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PHBV cycle of life using waste as a starting point: from production to recyclability

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Global concern about plastic pollution is forcing new policies and modifications of human consumption as well as promoting new research lines aiming at the replacement of non-degradable plastics with other polymers more environmentally friendly. Addressing food waste and promoting circular economy strategies, among other approaches, are crucial in reducing environmental impacts and fostering sustainability in several sectors like the agri-food industry. The European Union's Circular Economy Action Plan is a significant initiative in this direction. Biotechnological processes, especially the valorisation of agri-food waste to produce highly marketed biomolecules like poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) using microorganisms as cellular factories, offer promising avenues for achieving these goals. PHBV is a biodegradable polymer firstly characterised as an isolated biopolymer from bacterial biomass. This biopolymer shows interesting physicochemical properties making possible immense potential in various applications due to its biocompatibility and sustainability, thus revealing it as a good candidate to replace plastics produced by chemical synthesis from petroleum (which are highly recalcitrant and consequently pollutants). This review critically analyses the PHBV synthesis and end-of-life scenarios from their synthesis using chemical and biological pathways, through the forms of biotechnological operation and production, to the forms described until the moment of recycling.

KEYWORDS

poly (3-hydroxybutyrate-co-3-hydroxyvalerate), polyhydroxyalkanoates, microbial cell factories, circular economy, biopolymers, waste valorisation, recyclability

1 Introduction

The plastic era began in the mid-1800s when John Hyatt created the first synthetic polymer derived from cellulose nitrate as a substitute for ivory (Rasmussen, 2021). In the 1940s the massive production of plastics increased exponentially (Adjani Diankristanti et al., 2024; Liwarska-Bizukojc, 2021), reaching a global amount of 400.3 million tons (Mt) in 2022 (Plastics Europe, 2022). Among them, the use of petroleum-derived plastics has become a problem worldwide, due to the well-known impact on human health and the environment (González-Rojo and Díez-Antolínez, 2023; Pinho et al., 2022; Simó-Cabrera et al., 2021). In fact, these materials are widely distributed around the world, contaminating diverse

ecosystems, also including the deepest marine ecosystems (de Mello et al., 2024) and one of the most critical problems are their long persistence in these environments (Amabile et al., 2024; Binelli et al., 2022; Pinho et al., 2022). The scientific community has focused on finding sustainable alternatives, including bioplastics, whose properties are similar to conventional plastic and whose production comes from renewable sources or biological matter (Atiwesh et al., 2021; Ciftcioglu-Gozuacik et al., 2023). Indeed, bioplastic production has been growing these last years from 1.792 Mt in 2021 to 2.217 Mt in 2022; in only 1 year, the production has increased by 123.72% globally. Additionally, global bioplastics production capacity is expected to increase significantly from around 2.18 million tons in 2023 to approximately 7.43 million tons in 2028 (European Bioplastics, 2022). However, it should be noted that not all bioplastics have biodegradable properties even though they all come from renewable sources, as in the case of some types of polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA) and polytrimethylene terephthalate (PTT) (Atiwesh et al., 2021; Di Bartolo et al., 2021; Venâncio et al., 2022; Arif et al., 2023). In this context, the group of biopolymers known as polyhydroxyalkanoates (PHAs) stand out for its biodegradability, biocompatibility, sustainability, and thermoplastic properties, making them valuable in various industries (Simó-Cabrera et al., 2021; Zhang et al., 2021). PHAs, discovered by Lemoigne in 1925 inside *Bacillus megaterium* cells (Doudoroff and Stanier, 1959), are linear polyesters of (R)-hydroxyl fatty acid monomers (Tan et al., 2014b; Kumar et al., 2017). They accumulated in the cytoplasm of various microorganisms in the form of granules, serving as a source of energy and carbon, particularly in the presence of excess carbon and limited essential nutrients such as nitrogen, phosphorus, or oxygen (Cánovas et al., 2021; Koller et al., 2007; Kumar et al., 2017; Obruca et al., 2018; Rivera-Briso and Serrano-Aroca, 2018). It is documented that granules are composed of 97.5% PHA, 2% proteins (PHA synthases, PHA depolymerases, phasins, and regulatory proteins) and 0.5% lipids (Cai et al., 2012; Cai et al., 2015; Amabile et al., 2024). More than 150 different monomers have been described as building blocks in the structure of PHAs, which can be classified according to them and the length of the radical group (Tan et al., 2014b) as short-chain- length PHAs (scl-PHAs), medium-chain-length PHA (mcl-PHA) and long-chain-length PHA (lcl-PHA) (Obruca et al., 2018; Neoh et al., 2022). Among the various types, polyhydroxybutyrate (PHB) stands out as the most widely studied PHA due to its production by numerous microorganisms, including Gram-negative and Gram-positive bacteria. However, one of the main drawbacks associated with the use of PHB is the narrow working range, as the difference between its melting point and degradation temperature is 20°C (180°C and 200°C) (Priya et al., 2022). However, to improve its physicochemical properties for potential uses in different industrial and medical sectors, it is often necessary to incorporate secondary monomers to form copolymers. Although several types of PHB derivatives exist, one of the most promising for various applications is poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Rivera-Briso and Serrano-Aroca, 2018).

PHBV is formed by the incorporation of 3-hydroxyvalerate (3HV) monomers into the PHB structure (Figure 1). PHBV is distinguished from other PHAs by several important characteristics such as zero toxicity, total biodegradability, proven in soils, water and compost, biocompatibility with many cell types, resistance to UV radiation, and better mechanical characteristics, such as lower degree of crystallinity, higher flexibility, and strength (Rivera-Briso and Serrano-Aroca, 2018; Rodríguez-Cendal et al., 2023). These improvements directly depend on the amount of 3HV incorporated in its structure. It has even been observed that the higher the concentration of 3HV, the higher the degradation rate and the lower the melting temperature, making it easier to process (Rivera-Briso and Serrano-Aroca, 2018; Policastro et al., 2021; Rodríguez-Cendal et al., 2023).

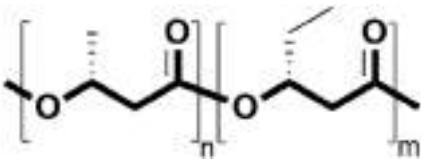

FIGURE 1
Structure of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (adapted from Rivera-Briso and Serrano-Aroca, 2018).

Although PHBV was first commercialised in 1980 by Imperial Chemical Industries under the name "Biopol", the cost of production is still high compared to the conventional production of plastic from petroleum for instance, making it very difficult to compete with the petrochemical industry (Obulisamy and Mehariya, 2021; Koller and Rittmann, 2022; Rodríguez-Cendal et al., 2023). The high cost associated with PHBV production is attributed to several factors related to the initial carbon source, fermentation costs, extraction and purification if it is necessary. These latter processes, account for approximately half of the total production cost (Zhang et al., 2024). Nevertheless, due to the growing concern about global pollution due to non-biodegradable plastics as well as growing interest in PHBV application in different biomedical areas and as a substitute for conventional plastic products, multiple research projects are being carried out to look for microorganisms able to produce PHBV efficiently and/or develop processes aiming at the reduction of the cost of their production (Hammami et al., 2022).

PHBV is produced by different Gram-negative and Gram-positive bacteria, however, the use of bacteria for the production has certain disadvantages. Gram-negative bacteria, such as *Cupriavidus necator*, which serves as a model for the study of PHA production, produce the well-known lipopolysaccharide, which is an endotoxin that copurifies with PHA extraction and can cause immunological reaction and limit its applications in the medical sector. Therefore, to remove toxins, the downstream process becomes more expensive (Tan et al., 2014b; Tebaldi et al., 2019; Kumar et al., 2020; Diankristanti et al., 2023). Gram-positive bacteria such as those belonging to the genera *Nocardia* or *Rhodococcus* are of limited interest due to their low production of the polymer compared to Gram-negative bacteria and their difficult purification (Guo et al., 2013; Tan et al., 2014b).

Therefore, scientists are currently focusing their attention on the use of extremophilic archaea, such as haloarchaea, to produce PHBV. The advantages of their use are numerous, such as easy lysis, no need for media sterilisation and the possibility of using a wide range of inexpensive raw materials for growth (Koller and Rittmann, 2022; Obruca et al., 2018).

