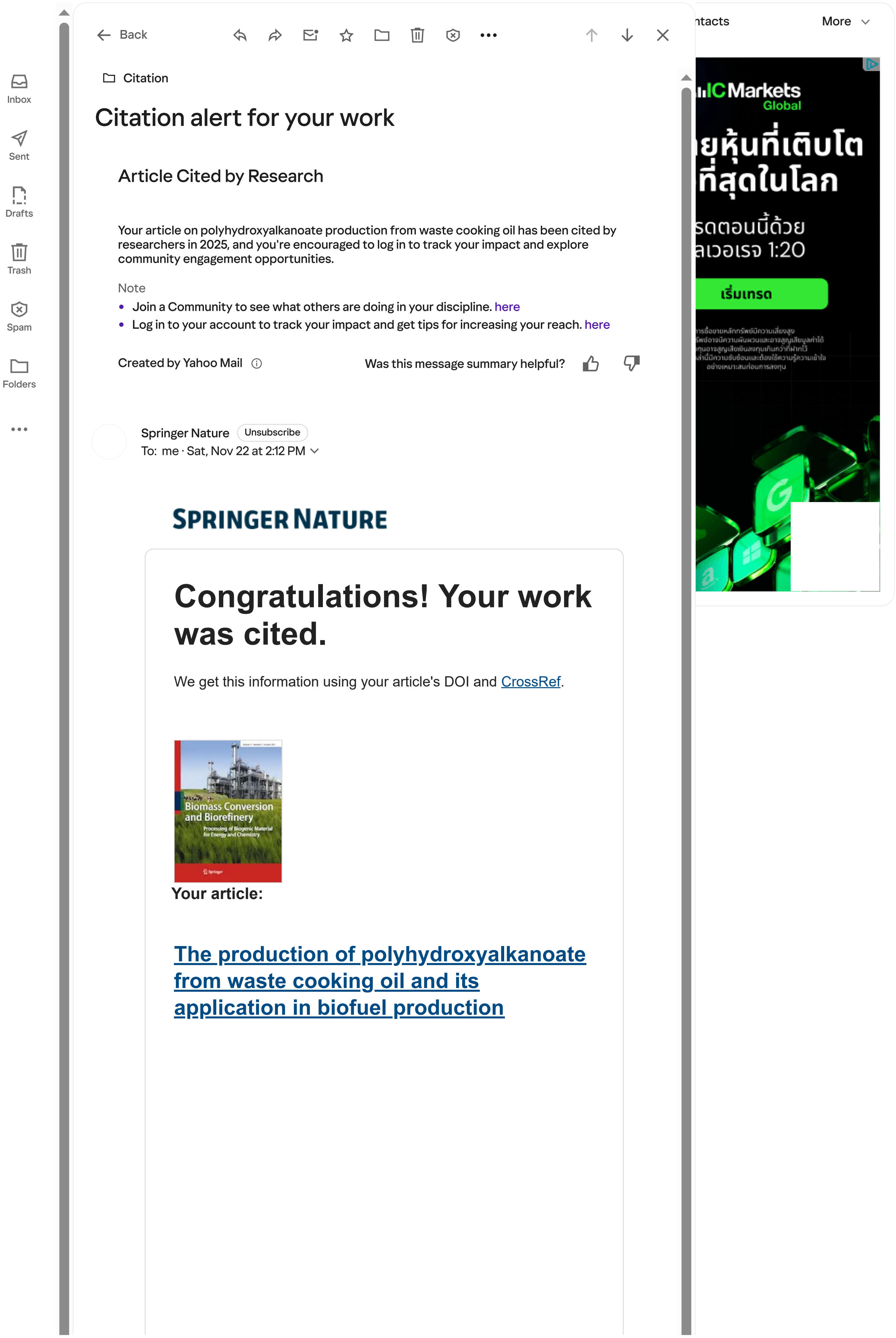

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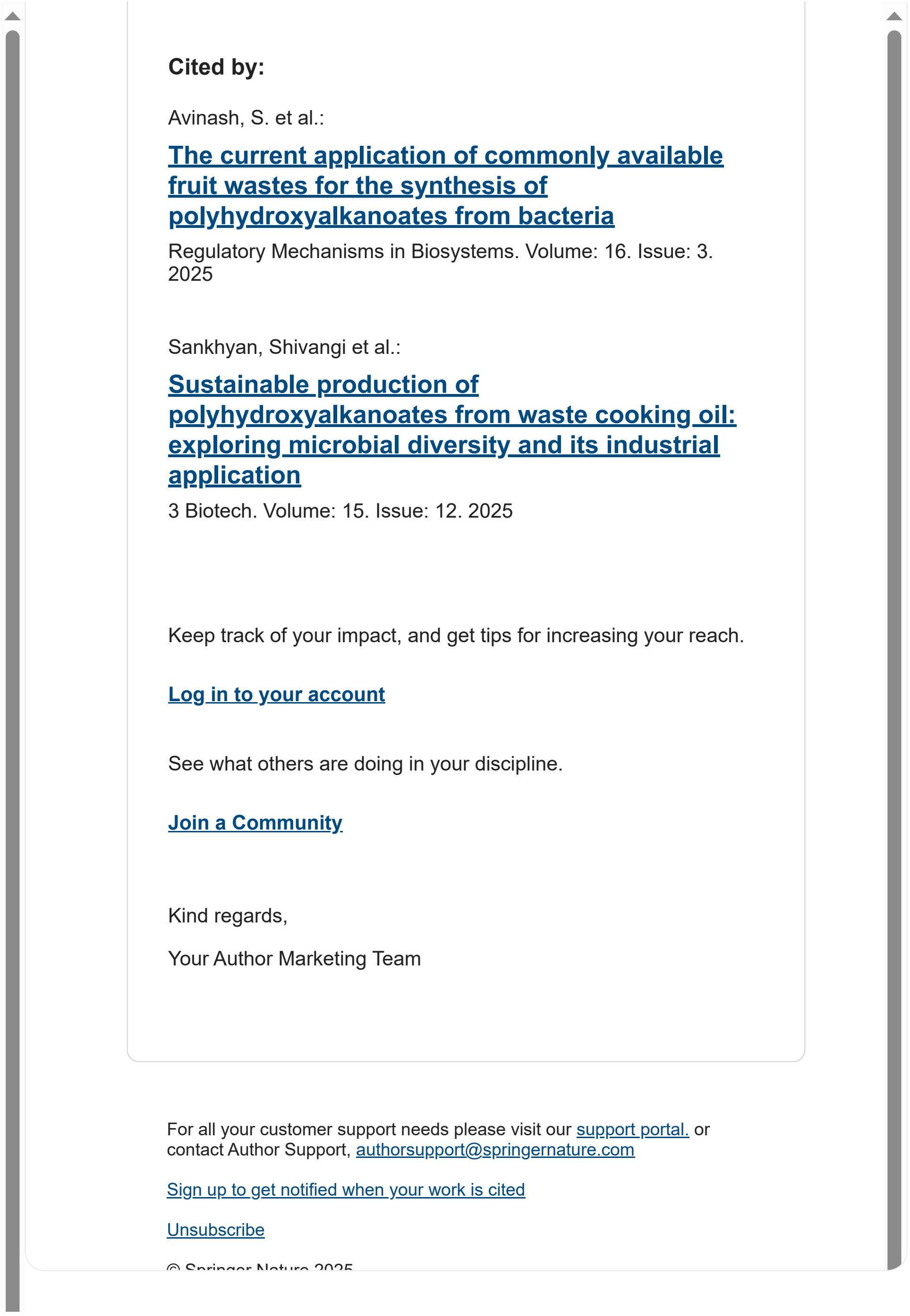

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The current application of commonly available fruit wastes for the synthesis of polyhydroxyalkanoates from bacteria

S. Avinash
School of Biomolecular Engineering and Biotechnology

C. Aakriti
School of Biomolecular Engineering and Biotechnology

S. Mritunjai
School of Biomolecular Engineering and Biotechnology

T. Riddhi
School of Biomolecular Engineering and Biotechnology

T. Archana
School of Biomolecular Engineering and Biotechnology

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Abstract

The rapid depletion of fossil fuel and its hazardous effect on the environment encourages research on the synthesis of bi o based plastics such as polyhydroxyalkanoates (PHAs) for the replacement of traditional plastics. Polyhydroxyalkanoates (PHA s) are intracellularly synthesized biopolymer s that are non-toxic and biologically degradable in nature. Their physicochemical and mechanical properties are mostly similar to petrochemically derived plastics. A major limitation of the commercialization of polyhydroxyalkanoates is the high cost in comparison with petroleum-derived polymers. A lot of research is in progress towards searching for the cheapest carbon source for the culture of bacteria. Among various carbon sources available , biosynthesis of PHA from fruit wastes is still in its initial stage and several challenges remain to be resolved. In order to increase the availability of fermentable sugars and increase microbial intake, screening of more fruit waste

