl-CoA hydratase are co-regulated but are not situated within the PHA synthase operon [60, 61]. *Bacillus thermoamylovorans* strain PHA005, isolated from palm oil mill wastewater effluent, demonstrates the ability to produce *mcl*-PHA at 50.77% of dry cell weight (DCW), which increases to 63.27% under optimal growth conditions, including a C/N ratio of 5:1 at 45 °C [62]. Mixed microbial cultures (MMCs) have been utilized for PHA production, yielding both short-chain-length (*scl*-PHA) and

mcl-PHA using enzymatically pretreated palm oil wastewater as feedstock [63]. The success of PHA production from wastewater relies on the microorganisms' capacity to accumulate intermediates and polymers. To enhance this process, enrichment techniques can be employed. MMCs enriched with a mixture of volatile fatty acids (VFA$_{mix}$) have shown improved accumulation results, with the maximum reported accumulation capacity achieved using the VFA$_{mix}$ system (54.5±8.0 wt%) [64]. It can be concluded that substrate switching influences accumulation and enrichment with mixed VFAs leading to maximum yield. Integrating wastewater treatment with PHA copolymer production presents the advantage of repurposing environmental waste to generate an environmentally friendly end product however, the process needs to optimize the waste processing conditions and process parameters to attain the maximum conversion.

Polyhydroxyalkanoates production utilizing wastewater resources

Polyhydroxyalkanoates constitute a class of biodegradable polymers synthesized by microorganisms as a means of intracellular carbon storage [65]. They present a sustainable and environmentally friendly alternative to traditional plastics and packaging materials. Certain bacteria have the capability to accumulate PHA from organic substrates, converting these carbon sources into valuable biopolymers [66, 67]. Recent studies have revealed a mutually beneficial relationship between wastewater

treatment and PHA production, signaling a shift towards sustainable practices that leverage waste streams to produce biodegradable bioplastics, thereby promoting a circular economy [68]. The process of wastewater treatment, resource recovery, and conversion into PHA entails various benefits and challenges, as elucidated by recent research [69, 70]. To harness the nutrients available in wastewater for valorization, several strategies and approaches have been developed, as discussed below (Fig. 2).

Wastewater composition and treatment challenges

Wastewater, sourced from domestic, industrial, and agricultural activities, presents a complex mixture of pollutants, both organic and inorganic, requiring sophisticated treatment methods to mitigate its adverse effects on ecosystems and human health [71, 72]. These compounds pose significant risks to lives and contribute to high oxygen demand [73]. Elevated levels of nutrients such as nitrogen and phosphorus, originating from human waste, detergents, and agricultural runoff, can induce eutrophication, leading to algal blooms and degradation of water quality [74].

Industrial effluents from textiles, pharmaceuticals, paper and pulp, and petroleum, etc., introduce a wide array of chemicals including heavy metals, pharmaceuticals, pesticides, and other toxic substances (Table 1), posing risks to ecosystems and human health [75, 76]. The paper and pulp industries rely on plant biomass as feedstock hence effluent [77] and granular sludge bed [78] from paper and pulp industries have high Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) mainly contributed by phenols along with heavy metals. Asphalt reclamation and production sites generate highly toxic and polluted wastewater referred as Bitumin fume condensate wastewater which has shown high COD due to high organic load [79]. In contrast to food and paper industries, petrochemical industries effluent is also rich in COD, and total organic carbon (TOC) but the COD mainly contributes to processing waste like terephthalic acid, NH_3-N, and volatile fatty acids [80].

The release of such pollutants is mainly responsible for deteriorating the environment, therefore, the treatment of wastewater for the removal or degradation becomes mandatory [24, 92, 93]. Based on the nature of the treatment technique, wastewater treatment techniques are divided into physical, chemical, and biological treatments [94]. Physical treatment methods like screening, sedimentation, and filtration are employed to remove solid particles and larger pollutants but may not effectively eliminate dissolved substances. However, in chemical
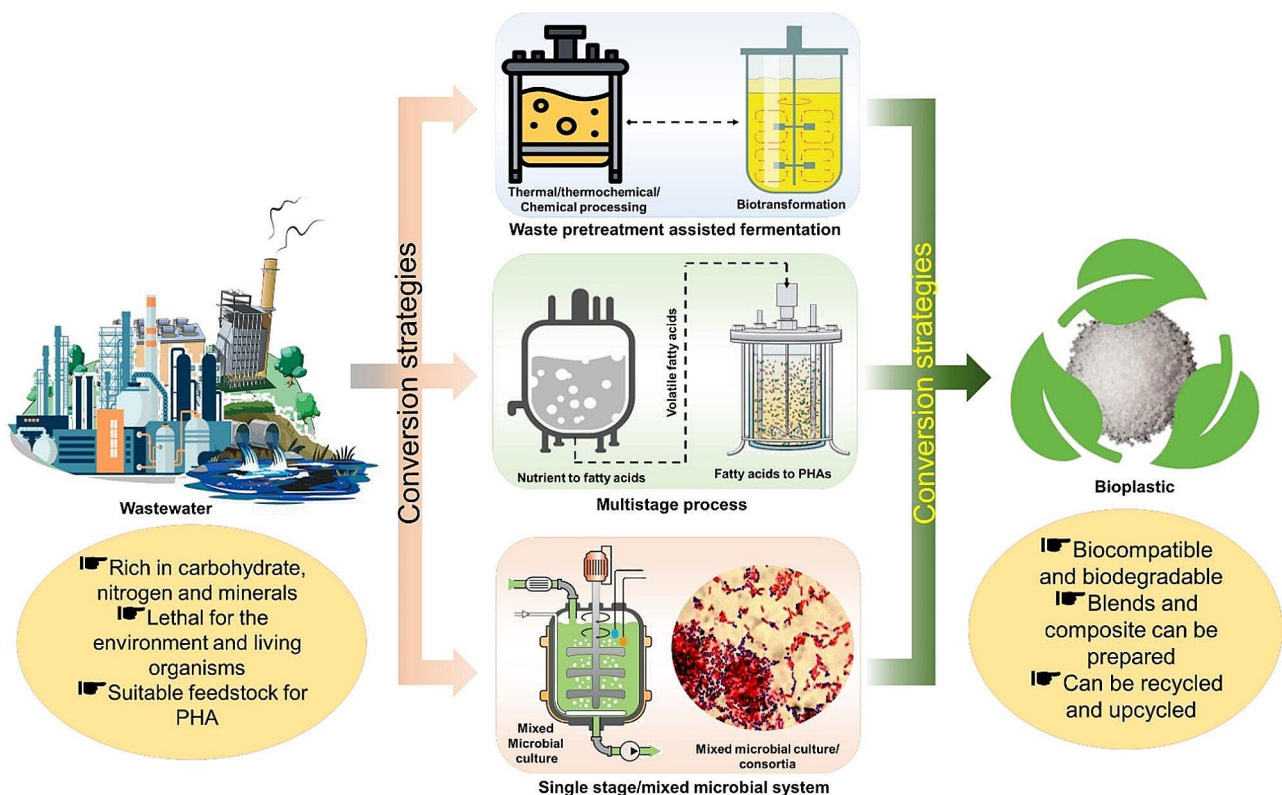

Fig. 2 Different strategies for bioplastic production from wastewater

Table 1 Wastewater from different industrial sources

Wastewater	Site	Main components and conductivity (EC)	Bio-chemical characteristics	References
Olive mill wastewater	-	Organic acids (formic and acetic acid), flavonoids, tannins, polyphenols	pH 2–6; high solid matter and organic load	[81]
Anaerobic digest of industrial wastewater from Candy production plant	InnovEn S.R.L. Turin, Italy	Lactic acid, propionic acid, acetic acid	TOC: 1.8% w/w	[82]
Season seed wastewater	Kasih Canned Food Production Co Amman (Jordan)	EC 2.5 ms.cm^{-1}	pH: 5.4; COD 3750 mg.L^{-1}, NH$_4$ 70 mg.L^{-1}, TS: 10,245 mg.L^{-1}	[83]
Dairy wastewater	-	EC 2.2 ± 0.1 ms	pH 8.62 ± 0.02, TSS: 265 ± 3 mg.L^{-1}, TDS 934 ± 3 mg.L^{-1}, COD 1,999 ± 10 mg.L^{-1}, TS: 1,889 ± 4 mg.L^{-1}	[84]
Palm oil industries' wastewater	-	EC 14.3 ± 0.1 ms	pH 2.05 ± 0.02, TSS: 518 ± 4 mg.L^{-1}, TDS 9,889 ± 4 mg.L^{-1}, COD 1,596 ± 9 mg.L^{-1}, TS: 10,415 ± 5 mg.L^{-1}	[84]
Vegetable industry wastewater	-	Cadmium, nickel, lead, cobalt, *E. coli* 2336.67 CFU.g^{-1}, EC 2.54 ms,	pH 6.90, VSS: 899 mg.L^{-1}, TP 39.02 mg.L^{-1}, COD 5392 mg.L^{-1}, toxicity 29.167 Eqtox.m^{-3}, TSS: 1281 mg.L^{-1}	[85]
Domestic wastewater sludge	Wastewater treatment Palermo University Italy	-	TSS: 6.15 ± 1.22 mg.L^{-1}, VSS 4.35 ± 1.11 mg.L^{-1}, sCOD 133.59 ± 93.91 mg.L^{-1}, TCOD 6320 ± 2217.93 mg.L^{-1}	[86]
Cashew industry wastewater	-	Microbial load 4.8 × 10^6 CFU.mL^{-1}, oil and grease 940 mg.L^{-1}	TSS: 551.43 mg.L^{-1}, pH 5.34, BOD 1161 mg.L^{-1}, COD 5740 mg.L^{-1}, total phenol 294 mg.L^{-1}	[87]
Winery wastewater (Vintage)	Niagara	Heavy metals including Hg 12,600 µg.L^{-1}, Cu 28,400 µg.L^{-1}, Mn 6100 µg.L^{-1}, Ni 3000 µg.L^{-1}, EC 1200–3400 µS.cm^{-1}	pH 4.24, COD 360,000 mg.L^{-1}, TSS: 70,300 mg.L^{-1}, BOD 96,000 mg.L^{-1}, TP 714 mg.L^{-1}, TVS 128,000 mg.L^{-1}	[88]
Winery wastewater (Non-vintage)	Niagara	Heavy metals including Hg 4170 µg.L^{-1}, Cu 29,600 µg.L^{-1}, Mn 7000 µg.L^{-1}, Ni 500 µg.L^{-1}, EC 355-12800 µS.cm^{-1}	pH 4.76, TSS: 84,400 mg.L^{-1}, COD 55,852 mg.L^{-1}, BOD 3160 mg.L^{-1}, TP 102 mg.L^{-1}, TVS 112,000 mg.L^{-1}	[88]
Petrochemical wastewater	-	Phenols 23 mg.L^{-1}, mercury, silver, arsenic, titanium, lead, copper, chromium, cadmium	pH 4.3–10.0, TOC: 1500 mg.L^{-1}, COD 1220 mg.L^{-1}, TSS 1.2–1000 mg.L^{-1}, NVFAs 275, VFAs 4900 mg.L^{-1}, TSS 1000 mg.L^{-1}	[89]
Raw winery wastewater	West Coast of South Africa	Turbidity 570–639 NTU, conductivity 175–230 mS.m^{-1}	pH 4-4.5, TSS: 1840–2275 mg.L^{-1}, TDS 1476–3546 mg.L^{-1}, COD 12,400–22,620 mg.L^{-1}	[90]
Petrochemical wastewater	-	Oil 500 mg.L^{-1}, salt 2157 mg.L^{-1}	pH 7.78, SS 200 mg.L^{-1}, CODcr 745.87 mg.L^{-1}, N$_{ammonia}$ 40 mg.L^{-1}, TDS 2000 mg.L^{-1}	[91]

treatment, advanced oxidation processes and chemical coagulation aim to degrade or remove persistent pollutants. The methods are effective but also cost-intensive and generate secondary contaminants. In contrast, biological treatment by activated sludge, biofiltration, and constructed wetlands harness microbial activity to break down organic matter but may be less effective for certain pollutants [74, 95, 96]. The challenges and limitations of each wastewater treatment technique vary, not just in terms of initial capital and operational running costs, but also in terms of operatability, effectiveness, reliability, pre-treatment needs, environmental impact, and the generation of sludge and toxic by-product waste. Besides high and recalcitrant pollutant load and lack of standard processes, wastewater treatment facilities globally suffer from aging infrastructure, inadequate capacity, outdated technologies, limited funding, lack of skilled labor, and poor maintenance of treatment facilities. In addition to wastewater treatment, microbial metabolism also provides a chance to transform the organic nutrients in waste into high-valued commodity products like bioplastic and related precursors including PHA that not only lower the pollutant load but also provide an opportunity to generate revenue and employment that ultimately contribute to a circular economy.

Strategies for wastewater-to-PHA conversion

The selection of suitable microbial strains, their metabolic capabilities, and their adaptation to the wastewater environment contribute to PHA production variability. The design and configuration of the employed bioreactor system also impact overall efficiency in wastewater treatment [97, 98]. On-going research focuses on advancing bioreactor design, optimizing microbial consortia, and exploring novel microbial species to improve wastewater-to-PHA conversion efficiencies [61, 99, 100]. The integration of wastewater treatment with PHA production embodies a sustainable approach aligned with circular

economy principles, where waste is redefined as a valuable resource. Therefore, the optimization of the process and the adoption of various strategies become imperative. Some of these strategies include the Feast-Famine process, batch, and continuous-flow systems, the utilization of microbial consortia, co-feeding, and single and multistage or integrated processes, which are discussed below.