Because of that, for an effective and competitive production, many efforts are being made such as the selection of efficient producer microorganisms, as well as the development of different strategies for the modification of PHBV to improve its properties. Despite all the technical difficulties that medium and large-scale PHBV production still has, the relevant benefits of the use of PHBV in biomedicine make the polymer of high interest to the global scientific community and companies aiming at the synthesis of bioplastics. Thus, its beneficial use has been reported in different fields of medicine, such as in tissue regeneration (bone, muscle, epithelial, among others) (Ren et al., 2023), in the controlled release of drugs using PHBV nanoparticles (tested in different treatments for various types of cancer, such as hepatocellular carcinoma, colon cancer, breast cancer, etc.) (Rodríguez-Cendal et al., 2023), in its use for sutures and in the manufacture of prostheses and pericardial patches, as well as its use for new vaccines against COVID-19. Other potential applications in different industries are mainly related to the packaging of food (Policastro et al., 2021; Koller and Rittmann, 2022; Urtuvia et al., 2022; Ren et al., 2023; Rodríguez-Cendal et al., 2023).

Considering the innovation and the current impact of the potential uses of PHBV, this review summarises insights into PHBV production by using different strategies (use of different carbon sources, microorganisms, and various industrial-scale production approaches), along with insights into its recyclability.

2 Pathways for the synthesis of polyhydroxyalkanoates (PHAs)

2.1 Chemical pathways

In the last decades, several chemocatalytic pathways have been investigated to synthesise different copolymers of PHAs. These pathways have attracted great attention since the thermal and mechanical properties of PHAs can be modulated by chemical approaches, manipulating to a greater extent the polymer stereomicrostructure. In this sense, several advantages can be reached, which include: i) precision in synthesis, considering both chain length control and comonomer sequence; ii) tunability of microstructure, which entails tacticities and R or S configurations; iii) ease scalability of the process, due to the fast reaction kinetics associated with ring-opening-polymerization (ROP) procedures. Nevertheless, the preparation of the cyclic monomers needed to conduct ROP mechanisms is not straightforward, and complex steps are required. Hence, the main constraint that it presents is caused by the need to develop a cost-effective synthesis of monomers to efficiently establish a "monomer-polymer-monomer" closed-loop lifecycle (Westlie et al., 2022). Considering their relevance, two different chemocatalytic pathways have been identified in this review as the most representative in chemical synthesis: i) direct route and ii) ring-opening polymerization (Gabirondo et al., 2020).

2.1.1 Direct route

The direct route, also known as polycondensation, is considered the most industrially widespread relevant strategy to produce polyesters. In this case, the availability of the monomers does not imply an obstacle. Herein, two different mechanisms are recognised: *step growth polymerization*, which mainly consists of the polymerization between dicarboxylic acids and diols, and *self-condensation polymerization*, which is focused on hydroxy acids, since they have, at least, one nucleophile (alcohol group) and one electrophile (carboxylic acid group) in their structure. The conditions required for polycondensation of (R)-3-hydroxybutyric acid make it prone to produce side reactions, such as elimination/termination reaction, giving rise to crotonization which entails the formation of crotonate end groups (Kobayashi et al., 1993; Ono et al., 2023). Self-polycondensation presents an important advantage in comparison with conventional condensation (from dicarboxylic acids and diols) since it does not confer the need to strictly control the stoichiometry and what is more, it allows obtaining high M_w polymers.

2.1.2 Ring-Opening-Polymerisation (ROP) (chain-growth polycondensation)

ROP, which is considered a simple method to obtain polyesters, is of utmost interest since its paramount advantage is related to its ability to obtain polymers with not only narrow polydispersity, but also both high number and weight-average molecular values. Regarding the availability of target monomers, ROP of 4-membered β-butyrolactone (β-BL) is the most convenient and promising approach toward PHB synthesis. The ROP mechanism of β-BL results in a retention of stereochemistry by scission of the O-acyl bond and chiral conversion or racemization by scission of the O-alkyl bond. Nevertheless, the use of enantiopure monomers for producing isotactic PHB is not cost-efficient hampering its industrial scale (Tang and Chen, 2018; Tang et al., 2019). A study conducted by Bruckmoser and coworkers shows the stereocontrol of the catalytic system *in situ* generated consisting of Ys $[N(SiHMe_2)_2]_3$ $(THF)_2(Y)$ and a salan-type pro-ligands, which allows the production of PHB with high productivity and isoselectivity (Figure 2) (Bruckmoser et al., 2023).

Moreover, the ROP of the racemic BL has been studied using aluminoxane (AlR_3/H_2O) catalyst, generating a mixture of different tacticities (Zintl et al., 2008). Other catalysts, such as salan-ligand rare-earth metal amide complexes and lanthanum aminobisphenolate, have been reported as initiators for the ROP of rac-BL, which results in switching from iso-tactic to syndio-tactic polymerization (Zhuo et al., 2018; Dong and Robinson, 2020). The production of an isotactic PHB via ROP of 8-membered diolide monomers has also been reported including the description of how by varying starting ratios of chiral racemic and achiral meso diastereomers enable the direct polymerization into stereosequenced crystalline PHA with isotactic and syndiotactic stereodiblock microstructures which enhance ductility and toughness. Besides, it has also been described how the design of unsymmetrical disubstituted eight-membered diolides (*rac*-$8DL^{R1\text{-}R2}$) and their stereoselective ROP with discrete chiral catalysts enable the synthesis of alternating isotactic PHA (Figure 3), specifically poly-(3-hydroxybutyrate-*alt*-3-hydroxyvalerate) (*alt*-P3HBV) and poly-(3-hydroxybutyrate-*alt*-hydroxyheptanoate) (*alt*-P3HBHp) (Tang et al., 2019).

More recently, the synthesis of enantiomeric (R)-*alt*-P3HBV and (S)-*alt*-P3HBV by ROP of *rac*-$8DL^{Me\text{-}Et}$ with an enhanced T_m has been described (Zhang et al., 2022). What is more, a recent report exhibits the potential of synthesizing unexplored

FIGURE 2
ROP of 4-membered butyrolactone (adapted from Dhaini et al., 2023).

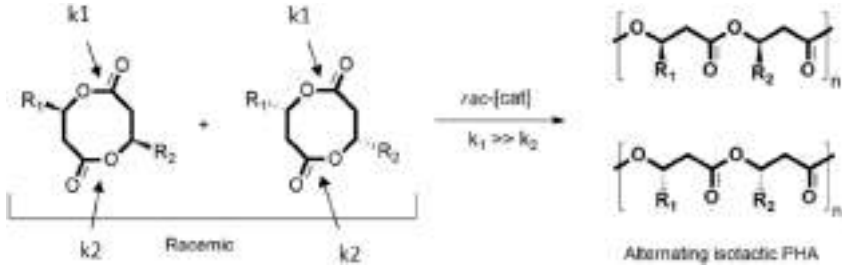

FIGURE 3
Alternating isotactic PHAs using the same diolide, rac-8DL (adapted from Zhang et al., 2022).

triblock copolymers (tri-BCPs) of PHA. Since hard-soft-hard ABA tri-blocks combine the elasticity of elastomers and the thermal processability of plastics, the resulting all-PHA tri-block shows different thermal properties, polydispersity, and crystallinity. Based on the composition of the soft B block, which is associated with R substituents of the ring, these properties are tuned (Westlie et al., 2023).

2.2 Biological synthesis of PHAs

Although the chemical synthesis *in vitro* of PHAs is currently possible and efficient, those biopolymers were first discovered and described as biomolecules produced by several microbial species. Specifically, it was first described from *B. megaterium* cells (Doudoroff and Stanier, 1959). Since that date, the number and diversity of microorganisms (belonging to the three domains of life) reported as PHAs-producers is significant. Most of them accumulate PHAs in their cytoplasm as granules at a high productivity rate. The cells produce and accumulate PHAs within the cells as a subcellular energy source and these biopolymers are usually produced when the microorganisms showing this metabolic capability are cultured with nutrient-limiting concentrations of nitrogen, phosphorus, sulphur, or oxygen and excess carbon sources (Cánovas et al., 2021; Simó-Cabrera et al., 2021). The most produced PHAs by microbes are polyhydroxybutyrate (PHB) although the most marketed is PHBV due to its physicochemical characteristics.