materials and substrate pre-treatment procedures must be improved. For PHA recovery and purification, the development of effective and economical downstream processing methods is an additional area that needs focus. The economic feasibility of PHA manufacturing might be significantly increased by advancements in this area, as these processes now account for a significant amount of the total production cost. The systematic screening of various fruit waste products to determine which have the greatest potential for PHA generation should be the main focus of future studies. Furthermore, improvements in genetic engineering and the optimization of the metabolic pathways of microorganisms that produce PHA may increase production and lower expenses. This article presents a thorough analysis of the value-adding of different kinds of fruit wastes for the production of biopolymers, stressing the various strategies used thus far, their drawbacks, and possible future development paths. The large-scale synthesis of PHAs from fruit waste may prove to be a sustainable and profitable way to lessen the environmental effects of conventional plastics by tackling current issues and utilizing cutting-edge technologies.

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Sustainable production of polyhydroxyalkanoates from waste cooking oil: exploring microbial diversity and its industrial application

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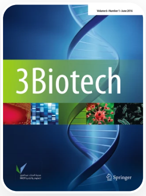

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Abstract

The increasing harm to the environment from synthetic plastics has increased the need for sustainable alternatives, resulting in increased interest in biopolymers such as polyhydroxyalkanoates (PHAs). PHAs have the potential to serve as an alternative plastic derived from petrochemicals. This eco-friendly plastic is produced through microbial fermentation using various carbon sources. Since the cost of PHA production is significantly influenced by the carbon source, researchers are currently exploring cost-effective and more sustainable alternatives to conventional sugar-based feedstocks, including waste lipid streams such as waste cooking oil (WCO). WCOs have been demonstrated to be an excellent carbon source for the production of PHAs. WCOs are vegetable oils discarded after the food frying process and accumulate in larger quantities. Its management is a challenge due to the environmental risk of illegal disposal into rivers and landfills. The individual arrangement of biodegradability and biocompatibility, along with mechanical and thermal characteristics, makes PHA an excellent option for different uses, including paper coatings, films, packaging, adhesives, drug delivery, tissue engineering, and the development of artificial organs in the medical field. In this review we have discussed the potential use of WCO in the microbial fermentation of PHA production and its industrial applications.

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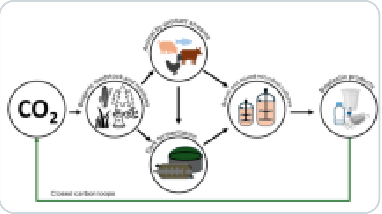

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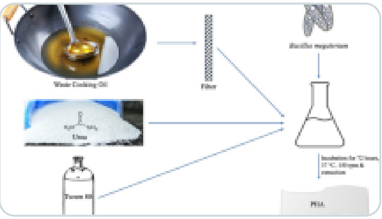

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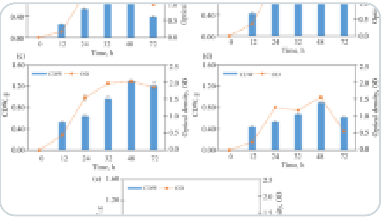

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Introduction

The increase in food items with population growth, leading to the generation of kitchen waste (Chavan et al. 2023). Waste cooking oil (WCO) (also referred to as used cooking oil – UCO) is a significant portion of this residue, which is often disposed of in the ecosystem or reused during frying processes. Unfortunately, this practice promotes environmental pollution and poses a risk to human well-being (Kongpeng et al. 2017). To address this issue and effectively manage waste products globally, the most recent strategies revolve around either creating products with value added or using energy from WCO. In Canada, an estimated annual production of 135,000 tons of WCO has been recorded (Chhetri et al. 2008). The United Kingdom and countries within the European Union (EU) exhibit annual WCO production levels of 200,000 and 700,000–1,000,000 tons, respectively (Kulkarni and Dalai 2006). The Asian region collectively generates an annual volume of 5.5 million tons of WCO, with Thailand alone contributing 117,000 tons of untreated WCO to this total on an annual basis (Maddikeri et al. 2012). Such large-scale WCO production worldwide poses challenge in terms of its collection, treatment, and disposal.