Feast-famine process

It represents a dynamic biological approach to wastewater treatment, embodying an innovative strategy that leverages the inherent resilience and adaptability of microbial communities to varying growth conditions, effectively eliminating pollutants and contaminants from wastewater sources. This approach has given rise to the evolution of a cyclic feast-famine regime, optimizing biological nutrient removal while reducing energy consumption and operational costs [101]. The Feast-Famine (F/F) process operates by alternating cycles of nutrient excess and limitation, primarily carbon and nitrogen or phosphorus, within wastewater treatment systems [102]. During feast periods, characterized by an abundance of nutrients, microbial growth, and substrate uptake are rapid, with microorganisms adapting to store excess nutrients. In contrast, famine periods induce the utilization of stored compounds and reserves for growth [103]. This cyclic F/F approach enhances biological nutrient removal efficiency, particularly for nitrogen and phosphorus, through mechanisms like denitrification and enhanced biological phosphorus removal.

The comparative assessment of F/F (0.2 and 0.6) revealed that biomass was able to accumulate higher PHB (approximately 500 mg.L^{-1}) at F/F 0.6 from acetate as feed while [103] for PHA production from VFAs, optimum F/F ratio was close to 0.2 d$_{feast}$/d$_{famine}$ [104, 105]. Similar observations were represented as maximum PHA accumulation (around 80%) was reported at F/F 0.2 [106]. It is noteworthy that the effect of F/F ratio on PHA accumulation may also be influenced by low dissolved oxygen (DO) concentrations. It was suggested that high DO becomes critical for biomass production while low DO supports PHA accumulation when butyrate and valerate are used as feed [107]. It is a general observation that DO becomes crucial for feed uptake by cells and hence there is a sudden spike in DO at the end of feast period. As per the reports, F/F affects PHA accumulation when DO is below 2 mg.L^{-1} [108, 109] while there is no effect of F/F on PHA production if DO is higher than 3 2 mg.L^{-1} [110, 111]. The adaptability of the F/F process makes it suitable for various wastewater types, including municipal wastewater, industrial effluents, and decentralized treatment systems. Maintaining stable microbial communities and preventing process upsets, such as bulking or foaming,

remains a challenge that requires robust control strategies [112]. The F/F process typically requires less energy for aeration and promotes efficient nutrient removal, reducing operational costs in wastewater treatment plants [103]. Therefore, combining the F/F process with emerging technologies, such as bioinformatics, sensor advancements, and process automation, holds promise for enhanced efficiency and control [102, 103].

The utilization of the F/F strategy in PHA production may encounter challenges due to organic load variability [103]. Normally, the implementation of this culture strategy occurs in a Sequential Batch Reactor (SBR), where external substrates are alternated between excess and deficiency [113]. Typically, the operation cycles of SBRs follow fixed time intervals [106, 114]. Consequently, variations in operational conditions can lead to adjustments in the relationship between the Feast and Famine periods. For instance, an increase in organic load can extend the feast period, resulting in a shorter famine period if the total cycle time remains constant. Previous studies have demonstrated that changes in operational conditions, such as organic load rate and cycle time, significantly impact the performance of SBRs and PHA production [112, 115]. Therefore, it is evident that applying different organic loads would necessitate adjustments to feast and famine times. Based on the operation, the process can be classified into batch, fed-batch, and continuous operation. The comparative assessment of batch and fed-batch process with VFAs as a feed from synthetic and wastewater suggested that under the same operating conditions higher PHA content accumulation (%) was reported from fed-batch mode. The PHA yield was increased from 0.48±0.006% (batch) to 0.52±0.03% (fed-batch) in a much lower time [54].

Continuous flow system

Continuous-flow system is another approach to achieve high throughput bioproduction as well as waste treatment. It represents a progressive approach that streamlines and optimizes the processing of effluents with consistent and uninterrupted flow. In contrast to batch processes and fed-batch processes, it offers advantages in efficiency, stability, and adaptability, making them pivotal in addressing the ever-growing challenges of managing and treating wastewater [116]. Continuous-flow-activated sludge processes utilize microbial activity in aeration tanks to biologically treat wastewater, achieving efficient organic matter and nutrient removal [117]. Semi-continuous mode for PHA production from different types of sludge recovered from wastewater treatment plant yielded 28.4% in 24 h cycle at 20 °C, neutral pH, and lower substrate concentration. Theoretical assessment of the process suggested that German wastewater treatment plants alone can compensate for 19% of global

biopolymers production [118]. A substantial portion of the costs associated with PHA production involves creating sterile conditions, refining substrate carbon sources, using and disposing of solvents for polymer extraction, and dealing with low productivity, yields, and limitations such as short production campaigns and downtime during batch changeovers in batch/fed-batch operation modes [37, 119, 120]. To address these challenges, halophilic microorganisms have been proposed as cost-effective PHA producers. The high salinity required for halophiles to thrive in the production medium (150–200 $g.L^{-1}$ of salts) minimizes contamination risks, eliminating the need for sterile conditions [121]. This allows for open vessels and a low contamination risk continuous process [119, 122, 123]. Parroquin-Gonzalez and Winterburn [117] used a halophile *Haloferax mediterranei* for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production from VFAs in continuous mode and reported an increase in cell density from 0.29 - 0.38 $mg.L^{-1}.h^{-1}$ (fed-batch fermentations) to 0.87–1.43 $mg.L^{-1}.h^{-1}$ (continuous fermentation). Additionally, the downstream process can be significantly simplified by extracting the polymer through a straightforward osmotic shock when transferring cells into an isotonic media, thus eliminating the necessity for toxic and expensive solvents [124, 125].

Mixed microbial culture

Microbial consortia or mixed microbial culture (MMC) offers an advantage over monoculture as it allows the exploitation of synergistic dynamics, diverse metabolic capabilities, and pathways to deal with high organic load or complex pollutants present in wastewater [126]. The interactions among microorganisms, including mutualism, competition, predation, and syntrophy, drive the functionality and resilience of mixed cultures in various environments [127]. MMCs also become useful for PHA production from waste residues along with achieving maximum productivity, aiming for high mass polymer production per unit volume with high efficiency and lowered costs [128, 129]. Various studies have reported the production of PHA through MMCs involving *Proteobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Candidatus, Saccharibacteria* from activated sludge substrates [130], *Plasticicumulans acidivorans*, and *Methylobacillus flagellates* from aerobic activated sludge and synthetic carbon source [131], *Alphaproteobacteria* and *Betaproteobacteria* from activated sludge and crude glycerol [109], and *Selenomonadales, Anaerobaculum*, and *Coprothermobacter* from raw sludge and thermally hydrolyzed sludge [36].

MMC also offers higher stability and functionality that can withstand environmental fluctuations and changing conditions [126]. Advancements in omics technologies and computational modeling offer insights into the intricate networks and metabolic interactions within mixed microbial communities [132]. Integrating mixed culture-based approaches with emerging technologies, such as artificial intelligence and high-throughput screening, holds promise for optimizing and scaling up biotechnological processes [133]. The limited competitiveness of PHA output compared to pure culture fermentation poses a significant challenge to the industrial scaling-up of the MMCs process [134, 135]. Nonetheless, an industrial-scale viable alternative lies in the PHA production process based on extended cultivation. For instance, Huang et al. [136] introduced an extended cultivation strategy for PHA-accumulation using MMCs. In their study, batch assays demonstrated high PHA content in cultivated MMCs, reaching 71.4% and 66.7% (higher than the 62.1% in the seed biomass) after 10 days of extended cultivation with and without sludge discharge, respectively. Incorporating this extended cultivation process achieved an overall PHA storage yield of 0.49 g COD PHA/g COD VFA and a volumetric productivity of 1.21 g PHA $L^{-1}d^{-1}$ with a final cell density of 17.22 gL^{-1}. Furthermore, the PHA accumulation ability was significantly enhanced by enrichment, irrespective of temperature and pH. Enrichment at 20–28 °C without pH control appeared most suitable for robust PHA accumulation [137]. Analysis of PHA accumulating microorganisms composition using the clone library method targeting *phaC* genes revealed that Burkholderiales dominated the seed sludge. However, after enrichment without pH control, Rhodocyclales, specifically *Azoarcus* spp. and *Thauera* spp., emerged as dominant, showcasing a robust ability to accumulate PHA.

Single and two-stage fermentation

The major application of the multistage process is the utilization of complex nature pollutants that obstruct their degradation [138]. In a single-stage process, PHA is generated directly from wastewater, whereas in a two-stage process, waste resources undergo initial fermentation into volatile fatty acids (VFAs) before being employed for PHA production. Municipal wastewater treatment plants (WWTPs) generate sludge as a by-product, and its effective management is crucial for controlling operating costs. Large WWTPs commonly employ anaerobic digestion (AD) to convert sludge into methane, reducing its mass. However, the current low market price of methane suggests an opportunity to explore alternative high-value products from sludge organic matter, such as PHA directly or via VFAs [139, 140]. Among the available wastewater, acidogenic effluents are a feasible option, as these can be derived from readily available regional sources of municipal and industrial organic wastewater and sludge. However, some waste has low concentrations of total VFAs i.e. 0.5 to 10 gL^{-1} [126] while higher VFAs

Ahuja *et al. Microbial Cell Factories*   (2024) 23:187

Page 9 of 22

concentrations can be found in specific cases such as dairy [141] and fishing [142] industry residues, these may not provide a sufficiently large and widespread source for industrial-scale MMC PHA production. Colombo et al. [143]., valorized organic acids from municipal waste and yielded PHA production of 223 ± 28 g.kg^{-1} total OA fed. PHA, produced from organic fraction has a molecular weight of $8\cdot105$ kDa and is comprised of hydroxybutyrate/hydroxyvalerate 53/47 (%). In another work, Ospina-Betancourth et al., [144] used yeast production industry wastewater (WWY) for the production of polyhydroxy-alkanoates in sequential anaerobic reactors (reactor A) followed by two aerobic reactors (reactor B and C). VFAs produced in an anaerobic batch reactor (for 78 days), raw as well as distilled effluent from reactor A were used as feed PHA-production in reactors B and C (aerobic). The sequential process with mixed culture has yielded a maximum PHB accumulation of 17% of cell dry weight (1.2 g$_{PHB}$.L^{-1}) from distilled effluent. Roche 454 16 S rRNA gene amplicon pyrosequencing identified *Paracoccus alcalophilus* (32%) and *Azoarcus* sp. (44%) as a dominant microbial population for PHB production. However, with widespread implementation and operation of the process, the availability of VFAs becomes crucial and hence waste collection, VFAs extraction, and processing are necessary parts of the process. In contrast to single stage process, two-stage processes compartmentalize operations into distinct stages or reactors, enabling a sequential or parallel execution of specific reactions or treatments and offering enhanced control over individual reaction kinetics or treatment conditions, enabling optimization of specific steps or facilitating intricate reactions [145].

Co-substrate feeding

The wastewater from different origins has a diverse composition which may become insufficient to maintain the stability of bioprocess. Hence use of multiple types of wastewater together might offer an advantage over monosubstrate systems [146]. The multisubstrate system would have higher productivity and stability due to nutrient balance, enhanced microbial metabolic diversity, and promotion of more comprehensive waste breakdown [147]. Usually waste from different industries has to be treated with chemicals or thermochemical treatments which encounters numerous technical and economic challenges related to product selectivity, conversion kinetics, yields, and potential applications. In comparison to chemical or thermochemical treatment, co-feeding, involving the simultaneous use of various feedstocks, offers synergistic benefits to enhance product yield and quality during the conversion process [148]. Valentino et al. [149]., combined municipal solid sludge and an organic fraction of municipal solid waste from the same urban area and used it for PHA production at a pilot

scale using a three-step mixed microbial culture (MMC) process. Under optimum conditions, PHA specific storage capacity was 258 mg COD$_{PHA}$.gCOD$_{Xa}$.h^{-1}. PHA accumulation capacity of mixed culture via fed-batch process was 46 wt % PHA (dry cell weight) and offered an overall yield of 65 g$_{PHA}$.Kg^{-1} $_{TVS}$. Caproic acid is one of the intermediates for PHA production. Iglesias-Iglesias et al. [150]., have reported caproic acid production by co-digestion of cheese whey and sewage sludge. In a continuous mode of operation, maximum acidification of 44% was achieved at hydraulic retention times (HRT) of 10 days and 2 feeding cycles per day. Under optimum conditions, caproic acid rich stream resulted in PHA, copolymer of HB-*co*-HV-*co*-HHx. Owusu-Agyeman et al., [151] also reported the volatile fatty acid production from codigestion of sewage sludge and organic waste. An increase in organic load shift the VFAs composition towards caproic acid as a dominant proportion (>55%). The major advantage of codigestion is the availability of nutrients by using a diverse range of waste as feed that possibly eliminates the shortcomings of individual wastewater however optimization of parameters is necessary to prevent the toxic or inhibitory effect of pollutants.

Bioprocess parameter affect and scaleup studies
Process parameter effect

The production of PHA from wastewater is a complex process influenced by various factors. One crucial factor is the composition of wastewater, with different organic substrates serving as feedstocks for PHA-producing microorganisms [45]. Calero et al., [152] compared organic loading rate (OLR) and VFAs production from cheese whey (estimated via the degree of acidification (DA)) via up-flow anaerobic sludge blanket reactor (UASB; continuous process) and sequencing batch reactor (SBR; discontinuous process). Both reactors have a maximum DA of 98% with an OLR of 2.7 g$_{COD}$.L^{-1}.d^{-1} in SBR and 97% with an OLR of 15.1 g$_{COD}$.L^{-1}.d^{-1} in UASB. It is distinct that continuous process would be able to handle higher OLR while in both cases product was mainly dominated by acetate, butyrate, propionate, and valerate. The availability and concentration of these substrates, such as VFAs and sugars, play a pivotal role in determining microbial growth and subsequent PHA accumulation. As per the literature, VFAs are the favorable substrate in comparison to sugar or other organic molecules for PHA production. Another observation suggested that not only VFAs amount but also composition has a direct effect on the productivity as well as composition of PHA [153]. A study revealed that mixed VFAs have higher productivity in comparison to monosubstrate systems. In context to composition, butyrate-rich VFAs feed has a maximum PHA accumulation of 72.08% of VSS followed by valerate-rich feed (61.57%).