In the Bacteria domain, *C. necator* (Aramvash et al., 2016; Aramvash et al., 2018; Flores-Sánchez et al., 2017; Dalsasso et al., 2019; Novackova et al., 2019; Zhang et al., 2015) and *B. megaterium* are the better characterised bacterial species in terms of PHAs production (Blunt et al., 2023; Patil et al., 2024; Rivas-Castillo et al., 2024). Regarding yeast, it is worth mentioning that this is a group of organisms from which more literature has been reported related to PHAs production apart from bacteria (Kurian and Das, 2021). For example, *Yarrowia lipolytica* is a yeast able to accumulate until 25% (w/w) of mcl-PHA (Rigouin et al., 2019), while *Wickerhamomyces anomalus* cells could reach a production of 19.5 g/L. However, yeasts are not as efficient as bacteria in the yield of PHA production (Kurian and Das, 2021). In the case of algae, several cyanobacteria and green unicellular algae are highlighted as PHA producers. *Spirulina plantesis* can produce up to 10% (w/w) of PHA under the stress experienced by the presence of acetate and CO_2 in the medium (Toh et al., 2008). In the case of *Chrollella* sp. PHAs production of around 27% (w/w) has also been reported. Finally, in the Archaea domain, haloarchaea are the most promising microorganisms showing metabolic capabilities for PHA. There are advantages to unnecessary strict sterile conditions because of the high salt concentrations of the media where these species are grown (thus avoiding the growth of other microbial strains) (Koller, 2019; Pacholak et al., 2021; Simó-Cabrera et al., 2021). Their metabolism is versatile thanks to molecular adaptations to extreme conditions in terms of environmental parameters or nutrient availability. Additionally, they produce other biomolecules with high biotechnological interest, like carotenoids (Flemming, 2016; Giani et al., 2019; Giani et al., 2022; Giani et al., 2021; Pfeifer et al., 2021) and exopolysaccharides (Flemming, 2016; Costa et al., 2018; Blackburn and Green, 2022). Besides, some haloarchaea can be used in the bioremediation processes to remove heavy metals and inorganic anions from brines and salty water (Oren, 2010; Nájera-Fernández et al., 2012; Martínez-Espinosa et al., 2015; Hou and Cui, 2018; Zuo et al., 2018; Pacholak et al., 2021; Pfeifer et al., 2021; Martínez et al., 2022; Priya et al., 2022; Moopantakath et al., 2023). Within haloarchaea, the most promising microorganism is *Haloferax mediterranei* (Pfeifer et al., 2021; Hagagy et al., 2022; Costa et al., 2023; Diankristanti et al., 2023), because it can synthesize PHBV without using any HV precursor (as required by other microorganisms) and can use different carbon sources to accomplish it (Tan et al., 2014b; Han et al., 2015; Parroquin-Gonzalez and Winterburn, 2023).

The metabolic pathways that allow the production of PHA are diverse if they are analyzed and compared in all groups of microorganisms, but essentially, all of them share the following reactions. The main building block required for their synthesis is acetyl-CoA. In principle, all the reactions and molecules involved in the production of acetyl-CoA can be considered the starting reactions required to produce PHAs. Thus, the Wood-Ljungdal pathway, Calvin–Benson–Bassham (CBB) cycle, Serine cycle, Ribulose monophosphate (RuMP) cycle, carbohydrates and fatty acids are involved in the PHA synthesis (Goswami et al., 2023; Banu et al., 2021; Salem et al., 2021). Once the microorganism has enough free acetyl-CoA within the cytoplasm, the enzyme 3-Ketothiolase (PhaA) bonds two units of acetyl-CoA, making acetoacetyl-CoA (AcAc-CoA)). Then, NADPH-linked acetoacetyl-CoA reductase (PhaB) reduces AcAc-CoA into 3-hydroxybutyryl-CoA (3HB-CoA). Finally, PHA synthase (PhaC) polymerises the molecules of 3HB-CoA into short-chain length PHA (Aghaali and Naghavi, 2023; Rodriguez-Perez et al., 2018; Banu et al., 2021; Han et al., 2010; Salem et al., 2021; Shahid et al., 2021; Zher Neoh et al., 2022). PhaC is also the enzyme needed to produce the medium-chain length, although the metabolic pathways promoting this reaction are the fatty acids β-oxidation and *de novo* fatty acids synthesis (Aghaali and Naghavi, 2023; Rodriguez-Perez et al., 2018; Banu et al., 2021; Salem et al., 2021; Shahid et al., 2021; Zhang et al., 2015; Zher Neoh et al., 2022).

3 Operational conditions for biological PHBV synthesis

In the last years, several studies focused on enhancing PHBV production while decreasing its economic costs (Tebaldi et al., 2019), since the high sale price of this copolymer entails a substantial limitation (Yu et al., 2006). It is necessary to address different strategies to reduce the competitiveness gap between PHBV and traditional plastics given the expenses derived from production costs regarding equipment, raw materials and substrates purchase, waste treatment or disposal, and operational conditions, among others (Policastro et al., 2021). Approximately estimated production costs of PHBV range from 1.50 to 10 $/Kg$_{PHA}$ strongly depending on production factors, as well as location and general capital operating costs (Garcia et al., 2011; Hermann-Krauss et al., 2013; Bhattacharyya et al., 2015). The addition of 3HV precursors significantly increases the hydroxyvalerate fraction in the copolymer and the PHBV production by several species, but due to its high costs, wild-producing species can be considered. Metabolic engineering also involves an interesting approach when improving PHBV production, as well as the use of mixed cultures, extremophile species, the utilization of organic waste as a substrate or the modification of abiotic factors (Policastro et al., 2021).

As previously mentioned, running out of an essential nutrient, such as nitrogen or phosphorous, with an excess of carbon are the nutritionally unbalanced conditions that induce the synthesis of PHBV. The biological production of this polymer is also affected by several operational parameters, such as temperature, pH, dissolved oxygen concentration and process regime. When Gram-negative bacteria produce these polymers, they can contain high levels of endotoxins that need to be removed in the purification process (Rivera-Briso et al., 2018). On the other hand, PHBV production by Gram-positive bacteria entails a higher economic cost due to difficulties in the purifying steps (Guo et al., 2013).

Nowadays the main goal is to identify microorganisms able to produce PHBV avoiding the use of precursors, and showing high growth rates, metabolic versatility (to use different sources of nutrients including wastes of different industrial processes), and genetic stability. Some extremophilic microbial strains have been recently revealed as good candidates to be used as cellular factories to produce PHAs, because they fit all these requirements. In the case of halophilic bacteria and archaea, the high salt concentrations favouring their growth avoid the growth of other microorganisms in non-sterile conditions, thus significantly reducing process costs. It is possible to design a production process operating at low energy requirements, modifying the culture composition deriving the carbon flux towards PHBV accumulation, supplementing with nitrogen and phosphate sources, and using optimal temperatures (Koller et al., 2015). For example, the haloarchaeon *H. mediterranei* has been used for the conversion of different organic waste materials, as follows: whey (Koller et al., 2008; Pais et al., 2016); raw glycerol (Hermann-Krauss et al., 2013); cornstarch (Chen et al., 2006; Huang et al., 2006); vinasse and stillage (Bhattacharyya et al., 2012; Bhattacharyya et al., 2014); rice bran (Huang et al., 2006); crop waste (Alsafadi et al., 2020); silkworm excrement (Cai et al., 2022); or ricotta cheese exhausted whey (Raho et al., 2020). High PHBV amount can be produced even without substrate pre-treatment reducing the production costs (Hermann-Klaus et al., 2013; Bhattacharyya et al., 2014; Alsafadi and Al-Mashaqbeh, 2017). *H. mediterranei* can also produce high molecular weight PHBV from volatile fatty acids (VFA) (Ferre-Guel et al., 2018). Similarly, the purple non-sulphur bacteria (PNSB) *Rhodospirillum rubrum* can also synthesize PHBV when fed with sugars and wastes without the need for a precursor supply (Smith et al., 2008; Liu et al., 2019). Godoy and coworkers boosted this species of PHBV producer by performing an aerobic-anaerobic transition (Godoy et al., 2023). Other species such as *B. cereus FA11* and *Bacillus flexus* can produce PHBV from glucose (Masood et al., 2012; Wagle et al., 2019). *B. cereus* can also produce PHBV using wheat starch wastewater (Sinaei et al., 2021). Pure cultures of obligate methanotrophs can convert the two primary components of natural gas (methane and ethane) into PHBV, mitigating methane greenhouse gas impact (Myung et al., 2024). The bacterium *Pseudomonas oleovorans* was found able to use *Jatropha curcas* seed oil as the only carbon source to synthesize PHBV, a renewable substrate that can be cheaply produced at large scale (Allen et al., 2010). Activated sludge also contains a versatile microbiome capable of converting waste into valuable products such as PHBV. Employing a hydrolysate derived from rice straw as substrate, *Corynebacteriaceae* and *Bacillaceae* were found to be the main microorganisms in this microbial population to produce PHBV thriving in repeated feast and famine phases (Morya et al., 2023). The marine bacterium *Rhodovulum sulfidophilum* DSM-1374 can be used as cellular factories enhancing PHBV production under a light-dark cycle using lactate as substrate and with the limitation of N-P nutrients (Carlozzi et al., 2022).