WCOs are produced when vegetable oils such as soybean, coconut, palm, sunflower, olive, cottonseed, and rapeseed oils are used in cooking or food processing (Onn et al. 2024). These oils are employed for domestic cooking applications in the domestic and hotel, restaurant, and catering (HORECA) sectors. Nevertheless, they undergo degradation to such an extent that they become unsuitable for human dietary consumption. In the HORECA industry, fast food establishments, especially those that prepare chicken and hamburgers, contribute significantly to the production of WCOs (Del Flores-Álvarez et al. 2012; Koh and Surh 2015; Rincón et al. 2019; Manzoor et al. 2023). During the frying process of vegetable oils containing triacylglycerols (TAGs), several physical and chemical changes occur when exposed to elevated temperatures ranging from 160 to 200 °C (Tsoutsos et al. 2016). These changes result in the generation of harmful compounds through mechanisms involving hydrolysis, polymerization of TAGs, and oxidation reactions. After the frying process, cooking oils undergo oxidation reactions, resulting in the formation of hydroperoxides. These hydroperoxides are susceptible to further oxidation, ultimately generating harmful byproducts, particularly 4-hydroxy-2-alkenals. Furthermore, the presence of water molecules within the food matrix can instigate attacks on the ester linkages of TAGs, leading to the formation of free fatty acids (FFAs), glycerol, diacylglycerols, and monoacylglycerols (Olu-Arotiowa et al. 2022). Hydrolytic changes occurring within the vegetable oil matrix involving short-chain and unsaturated fatty acids show greater prominence, primarily due to the increased aqueous solubility of these components relative to their long-chain and saturated counterparts. As a result, the hydrolysis byproducts exhibit higher reactivity and an enhanced propensity toward oxidation reactions in contrast to TAGs contained in vegetable oils. The presence of FFAs gives rise to smoke, noxious olfactory emissions, and unpleasant flavours, thereby adversely affecting the subsequent utilization of WCO. Polymerization of TAG results in the formation of nonpolar dimers and various oligomer compounds. The polymerization level is affected by the fatty acid composition of the frying temperature, the duration of the frying process, and the vegetable oil (Tompkins and Perkins 2000).

Oxidation of oils with high concentrations of oleic acid results in increased levels of unsaturated aldehydes, such as 2-decenal and 2-undecenal. However, it produces comparatively small amounts of saturated aldehydes and hydrocarbons. Oxidation of linoleic acid produces hexanal, 2,4-nonadienal, 2,4-decadienal, 2,4-hexadienal 2-heptanal, 2,4-octadienal, 2-octanal, butanal, propanal, 2,4-heptadienal, and 2-propanal (commonly known as acrolein) (Chang et al. 2020). Various substances produced by the breakdown of cooking oils pose health risks to humans, which are recognized for their hepatotoxic, neurotoxic, carcinogenic, and mutagenic effects, along with other harmful effects (Akaraonye et al. 2010; Abdulbari and Zuhan 2018). When continuously exposed to heat, oil undergoes both physical and chemical changes due to degradation reactions such as polymerization, oxidation, and hydrolysis that occur under these conditions. These reactions cause increases in specific heat, density, viscosity, and saturated fatty acid content. These degradation processes lead to a reduction in the amount of double bonds or the ratio of monounsaturated and polyunsaturated components, as well as measures such as peroxide value, iodine value, saponification value, volatile components, antioxidants (such as vitamin E), and smoke point. The acidic elements found in WCO are further classified into saturated, monounsaturated, and polyunsaturated compounds (Zahoor et al. 2021a, b). Saturated fatty acids include stearic acid, myristic acid, and palmitic acid. Monounsaturated fatty acids contain oleic acid, as well as cis-11-eicosenoic acid. Meanwhile, polyunsaturated fatty acids contain ɣ-linolenic acid, linolenic acid, and linoleic acid. Therefore, the major constituents for WCO composition are palmitic acid, linoleic acid and oleic acid, which consist of 38.35%, 11.39%, and 43.67% respectively (Mann et al. 2024) (Table 1). WCOs encompass a broad array of oils utilized in diverse cooking methods such as frying and sauteing. As a result of their prior culinary usage, WCOs can exhibit diverse textures and contain varying levels of food residues. Several factors contribute to the texture disparity in WCOs, including the type of cooking oil used, cooking temperature, food types cooked, and the presence of dissolved food particles. These particles, coming from ingredients cooked in the oil, encompass starches, proteins, and moisture, impacting the oil’s appearance, aroma, and consistency. Understanding these variations is crucial for optimizing WCOs’ treatment and conversion into valuable resources, as their suitability for recycling and repurposing hinges on these characteristics (Awogbemi et al. 2021). Efforts have been made to include WCO as an ingredient in animal feed. Nevertheless, the European Union has imposed a ban on the use of this substance since 2002 due to the presence of hazardous components within WCO, which can potentially spread into the animal muscle tissue. Numerous chemical or enzymatic methods have proven effective in utilizing WCO to produce valuable new chemical compounds. However the research efforts towards bioconversion of WCO to PHA gained momentum recently as indicated by the articles published in the past two decades (Fig. 1). A structured literature search was conducted to compile relevant studies on WCO valorization for PHA production. Publications were retrieved from Scopus up to April 2024. The search strategy employed the following keywords and Boolean operators: ("waste cooking oil" OR "used cooking oil") AND ("polyhydroxyalkanoates"). Only peer-reviewed journal articles, reviews, and patents in English were considered. Conference abstracts, non-English studies, and grey literature were excluded. After removal of duplicates, titles and abstracts were screened for relevance, and full texts were reviewed when necessary. For Fig. 1, bibliometric data were extracted from Scopus on April 15, 2024, using the exact query string: TITLE-ABS-KEY ("waste cooking oil" AND "polyhydroxyalkanoates"). WCO has also found application in the production of biodiesel (Kamilah et al. 2013). However, the high content of FFA poses a challenge in the biodiesel production process. To overcome this limitation, WCO can be used in the context of PHA manufacturing. The use of WCO for this specific application has the potential to reduce the issue of improper WCO disposal (Cai et al. 2015).