Ahuja *et al. Microbial Cell Factories*   (2024) 23:187

Page 10 of 22

However, the bioplastic produced was more robust in the case of valerate-rich feed and was mainly comprised of 3-hydroxyvalerate (HV) (more than 20%) [154]. Further, the presence of HV in PHA increased in average molecular weight and crystallinity [155]. The concentration of HV in PHA might be responsible for the amorphous nature of bioplastic as its increasing concentration lowers the crystallinity and melting temperature and makes it sensitive to thermal degradation. VFAs stream produced from chicken manure (VFA_{CM}) and potato peels (VFA_{PP}) have been compared for PHA production and composition based on feed composition. VFA_{PP} is rich in acetic acid, and ammonium nitrogen while VFA_{CM} was rich in butyric acid, and valeric acid. The maximum bioplastic production was reported from potato peels but comparative analysis revealed that PHA from chicken manure has lower volatile mass and higher dehydration temperatures while PHA from VFA_{PP} has higher thermal degradation temperature [156].

Nitrogen and phosphorus concentration also play an important role in PHA accumulation. To study the C/N ratio's effect on PHA production, Valencia et al. supplemented activated sludge with acetate and ammonia in a sequencing batch reactor (SBR) to maintain C/N (13.3–42.1) and found C/N of 23.3 results in higher PHA accumulation, and beyond this, there is negative effect [157]. Zhang et al., studied PHA accumulation from activated sludge at pH (7.5 ~ 8.5) with various C/N and C/P ratios and observed 150 as the optimum ratio for maximum PHA accumulation i.e. 50.39% and 36.07% respectively [158]. Tu et al., [159] studied the phosphorus limitation effect on PHA accumulation from thermal hydrolyzed sludge and reported an increase in PHA content from 23 to 51% when phosphorus concentration decreased from 127.5 to 1.35 mgL^{-1}.

Furthermore, the operational conditions of the wastewater treatment process, including temperature, pH, and dissolved oxygen levels, influence microbial activity and metabolic pathways leading to PHA synthesis. Temperature has a direct influence on biomass hydrolysis and results in increased microbial biomass production which ultimately leads to increased PHA accumulation. The availability of acetate and temperature also affected the microbial diversity as in the beginning sludge was denoted with 29 species which was reduced to 16 after community selection. Mostly non-defined genera were eliminated and post-selection, the community composition was represented by Mucilaginibacter. The reason for elimination was also the unavailability of nutrients to non-PHA accumulating microorganisms. In contrast, De Grazia et al., [160] used mixed microbial culture that was much more stable and tolerant and hence was able to accumulate around 60–65% $g_{PHA}.g_{VSS}^{-1}$ from acetic acid

at 15–25 °C which supported the fact that mixed microbial culture was stable in seasonal variation.

Availability of oxygen becomes critical for microbial cell growth as well as PHA production and characteristics. Available oxygen also affects the pollutant's removal from wastewater especially in the context of nitrification. At higher oxygen availability, both PHA production and nitrification occur in the reactor while in reduced dissolved oxygen (DO) nitrification process is halted and only PHA production occurs. However, high DO levels support PHA accumulation and acetate, butyrate, propionate, and valerate as dominant VFAs while at lower DO acetate and propionate dominate [107, 161]. At lower DO and bioreactor oxygen transfer rate production of short-chain length PHA induced [162].

Scale-up studies

Maximum bioplastic and intermediates like VFAs production from wastewater can be achieved under optimum growth conditions but commercial application of bioplastic needs to scale up with maximum resource utilization along with maintenance of bioplastic yield. However, some of the researchers have shown the sincerest effort to scale up the PHA production to pilot or industrial scale from industrial wastewater at high organic load (mostly fat and lipids). At the laboratory scale, *Ralstonia eutropha* proved to be an efficient PHA producer from glucose and fructose while *Bacillus megaterium* was better in the case of whey as feed [163]. The overall assessment led to the selection of *R. eutropha* for upscaling the PHA production at 3 L working volume. At 2 L production scale, *R. eutropha* attained a maximum PHA content of 4.19 gL^{-1} (74.2%) with substrate consumption of 79.0%. The overall process has a PHA yield of 0.72 (YP/x) and productivity of 0.19 $g.L^{-1}.h^{-1}$ [164]. PHA production from sewage sludge at a continuous stirred tank pilot scale bioreactor (225 L) showed that domestic sewage sludge can be a good feed as a PHA yield of 0.37 $g_{PHA}.g_{VFAs}$ was obtained even with low organic loading of 0.06 $Kg_{BOD}.Kg_{SS}^{-1}.day^{-1}$ [86].

The major issues with wastewater-based PHA production at pilot and industrial scales were operating conditions, the composition of wastewater, salt concentration, and pH of the medium. The seasonal variation in wastewater has a major influence on PHA production as VFAs are the prime source for PHA production. In the case of lower concentration of VFAs in wastewater, non-PHA accumulating microorganisms biomass increased while PHA accumulating and VFAs consuming microbial diversity reduced. Hence lower VFAs (0.35–1.00 $g_{VFA-COD}/gs_{COD}$) was not preferred [165]. The influence of temperature on the enrichment of biomass and PHA production by activated sludge was evaluated within a practical case study. Two laboratory-scale sequencing batch reactors

Ahuja *et al. Microbial Cell Factories*   (2024) 23:187

Page 11 of 22

(SBRs) were operated at different temperatures (15 and 25 °C) in parallel over 131 days to treat wastewater from a potato-starch modification facility and produce surplus activated sludge biomass with PHA accumulation potential. Temperature did not influence wastewater treatment performance (average 97% COD removal). Several other researchers have summarised the efforts for PHA production from wastewater from different sources at various operational scales are summarised in Table 2.

Advances in PHA recovery and purification

PHA recovery and purification are also one of the challenges as it alone accounts for 30–50% of the total costs [179] and determines the process feasibility for industrial applicability. The product recovery underwent five crucial stages including biomass recovery/harvesting, pretreatment of biomass, PHA recovery, and formulation [37]. Several strategies including solvent extraction, and microbial cell disruption have been suggested (Fig. 3).

The PHA recovery is a multi-step process that begins with cell mass separation followed by extraction or cell lysis. For PHA recovery, cell lysis can be achieved by physical, chemical, or enzymatic methods. Physical methods employ mechanical and shear forces like crushing and bead mills-based treatment for cell disruption [180]. In comparison, chemical methods use different chemical agents to degrade or depolymerize the cell coverings and expose the inner chamber. The methods have respective pros and cons like physical methods need high energy input and have questionable efficiency on a commercial scale. While chemical methods involve the use of toxic chemicals and generate inhibitory by-products e.g. furans and phenolics. Hence selection of a treatment strategy becomes an important aspect of downstream processing.

It has also been observed that integrated and combined treatment of physical and chemical approaches improves the cell lysis and recovery rate. Pillai et al., [181] have combined microwave treatment with EDTA followed by CHCl$_3$ and NaOCl-assisted cell lysis for PHA recovery. The use of microwave-EDTA treatment has improved the molecular weight of the recovered polymer by 2.9 folds with 93.75% recovery and 97.21% purity. Another study also showed that microwave-based extraction has a higher rate of energy transfer in comparison to heat which improved the recovery rate in a lower time [182]. In comparison to physical cell lysis-based methods, solvent-based methods have higher applicability due to their ability to handle large volumes and can be used at an industrial scale and the possible recyclability of solvents that reduce the process cost. Solvent-based extraction of bioplastic and associated polymers includes treatment of microbial biomass with solvent or extraction agents followed by heating to the optimized temperature which

is commonly below 200 °C (usually below PHA decomposition) that solubilizes PHA in the organic phase and removes non-PHA mass in the aqueous phase. PHA from the solvent phase can be recovered by evaporation-facilitated distillation or filtration or using antisolvents to precipitate the PHA [38]. Solvents systems can be categorized based on the solvent nature (Table 3).

As suggested in the table, each type of solvent system has its respective advantages and disadvantages. Chloroform is the standard solvent used for PHA recovery which is under chlorinated solvent that may have a toxic impact on recovered product thus non-chlorinated and green solvents are preferred. Comparative evaluation of non-chlorinated solvents (cyclohexanone and γ-butyrolactone) showed higher extraction efficiency (>95%) with cyclohexanone in comparison to γ-butyrolactone [189]. Extraction conditions for solid loading, temperature, and appropriate solvent are crucial for product recovery. It has been observed that extraction at higher temperatures, has a higher recovery yield. Vermeed et al., [190] selected 6 solvents including 1-butanol, 2-butanol, 2-ethyl hexanol, dimethyl carbonate (DMC), methyl isobutyl ketone (MIBK), and acetone from 35 solvents based on toxicity to extract poly 3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV), produced from organic waste streams using mixed microbial communities at pilot scale. Among the selected 6 solvents, acetone and DMC have offered a maximum yield of 91–95% with 93–96% purity. The selection of solvents also becomes critical due to the differential solubility of PHA followed by the removal and recycling of solvents. Over the conventional solvent system, the use of solvent-antisolvents system has shown higher efficiency as well as PHA purity. Mongili et al., [191] compared two solvent/antisolvent systems comprised of DMC/ethanol and chloroform/hexane for the extraction of PHB from wet and dry *Escherichia coli* biomass. In comparison to dry biomass, PHB yield was stable with DMC as well as chloroform-based systems while crystallinity was higher with dry biomass (53%) even higher than chloroform-based extraction (41%). The system has shown commendable properties however, the complexity of product recovery increased due to the use of multiple solvents.

Advanced extraction systems and the use of non-ionic surfactants and biobased solvents have represented greener and more cost-effective alternatives to conventional solvents for PHA extraction. Comparative evaluation between non-ionic surfactants (Tween® 20, Brij® L4, and Triton™ X-114) with DMC and CHCl$_3$ have shown maximum yield with CHCl$_3$ (63%) followed by Tween® 20 (50%) while the purity of extracted polymer was higher than 90% [179]. In another work, Elhami et al., [39] recovered around 62±3% 3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV) with >99% purity from mixed

Table 2 Polyhydroxyalkanoates production from different substrates

Organisms	Source	Scale	Process conditions	Major outcome	PHA yield % w/w	Productivity g.L^{-1}	References
Synechocystis MT_a24	Urban wastewater	100 L	Thin-layer raceway pond; light intensity 100–800 μmol photons m^{-2}.s^{-1}; pH 7.5-8.0; night temp 18–22 °C and morning 27–39 °C.	Maximum PHB was at a late stationary phase (under nutrient-limited conditions) *Poterioochromonas malhamensis* contamination was eliminated by *Synechocystis* cultivation in an alkaline environment.	23.7 ± 2.2	0.62	[166]
Aerobic granular sludge	Epichlorohydrin wastewater	1 L	Batch process; working volume 1 L; temp 25 °C; aeration 1.5 L.min^{-1}.	COD removal efficiency 70%; sludge dominated by *Halomonas* sp. (86 ± 0.50%).	52.67	-	[167]
Nordic sp.	Wastewater	250 mL	Temp 30 °C; mixing rate 180 rpm; production 72 h; pH 7.0.	Wastewater pretreated by thermochemical treatment with 1% H$_2$SO$_4$ increases the solubility of total sugars.	27	3.83 ± 0.24	[168]
Synechocystis sp.	Secondary effluent	10 L	pH 7.99; conductivity 2.47 ± 0.08 mS.cm^{-1}; COD 96.65 ± 40.70 mgO$_2$.L^{-1}	Hydraulic retention time (HRT) 8 d; lipid accumulation 44.7% (30 d)	4.8	-	[169]
Bacillus sp. CYR1	Cheese whey + raw sewage (Rantoh wastewater treatment plant, Japan)	500 mL	4% *Bacillus* sp. CYR1 inoculum; 30 °C and 120 rpm for 96 h.	Cell mass of 692.4 ± 7.62 mg.L^{-1}, sewage has low organic content hence addition of high organic carbon is needed for PHA accumulation.	24.1 ± 0.70	0.147	[170]
Bacterial isolate	Cashew industry wastewater	250 mL	Dried microbial biomass suspended in 4% sodium hypochlorite solution and hydrolysis was conducted at 37 °C for 1 h. PHA was extracted from a cell pallet with chloroform	Maximum PHA accumulation was 34.04% with 20% wastewater. An increase in wastewater beyond this, reduced PHA accumulation. Phenol can also be used as a carbon source	34.04	0.32	[87]
P. aminovorans	Lactic acid fermentation broth, swine manure, and apple waste	10 L	Temp 35 ± 1 °C and time 10 days; mixing rate 100 rpm and aeration 1 mL.min^{-1}	PHA content increased with a reduction in lactic acid concentration in the medium. At higher lactic acid concentrations, cells have competitive interaction and available carbon source used for proliferation rather than PHA	55.4	1.04	[171]
Waste activated sludge	Bath wastewater treatment plant (Rilland-Bath, Netherlands)	167 L	Temp 25 °C; mixing rate 230 rpm and aeration rate 50 Lmin^{-1}.	No PHA accumulation was detected in fresh activated sludge. Nile Red has better PHA accumulation signal generation than Nile Blue A	48 ± 2	-	[172]
Bacillus pumilus NMG5 strain	Paper factories wastewater (Minh Hung paper factory)	30 L	Dissolved oxygen 4 mg.L^{-1}	COD removal was 95.93%. Phosphorus and nitrogen removal efficiencies were 83.55% and 79.36%	42.28	-	[173]
Haloferax mediterranei	Sesame seed wastewater	5 L	Mixing rate 230 rpm, at 37 °C for 4 d, pH 7.0, aeration 0.75 VVM	6 g.L^{-1} yeast extract and 100 g.L^{-1} NaCl was optimum for PHA accumulation.	75	0.53	[83]
Mixed inoculum	Wastewater	10 L	Volatile suspended solids 100 mg L^{-1}; Temp 24 ± 4 °C; dissolved oxygen < 0.2 mg L^{-1}; pH 7 and time 5 days	Inorganic acid inactivation of metabolism increased the yield from 20–33%	35–38	-	[174]
Pseudomonas mendocina CH50	Waste frying oil	14 L	Temp 30 °C, mixing rate 200 rpm, air flow 1 vvm, and time 48 h	Biomass accumulation 1.03 g.L^{-1} AuNPs/PHA composite reduced viral infectivity against SARS-CoV-2 pseudotype virus by 75%.	–	0.23	[175]
Microalgae	Aquaculture effluent	22 L	pH 6.8; period 14 d;	The high light intensity increased PHB accumulation while biomass yield lowered; no effect of light on lipid content; low-intensity red light was carbon neutral.	0.014	-	[176]