Apart from the natural metabolic capabilities, metabolic engineering is a strategy used to produce PHBV in recombinant strains or to increase its production. Since the supplementation of precursors is associated with high costs, this strategy can

be considered to biosynthesize this copolymer. *Escherichia coli-engineered* strains can produce PHBV from unrelated carbon sources such as glucose and glycerol using enzymes involved in the 3HV biosynthetic pathway (Miscevic et al., 2019). The recombinant strain of *Corynebacterium glutamicum* in which the *phaCAB* gene cluster was inserted, produced high PHBV concentrations from glucose with high 3HV fraction (Ma et al., 2018). A recombinant *Halomonas TD01* species was generated by overexpressing the threonine synthesis pathway and threonine dehydrogenase, producing PHBV using carbohydrates as the sole carbon source (Tan et al., 2014a). Choi and coworkers studied a threonine overproducing mutant of *Alcaligenes* sp. *SH-69* able to increase the production of PHBV up to 6 times compared with the wild type, using glucose as substrate (Choi et al., 2023). *Halomonas bluephagensis* tricarboxylic acid (TCA) cycle was engineered via CRISPR/Cas9 producing PHBV with different 3HV fractions (0–25 mol%) using glucose as a carbon source (Chen et al., 2019). Yang and coworkers demonstrated that the mutant strain *H. mediterranei* Δ123 with a replication origin deletion produced a higher amount of PHBV, providing an advance in the production of bio-based chemicals by modulating chromosome replication (Yang et al., 2022). This biopolymer production was also optimised in *H. mediterranei* via CRISPRi-mediated redirection of the carbon flux. Specifically, plasmid-mediated CRISPRi downregulation of the citrate synthase genes (*citZ* and *lgtA*) improved the PHBV by 76.4% (Lin et al., 2021). The chromosomal expression system also showed advantages in PHBV production by *Escherichia coli* and the mutant *Halomonas* TD08 compared to the plasmid expression system (Yin et al., 2015).

As mentioned before, the addition of 3HV precursors is a strategy that implies an economical cost, but several species are only capable of producing PHBV when specific precursors are available. These compounds are also used to modify the polymer properties adjusting the 3HV fraction. Valeric acid and propionic acid have been the most widely studied precursors. *Photobacterium* sp. TLY01, a novel strain in this genus, could effectively utilize a series of sustainable substrates including plant oils, glycerol, and volatile fatty acids when propionate or valerate were added as the secondary carbon sources to produce PHBV (Tian et al., 2022). *Bacillus megaterium* could synthesize PHBV using cheese whey permeate, a processed by-product of the dairy industry, adding propionic acid and optimizing other parameters such as time or C/N ratio (Suhazsini et al., 2020). The addition of propionic acid and valeric acid was also studied in a thermophilic mixed methane-utilizing culture resulting in the production of PHBV with higher 3HV values (Luangthongkam et al., 2019). The addition of valerate with various combinations of methane was also tested in a methanotrophic consortium showing the same positive results (Myung et al., 2015). *Caldimonas taiwanensis* was also able to produce PHBV in a sugar-rich medium supplemented with different concentrations of valerate increasing the 3HV fraction up to 90% (Sheu et al., 2009). Choi and coworkers performed a comparison between the utilisation of propionic acid, valeric acid, and levulinic acid using the bacterium *Alcaligens* SH 69 with glucose as substrate, showing an increase of 3HV in all cases, with levulinic acid producing the highest value (Choi et al., 2023). The modulation of 3HV monomer is not only important for the quality of PHBV produced but also for enhancing the quality of the final product (Koller et al., 2008; Zinn et al., 2023). For this purpose, the addition of precursors can be combined with engineered strains, as well as other strategies, to obtain optimal results. *phaCp* and *phaABp* genes from *Propylenella binnzhouense* have been cloned in *E. coli* to produce PHBV, improving its synthesis activity and polymer performance at higher processing temperatures (Meng et al., 2022). *Rhodoligotrophos defluvii* was proved to be able to synthesize PHBV in the presence of propionate through the successful construction of a PHA synthesis pathway in recombinant *E. coli* (Miao et al., 2021).

PHA extraction is also a crucial aspect in terms of economic approach. The whole PHA production life cycle requires high energy consumption, especially during the downstream operations, which can represent half of the total cost (Del Oso et al., 2021). Isolation and purification of the polymers must be carried out by efficient methods in order to reduce not only costs, but also the environmental impact of the process. These methods can be mechanical, chemical, or biological (Zhang et al., 2024).

Mechanical methods minimize environmental pollution since they do not involve any chemicals (Jacquel et al., 2008). Among them, bead mill disruption requires less power supply when compared to other techniques and is easy to escalate but involves a large number of steps (Tamer et al., 1998); high pressure homogenization (HPH) is also easily scalable, less contaminant and does not require pre-treatment, but the energy consumption is high and PHA can be micronized; sonication presents low costs and is environmentally friendly (Divyashree et al., 2009), but must be combined with other methods as well as gamma irradiation, which can improve polymer properties (Zhang et al., 2024).

Chemical methods include solvent extraction, the most extended one due to its simplicity and rapidity, offers high purity levels and low levels of endotoxin content, but it is not suitable for large scale processing, presents high costs and most of the used solvents are harmful to the environment and human health (Kunasundari and Sudesh, 2011). Both halogenated and non-halogenated solvents offer a high recovery yield and purity, but the first one is toxic to the environment, while the latter has an elevated cost (Zhang et al., 2024). Aqueous two phase systems (ATPS) is a technique in which two immiscible phases (polymers and/or inorganic salts) coexist (Yang et al., 2008), presenting low toxicity, energy consumption and material cost, as well as good resolution and large operating capacity, but it has low reproducibility and the mechanism is not well known; surfactants are also widely used for PHA recovery as they allow a direct extraction of the polymer but the cost is elevated and the removal of SDS from the isolated polymer is challenging (Zhang et al., 2024); supercritical fluids (SCF) emerged as a technique for PHA recovery due to its advantages such as low toxicity, when compared to others, and low cost (Khosravi-Darani and Mozafari, 2009), but the process requires strict parameters, extraction of polar analytes and frequent cleaning up (Kunasundari and Sudesh, 2011); sodium hypochlorite is used as a method to extract PHA based on the solubilization of non-PHA cellular mass (NPCM) for which cell drying is not necessary, presenting strong oxidizing properties although the molecular weight of the extracted polymer is reduced (Yu and Chen, 2006). NPCM solubilization can also be carried out using acids, also resulting in a low molecular weight (Zhang et al., 2024).