Various factors are employed to evaluate the progression of chemical reactions and degradation of the quality of reused frying oil. These include saponification index (which measures the average molecular mass of fatty acids), iodine value (measuring the degree of oil unsaturation), acid value (which determines the percentage of free fatty acids), p-anisidine value (which assesses oil oxidation), peroxide value (indicating initial oxidation), and total oxidation value (which provides oxidative degradation of oil lipids). The deep-frying process affects the physical characteristics of vegetable oils, including viscosity, density, colour, and surface tension. Visual inspection allows quick assessment of certain physical changes, which serve as indicators of the quality of the cooking oil used. Darkness of cooking oil is caused by the development of pigments, which result from the oxidation of fatty acids, Maillard reactions, and the oxidation of phenolic compounds found in vegetable oils (Félix et al. 2017; Kosseva and Rusbandi 2018). The viscosity of the WCO increases as the frying process progresses, which is a result of the formation of non-polar dimers and high-molecular-weight polymer compounds as the triglycerides undergo polymerization (Ahmad Tarmizi et al. 2013). Disposal of WCO is a major challenge due to its substantial quantity, and incorrect discharge into sewers or drains can result in problems such as blockage, as well as unpleasant odor or attraction of insects. WCOs contain substances that persist in the environment for long periods of time, increasing the organic burden in water bodies. These compounds form a fine film on the surface of the water, reducing the levels of dissolved oxygen needed by subaquatic species and thus altering the ecosystem. The presence of lipid-rich residues can hamper the effectiveness of wastewater treatment by giving rise to issues such as the attachment of long-chain fatty acids (LCFAs) to the biomass, which can lead to problems such as sludge flocculation and challenges in mass transfer. Additionally, formation of undissolved LCFAs may lead to foam formation, and increased LCFA concentration may have negative effects on anaerobic microbial communities. The primary focus of biorefinery concepts related to WCO revolves around biodiesel however, various other possibilities have been explored. These include the use of WCO as a key ingredient in the manufacturing processes of biopolymers such as polyhydroxyalkanoates (PHAs), soaps, resins, polyurethane, and grease lubricants (Appels et al. 2011; Sipaut et al. 2012). WCO can serve as a resource to generate energy in various forms, including hydrocarbons through gasification, pyrolytic oil, biohydrogen, and electricity through direct combustion of hydrocarbons through gasification (Chen and Wang 2019; Xiong et al. 2019). In this review, PHAs production from WCO has been explored with different applications associated with industrial, medical, and agricultural.