Table 2 (continued)

Organisms	Source	Scale	Process conditions	Major outcome	PHA yield % w/w	Productivity g.L^{-1}	References
Haloferax mediterranei	Ricotta Cheese exhausted whey	3 L	pH 7.2, temperature 37 °C for 24 h and mixing rate 150 rpm, inoculum 20% (v/v)	The melting temperature was 146.2 °C lower than commercial PHBV.	9.36	1.18±0.06	[177]
Cupriavidus necator DSM 13,513	Dairy wastewater	4 L	Fed-batch fermentation, temp 30 °C, for 48 h, mixing rate 200 rpm	Yield was optimum in 12 h with aeration both in batch and fed bath mode. Accumulation of acetyl CoA induces the PHB synthesis.	0.52	-	[54]
Mixed microbial culture	Rice winery wastewater	-	Batch experiment DO > 2 mg.L^{-1}, pH 7 temperature 25–30 °C	Maximum PHA yield at OLR of 2.4 g$_{COD}$.L^{-1}.D^{-1} *Zoogloea* was the most dominant PHA-accumulating organism.	23	135.6	[178]
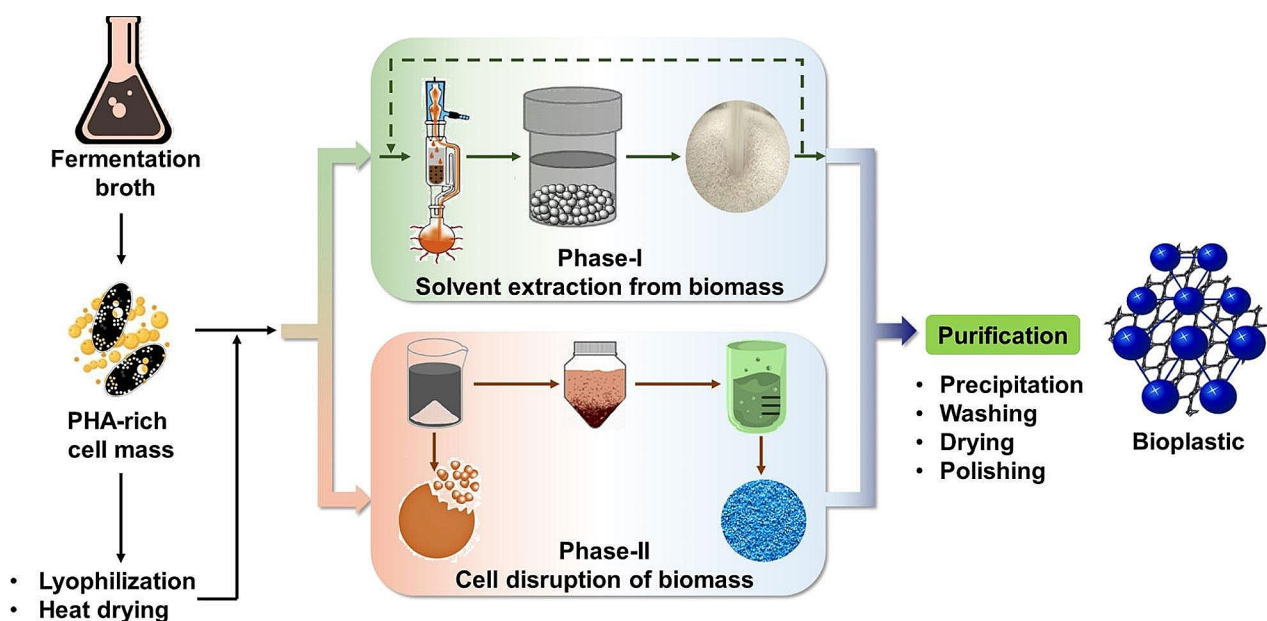
Fig. 3 Downstream processing and product recovery of polyhydroxyalkanoates

microbial culture (grown in wastewater) with methyl-tetrahydroxyfuran (2-MTHF) at 80 °C in 1 h. Table 4 summarises major outcomes for PHAs recovery using different techniques.

Process economics and energy investment also suggested solvent-based extraction as a low-energy operation and cost-effective approach. In terms of energy investment, the heat capacity of solvents/biomass is usually lying around 1–2 kJ.Kg^{-1} K^{-1} while the heat capacity of water is 4.2 kJ.Kg^{-1} K^{-1} (microbial slurry with 10–20 wt %). As the required energy for extraction operation is normally around 0.5–2 MJ.Kg^{-1} PHA which showed the process is low energy intensive [38]. In comparison to conventional solvents, biobased solvents have superiority due to cost-effectiveness and low carbon footprint [179]. Besides, operational feasibility, solvent recyclability can further improve the economics and process life

cycle as the same solvent can be used multiple times and reduces the residues and by-products generation from the process. Moreover, standardization of the extraction process becomes easier and makes it possible to adopt at an industrial scale as well.

Sustainability considerations and techno-economic analysis

Plastic pollution has become a serious concern on global platforms and raised the alarm for future sustainability. The United Nations has put the concern related to plastic pollution in front of 187 UN members during the 73rd session (2018–2019) and suggested the requirement for a transparent and regulatory framework besides including plastic waste in global hazardous materials with the amendment of the directives of the 1989 Basel Convention [192]. Along with this the UN Industrial

Table 3 Different solvent systems used for solvent extraction

Categories	Example	Advantage	Disadvantage
Halogenated solvents	Chloroform, di-chloromethane, 1,2-dichloroethane, 1,1,2-trichloroethane, and 1,1,2,2-tetrachloro-ethane.	High molecular weight PHA (1.2 MDa) can be extracted. Extracted PHA will be of high purity.	High risk of environmental pollution.
Alkanes	Heptane, octane, and hexadecane.	Recovered PHA has a high purity.	Low yield of PHA. Polymer deg-radation dur-ing recovery.
Alcohols	Methanol, ethanol, n-propanol, iso-propanol	Short-me-dium chain polymer can be recovered.	Too polar for high-length polymers hence recov-ery will be low.
Esters	Ethylic esters	Purity will be high	Operated at high tempera-tures (90–100 °C)
Carbonates	Diethyl carbonate, propylene carbonate, and ethyl acetate	Eco-friend-ly alterna-tives have minimal toxicity, and biodegrad-able	Low stability to temperature.
Ketones	Methyl ethyl ketone	Suitable for all tempera-tures and pressure.	Problematic and may be toxic.
Biobased solvents	2-methyltetrahydroxyfu-ran and dihydrolevoglu-cosenone (cyrene)	Non-toxic, greener, and sustainable.	Long extrac-tion times and low extraction selectivity and yield.

Development Organization and G20 nations imposed a ban on plastic materials and ensured widespread participation in waste management by providing financial incentives [193]. Biopolymers have shown possible routes to reduce or eliminate petroleum-based plastic. Furthermore, the use of microbial fermentation using waste resources as feedstocks lowers the production cost of biopolymers. National and international government bodies are also emphasizing the shift to circular economy principles and utilization of waste resources to maintain sustainability.

The World Economic Forum, McKinsey & Company, and Ellen MacArthur Foundation proposed some initiatives like EPR (Extended producer responsibility)

schemes to reduce the ocean leakage rates by 80% by 2040. The strategies include the prevention of waste exports into countries having high leakage rates, and improving the waste recycling capacity from 21 to 54% apart from eliminating the major microplastic sources. The European Union (EU) has also suggested policies framework to amend the European Green Deal and Circular Economy Action Plan. These suggestions include recycling around 50% of packaging plastic by 2030. As a result, the use of single-use plastic items like polysty-rene-based beverage containers, cutlery, food and straws, cotton bud sticks, and all oxo-degradable plastics have been prohibited in the EU since January 2021 along with restricted export of low-grade plastic outside EU borders (as mentioned in Basel agreements). As a financial restriction, a high amount of tax (around €800 per tonne) has also been imposed on non-recycled plastic to suppress the use of non-recyclable plastic and to promote industries manufacturing eco-friendly, recyclable, biodegradable, and reusable plastic alternatives [194].

As per the report published, China is the largest producer of single-use plastics across the globe. With international commitments, China also announced the ban on the production of non-recyclables plastic by 2025 and shifted to degradable bioplastic [195]. To achieve the planned target, Chinese manufacturers have boosted PLA production to 700,000 tons per year and target the total outcome of polybutylene adipate-*co*-terephthalate (PBAT) and polybutylene succinate (PBS) to 1.24 million tonnes per year by 2023. On a similar track, other countries including Japan, Malaysia, Singapore, and South Korea have also proposed financial subsidies for bioplastics production [3, 194]. The main obstacle to the implementation of bioplastic as the main alternative to plastic at the global level is the associated cost. The assessments have been conducted to evaluate the production of different types of bioplastics under different environments and scales. Waste materials and resources are the prime feedstock for the cost-effective production of various products. Rajendran and Han [196] used food waste as low-cost raw material for poly (butylene succinate) (PBS) production and economic feasibility was assessed. The process suggested the minimum selling price (MSP) of PBS, produced from food waste was 3.5 $ Kg^{-1} (determined by the Monte Carlo simulation). The process offered the plant's return on investment (ROI) of 15.79%, with a payback period, and internal rate of return (IRR) of 6.33 years and 16.48% respectively. The process has a net present value of 58,879,000 USD. The analysis also revealed GHG emission of 5.19 Kg CO_2eq Kg^{-1} which was much lower than the conventional production process for PBS. PHA production from molasses by mixed microbial culture was evaluated which showed the PHA manufacturing process cost $994,143 with an annual

Table 4 Downstream processing and recovery of PHA from microbial biomass at different scales

Source	Scale	Extraction conditions	PHA recovery %	PHA purity%	Molecular weight	Major outcome	References
Activate sludge	3 L	Supercritical CO_2, pressure 200 bar, time 15 min at 40 °C.	80%	80%	0.27×10^6	Biomass density of 57 $g.L^{-1}$. The presence of methanol as a modifier increased the molecular weight of the polymer.	[183]
Organic waste (Treviso plant)	380 L	Wet biomass acidified with H_2SO_4 centrifuged and undergoes aqueous phase extraction with inorganic reagents	-	98.9±0.7%	424.8±20.6 KDa	PHA contains 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) (92.6–79.8 and 7.4–20.2 w/w)	[184]
Lisbon plants	100 L		-	98.9±0.7% w/w	224.9±21.9 KDa	PHA contains 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) (92.6–79.8 and 7.4–20.2 w/w relative percentage)	
Carbonera plants	2800 L	Dried biomass undergoes aqueous phase extraction with inorganic reagents.	-	85.5±3.6% w/w	424.8±20.6 KDa	PHA contains 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) (44–13 and 56–87 w/w relative percentage).	
Fermented sewage sludge	100 L	Temp 115 °C and pressure around 300 kPa	70%	92%	571 KDa	$NaOH + H_2O_2$-based extraction has the highest recovery. It facilitates hydrolysis and oxidation.	[185]
Wastewater	10 L	Inactivation with 0.2% formaldehyde then cell mass recovery and lyophilization and extraction with chloroform.	20%	-	-	Comparatively economic and can be used at a higher scale as well accumulation was 33% dry cell weight.	[174]
PHARIO project activated sludge	-	Extraction with 10 mL dimethyl carbonate at 125 °C for 60 min.	100%	95±2	-	Thermal treatment of dry PHA-rich biomass lowers PHA molecular weight predictably.	[186]
Microbial sludge (food pulp waste feed)	60–100 L	Biomass dried and treated with 0.3 M NaOH at 30 °C for 300 min	100	99	-	Standard protocol with $CHCl_3$ has recovery of 81–83% recovery The purity of the final product increased with intracellular concentration and irrespective of extraction agent.	[187]
	60–100 L	Biomass dried and treated with 9.0% NaClO at 30 °C for 3.4 h	90	100	-		
Secondary sedimentation tank (STP-300 KLD) Jawaharlal Nehru University, India	500 mL	2 g sludge biomass 200 mL $CHCl_3$ at 70 °C, 180 rpm for 72 h with continuous agitation.	8.3	96		90% 3-hydroxy-butyrate and 10% 3-hydroxy-valerate.	[188]

process operation cost of $159,711 and a payback period of 6.79 years. The process has an internal return rate of 16%. It was also suggested that the benefit from the process could be increased by 25% if the product costs were reduced by 20% [197]. The process seems greener in terms of GHG emission while process parameters optimization and byproduct re-valorization might reduce the process cost. Besides food waste, other waste including agricultural residues process byproducts, and wastewater from residential as well as industrial areas can also be used as feed.