Biological extraction methods are being studied focusing on the economic aspect of these technologies, as well as their

environmental impact, in order to reach scalability goals of industrial biotechnology (Mannina et al., 2019). Enzymatic digestion is a method in which NPCM is solubilized with a high PHA recovery yield, purity and low toxicity, but it involves a complex procedure, and enzymes entail a high cost (Kapritchkoff et al., 2006); cell fragility provides an efficient polymer release obtaining a high yield and purity without harming the environment, but genetically engineered strains are required (Zhang et al., 2024); predatory bacteria can also be used to extract the biopolymers, as they can attack Gram-negative bacteria by using their cytoplasmic contents as nutrients. This method is economical and requires almost no chemical reagents, but PHA may be degraded (Im et al., 2018); the construction of genetically engineered strains with a PHA extracellular secretion system is another method that, despite the complexity of the process, needs no chemicals, does not harm the environment and offers a continuous production of the polymer (Zhang et al., 2024).

Despite all the environmental advantages of the PHBV copolymer, its wide production and utilization are limited by the high production and downstream process costs, as well as the low productivity rate. Therein lies the importance of implementing new efficient, low-cost PHBV production processes considering techno-economic factors. The evaluation of all the different mentioned strategies, along with their possible combinations and operational conditions are crucial to enhance the economic competitiveness of PHBV compared to petroleum-based plastics.

4 Valorisation of agri-food waste for PHBV production

In the European Union (EU), between 118 and 138 million tonnes of biowaste are generated annually, of which over 58 million tonnes are food waste (131 kg/inhabitant) with associated costs estimated at €143 billion (Santiago et al., 2023). Within the food value chain, it is estimated that consumption generates 52% of food waste, followed by production (23%), handling and storage (11%), distribution and sale (9%) and processing (5%). Of the total food waste generated globally, the beverage industry accounts for 26%, followed by the dairy and ice cream industry (21.3%), fruit and vegetable production and preservation (14.8%), the manufacture of grain and starch products (12.9%), and meat production, processing, and conservation (8%) (Capanoglu, et al., 2022; Santiago et al., 2023).

Most of the wastes generated are seeds, leaves, roots, tubers, fruits, and pomace, among others. Unfortunately, despite retaining a high nutrient content in carbohydrates (30%–60%), lipids (15%–40%) and proteins (5%–20%), most food waste is considered a disposal problem and about 40% is currently being landfilled or incinerated. Intending to reduce the environmental impact of food production and reducing food waste, the EU has adopted The Circular Economy Action Plan (European Commission, 2020), which promotes the circularity of food waste/by-products by adopting strategies to close the loop in industrial production systems. In this sense, the biotechnological processes play a fundamental role in implementing circular economy strategies in key industries, such as the agri-food industry, through the valorisation of organic waste or by-products to obtain value-added products that can be later launched on the market.

Agri-food wastes are rich in lignocellulose, which can be hydrolysed to obtain fermentable sugars from the cellulose and hemicellulose fractions. Therefore, these fermentable sugars can replace the pure derived sugars as substrates in the fermentation process, such as the PHBV production, promoting the sustainability and circularity of this waste stream (González-Rojo and Díez-Antolínez, 2023). The carbon substrate in PHBV production contributes to about 40%–50% (Pérez et al., 2020) of the total cost of the process, increasing the price of PHAs by six times (Del Oso et al., 2021) compared to fossil-based plastics. The high cost of PHA production reduces the market applications to those with high-added value, such as biodegradable scaffolds for biomedical applications. Therefore, substituting commercial carbon sources with agri-food wastes would reduce the overall cost of PHBV production, which is the primary constraint to industrial scale-up, and the production will be affordable for many different applications, such as packaging. In this way, the process will be moved towards a biorefinery scenario using abundant and inexpensive materials, also considering the utilisation of waste-derived hydroxyvalerate precursors.

Life cycle assessment (LCA) is a systematic methodology to quantify the potential environmental impacts of a product system throughout its life cycle. LCA encompasses the complete life cycle of a product, process, or activity, comprising: the extraction and refinement of raw materials; manufacturing, transportation, and distribution; utilization, reutilization, and preservation; and recycling and disposal (Banerjee and Ray, 2022). They can be performed with different boundaries, which outcome in dissimilar calculations (Pawelzik et al., 2013). International LCA standards, like ISO 14040 and EB 16760, help guide the structure, conduct, limitations and assumptions for the general LCAs, although they are heterogeneous because of the different approaches and assumptions (Yates and Barlow, 2013; Spierling et al., 2018; Walker and Rothman, 2020).

The majority of LCA research has identified PHA downstream processing as the primary focus area. Studies often used either a cradle-to-gate or gate-to-gate methodology, overlooking the final stages of the bioplastic life cycle, including shaping, compounding, usage, and end-of-life (EoL) (Del Oso et al., 2023). Although PHA usage in food packaging typically imposes minimal burdens on life cycle impacts, given its limited energy consumption and environmental emissions (Nessi et al., 2021), disregarding the EoL phase fails to recognize the benefits associated with PHA's biodegradability and overlooks the lasting environmental consequences of traditional plastic pollution in marine ecosystems (Roibás-Rozas et al., 2022).

In a 2010 study, Tabone et al. (2010) compared twelve polymers of fossil and biological origin, including PHA derived from corn stalks and corn grain. This study stands out for its use of a wide range of ecological indicators, such as acidification, carcinogens, eutrophication, eco-toxicity, GWP, ozone depletion, and fossil fuel depletion. While bio-based materials generally outperform fossil-based ones in GWP, they exhibit similar or even worse performance in other impact categories. PHA from corn grain, for instance, ranks highest in acidification and ozone depletion potential, comparable to or worse than polyethylene, polycarbonates, and polypropylene

in eco-toxicity. However, many of these impacts are attributed to the agricultural production of raw materials. Similarly, a study by Kim and Dale (2008), analyzing the energy and GWP profiles of polyhydroxybutyrate (PHB) from corn grain, found PHB to have lower ecological impacts than fossil-based polymers. They suggest that implementing sustainable practices in corn cultivation, such as no-tillage and winter cover, could further reduce PHB's production impact by up to 72% (Kim and Dale, 2008).

PHA's recent LCA studies have been compared to harmonized product environmental footprint (PEF) (Cristóbal et al., 2016). The amount of kg CO_2/kg PHA in these studies ranged from 2.3–6.9 which compared to 2.72 with sugarcane feedstock and to 4.26 for corn starch in the PEF. In the cases where the value of kg CO_2/kg PHA is under 0.49 carbon storage in the polymer was counted as carbon sequestration from the atmospheric CO_2. In the cases in which the polymer temporarily captures atmospheric CO_2, forming its chains, kg CO_2-eq/kg PHA values lower than 0.49 were obtained (Dietrich et al., 2017).

Many researchers have investigated the valorisation of agri-food waste for PHBV production using *C. necator*, which was the first microorganism used for industrial PHA production by Imperial Chemical Industries (Wang et al., 2019). *C. necator* is a well-known PHB producer from a wide variety of carbon sources. In addition, with short-chain volatile fatty acids obtained from the degradation of organic waste, such as acetic acid, valeric acid, propionic acid or butyric acid, *C. necator* is also able to produce PHBV. In this way, Hathi and coworkers studied the production of PHBV by *C. necator* valorising food waste hydrolysate and VFAs as PHBV inducers (Hathi et al., 2022). They showed that the fermentations combining food waste hydrolysate with valeric acid produced the highest yield of PHBV (34.12%) per total amount of biomass and the highest molar 3HV content (50%) (Hathi et al., 2022). The use of food waste derived VFAs as the sole carbon source for PHBV batch production was also analysed reporting a yield of 1.02 g/L PHAs (Vu et al., 2022). *C. necator* has also been tested for PHBV production through the valorisation of waste rapeseed oil adding selected precursors of 3-HV, such as propanol, propionate and valerate, allowing the production of PHA (g/L) of 11.7, 7.3 and 6.5, respectively, with a 3-HV (%) content of 9, 13 and 18 (Obruca et al., 2010).

C. taiwanensis has also been reported as a PHBV producer. It is a thermophilic microorganism capable of accumulating PHBV granules directly from starch by modulating the 3-HV content with the addition of valerate as a mixed carbon source (Sheu et al., 2009). The authors tested different starches from cassava, corn, potato, sweet potato, and wheat, combined with 0.05% valerate, and the highest PHBV accumulation was found in the corn and cassava starch experiments, which gave a yield of 2.145 and 1.876 g/L PHBV with a 3-HV content of 10% and 13%, respectively. Koller and coworkers studied the use of whey permeate from the dairy industry for PHB synthesis combined with levulinic acid and sodium valerate from renewable resources for 3-HV synthesis in *Hydrogenophaga pseudoflava* fermentation, reporting production of 4.4 g/L with molar 3-HV content of 55% using levulinic acid and valerate as precursors and 2.2 g/L PHBV with 45% of 3-HV only using levulinic acid (Koller et al., 2017).