Polyhydroxyalkanoates as sustainable biopolymers

PHAs represent a class of biodegradable macromolecules synthesized by various microorganisms (Xu et al. 2019; Park et al. 2021). PHAs possess several properties, such as biodegradability, biocompatibility, and recyclability, making them a viable alternative to certain petroleum-based synthetic plastics used in the production of disposable items like bottles, containers, cups, and bags (Singh et al. 2015). Additionally, they have applications in the pharmaceutical, agriculture, and medical fields that include the development of heart valves, vascular stents, sutures, and skin substitutes. The accumulation of PHAs typically occurs when there is a limited supply of nutrients like nitrogen or phosphorous, while an excess of carbon is present (Ray and Kalia 2017a). Currently, more than 150 distinct PHA monomeric subunits have been characterized, with poly(3-hydroxybutyrate) (PHB) emerging as a prevalent biopolymeric component within natural environments (Pizzi et al. 2021).

Microbial communities capable of synthesizing PHAs encompass a diverse range of organisms, including both gram-positive and gram-negative bacteria such as *Burkholderia*, *Pseudomonas*, *Ralstonia*, *Halomonas*, and *Bacillus*, as well as archaeal counterparts (Altun et al. 2023; Fook et al. 2024). *Ralstonia eutropha* is renowned for its exceptional efficiency in PHA accumulation, achieving up to ~ 90% of cellular dry weight (CDW) under nutrient-limited conditions, while co-utilization of crude glycerol and biowastes results in about 60% of CDW as PHA (Ray and Kalia 2017b; Ray et al. 2018). These species exhibit versatile carbon substrate utilization capabilities through both heterotrophic and autotrophic metabolic pathways. While carbohydrates are suitable sources for PHA production, their high production cost remains a limiting factor when compared to synthetic plastics. Consequently, the high production cost restricts the widespread application of PHA. It would be ideal to discover inexpensive substrates capable of producing large quantities of PHA, thereby enhancing the economic attractiveness of its manufacturing process (Ray et al. 2019; Ruiz et al. 2019b). Low-cost carbon substrates, such as waste-derived oil, can be used to reduce the expenses associated with PHA biosynthesis (Parlato et al. 2020). The selection of the substrate holds significant importance for several reasons. Firstly, it can account for up to half of the total production expenses. Secondly, it plays a pivotal role in determining the composition of the polymer or copolymer (Fernandes et al. 2017). This is because different carbon sources undergo distinct metabolic pathways, resulting in the production of varied PHA monomers. Research endeavors are directed towards lowering production costs by utilizing diverse waste streams as carbon sources, such as whey, starch, spent coffee grounds, wastewaters, wheat and rice straw, glycerol, and lignin, among others. These efforts align with the biorefinery concept, integrating PHA production with other product manufacturing to valorize waste streams. However, a major concern is maintaining consistent characteristics of the polymerization feedstocks to prevent variability in biopolymer properties, like molecular mass and copolymer composition. This consistency is essential, especially for applications like medical use, where impurities from waste materials may jeopardize the biocompatibility of the resulting plastics (Medeiros Garcia Alcântara et al. 2020).