A simulation study for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production from cheese whey by a halophile '*Haloferax mediterranei*' was conducted by SuperPro Designer to assess the material flow and process economics. A local cheese plant was selected for the study that has around 168.7 metric tons of lactose.day^{-1} ($MT.d^{-1}$) and conversion process was divided into three scenarios i.e. (a) without recycling/reuse of salt and enzyme, (b) enzyme reuse + non-recycling of salts, and (c) recycling and reuse of salts and enzyme. The recovered whey stream was reused for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production by halophiles. The simulation showed the production of 9700 MT_{PHBV} $year^{-1}$ (0.2 $g_{PHBV} \cdot g_{lactose}^{-1}$) with a conversion efficiency of 87%. The breakeven price for the product was sensitive to enzyme and lactose prices as it offered the lowest price of 4 $.Kg^{-1}_{PHA}$ [198]. Another analysis with sewage sludge from municipal wastewater treatment plants for bioplastic production at two different scales and processes i.e. (a) sludge is dewatered only and small operation scale (small wastewater treatment plant) and (b) anaerobic digestion of sewage sludge and large operation scale (large wastewater treatment plant). PHA production was conducted in a two-stage process which offered a minimum PHA cost of 1.26 US.Kg^{-1}_{crudePHA}$ (large

wastewater treatment plant) and 2.26 US$.Kg$^{-1}_{crudePHA}$ (small wastewater treatment plant). In another scenario, a single-stage process was also studied in which secondary sludge was used for PHA accumulation. In this case, PHA production costs were further reduced by 19.0% and 15.9% for large and small wastewater treatment plants respectively which was mainly due to lowered capital investment [100]. Instead of conventional heterotrophic systems with bacteria, fungi, algae, and cyanobacterial systems have higher efficiency due to lower landscape and resource requirements. It allows the use of inorganic carbon from air (CO and CO_2) as well as organic carbon from wastewater. Process economics for PHBs production from wastewater revealed that the minimum selling price of PHB was around 135 € Kg_{PHB}^{-1} when PHB productivity was 12.5 g_{PHB} m^{-3} d^{-1} which makes around 50% of dry cell weight (dcw). However, productivity must be much higher (810 mg L^{-1} d^{-1}) to compete with the market cost (i.e. 4 € Kg_{PHB}^{-1}) [199] which emphasizes a more in-depth analysis of the process and investment of resources. The analysis not only identified wastewater as potential feed but also reported chemical oxygen demand and operation size as critical factors. In addition, supportive governmental policies and technical upgradation seem mandatory to reduce the process cost and wide acceptability of biopolymers as an alternative to petroleum-based plastic.

Future perspectives and challenges

In the past, polymers produced from petroleum have been extensively utilized in several applications, including textile, medical, transportation, chemical manufacturing, optical, and electrical devices. Nevertheless, the increasing demand for biopolymers can be attributed to various factors, including the volatility of oil prices, the environmental concerns associated with petroleum-derived biopolymers, advancements in biopolymer production technology, and the rapid development of biopolymer-based products. However, at present, bioplastics account for merely 1% of the total yearly plastic production [200]. The handfuls of start-ups producing bioplastic on a commercial scale are PHAXTEC, Inc. (Wake Forest, NC, USA), VEnvirotech Biotechnology SL (Barcelona, Spain), Verde Bioresins, Inc™ (Fullerton, CA, USA), Bhagirath Industries Private Ltd. (Gujarat, India) and Tianjin Green Bioscience Co., Ltd. (Tianjin, China).

The primary factor contributing to the low efficacy of bioplastic is its significantly higher production costs (2.2 to 5.0 €/kg), which are about three times greater than those of traditional synthetic plastics (less than 1.0 €/kg) such as polyethylene (PE) and polypropylene (PP) [99]. To compete with a petroleum-based plastic, it is necessary to address certain challenges, including the sustainable production of biopolymers. This involves overcoming issues such as the cost of production, scaling of the production process, and downstream of the products. The production cost can be reduced by up to 50% by considering locally available waste or residues as feedstocks for bioplastic production [201]. However, the variability of wastewater composition is contingent upon the source of feedstocks. Eventually, the variability in the efficiency of PHA production is observed. This issue can be solved to some extent by classifying and separating different wastes used as feedstocks for PHA production processes. Furthermore, wastewater is a complex substrate for microbial growth because it contains many different constituents, including nutrients and micropollutants. Therefore, strategies like acclimatization for extreme conditions, consortium, and multistage integrated processes have proved advantageous over pure culture for the efficient consumption of waste streams. In addition to that, naturally developed mixed culture reduces the cost of the process associated with sterilization [202]. In addition to high production cost, GHG emissions during production, use of complete degradation (complex polymers and composites), detailed analysis of negative ecological impacts (if traces remained in the system), unawareness of society, and insignificant resistance to water and hydrophilic environment are the other issues that affect bioplastic performance [203, 204].

Alternatively, genetic modification of PHA-producing microbes using CRISPR and Cas9 technologies could be promising for enhanced bioplastic production [204, 205]. For marketing, effective strategies need to be planned and implicated to enhance the adaptation of bioplastic by lower economy zones along with higher economy and middle economy zones [206]. Some of the recent works have shown the possible use of natural biopolymers from plants as plasticizers and reinforcement materials. It has been reported that the blending of starch lowered the use of non-renewable energy and GHG emissions by 60% and 80% respectively along with a 40% reduction in eutrophication potential 60% reduction in land use [203]. The research and marketing area have lots of opportunities as well as challenges that need continuous efforts for quick and efficient addressal. Moreover, alone replacement of petroleum-based plastic materials with bioplastic is not sufficient, and need to find some strategies to reuse plastic-based waste, accumulated in the environment.

Conclusion

Polyhydroxyalkanoates (PHA), a biodegradable polymer, presents a promising alternative to traditional plastics, offering potential solutions for reducing packaging waste. The wastewater generated by industrial, agri-horticultural, and municipal activities is rich in organic and inorganic compounds, including carbon, nitrogen, phosphorus, and minerals, serving as a natural resource

for microorganisms to produce PHA. The selection of microbes through methods like the feast and famine approach, along with the utilization of microbial consortia, enhances the efficiency of PHA production processes. However, challenges persist in the downstream processing of PHA, as existing extraction methods often yield lower quantities and purity, affecting the material's natural properties. Utilization of wastewater as feedstock is advantageous as it doesn't require any pretreatment like lignocellulosic biomass and the process can be more economical. However, long-term sustainability and feasibility must be scrutinized to prevent the further evolution of new pollutants. Further, wastewater characterization and selection of efficient PHA producers with easy and eco-friendly PHA recovery methods are areas that need attention to make the process feasible at a large scale.

Author contributions
V.A.: Conceptualization; Data curation; Formal analysis; Methodology; Software; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing, P.K.S: Roles/Writing - original draft; and Writing - review & editing, C.M: Roles/Writing - original draft; and Writing - review & editing, J.M.J: Roles/Writing - original draft; and Writing - review & editing, G.K: Roles/Writing - original draft; and Writing - review & editing, Yung-Hun Yang: Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision, S.K.B.: Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biotechnology, University Centre for Research & Development, Chandigarh University, Mohali, Punjab 140413, India
[2]Department of Agricultural and Biological Engineering, University of Illinois at Urbana- Champaign, 1304 W. Pennsylvania Avenue, Urbana 61801, USA
[3]Green & Sustainable Materials R&D Department, Research Institute of Clean Manufacturing System, Korea Institute of Industrial Technology (KITECH), Chungnam 331-825, Republic of Korea
[4]School of Civil and Environmental Engineering, Yonsei University, Seoul 03722, Republic of Korea
[5]Institute of Chemistry, Bioscience and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, Box 8600, Forus, Stavanger 4036, Norway
[6]Department of Biological Engineering, College of Engineering, Konkuk University, Seoul 05029, Republic of Korea
[7]Institute for Ubiquitous Information Technology and Application, Konkuk University, Seoul 05029, Republic of Korea

References
1. Blettler MCM, Mitchell C. Dangerous traps: macroplastic encounters affecting freshwater and terrestrial wildlife. Sci Total Environ. 2021;798:149317.
2. Geyer R. Chapter 2 - Production, use, and fate of synthetic polymers. In: Letcher TM, editor. Plastic Waste and Recycling [Internet]. Academic Press; 2020 [cited 2024 Jan 17]. pp. 13–32. https://www.sciencedirect.com/science/article/pii/B9780128178805000025.
3. Moshood TD, Nawanir G, Mahmud F, Mohamad F, Ahmad MH, Abdul Ghani A. Expanding policy for biodegradable plastic products and market dynamics of bio-based plastics: challenges and opportunities. Sustainability. 2021;13:6170.
4. UNEP. From Pollution to Solution: A global assessment of marine litter and plastic pollution [Internet]. United Nation Environmental Program. 2021. https://www.unep.org/resources/pollution-solution-global-assessment-marine-litter-and-plastic-pollution.
5. Kassab A, Al Nabhani D, Mohanty P, Pannier C, Ayoub GY. Advancing plastic recycling: challenges and opportunities in the integration of 3d printing and distributed recycling for a circular economy. Polymers. 2023;15:3881.
6. Zaghdoudi M, Kömmling A, Jaunich M, Wolff D. Scission, cross-linking, and physical relaxation during thermal degradation of elastomers. Polymers. 2019;11:1280.
7. Zeenat, Elahi A, Bukhari DA, Shamim S, Rehman A. Plastics degradation by microbes: a sustainable approach. J King Saud Univ - Sci. 2021;33:101538.
8. Wojnowska-Baryła I, Bernat K, Zaborowska M. Plastic waste degradation in landfill conditions: the problem with microplastics, and their direct and indirect environmental effects. IJERPH. 2022;19:13223.
9. Mohanan N, Montazer Z, Sharma PK, Levin DB. Microbial and enzymatic degradation of synthetic plastics. Frontiers in Microbiology. 2020;11. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2020.580709/full.
10. Auta HS, Emenike CU, Jayanthi B, Fauziah SH. Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment. Mar Pollut Bull. 2018;127:15–21.
11. Montazer Z, Habibi Najafi MB, Levin DB. Microbial degradation of low-density polyethylene and synthesis of polyhydroxyalkanoate polymers. Can J Microbiol. 2019;65:224–34.
12. Giacomucci L, Raddadi N, Soccio M, Lotti N, Fava F. Polyvinyl chloride biodegradation by *Pseudomonas citronellolis* and *Bacillus flexus*. New Biotechnol. 2019;52:35–41.
13. Peng B-Y, Chen Z, Chen J, Yu H, Zhou X, Criddle CS, et al. Biodegradation of polyvinyl chloride (PVC) in *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae. Environ Int. 2020;145:106106.
14. Landrigan PJ, Stegeman JJ, Fleming LE, Allemand D, Anderson DM, Backer LC, et al. Human health and ocean pollution. Annals Global Health. 2020;86. https://doi.org/10.5334/aogh.2831.
15. Bhatia SK, Kumar G, Yang Y-H. Understanding microplastic pollution: tracing the footprints and eco-friendly solutions. Sci Total Environ. 2024;914:169926.
16. Evode N, Qamar SA, Bilal M, Barceló D, Iqbal HMN. Plastic waste and its management strategies for environmental sustainability. Case Stud Chem Environ Eng. 2021;4:100142.
17. Kour H, Khan SS, Kour D, Rasool S, Sharma YP, Rai PK, et al. Microbes mediated plastic degradation: a sustainable approach for environmental sustainability. J Appl Biology Biotechnol. 2022;3:11.
18. Rosenboom J-G, Langer R, Traverso G. Bioplastics for a circular economy. Nat Rev Mater. 2022;7:117–37.
19. Bhatia SK, Patel AK, Yang Y-H. The green revolution of food waste upcycling to produce polyhydroxyalkanoates. Trends Biotechnol. 2024. https://doi.org/10.1016/j.tibtech.2024.03.002.
20. Mitra R, Xu T, Xiang H, Han J. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. Microb Cell Fact. 2020;19.
21. Katagi VN, Bhat SG, Paduvari R, Kodavooru D, Somashekara DM. Waste to value-added products: an innovative approach for sustainable production

of microbial biopolymer (PHA) - emphasis on inexpensive carbon feedstock. Environ Technol Reviews. 2023. https://doi.org/10.1080/21622515.2023.2250 066. https://www.tandfonline.com/doi/abs/.

22. Helmecke M, Fries E, Schulte C. Regulating water reuse for agricultural irrigation: risks related to organic micro-contaminants. Environ Sci Europe. 2020;32:4.

23. Kawashima N, Yagi T, Kojima K. How do bioplastics and fossil-based plastics play in a circular economy? Macromol Mater Eng. 2019;304:1900383.

24. Obaideen K, Shehata N, Sayed ET, Abdelkareem MA, Mahmoud MS, Olabi AG. The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. Energy Nexus. 2022;7:100112.

25. United Nation. Water Quality and Wastewater [Internet]. United Nation. 2023 [cited 2024 Apr 12]. https://www.unwater.org/water-facts/water-quality-and-wastewater.

26. Pratap B, Kumar S, Nand S, Azad I, Bharagava RN, Romanholo Ferreira LF, et al. Wastewater generation and treatment by various eco-friendly technologies: possible health hazards and further reuse for environmental safety. Chemosphere. 2023;313:137547.

27. Samir A, Ashour FH, Hakim AAA, Bassyouni M. Recent advances in biodegradable polymers for sustainable applications. Npj Mater Degrad. 2022;6:1–28.

28. Bengtsson S, Karlsson A, Alexandersson T, Quadri L, Hjort M, Johansson P, et al. A process for polyhydroxyalkanoate (PHA) production from municipal wastewater treatment with biological carbon and nitrogen removal demonstrated at pilot-scale. New Biotechnol. 2017;35:42–53.

29. Gouveia AR, Freitas EB, Galinha CF, Carvalho G, Duque AF, Reis MAM. Dynamic change of pH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: impact on performance and microbial population. New Biotechnol. 2017;37:108–16.

30. Campanari S, Augelletti F, Rossetti S, Sciubba F, Villano M, Majone M. Enhancing a multi-stage process for olive oil mill wastewater valorization towards polyhydroxyalkanoates and biogas production. Chem Eng J. 2017;317:280–9.

31. Liao Q, Guo L, Ran Y, Gao M, She Z, Zhao Y, et al. Optimization of polyhydroxyalkanoates (PHA) synthesis with heat pretreated waste sludge. Waste Manag. 2018;82:15–25.

32. Queirós D, Rangel C, Lemos PC, Rossetti S, Serafim LS. Impact of organic acids supplementation to hardwood spent sulfite liquor as substrate for the selection of polyhydroxyalkanoates-producing organisms. Fermentation. 2018;4.

33. Tamis J, Mulders M, Dijkman H, Rozendal R, Van Loosdrecht MCM, Kleerebezem R. Pilot-scale polyhydroxyalkanoate production from paper mill wastewater: process characteristics and identification of bottlenecks for full-scale implementation. J Environ Eng. 2018;144.