However, the use of microorganisms which do not require 3-HV precursors is advisable to reduce production costs and control to keep their concentration in non-inhibiting thresholds. Within this group, it is worth mentioning some halophilic microorganisms, which can produce PHBV from waste and without the contribution of external precursors and have been widely studied in the literature. For example, starch and cassava waste were tested for PHBV production by *Halogeometricum borinquense* fermentation reaching a maximum yield of 4.6 g/L and 1.52 g/L with 13.11% and 19.65% 3-HV units, respectively (Salgaonkar et al., 2018). The potential of *Halomonas alkaliantarctica* to produce PHAs from bioproducts generated by cheese manufacturing has also been described finding that this microorganism could produce PHBV and PHB polymers utilising cheese whey and cheese whey mother liquor as the only carbon sources, obtaining the highest yield of 0.42 g/L using cheese whey mother liquors (Mozejko-Ciesielska et al., 2023).

In this context, *H. mediterranei* is the better-studied and most promising halophilic microorganism to produce PHBV. Table 1 summarises the most relevant works on the production of PHBV by *H. mediterranei* using wastes from different industries. By comparing these works, the main conclusion is that the batch cultivation mode is the most used. In this type of process, all media are added to the bioreactor at the beginning of the process and there are no inlet or outlet streams. The fermentation process is running until the desired cell stage or product concentration is reached (Policastro et al., 2021). The highest production described in the literature is 77.8 g PHBV/L with a fed-batch fermentation, thus indicating that this methodology for PHBV production usually produces more PHBV than batch fermentations (Huang et al., 2006). This comparison has been described by Alsafadi and coworkers: PHBV production was monitored using the same carbon waste (date waste extract) in a fed-batch fermentation against a batch fermentation (Alsafadi et al., 2020). In the first one, the production reached 4.5 g PHBV/L, while with the batch fermentation a final production of 3.20 ± 0.07 g PHBV/L was quantified (Alsafadi et al., 2020). Regardless, the batch fermentation type also has high production rates, reaching 19.7 g PHBV/L, 17.4 g PHBV/L (Bhattacharyya et al., 2012), 16.42 ± 0.02 g PHBV/L (Bhattacharyya et al., 2015) and 13.12 ± 0.05 g PHBV/L (Bhattacharyya et al., 2014). Consequently, a fed-batch fermentation increases the PHBV production potential of a carbon source with already potential, increasing its yields.

As can be seen in Table 1, none of the PHBVs obtained present an HV concentration higher than 20%, which provides properties similar to polypropylene (one of the most marketed polymers) (Lee et al., 2023). Even if the polymer is produced with an HV precursor, *H. mediterranei* cannot achieve this copolymer concentration based on the information reported in the bibliography. However, in some cases, the concentration of 20% HV is almost attained (Bhattacharyya et al., 2014; Bhattacharyya et al., 2015; Alsafadi et al., 2020; Priya et al., 2022; Wang et al., 2022).

5 End-of-life of polyhydroxyalkanoates

Unquestionably, the disposal of biodegradable bioplastics must be considered, setting the basis for developing an optimal end-of-life pathway in terms of maximizing the circular economy. An ideal strategy to address the end-of-life key challenges of plastics is to develop next-generation polymers with closed-loop

TABLE 1 PHBV produced by *H. mediterranei* using wastes as a carbon source.

Waste	CDW (g/L)	PHBV (g/L)	HV (%)	References
Hydrolysed rapeseed meal	11.085 ± 2.478	0.512 ± 0.164	10.000 ± 0.007	Khamplod et al. (2023)
Stillage from rice-based ethanol	20.825[a]	13.12 ± 0.05	17.9	Bhattacharyya et al. (2015)
Stillage from rice-based ethanol	23.127[a]	16.42 ± 0.02	15.4	Bhattacharyya et al. (2014)
Whey lactose	11[a]	5.5	8–10	Koller et al. (2008)
Whey lactose	14.795[a]	10.8	6	Koller et al. (2008)
Extruded cornstarch and extruded rice bran	140.0[bc]	77.8[c]		Huang et al. (2006)
Extruded cornstarch and extruded rice bran	131.0 [bc]	52.7[c]		Huang et al. (2006)
Extruded cornstarch and native wheat bran	68.4 [bc]	28.0[c]		Huang et al. (2006)
Extruded cornstarch	62.6 [bc]	24.2[c]		Huang et al. (2006)
Extruded cornstarch	62.6[b]	24.2		Huang et al. (2006)
Ricotta cheese exhausted whey	18.32 ± 0.15	1.27 ± 0.09		Raho et al. (2020)
Wasted bread and seawater	6.37 ± 0.92	1.187 ± 0.198	12.95 ± 0.04	Montemurro et al. (2022)
Date waste extract	12.8 ± 0.8	3.20 ± 0.07		Alsafadi et al. (2020)
Date waste extract	18[c]	4.5[c]	18	Alsafadi et al. (2020)
Food waste (meat, noodles, rice, and vegetables) hydrolysed	2.59	1.469[a]	18.55[d]	Alsafadi and Al-Mashaqbeh (2017)
Olive mill wastewater	10	0.2	6.5	Alsafadi and Al-Mashaqbeh (2017)
Starch	7.01 ± 0.35	1.74 ± 0.04	13.37 ± 0.73	Lu et al. (2008)
25% pre-treated vinasse	28.143[a]	19.7	12.6	Bhattacharyya et al. (2012)
50% pre-treated vinasse	26.364[a]	17.4	14.09	Bhattacharyya et al. (2012)
CGP from tallow-base biodiesel		5.76	10	(Hermann-Krauss et al., 2013)
Fermented food waste feedstock collected from READ	7.0 ± 0.7	4.5 ± 0.2	17.1 ± 0.8[d]	Wang et al. (2022)
Food waste derived	0.6–3.0	0.41–0.54 (g/g)[b]	6.1–10.7[d]	Wang and Zhang (2021)
Seaweed hydrolysates	3.90 ± 0.25	2.08 ± 0.34	10.6	Ghosh et al. (2019); Ghosh et al. (2021); Ghosh et al. (2022)

[a]Data calculated with paper information.
[b]Maximum value obtained.
[c]Fed-batch process.
[d]Culture medium presents HV, precursor.

life cycles. Currently, there are no recycling strategies commercially implemented to give biodegradable bioplastics a second life, since only biodegradation is seen as a suitable end-on-life option for these bioplastics (Fredi and Dorigato, 2021; Renard et al., 2004). Nevertheless, the fact that bioplastics are biodegradable does not necessarily imply that they can degrade in all environments (domestic and industrial compost, soil, marine and freshwater). Moreover, existing standards (Silva et al., 2023) that regulate certifications of biodegradation in open environments exhibit an obvious weakness related to their poor reproducibility since several factors (such as biodiversity, soil, pH, water content and temperature) can affect the reproducibility of the results, causing the alteration of biodegradation. Most commercial biodegradable bioplastics comprise other substances, such as additives, adhesives, and coatings, which makes biodegradation challenging since initially its definition only considers the organic components of the plastics. Therefore, all the above-mentioned brings to light the need to develop real recycling routes, including for biodegradable bioplastics, to close the loop and thus fostering the main principles of Plastics Strategy regulation (Plastics Strategy, 2022).

Regarding bioplastics, selective collection has not yet been implemented in Europe, so it comes as no surprise that, at the end-of-life of biobased biodegradable plastics, it usually ends up landfilled together with organic residues, leading to the loss of high-quality materials. Hence, to efficiently involve these materials in the Circular Economy, it is crucial to develop feasible recycling routes. Up to now, very few papers have been reported to evaluate the performance of different recycling pathways of PHAs, but the main strategies involved can be summarised as follows: i) primary recycling, considered as mechanical recycling to obtain similar properties compared to initial products; ii) secondary recycling, or mechanical recycling thereby products of a lower quality are obtained; iii) tertiary recycling, chemical recycling or raw material recovery, that commonly requires depolymerisation to the constituent monomers; iv) quaternary recycling or energy recovery (Hahladakis et al., 2018).