As per the Global Bioplastics Market Report 2020, bio-based plastics represent only 1% of the total annual plastic production exceeding 360 million tons, despite a yearly growth rate of 20–30% (Fernández-Dacosta et al. 2015). The rising demands in food, beverage, and e-retail industries are expected to substantially increase packaging needs in the foreseeable future. Successful ventures in PHA manufacturing within a bio-economy necessitate rigorous regulatory oversight concerning greenhouse gas emissions, extraction process toxicity, and proof of compostability to enhance its techno-economic and environmental performance. A study assessing the techno-economic and environmental aspects of commercial PHB production through different downstream processing routes and fermentation processes revealed insights. Among the cases studied, Case 1 employing alkali treatment emerged as the most cost-effective and sustainable option, with a total production cost of 1.40 €/kg PHB and a global warming potential (GWP) of 2.4 kg CO_2e/kg PHB (Colombo et al. 2017). Conversely, Case 2, utilizing surfactant hypochlorite treatment, resulted in a production cost of 1.56 €/kg PHB with a GWP of 2.06 kg CO_2e/kg PHB (Fernández-Dacosta et al. 2015). Case 3, involving dichloromethane solvent extraction, achieved the highest PHB yield of about 82.2%, yet incurred the highest costs in distillation processes, totalling 1.95 €/kg PHB economically and 4.30 kg CO_2e/kg PHB environmentally (Basnett et al. 2017). Utilizing wastewater as a feedstock proves more favourable than conventional pure culture-based PHB production from sugars, especially for large-scale setups. Optimization of acidogenic fermentation processes can yield significant quantities of organic acids from municipal solid waste, serving as a substrate for PHA production. This approach could potentially generate substantial gross revenue, estimated at $7.6–16.9 billion, emphasizing the importance of multiproduct biorefinery concepts for maximizing economic value and waste management efficiency (Kaur et al. 2023). For example, using cheese whey as a low-cost feedstock for PHB production in different capacity production units has shown efficiency at 1000 ton/h, with costs around US$10/kg (Peña-Jurado et al. 2019). Another illustration of economic feasibility involves biorefinery production of PHB, succinic acid, and electricity using sugarcane bagasse and lignocellulosic wastes. Optimized configurations suggest an internal rate of return of approximately 24% and a net present value of US$477 million (Bhola et al. 2021). PHAs consist of linear polyesters made from hydroxyalkanoic acid monomers linked together by ester bonds (Możejko-Ciesielska et al. 2023). The general structure of PHA is [$O-CH_2-CH(R)-CO$]. The most common PHA is polyhydroxybutyrate (PHB), which contains a methyl group (CH_3) as the R group. Here, the R group represents an alkyl group, whose chain length ranges from three to fourteen carbon atoms. Table 2 shows the physical properties of PHAs (Zhang et al. 2006).

Bacterial biosynthesis routes for PHA production from WCO

Bacteria convert WCO into PHAs through distinct biosynthetic routes, which vary according to chain length and enzymatic pathways. PHAs can be systematically classified based on their monomeric structure and chain length. According to the chain length, PHAs can be divided into three primary categories: short-chain length (scl-PHAs) containing 3–5 carbon atoms, medium-chain length (mcl-PHAs) containing 6–14 carbons, and long-chain length (lcl-PHAs) containing more than 14 carbon atoms (Dietrich et al. 2019; Lim et al. 2023). Scl-PHAs exhibit a remarkable degree of crystallinity ranging from 50% to 70% (Hong et al. 2019). They are found to be brittle in nature and with low elasticity. In contrast, mcl-PHAs exhibit low crystallinity and higher elongation at break (Feng et al. 2018). PHAs are also classified as homopolymers, copolymers, and heteropolymers. Examples of PHA homopolymers include poly-4-hydroxybutyrate (P4HB) and poly-3-hydroxybutyrate (P3HB) (George et al. 2020). In contrast, heteropolymers include mixtures of mixed polymer varieties, typically scl-PHA or scl-PHA combined with mcl-PHA monomers, based on poly-3-hydroxybutyrate-co-4-hydroxybutyrate P(3HB-co-4HB) (Arumugam et al. 2018), poly-3-hydroxybutyrate-co-3-hydroxyvalerate P(3HB-co-3HV) and poly-3-hydroxyoctanoate and 3-hydroxydecanoate P(3HO-co-3HD) (Mohamad Fauzi et al. 2019). The particular polymer synthesized is dependent on the species of bacterial strains and the selection of lipid substrates employed. The ability of PHA synthase is involved in copolymerization for both scl and mcl monomers.

In the initial metabolic pathway, Acetyl-CoA is produced as a result of the absorption of a carbon source such as fatty acids, amino acids, or sugar. Next, the two Acetyl-CoA molecules undergo a series of enzymatic transformations, leading to their condensation into acetoacetyl-CoA, which is facilitated by the enzyme β-ketothiolase (PhaA) (Ray et al. 2023). This intermediate is then reduced by NADPH-dependent reductase (PhaB), which lead to the generation of R-3HB-CoA. Finally, R-3HB-CoA converts to PHA, by PHA synthase (PhaC). This particular metabolic pathway leads to the production of scl-PHA composed of monomers containing 3–5 carbon atoms (Chen et al. 2015). In the second pathway, β-oxidation of fatty acids leads to the production of PHAs. This pathway leads to the biosynthesis of mcl-PHA molecules containing carbon atoms (6–14). Key enzymes participating in this pathway include an (R)-enoyl-CoA hydratase, which facilitates the conversion of Acyl-CoA to (R)-3HB-CoA, and PHA synthase (PhaC). The third pathway involves the synthesis of 3-hydroxyacyl monomers derived from fatty acids. The above process is regulated by 3-hydroxyacyl-ACP-CoA transferase, denoted as PhaG, which converts 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA. Subsequently, this intermediate is used by PhaC to initiate the biosynthesis of PHAs (Acharjee et al. 2024). In a broader context, the production of heteropolymers depends on the careful selection of bacterial strains, appropriate lipid substrates, and precise enzymatic pathways involved in the synthesis of both scl-PHAs and mcl-PHAs (Fig. 2).