34. Zhang T, Wang X, Zhou J, Zhang Y. Enrichments of methanotrophic–heterotrophic cultures with high poly-β-hydroxybutyrate (PHB) accumulation capacities. J Environ Sci. 2018;65:133–43.

35. Fradinho JC, Oehmen A, Reis MAM. Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int J Biol Macromol. 2019;126:1085–92.

36. Zhang D, Jiang H, Chang J, Sun J, Tu W, Wang H. Effect of thermal hydrolysis pretreatment on volatile fatty acids production in sludge acidification and subsequent polyhydroxyalkanoates production. Bioresour Technol. 2019;279:92–100.

37. Kourmentza C, Plácido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN et al. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering. 2017;4.

38. Pagliano G, Galletti P, Samorì C, Zaghini A, Torri C. Recovery of polyhydroxyalkanoates from single and mixed microbial cultures: a review. Front Bioeng Biotechnol. 2021;9.

39. Elhami V, van de Beek N, Wang L, Picken SJ, Jelmer T, Sousa JAB, et al. Extraction of low molecular weight polyhydroxyalkanoates from mixed microbial cultures using bio-based solvents. Sep Purif Technol. 2022;299:121773.

40. Didion YP, Vargas MVGA, Tjaslma TG, Woodley J, Nikel PI, Malankowska M, et al. A novel strategy for extraction of intracellular poly(3-hydroxybutyrate) from engineered *Pseudomonas putida* using deep eutectic solvents: comparison with traditional biobased organic solvents. Sep Purif Technol. 2024;338:126465.

41. Lavagnolo MC, Poli V, Zampini AM, Grossule V. Biodegradability of bioplastics in different aquatic environments: a systematic review. J Environ Sci. 2024;142:169–81.

42. Gong L, Passari AK, Yin C, Kumar Thakur V, Newbold J, Clark W, et al. Sustainable utilization of fruit and vegetable waste bioresources for bioplastics production. Crit Rev Biotechnol. 2024;44:236–54.

43. Ali Z, Abdullah M, Yasin MT, Amanat K, Ahmad K, Ahmed I, et al. Organic waste-to-bioplastics: Conversion with eco-friendly technologies and approaches for sustainable environment. Environ Res. 2024;244:117949.

44. Bhat SG, Thivaharan V, Divyashree MS. Sustainable opportunities in the downstream processing of the intracellular biopolymer polyhydroxyalkanoate. ChemBioEng Reviews. 2024;11:79–94.

45. Obruča S, Dvořák P, Sedláček P, Koller M, Sedlář K, Pernicová I, et al. Polyhydroxyalkanoates synthesis by halophiles and thermophiles: towards sustainable production of microbial bioplastics. Biotechnol Adv. 2022;58:107906.

46. Jung HJ, Kim SH, Shin N, Oh S-J, Hwang JH, Kim HJ, et al. Polyhydroxybutyrate (PHB) production from sugar cane molasses and tap water without sterilization using novel strain, *Priestia* sp. YH4. Int J Biol Macromol. 2023;250:126152.

47. Kim B, Oh SJ, Hwang JH, Kim HJ, Shin N, Bhatia SK, et al. Polyhydroxybutyrate production from crude glycerol using a highly robust bacterial strain *Halomonas* sp. YLGW01. Int J Biol Macromol. 2023;236:123997.

48. Chen S, Dai X, Yang D, Dai L, Hua Y. Polyhydroxyalkanoate synthesis from primitive components of organic solid waste: comparison of dominant strains and improvement of metabolic pathways. Appl Energy. 2023;344:121245.

49. Sehgal R, Gupta R. Polyhydroxyalkanoate and its efficient production: an eco-friendly approach towards development. 3 Biotech. 2020;10.

50. Zhou L, Zhang Z, Shi C, Scoti M, Barange DK, Gowda RR, et al. Chemically circular, mechanically tough, and melt-processable polyhydroxyalkanoates. Science. 2023;380:64–9.

51. Urtuvia V, Villegas P, Fuentes S, González M, Seeger M. Burkholderia xenovorans LB400 possesses a functional polyhydroxyalkanoate anabolic pathway encoded by the pha genes and synthesizes poly(3-hydroxybutyrate) under nitrogen-limiting conditions. Int Microbiol. 2018;21:47–57.

52. Adebayo Oyewole O, Usman Abdulmalik S, Onozasi Abubakar A, Ishaku Chimbekujwo K, Dorcas Obafemi Y, Oyegbile B, et al. Production of polyhydroxyalkanoate (pha) by *pseudomonas aeruginosa* (ol405443) using agrowastes as carbon source. Clean Mater. 2024;11:100217.

53. Pagliano G, Ventorino V, Panico A, Romano I, Pirozzi F, Pepe O. Anaerobic process for bioenergy recovery from dairy waste: meta-analysis and enumeration of microbial community related to intermediates production. Front Microbiol. 2019;9. https://www.frontiersin.org/journals/microbiology/articles/https://doi.org/10.3389/fmicb.2018.03229.

54. Pagliano G, Gugliucci W, Torrieri E, Piccolo A, Cangemi S, Di Giuseppe FA et al. Polyhydroxyalkanoates (PHAs) from dairy wastewater effluent: bacterial accumulation, structural characterization and physical properties. Chem Biol Technol Agric. 2020;7.

55. Lai C-W, Bhuyar P, Shen M-Y, Chu C-Y. A two-stage strategy for polyhydroxybutyrate (PHB) production by continuous biohydrogen fermenter and sequencing batch reactor from food industry wastewater. Sustain Energy Technol Assess. 2022;53:102445.

56. Hassemer G, de Nascimento S, Lin LH, Steffens Y-H, Junges C, Valduga A. Influence of redox potential on the accumulation of poly(3-hydroxybutyrate) by Bacillus megaterium. Bioprocess Biosyst Eng. 2023;46:1221–30.

57. Lee HS, Lee SM, Park SL, Choi TR, Song HS, Kim HJ et al. Tung oil-based production of high 3-hydroxyhexanoate-containing terpolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) using engineered *Ralstonia eutropha*. Polymers. 2021;13.

58. Chien Bong CP, Alam MNHZ, Samsudin SA, Jamaluddin J, Adrus N, Mohd Yusof AH, et al. A review on the potential of polyhydroxyalkanoates production from oil-based substrates. J Environ Manage. 2021;298:113461.

59. Yang M, Zou Y, Wang X, Liu X, Wan C, Harder M, et al. Synthesis of intracellular polyhydroxyalkanoates (PHA) from mixed phenolic substrates in an acclimated consortium and the mechanisms of toxicity. J Environ Chem Eng. 2022;10:107944.

60. Yasin AR, Al-Mayaly I. k. Biosynthesis of polyhydroxyalkanoate (PHA) by a newly isolated strain *Bacillus tequilensis* ARY86 using inexpensive carbon source. Bioresource Technology Reports. 2021;16:100846.

61. Zhou W, Bergsma S, Colpa DI, Euverink G-JW, Krooneman J. Polyhydroxyalkanoates (PHAs) synthesis and degradation by microbes and applications towards a circular economy. J Environ Manage. 2023;341:118033.

62. Choonut A, Prasertsan P, Klomklao S, Sangkharak K. Study on mcl-PHA production by Novel Thermotolerant Gram-positive isolate. J Polym Environ. 2020;28:2410–21.

63. Gao M, Li Y, Ma X, Li D, Li J. Biotransformation of palm oil wastewater to scl- and mcl-polyhydroxyalkanoates by mixed microbial consortia using different nitrogen and phosphorus sources. Biomass Conv Bioref. 2023;13:12961–73.

64. Palmeiro-Sánchez T, Val del Rio A, Fra-Vázquez A, Luis Campos J, Mosquera-Corral A. High-yield synthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymers in a mixed Microbial Culture: effect of substrate switching and F/M ratio. Ind Eng Chem Res. 2019;58:21921–6.

65. Jaffur BN, Kumar G, Jeetah P, Ramakrishna S, Bhatia SK. Current advances and emerging trends in sustainable polyhydroxyalkanoate modification from organic waste streams for material applications. Int J Biol Macromol. 2023;253:126781.

66. Jung HJ, Shin Y, Hwang JH, Shin N, Kim HJ, Oh S-J, et al. Establishment of an optimized electroporation method for Halomonas sp. YK44 and its application in the coproduction of PHB and isobutanol. Biotechnol Bioproc E. 2024;29:339–51.

67. Oh SJ, Choi T-R, Kim HJ, Shin N, Hwang JH, Kim HJ, et al. Maximization of 3-hydroxyhexanoate fraction in poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) using lauric acid with engineered *Cupriavidus necator* H16. Int J Biol Macromol. 2024;256:128376.

68. Bhatia SK, Otari SV, Jeon J-M, Gurav R, Choi Y-K, Bhatia RK, et al. Biowaste-to-bioplastic (polyhydroxyalkanoates): Conversion technologies, strategies, challenges, and perspective. Bioresour Technol. 2021;326:124733.

69. Melchor-Martínez EM, Macías-Garbett R, Alvarado-Ramírez L, Araújo RG, Sosa-Hernández JE, Ramírez-Gamboa D, et al. Towards a circular economy of plastics: an evaluation of the systematic transition to a new generation of bioplastics. Polymers. 2022;14:1203.

70. Park H, He H, Yan X, Liu X, Scrutton NS, Chen G-Q. PHA is not just a bioplastic! Biotechnol Adv. 2024;71:108320.

71. Ahmed I, Zia MA, Afzal H, Ahmed S, Ahmad M, Akram Z et al. Socio-economic and environmental impacts of biomass valorisation: a strategic drive for sustainable bioeconomy. Sustainability. 2021;13.

72. Nishat A, Yusuf M, Qadir A, Ezaier Y, Vambol V, Ijaz Khan M, et al. Wastewater treatment: a short assessment on available techniques. Alexandria Eng J. 2023;76:505–16.

73. Chandel N, Ahuja V, Gurav R, Kumar V, Tyagi VK, Pugazhendhi A, et al. Progress in microalgal mediated bioremediation systems for the removal of antibiotics and pharmaceuticals from wastewater. Sci Total Environ. 2022;825:153895.

74. Akinnawo SO, Ayadi PO, Oluwalope MT. Chemical coagulation and biological techniques for wastewater treatment. Ovidius Univ Annals Chem. 2023;34:14–21.

75. Cverenkárová K, Valachovičová M, Mackuľak T, Žemlička L, Bírošová L. Microplastics in the Food Chain. Life. 2021;11:1349.

76. Samal K, Mahapatra S, Hibzur Ali M. Pharmaceutical wastewater as emerging contaminants (EC): treatment technologies, impact on environment and human health. Energy Nexus. 2022;6:100076.

77. Sharma P, Tripathi S, Chaturvedi P, Chandra R. Characterization of autochthonous bacteria capable for degradation of residual organic pollutants of pulp paper mill effluent by biostimulation process. J Pure Appl Microbiol. 2020;1181–94.

78. Bakraoui M, Karouach F, Ouhammou B, Aggour M, Essamri A, El Bari H. Biogas production from recycled paper mill wastewater by UASB digester: optimal and mesophilic conditions. Biotechnol Rep. 2020;25:e00402.

79. García Rea VS, Egerland Bueno B, Muñoz Sierra JD, Nair A, Lopez Prieto IJ, Cerqueda-García D, et al. Chemical characterization and anaerobic treatment of bitumen fume condensate using a membrane bioreactor. J Hazard Mater. 2023;447:130709.

80. Atukunda A, Ibrahim MG, Fujii M, Ookawara S, Nasr M. Dual biogas/biochar production from anaerobic co-digestion of petrochemical and domestic wastewater: a techno-economic and sustainable approach. Biomass Conv Bioref [Internet]. 2022 [cited 2024 Feb 14]; https://doi.org/10.1007/s13399-022-02944-w.

81. Mouzakitis Y, Adamides ED. Techno-Economic Assessment of an Olive Mill Wastewater (OMWW) Biorefinery in the Context of Circular Bioeconomy. Eng. 2022;3:488–503.

82. Hernández-Herreros N, Rivero-Buceta V, Pardo I, Prieto MA. Production of poly(3-hydroxybutyrate)/poly(lactic acid) from industrial wastewater by wild-type *Cupriavidus necator* H16. Water Res. 2024;249:120892.

83. Alsafadi D, Aljariri Alhesan JS, Mansoura A, Oqdeha S. Production of polyhydroxyalkanoate from sesame seed wastewater by sequencing batch reactor cultivation process of *Haloferax mediterranei*. Arab J Chem. 2023;16:104584.

84. Sringari SS, Raja VK. Treatment of food processing industries wastewater using a novel Fuller's earth clay-based tubular ceramic membrane. Water Sci Technol. 2023;88:2533–46.

85. Martín-Marroquín JM, Garrote L, Hidalgo D, Moustakas K, Barampouti EM, Mai S. Solar-powered algal production on vegetable processing industry wastewater at pilot scale. Clean Technol Environ Policies. 2023. https://doi.org/10.1007/s10098-023-02505-3.

86. Mannina G, Mineo A. Polyhydroxyalkanoate production from fermentation of domestic sewage sludge monitoring greenhouse gas emissions: a pilot plant case study at the WRRF of Palermo University (Italy). J Environ Manage. 2023;348:119423.

87. Katagi VN, Manasa S, Raghavendra P, Bhat SG, M SD. Valorization of cashew industry wastewater as a carbon and nutrient source for the microbial growth and production of the polyhydroxyalkanoates: a potential biopolymer by Bacillus species. Cogent Eng. 2023;10:2269652.

88. Johnson Mb, Mehrvar M. Characterising winery wastewater composition to optimise treatment and reuse. Aust J Grape Wine Res. 2020;26:410–6.

89. Ghimire N, Wang S, Ghimire N, Wang S. Biological treatment of petrochemical wastewater. Petroleum chemicals - recent insight. IntechOpen; 2018. https://www.intechopen.com/chapters/62888.