5.1 Mechanical recycling

Mechanical recycling is the most extended recycling pathway, considering its use for post-consumer and post-industrial plastics. It consists mainly of several cycles of extrusion or injection moulding, where the main aim is to preserve the original value of mechanical properties without modifying chemical structure. Indeed, it is the most developed alternative for plastics recycling, independently of its origin. Typical mechanical recycling process involves several steps such as cleaning, sorting, melting, shredding and finally, the re-extrusion process into recycled plastic pellets. If the final output is sensitive to be used in food-contact grade applications, an additional step of decontamination is required (Das et al., 2021). To be treated by mechanical recycling, the following conditions must be accomplished: i) a low degree of degradation; ii) efficient and suitable previous separation of PHAs from other types of plastics (such as fossil-based ones), for which selective collection is essential; iii) free from impurities, materials or particles that may interfere with the process or the final characteristics of the quality product and collection of sufficient quantities to achieve industrial, and iv) economic viability of the process.

Due to the high production costs and the poor mechanical properties, PHB is usually blended with other polymers to enhance their properties, which explains the few reported publications associated with pure PHAs reprocessing. In 2017, the recyclability of PHB was evaluated through multiple processing cycles, and after each cycle, mechanical and thermal properties were assessed. These properties were drastically decreased after two processing cycles, including the increase in the degree of crystallinity owing to the chemical crystallisation by chain scission (Rivas et al., 2017). Some authors (El-Taweel et al., 2004) noticed the interdependence between crystallization degree of PHB and tensile strength values so it is no wonder that the more cycles the worse mechanical properties. Four commercial grades of PHAs from different manufacturers were investigated via injection moulding (Corre et al., 2012) where the results showed a decrease in the molecular weight and dispersity value for all the PHAs studied. As a consequence, the degradation mechanism that could be expected is driving by a random process such as free radical mechanism. Several studies (Moraczewski et al., 2015) (Main et al., 2023) support the fact that the melt flow index (MFI) of virgin PHB increases with each cycle of reprocessing, resulting in a molecular weight decrease. Regarding mechanical properties, it was noticed that after five cycles of extrusion, virgin PHB becomes progressively brittle with a reduction of 50% in impact strength. Even if the results were not promising, other studies pointed out that mechanically recycled and degraded PHB could be used as a plasticizer for PLA (Yang et al., 2015). On the other hand, Zaverl evaluated the mechanical recycling of PHBV, which results in tensile and flexural strength decreased after five cycles (Zaverl et al., 2012). Moreover, the recyclability of blends of PHBV and PLA has also been investigated and, in comparison with the pure PHBV, the mixture shows higher mechanical properties (tensile strength, elongation at break and impact strength) stability after 6 cycles of processing (Zembouai et al., 2014).

Nevertheless, mechanical recycling presents the following limitations: i) those plastic materials that are excessively degraded, are not sensitive to be mechanically recycled, because of their poor mechanical and thermal properties; ii) plastic materials mixed or contaminated with other substances are not mechanically recyclable and iii) plastic colour, that hampers recycling since over pigmented plastics materials have a lower value than those which are not pigmented as well as the difficulty to treat pigmented materials which need to be sorted using optical methods.

5.2 Enzymatic recycling

The implementation of enzymes for the controlled degradation of plastics is emerging strongly in the research for new environmentally friendly technologies (Roohi and Kuddus, 2018). The natural biodegradation procedure for bioplastics is as follows: i) depolymerisation, cleavage of the polymer chain to small pieces occurs for better assimilation by microorganisms when polymer chains reach sufficiently low molecular weight (Webb et al., 2000); ii) microorganisms assimilate these small pieces to subsequently transform them in biomass and CO_2. Microbial biodegradation can occur via aerobic or anaerobic; aerobic degradation, in the absence of oxygen, takes place to produce CO_2 and H_2O while in anaerobic degradation, the cleavage happens to obtain CH_4, CO_2 and H_2O (Eubeler et al., 2010). The enzymatic degradation via hydrolysis is a two-step process, where firstly enzyme is tied up to the polymer substrate by catalysing the hydrolytic cleavage so the adherence, as a limiting step, comes before the colonization of the exposed surface (Amobonye et al., 2021; Shan et al., 2008).

PHA degradation has been investigated with different biopolymer blends and different enzymes. Even if numerous investigations have demonstrated its degradation through catalysed basic media, their degradation products have not been yet uniformly reported (Estelle et al., 2004). For instance, the viability of PHB depolymerization by PHA enzyme depolymerase from different sources at 37°C was investigated, obtaining dimers and monomers of 3-hydroxybutyric acid. Depending on the source of PHA depolymerase, such as *P. picketti,* greater yields are achieved for 3HB monomers (Mukrai et al., 1993). Furthermore, the degradation of P (3HB-*co*-4HB) to obtain its pertinent low molecular weight oligomers (5,000–10,000 Da) is also demonstrated through sustainable controlled enzymatic depolymerization using commercial triglyceride lipases, considering that the driving

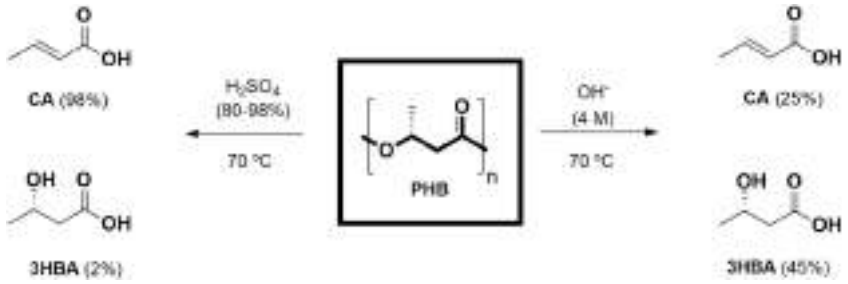

FIGURE 4
Hydrolysis of PHA in different conditions (adapted from Bruckmoser et al., 2023).

factor of the degradation is the enzymatic activity (Rodríguez-Contreras et al., 2012). Various microorganisms have found to be sensitive to produce depolymerase enzymes to degrade PHB and PHBV, among which are include: *Pseudomonas lemoignei* (Jendrossek et al., 1996), *Streptomyces venezuelae* (Santos et al., 2013), *B. megaterium* (Sanchez et al., 2000), *Penicillium oxalicum* strain (Li et al., 2012).

Normally, enzymatic recycling provides a wide range of degradation products with different molecular weights, which makes its subsequent repolymerization difficult since it requires a separation and purification process that would not make the recycling process viable.

5.3 Chemical recycling

It is urgent to develop a recycling closed loop to boost the circular economy, which can overcome current constraints in mechanical recycling. Chemical recycling tends to depolymerize into intermediate products that can be used as building blocks to synthesize the same material or to transform them into new high (upcycling)/low (downcycling) value products (Dhaini et al., 2023).

Most widespread chemical recycling methods consist of i) *solvolysis* and ii) *the application of heat approach*. The expression *solvolysis* includes total and partial depolymerization, which can be achieved by a wide range of solvents to depolymerize up to the pertinent monomers or other degradation products (such as oligomers). *Solvolysis* can be carried out using different approaches: hydrolysis (water-based), alcoholysis (alcohol-based), glycolysis (glycol-based) and lastly, aminolysis (amine-based). Most papers disclosed in this review are focused on hydrolysis, both acid and basic-based (Figure 4).