90. Vlotman D, Key D, Cerff B, Bladergroen BJ. Shear enhanced flotation separation technology in Winery Wastewater Treatment. Water. 2023;15:2409.

91. Jia X, Jin D, Li C, Lu W. Characterization and analysis of petrochemical wastewater through particle size distribution, biodegradability, and chemical composition. Chin J Chem Eng. 2019;27:444–51.

92. Bhatia SK, Ahuja V, Chandel N, Mehariya S, Kumar P, Vinayak V, et al. An overview on microalgal-bacterial granular consortia for resource recovery and wastewater treatment. Bioresour Technol. 2022;351:127028.

93. Singh BJ, Chakraborty A, Sehgal R. A systematic review of industrial wastewater management: evaluating challenges and enablers. J Environ Manage. 2023;348:119230.

94. Saleh TA, Mustaqeem M, Khaled M. Water treatment technologies in removing heavy metal ions from wastewater: a review. Environ Nanotechnol Monit Manage. 2022;17:100617.

95. Chalaris M, Gkika DA, Tolkou AK, Kyzas GZ. Advancements and sustainable strategies for the treatment and management of wastewaters from metallurgical industries: an overview. Environ Sci Pollut Res. 2023;30:119627–53.

96. Sharma M, Yadav A, Mandal MK, Pandey S, Pal S, Chaudhuri H et al. Chapter 7 - Wastewater treatment and sludge management strategies for environmental sustainability. In: Stefanakis A, Nikolaou I, editors. Circular Economy and Sustainability. 2022; 97–112. https://www.sciencedirect.com/science/article/pii/B9780128216644000273.

97. Behera S, Priyadarshanee M, Vandana DS. Polyhydroxyalkanoates, the bioplastics of microbial origin: Properties, biochemical synthesis, and their applications. Chemosphere. 2022;294:133723.

98. Singha S, Mahmutovic M, Zamalloa C, Stragier L, Verstraete W, Svagan AJ, et al. Novel bioplastic from single cell protein as a potential packaging material. ACS Sustainable Chem Eng. 2021;9:6337–46.

99. Sabapathy PC, Devaraj S, Meixner K, Anburajan P, Kathirvel P, Ravikumar Y, et al. Recent developments in Polyhydroxyalkanoates (PHAs) production - A review. Bioresour Technol. 2020;306:123132.

100. Crutchik D, Franchi O, Caminos L, Jeison D, Belmonte M, Pedrouso A et al. Polyhydroxyalkanoates (PHAs) production: a feasible economic option for the treatment of sewage sludge in municipalwastewater treatment plants? Water (Switzerland). 2020;12.

101. Corsino SF, Di Bella G, Traina F, Montes LA, Val Del Rio A, Corral AM, et al. Membrane fouling mitigation in MBR via the feast-famine strategy to enhance pha production by activated sludge. Membr (Basel). 2022;12:703.

102. Sruamsiri D, Thayanukul P, Suwannasilp BB. In situ identification of polyhydroxyalkanoate (PHA)-accumulating microorganisms in mixed microbial cultures under feast/famine conditions. Sci Rep. 2020;10.

103. Cabrera F, Torres-Aravena Á, Pinto-Ibieta F, Campos JL, Jeison D. On-line control of feast/famine cycles to improve phb accumulation during cultivation of mixed microbial cultures in sequential batch reactors. IJERPH. 2021;18:12611.

104. Lorini L, di Re F, Majone M, Valentino F. High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding. New Biotechnol. 2020;56:140–8.

105. Zheng Y, Guo L, Liu Y, She Z, Gao M, Jin C, et al. Effects of chemical oxygen demand concentration, pH and operation cycle on polyhydroxyalkanoates synthesis with waste sludge. Environ Technol. 2021;42:1922–9.

106. Pinto-Ibieta F, Serrano A, Cea M, Ciudad G, Fermoso FG, Beyond PHA. Stimulating intracellular accumulation of added-value compounds in mixed microbial cultures. Bioresour Technol. 2021;337.

107. Wang X, Oehmen A, Freitas EB, Carvalho G, Reis MAM. The link of feast-phase dissolved oxygen (DO) with substrate competition and microbial selection in PHA production. Water Res. 2017;112:269–78.

108. Cui Y-W, Zhang H-Y, Lu P-F, Peng Y-Z. Effects of carbon sources on the enrichment of halophilic polyhydroxyalkanoate-storing mixed microbial culture in an aerobic dynamic feeding process. Sci Rep. 2016;6:30766.

109. Mohamad Fauzi AH, Chua ASM, Yoon LW, Nittami T, Yeoh HK. Enrichment of PHA-accumulators for sustainable PHA production from crude glycerol. Process Saf Environ Prot. 2019;122:200–8.

110. Freches A, Lemos PC. Microbial selection strategies for polyhydroxyalkanoates production from crude glycerol: Effect of OLR and cycle length. New Biotechnol. 2017;39:22–8.

111. Fra-Vázquez A, Pedrouso A, Palmeiro-Sánchez T, Moralejo-Gárate H, Mosquera-Corral A. Feasible microbial accumulation of triacylglycerides from crude glycerol. J Chem Technol Biotechnol. 2018;93:2644–51.

112. Cabrera F, Torres Á, Campos JL, Jeison D. Effect of operational conditions on the behaviour and associated costs of mixed microbial cultures for PHA production. Polymers. 2019;11.

113. Oliveira CSS, Silva CE, Carvalho G, Reis MA. Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: feast and famine regime and uncoupled carbon and nitrogen availabilities. New Biotechnol. 2017;37:69–79.

114. Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V, Polyhydroxyalkanoate. (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. Express Polym Lett. 2014;8:791–808.

115. Raza ZA, Abid S, Banat IM. Polyhydroxyalkanoates: characteristics, production, recent developments and applications. Int Biodeterior Biodegradation. 2018;126:45–56.

116. Tayou Nguemna L, Marzulli F, Scopetti F, Lorini L, Lauri R, Pietrangeli B, et al. Recirculation factor as a key parameter in continuous-flow biomass selection for polyhydroxyalkanoates production. Chem Eng J. 2023;455:140208.

117. Parroquin-Gonzalez M, Winterburn J. Continuous bioreactor production of polyhydroxyalkanoates in Haloferax mediterranei. Front Bioeng Biotechnol. 2023;11:1220271.

118. Pittmann T, Steinmetz H. Polyhydroxyalkanoate production on wastewater treatment plants: process scheme, operating conditions and potential analysis for German and European municipal waste water treatment plants. Bioengineering. 2017;4.

119. Chen G-Q, Jiang X-R. Next generation industrial biotechnology based on extremophilic bacteria. Curr Opin Biotechnol. 2018;50:94–100.

120. Kumar P, Mehariya S, Ray S, Mishra A, Kalia VC. Biotechnology in Aid of Biodiesel Industry Effluent (Glycerol): Biofuels and Bioplastics. In: Kalia VC, editor. Microbial Factories: Biofuels, Waste treatment: Volume 1 [Internet]. New Delhi: Springer India; 2015 [cited 2024 Jan 17]. pp. 105–19. https://doi.org/10.1007/978-81-322-2598-0_7.

121. Park YL, Song HS, Choi TR, Lee SM, Park SL, Lee HS, et al. Revealing of sugar utilization systems in Halomonas sp. YLGW01 and application for poly(3-hydroxybutyrate) production with low-cost medium and easy recovery. Int J Biol Macromol. 2021;167:151–9.

122. Kasirajan L, Maupin-Furlow JA. Halophilic archaea and their potential to generate renewable fuels and chemicals. Biotechnol Bioeng. 2021;118:1066–90.

123. Mahler N, Tschirren S, Pflügl S, Herwig C. Optimized bioreactor setup for scale-up studies of extreme halophilic cultures. Biochem Eng J. 2018;130:39–46.

124. Oren A. Industrial and environmental applications of halophilic microorganisms. Environ Technol. 2010;31:825–34.

125. Koller M, Maršálek L, de Sousa Dias MM, Braunegg G. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. New Biotechnol. 2017;37:24–38.

126. Werker A, Lorini L, Villano M, Valentino F, Majone M. Modelling mixed Microbial Culture Polyhydroxyalkanoate Accumulation Bioprocess towards Novel methods for Polymer Production using dilute volatile fatty acid Rich Feedstocks. Bioengineering. 2022;9:125.

127. Weiland-Bräuer N. Friends or foes—Microbial. Interact Nat Biology. 2021;10:496.

128. Feng R, Chen L, Chen K. Fermentation trip: amazing microbes, amazing metabolisms. Ann Microbiol. 2018;68:717–29.

129. Magocha TA, Zabed H, Yang M, Yun J, Zhang H, Qi X. Improvement of industrially important microbial strains by genome shuffling: current status and future prospects. Bioresour Technol. 2018;257:281–9.

130. Huang L, Chen Z, Wen Q, Zhao L, Lee DJ, Yang L, et al. Insights into feast-famine polyhydroxyalkanoate (PHA)-producer selection: microbial community succession, relationships with system function and underlying driving forces. Water Res. 2018;131:167–76.

131. Marang L, Jiang Y, van Loosdrecht MCM, Kleerebezem R. Impact of non-storing biomass on PHA production: an enrichment culture on acetate and methanol. Int J Biol Macromol. 2014;71:74–80.

132. Arıkan M, Muth T. Integrated multi-omics analyses of microbial communities: a review of the current state and future directions. Mol Omics. 2023;19:607–23.

133. Helleckes LM, Hemmerich J, Wiechert W, Von Lieres E, Grünberger A. Machine learning in bioprocess development: from promise to practice. Trends Biotechnol. 2023;41:817–35.

134. Cavaillé L, Albuquerque M, Grousseau E, Lepeuple A-S, Uribelarrea J-L, Hernandez-Raquet G, et al. Understanding of polyhydroxybutyrate production under carbon and phosphorus-limited growth conditions in non-axenic continuous culture. Bioresour Technol. 2016;201:65–73.

135. Valentino F, Morgan-Sagastume F, Campanari S, Villano M, Werker A, Majone M. Carbon recovery from wastewater through bioconversion into biodegradable polymers. New Biotechnol. 2017;37:9–23.

136. Huang L, Chen Z, Wen Q, Lee DJ. Enhanced polyhydroxyalkanoate production by mixed microbial culture with extended cultivation strategy. Bioresour Technol. 2017;241:802–11.

137. Inoue D, Suzuki Y, Sawada K, Sei K. Polyhydroxyalkanoate accumulation ability and associated microbial community in activated sludge-derived acetate-fed microbial cultures enriched under different temperature and pH conditions. J Biosci Bioeng. 2018;125:339–45.

138. Alvarez Chavez B, Raghavan V, Tartakovsky B. A comparative analysis of biopolymer production by microbial and bioelectrochemical technologies. RSC Adv. 2022;12:16105–18.

139. Kehrein P, Van Loosdrecht M, Osseweijer P, Garfí M, Dewulf J, Posada J. A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks. Environ Sci: Water Res Technol. 2020;6:877–910.

140. Owusu-Agyeman I, Plaza E, Elginöz N, Atasoy M, Khatami K, Perez-Zabaleta M, et al. Conceptual system for sustainable and next-generation wastewater resource recovery facilities. Sci Total Environ. 2023;885:163758.

141. Lagoa-Costa B, Kennes C, Veiga MC. Cheese whey fermentation into volatile fatty acids in an anaerobic sequencing batch reactor. Bioresour Technol. 2020;308:123226.

142. Bermúdez-Penabad N, Kennes C, Veiga MC. Anaerobic digestion of tuna waste for the production of volatile fatty acids. Waste Manag. 2017;68:96–102.

143. Colombo B, Favini F, Scaglia B, Sciarria TP, D'Imporzano G, Pognani M et al. Enhanced polyhydroxyalkanoate (PHA) production from the organic fraction of municipal solid waste by using mixed microbial culture. Biotechnol Biofuels. 2017;10.

144. Ospina-Betancourth C, Echeverri S, Rodriguez-Gonzalez C, Wist J, Combariza MY, Sanabria J. Enhancement of PHA production by a mixed Microbial Culture using VFA obtained from the fermentation of Wastewater from yeast industry. Fermentation. 2022;8:180.

145. Chang Y-C, Reddy M, Imura K, Onodera R, Kamada N, Sano Y. Two-stage polyhydroxyalkanoates (PHA) production from cheese whey using Acetobacter pasteurianus C1 and Bacillus sp. CYR1. Bioengineering. 2021;8:157.

146. Xu Z, Pan C, Li X, Hao N, Zhang T, Gaffrey MJ et al. Enhancement of polyhydroxyalkanoate production by co-feeding lignin derivatives with glycerol in Pseudomonas putida KT2440. Biotechnol Biofuels. 2021;14.

147. Favaro L, Basaglia M, Casella S. Improving polyhydroxyalkanoate production from inexpensive carbon sources by genetic approaches: a review. Biofuels Bioprod Biorefin. 2019;13:208–27.

148. Hakeem IG, Halder P, Dike CC, Chiang K, Sharma A, Paz-Ferreiro J et al. Advances in biosolids pyrolysis: roles of pre-treatments, catalysts, and co-feeding on products distribution and high-value chemical production. 2022;166:105608. https://doi.org/10.1016/j.jaap.2022.105608.

149. Valentino F, Moretto G, Lorini L, Bolzonella D, Pavan P, Majone M. Pilot-scale polyhydroxyalkanoate production from combined treatment of organic fraction of municipal solid waste and sewage sludge. Ind Eng Chem Res. 2019;58:12149–58.

150. Iglesias-Iglesias R, Portela-Grandío A, Treu L, Campanaro S, Kennes C, Veiga MC. Co-digestion of cheese whey with sewage sludge for caproic acid production: role of microbiome and polyhydroxyalkanoates potential production. Bioresour Technol. 2021;337.

151. Owusu-Agyeman I, Plaza E, Cetecioglu Z. Production of volatile fatty acids through co-digestion of sewage sludge and external organic waste: Effect of substrate proportions and long-term operation. Waste Manag. 2020;112:30–9.