Concerning basic-based PHB hydrolysis, it is demonstrated that depolymerization rates increased up to 70% when NaOH concentration reached to 4.0 M, leading to crotonate acid (25%) and 3-hydroxybutyric acid (45%) as major degradation products. Nonetheless, concerning acid-based hydrolysis, the exposure of PHB to acid solutions (up to 90wt% H_2SO_4) mainly produces crotonate acid (90%) and, as the minor degradation product, 3-hydroxybutyric acid (2%) (Yu and Marchessault, 2000). Furthermore, recent work confirms the direct dependence of degradation kinetics with temperature as the higher the temperature, the greater the extent of weight loss (Yu et al., 2005). The main advantage that catalytic processes present in comparison with other recycled methods is the notable selectivity towards a specific product (Westhues et al., 2018). Initially, transformations of chemical recycling explored and boosted by catalysis can be classified as follows: (1) ester group hydrolysis and (2) ester group reductive rupture over metallic catalysts. Homogeneous catalysis has been addressed using acid catalysts as well as metallic complexes. It has been reported the depolymerization via a one-pot catalytic hydrogenolysis process of PHB, using Pd/C as catalyst at mild conditions where solvents are not needed (solid-solid reaction), leading to the formation of butyric acid, butanol, butyrate, and gaseous products, whereas a Cu/Zn/Al catalyst showed more selectivity to butyric acid (70%) (Zhang et al., 2021). More recently, the production of 3-hydroxybutyric and crotonate acid has been reported as degradation products when PHB is subject to hydrolysis in acids, bases and carboxylic acid salts conditions and followed by the dehydration and decarboxylation to propylene and CO_2 (Li and Strathmann, 2019). Moreover, Melchiors and coworkers described how depolymerised PHB into its corresponding cyclic trimer [(R,R,R)-4,8,12-trimethyl-1,5,9-trioxacyclododeca-2,6,10-trione, TBL] at 80% yield, in solution using p-toluenesulfonic acid (PTSA) acting as catalyst, and thus the polymerization of TBL through dibutyltin dimethoxide to produce high-quality PHB (Melchiors et al., 1996). Various catalysts (Ru or Re) have been screened for the heterogeneous degradation of PHB, where it was demonstrated that Ru deposited on ceria (CeO_2) exhibits promising yield (up to 79%) to achieve 3-HBA monomer (Lehnertz et al., 2022). Recently, the efficacy of $ZnCl_2$ and polyethylene glycol (PEG) has been demonstrated, as a mixture to catalyse polyesters and polycarbonates depolymerization reactions towards their corresponding rings formation, under vacuum conditions and high ceiling temperature (Tc) (Gallin et al., 2023). What is more, the catalytic system can selectively depolymerize polyesters in the presence of other plastics, thus it shows the effectiveness of different plastic mixture separations.

When it comes to the application of heat, two different techniques can be well defined: i) thermochemical gasification, which mainly produces H_2, CO, H_2O, CH_4 y CO_2, which are accompanied by high-boiling polycyclic aromatic hydrocarbons, and ii) pyrolysis, which describes the thermal decomposition of solid material in absence of oxygen which gives rise to CO, H_2, H_2O, CO_2 and simple hydrocarbons.

Ariffinet proposed, from a biorefinery point of view, a thermal catalyst degradation, where CaO and $Mg(OH)_2$ were used as catalysts to selectively depolymerized towards crotonic and 2-pentenoic acid at lower degradation temperatures (Ariffin et al.,

TABLE 2 Comparison between the different techniques developed for PHAs recycling reported nowadays in the literature.

Recycling type	Key technologies/features	Input	Output	Applications
Mechanical recycling	Extrusion and/or injection molding	High quality (low degradation)	Medium (upcycling) or low (downcycling) quality of PHA recycled	Food grade and non-food grade products
		Low degree of contaminants		Fillers, plasticizers
		Pure streams		
Enzymatic recycling	PHA enzyme depolymerase from different sources (*Pseudomonas lemoignei*, *Streptomyces venezuelae*, *B. megaterium*, *Penicillium oxalicum*)	Pure and blended streams	Wide range of monomers and oligomers	Downcycling applications
		Low degree of impurities		Building blocks
Chemical recycling	Solvolysis (hydrolysis, alcoholysis, glycolysis and aminolysis) and the application of heat (thermochemical gasification and pyrolysis)	Low or high quality	Depending on the type of recycling: solvolysis (oligomers, monomers, cyclic monomers) and the application of heat (pyrolysis oil, aromatic and simple hycrocarbons)	Food grade and non-food grade products
		Low degree of contaminants		Building blocks for high-value products (pharmaceutical and chemical synthesis)
		Pure and blended streams		

2010). In 1996, thermodynamics was investigated to satisfy the viability of different degradation products obtained (Melchiors et al., 1996). Two different pathways were examined: i) solution-based and ii) heat-applied. Heat depolymerization reached higher yields to obtain crotonic acid and linear oligomers from 3-hydroxybutyric acid, aligned with the expected results of esters pyrolysis reactions, independently of the catalyst used.

Interestingly, through solution-based depolymerization, cyclic oligomers are obtained mainly by back-biting mechanism-based reactions. Several parameters, such as catalyst/substrate rates, solvent nature, reaction time and temperature range were proven for both heat and solvent-based depolymerization. In any case, it required aggressive conditions as well as high reaction times and non-secure catalysts (such as *p*-TsOH or CF_3SO_3Me) leading to unsustainable processes which are against the circular economy principles.

5.4 Recycling technologies comparative

The current low recycling rates for plastics have produced an increased interest in developing different recycling strategies. Regarding plastics recycling, independently of its origin, several recycling technologies have been implemented during the last years to deal with the pollution concern. The most developed technologies have been mechanical, chemical and enzymatic techniques, intending to substitute current plastics end-of-life options, which are focused on landfill and energy recovery. In terms of bio-based biodegradable plastics, in particular, for the PHA family, recycling technologies are still at a very early stage, just like it can be seen on the previous sections.

The following table (Table 2) shows a narrow comparison between the different techniques developed for PHAs recycling so far. It must be considered that the technology maturity (Technology Readiness Level, TRL) is poor, which makes the comparison sparse on details.

6 Conclusion

Various studies have demonstrated the feasibility of using different waste streams and microorganisms like *C. necator*, *C. taiwanensis*, *H. pseudoflava*, *H. borinquense*, *H. alkaliantarctica*, and *H. mediterranei* for PHBV production from diverse carbon sources ranging from food waste to industrial byproducts. Of these microorganisms, the ones which stand out the most are *C. necator* because of their high yields and *H. mediterranei* because of their high 3-HV production. What makes *H. mediterranei* the most interesting microorganism for PHBV production is its capability to grow in highly salty mediums, where the contaminations are more unlikely to happen, reducing the restrictions for sterile conditions. Also, *H. mediterranei* metabolism is so versatile, making them suitable to produce PHBV using wastes of a wide range of chemical compositions.

Concerning production methods, fed-batch fermentation processes have enhanced microorganisms' PHBV production potential, emphasizing the importance of process optimization. While challenges remain in achieving higher 3-HV copolymer concentrations comparable to commercial standards, ongoing research and optimization efforts continue to show promising results, making PHBV a viable and sustainable alternative in various applications, such as packaging and biomedical materials.

Regarding PHAs end-of-life, different applications and end-of-life scenarios must be considered to settle on the proper pathway to follow. As PHAs are still at an early stage of investigation and development, it is not surprising that different end-of-life options

are not sufficiently described. Despite that, different routes including mechanical, enzymatic, and chemical recycling have been reported. Indeed, chemical recycling exhibits a promising environmental solution due to its ability to recover the constituent monomers and put them back into the PHAs life cycle. Refinement of LCA methodologies and standardization efforts are crucial for accurate environmental assessment. Continued research is essential to fully understand PHA's environmental impact throughout its lifecycle. Therefore, to have an accurate LCA, it is essential to consider all the processes around the PHA, from the feedstock generation to the PHA EoL.

Overall, this is an innovative field for research and industrial applications due to global concerns about plastic pollution and the advantages of using PHBV in specific fields like biomedicine due to their physicochemical properties. The advancements described here underscore the potential of biotechnological approaches in transforming waste streams into valuable resources, contributing to a more sustainable and circular economy in the agri-food sector.

Author contributions

SC: Investigation, Writing–original draft, Writing–review and editing. TM: Investigation, Writing–original draft, Writing–review and editing. MN: Investigation, Writing–original draft. FM: Conceptualization, Project administration, Writing–review and editing. AS: Investigation, Writing–original draft. LC: Investigation, Writing–original draft. RM-E: Conceptualization, Investigation, Project administration, Writing–original draft, Writing–review and editing.

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Conflict of interest

Authors SC and MN were employed by company Cetec Biotechnology S. L.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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