152. Calero R, Iglesias-Iglesias R, Kennes C, Veiga MC. Organic loading rate effect on the acidogenesis of cheese whey: a comparison between UASB and SBR reactors. Environ Technol. 2018;39:3046–54.

153. Bhatia SK, Gurav R, Choi TR, Jung HR, Yang SY, Song HS, et al. Poly(3-hydroxy-butyrate-co-3-hydroxyhexanoate) production from engineered *Ralstonia eutropha* using synthetic and anaerobically digested food waste derived volatile fatty acids. Int J Biol Macromol. 2019;133:1–10.

154. Zhang Z, Lin Y, Wu S, Li X, Cheng JJ, Yang C. Effect of composition of volatile fatty acids on yield of polyhydroxyalkanoates and mechanisms of bioconversion from activated sludge. Bioresour Technol. 2023;385:129445.

155. Albuquerque MGE, Martino V, Pollet E, Avérous L, Reis M, a. M. Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: effect of substrate composition and feeding regime on PHA productivity, composition and properties. J Biotechnol. 2011;151:66–76.

156. Aremu MO, Ishola MM, Taherzadeh MJ. Polyhydroxyalkanoates (PHAs) production from volatile fatty acids (VFAs) from organic wastes by pseudomonas oleovorans. Fermentation. 2021;7:287.

157. Sánchez Valencia AI, Rojas Zamora U, Meraz Rodríguez M, Álvarez Ramírez J, Salazar Peláez ML, Fajardo Ortiz C. Effect of C/N ratio on the PHA accumulation capability of microbial mixed culture fed with leachates from the organic fraction of municipal solid waste (OFMSW). J Water Process Eng. 2021;40.

158. Zhang K, Fang Q, Xie Y, Chen Y, Wei T, Xiao Y. The synthesis of polyhydroxy-alkanoates from low carbon wastewater under anaerobic-microaerobic process: effects of pH and nitrogen and phosphorus limitation. Environ Eng Res. 2022;27. http://www.eeer.org/journal/view.php?number=1352.

159. Tu W, Zhang D, Wang H. Polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge by mixed microbial cultures: the link between phosphorus and PHA yields. Waste Manag. 2019;96:149–57.

160. De Grazia G, Quadri L, Majone M, Morgan-Sagastume F, Werker A. Influence of temperature on mixed microbial culture polyhydroxyalkanoate production while treating a starch industry wastewater. J Environ Chem Eng. 2017;5:5067–75.

161. Wang X, Bengtsson S, Oehmen A, Carvalho G, Werker A, Reis MAM. Application of dissolved oxygen (DO) level control for polyhydroxyalkanoate (PHA) accumulation with concurrent nitrification in surplus municipal activated sludge. New Biotechnol. 2019;50:37–43.

162. Blunt W, Sparling R, Gapes DJ, Levin DB, Cicek N. The role of dissolved oxygen content as a modulator of microbial polyhydroxyalkanoate synthesis. World J Microbiol Biotechnol. 2018;34.

163. Bhatia SK, Gurav R, Choi TR, Jung HR, Yang SY, Moon YM, et al. Bioconversion of plant biomass hydrolysate into bioplastic (polyhydroxyalkanoates) using *Ralstonia eutropha* 5119. Bioresour Technol. 2019;271:306–15.

164. Patil TD, Ghosh S, Agarwal A, Patel SKS, Tripathi AD, Mahato DK, et al. Production, optimization, scale up and characterization of polyhydoxyalkanoates copolymers utilizing dairy processing waste. Sci Rep. 2024;14:1620.

165. Estévez-Alonso Á, Pei R, van Loosdrecht MCM, Kleerebezem R, Werker A. Scaling-up microbial community-based polyhydroxyalkanoate production: status and challenges. Bioresour Technol. 2021;327:124790.

166. Grivalský T, Lakatos GE, Štěrbová K, Manoel JAC, Beloša R, Divoká P, et al. Poly-β-hydroxybutyrate production by Synechocystis MT_a24 in a raceway pond using urban wastewater. Appl Microbiol Biotechnolology. 2024;108:44.

167. Wang L, Cui Y-W. Simultaneous treatment of epichlorohydrin wastewater and polyhydroxyalkanoate recovery by halophilic aerobic granular sludge highly enriched by *Halomonas Sp*. Bioresour Technol. 2024;391:129951.

168. Mehariya S, Plöhn M, Jablonski P, Stagge S, Jönsson LJ, Funk C. Biopolymer production from biomass produced by nordic microalgae grown in wastewater. Bioresour Technol. 2023;376:128901.

169. Senatore V, Rueda E, Bellver M, Díez-Montero R, Ferrer I, Zarra T, et al. Production of phycobiliproteins, bioplastics and lipids by the cyanobacteria *Synechocystis* sp. treating secondary effluent in a biorefinery approach. Sci Total Environ. 2023;857:159343.

170. Chang Y-C, Reddy MV, Tsukiori Y, Mawatari Y, Choi D. Production of polyhy-droxyalkanoates using sewage and cheese whey. Heliyon. 2023;9. https://www.cell.com/heliyon/abstract/S2405-8440(23)10338–0.

171. Zhou T, Wang S, Zhang W, Yin F, Cao Q, Lian T, et al. Polyhydroxyalkanoates production from lactic acid fermentation broth of agricultural waste without extra purification: the effect of concentrations. Environ Technol Innov. 2023;32:103311.

172. Pei R, Vicente-Venegas G, Tomaszewska-Porada A, Van Loosdrecht MCM, Kleerebezem R, Werker A. Visualization of polyhydroxyalkanoate accumulated in waste activated sludge. Environ Sci Technol. 2023;57:11108–21.

173. Minh HKQ, Thai ND, Khoa TVA, Thao NTN, Sichaem J. Isolation and production of polyhydroxybutyrate (PHB) from *Bacillus pumilus* NMG5 strain for bioplastic production and treatment of wastewater from paper factories. J Experimental Biology Agricultural Sci. 2023;11:351–8.

174. Srivastava P, Villamil JA, Melero JA, Martínez F, Puyol D. Using inorganic acids to stop purple phototrophic bacteria metabolism improves PHA recovery at a large scale. Biomass Convers Biorefinery. 2023. https://doi.org/10.1007/s13399-023-03810-z.

175. Paxinou A, Marcello E, Vecchiato V, Erman L, Wright E, Noble B, et al. Dual production of polyhydroxyalkanoates and antibacterial/antiviral gold nanoparticles. Front Nanatechnol. 2023;5. https://doi.org/10.3389/fnano.2023.1243056. https://www.frontiersin.org/articles/.

176. Wicker RJ, Autio H, Daneshvar E, Sarkar B, Bolan N, Kumar V, et al. The effects of light regime on carbon cycling, nutrient removal, biomass yield, and polyhydroxybutyrate (PHB) production by a constructed photosynthetic consortium. Bioresour Technol. 2022;363:127912.

177. Raho S, Carofiglio VE, Montemurro M, Miceli V, Centrone D, Stufano P et al. Production of the polyhydroxyalkanoate PHBV from ricotta cheese exhausted whey by haloferax mediterranei fermentation. Foods. 2020;9.

178. Fang F, Xu RZ, Huang YQ, Wang SN, Zhang LL, Dong JY et al. Production of polyhydroxyalkanoates and enrichment of associated microbes in bioreactors fed with rice winery wastewater at various organic loading rates. Bioresour Technol. 2019;292.

179. Colombo B, Pereira J, Martins M, Torres-Acosta MA, Dias ACRV, Lemos PC et al. Recovering PHA from mixed microbial biomass: using non-ionic surfactants as a pretreatment step. Sep Purif Technol. 2020;253.

180. Madkour MH, Heinrich D, Alghamdi MA, Shabbaj II, Steinbüchel A. PHA Recovery from Biomass. Biomacromolecules. 2013;14:2963–72.

181. Balakrishna Pillai A, Jaya Kumar A, Kumarapillai H. Enhanced production of poly(3-hydroxybutyrate) in recombinant *Escherichia coli* and EDTA–microwave-assisted cell lysis for polymer recovery. AMB Express. 2018;8:142.

182. Bocaz-Beltrán J, Rocha S, Pinto-Ibieta F, Ciudad G, Cea M. Novel alternative recovery of polyhydroxyalkanoates from mixed microbial cultures using microwave-assisted extraction. J Chem Technol Biotechnol. 2021.

183. Yılmaz Nayır T, Konuk S, Kara S. Extraction of polyhydroxyalkanoate from activated sludge using supercritical carbon dioxide process and biopolymer characterization. J Biotechnol. 2023;364:50–7.

184. Lorini L, Martinelli A, Capuani G, Frison N, Reis M, Sommer Ferreira B et al. Characterization of polyhydroxyalkanoates produced at pilot scale from different organic wastes. Front Bioeng Biotechnol. 2021;9.

185. Salvatori G, Alfano S, Martinelli A, Gottardo M, Villano M, Ferreira BS, et al. Chlorine-free extractions of mixed-culture polyhydroxyalkanoates produced from fermented sewage sludge at pilot scale. Ind Eng Chem Res. 2023;62:17400–7.

186. Werker A, Pei R, Kim K, Moretto G, Estevez-Alonso A, Vermeer C, et al. Thermal pre-processing before extraction of polyhydroxyalkanoates for molecular weight quality control. Polym Degrad Stab. 2023;209:110277.

187. Rodrigues AM, Franca RDG, Dionísio M, Sevrin C, Grandfils C, Reis MAM, et al. Polyhydroxyalkanoates from a mixed microbial culture: extraction optimization and polymer characterization. Polymers. 2022;14:2155.

188. Kumar V, Srivastava S, Thakur IS. Enhanced recovery of polyhydroxyalkanoates from secondary wastewater sludge of sewage treatment plant: analysis and process parameters optimization. Bioresource Technol Rep. 2021;15:100783.

189. Jiang G, Johnston B, Townrow DE, Radecka I, Koller M, Chaber P et al. Biomass extraction using non-chlorinated solvents for biocompatibility improvement of polyhydroxyalkanoates. Polymers. 2018;10.

190. Vermeer CM, Nielsen M, Eckhardt V, Hortensius M, Tamis J, Picken SJ, et al. Systematic solvent screening and selection for polyhydroxyalkanoates (PHBV) recovery from biomass. J Environ Chem Eng. 2022;10:108573.

191. Mongili B, Abdel Azim A, Fraterrigo Garofalo S, Batuecas E, Re A, Bocchini S, et al. Novel insights in dimethyl carbonate-based extraction of polyhydroxybutyrate (PHB). Biotechnol Biofuels. 2021;14:13.

192. United Nation. Report of the conference of the parties to the basel convention on the control of transboundary movements of hazardous wastes and their disposal on the work of its fourteenth meeting. Fourteenth meeting of the conference of the parties to the basel convention meeting documents. United Nation Environment Programme. 2019. pp. 1–123. https://www.basel.int/TheConvention/ConferenceoftheParties/Meetings/COP14/tabid/7520/Default.aspx.

193. Rosenboom JG, Langer R, Traverso G. Bioplastics for a circular economy. Nat Reviews Mater 2022. 2022;7(2):7:117–37.

194. Talan A, Pokhrel S, Tyagi RD, Drogui P. Biorefinery strategies for microbial bioplastics production: sustainable pathway towards circular bioeconomy. Bioresource Technol Rep. 2022;17:100875.

195. The Times Editorial Board. China is putting the U.S. to shame in the fight against plastic trash. Los Angeles Times. 2020; https://www.latimes.com/opinion/story/2020-01-27/us-shouldnt-let-china-win-the-plastic-trash-war.

196. Rajendran N, Han J. Techno-economic analysis and life cycle assessment of poly (butylene succinate) production using food waste. Waste Manag. 2023;156:168–76.

197. Shen M-Y, Souvannasouk V, Chu C-Y, Tantranont N, Sawatdeenarunat C. Pha production from molasses using mixed microbial cultures: techno-economic feasibility analysispha production from molasses using mixed microbial cultures: Techno-economic feasibility analysis. Research Square; 2023.

198. Wang K, Hobby AM, Chen Y, Chio A, Jenkins BM, Zhang R. Techno-Economic Analysis on an industrial-scale production system of polyhydroxyalkanoates (PHA) from cheese By-Products by Halophiles. Processes. 2022;10:17.

199. Rueda E, Senatore V, Zarra T, Naddeo V, García J, Garfí M. Life cycle assessment and economic analysis of bioplastics production from cyanobacteria. Sustainable Mater Technol. 2023;35:e00579.

200. Ali SS, Abdelkarim EA, Elsamahy T, Al-Tohamy R, Li F, Kornaros M et al. Bioplastic production in terms of life cycle assessment: a state-of-the-art review. Environ Sci Ecotechnology. 2023;15.

201. Thomas AP, Kasa VP, Dubey BK, Sen R, Sarmah AK. Synthesis and commercialization of bioplastics: Organic waste as a sustainable feedstock. Sci Total Environ. 2023;904:167243.

202. Carvalho JM, Marreiros BC, Reis MAM. Polyhydroxyalkanoates production by mixed microbial culture under high salinity. Sustainability. 2022;14:1346.

203. Abe MM, Martins JR, Sanvezzo PB, Macedo JV, Branciforti MC, Halley P, et al. Advantages and disadvantages of bioplastics production from starch and lignocellulosic components. Polym (Basel). 2021;13:2484.

204. Arora Y, Sharma S, Sharma V. Microalgae in Bioplastic production: a Comprehensive Review. Arab J Sci Eng. 2023;48:7225–41.

205. Cherry C, Sahoo G, Bioplastics. and CRISPR/Cas 9 mediated gene replacement to overcome the limitations of bioplastics. 2020. https://doi.org/10.13140/RG.2.2.14649.39523.

206. Klein F, Emberger-Klein A, Menrad K, Möhring W, Blesin J-M. Influencing factors for the purchase intention of consumers choosing bioplastic products in Germany. Sustainable Prod Consum. 2019;19:33–43.

Publisher's Note