The composition of WCO plays a pivotal role in determining PHA yield and polymer properties. WCO is primarily composed of triglycerides along with free fatty acids (FFAs), mono- and diacylglycerols, and minor degradation products. Variations in fatty acid profiles particularly the relative abundance of palmitic, oleic, and linoleic acids directly influence microbial uptake routes and the type of PHA synthesized. For instance, oils rich in unsaturated fatty acids (e.g., oleic and linoleic acids) tend to enhance β-oxidation fluxes, favoring the accumulation of medium-chain-length PHAs with improved elastomeric properties, whereas saturated fatty acid rich WCOs often support higher yields of short-chain-length PHAs such as PHB, which exhibit greater crystallinity but reduced flexibility (Song et al. 2008). Moreover, excessive FFAs in WCO can inhibit cell growth if not pretreated; however, at moderate concentrations, they serve as readily metabolizable substrates for PHA synthase, thereby enhancing process efficiency. Differences in frying duration and food matrix residues further alter WCO quality (peroxide value, acid value, polymerization products), which in turn can impact both microbial metabolism and final polymer purity. Addressing these variations through pretreatment (e.g., hydrolysis, emulsification) or metabolic engineering of strains allows more consistent PHA productivity.

Different types of bacteria that exploit WCO for PHA production

Gram – positive bacteria

Bacillus spp. is one of the most versatile gram-positive bacteria that has been gaining recognition as a microbial polymer producer due to its distinct metabolic characteristics. Also, several gram-positive bacteria such as *Corynebacteria*, *Nocardia*, *Staphylococcus*, *Streptomyces*, and *Rhodococcus* are also able to produce PHA polymers. These are the bacteria that can produce both homopolymers and copolymers of PHA. *B. cereus* STV1180 utilized rubber seed oil to produce PHA copolymer P (3HB-co-HV) with a PHA yield of 2.55 g/L (Kynadi and Suchithra 2017). *B. megaterium* R11 could produce PHB by utilizing oil palm empty fruit bunch with a PHA yield of 51.60% and 24.29 g/L CDW (Zhang et al. 2013). *B. thermoamylovorans* produced PHAs up to 87.5% by utilizing WCO as a substrate (Sangkharak et al. 2021) (Table 3). It has also been demonstrated that the presence of urea as nitrogen source and Tween-80 as surfactant enhances the oil utilization leading to higher PHA yield. Exploring this, *B. megaterium* MTCC 428 was able to accumulate upto 77.9% PHA using 2.45% WCO and 2% Tween-80 (Ghosh et al. 2025).

Gram – negative bacteria

Cupriavidus necator is the most common host for the production of PHA. It produces both homopolymer and copolymer. *C. necator* used spent palm oil with 1,4-butanediol to produce P (3HB-co-4HB) copolymer up to 15% mol 4HB and 6.0–6.2 g/L of CDW with a yield of 0.75–0.80 g/g (Table 3) (Rao et al. 2010). It was suggested that spent palm oil has low linolenic acid content but high levels of palmitic acid, oleic acid, and linoleic acid, leading to increased cellular proliferation and PHA production in *C. necator* (Rao et al. 2010). *C. necator* and *P. oleovorans* could produce PHB monomer up to 0.03–0.90 g/g with 3.2–9.0 g/L CDW from co-utilization of olive oil distillate, fatty acid by-product, and WCO (Cruz et al. 2016b). *C. necator* H16 produced PHB up to 79.2% with a CDW of 25.4 g/L by utilizing waste frying oil as a substrate (Abdullah 2015). On the other hand, when soyabean and rapeseed oil were used as co-substrates by *C. necator* H16, it produced 57% of PHA and CDW up to 6.1 g/L. Similarly, when *C. necator* H16 utilized palm oil and lard as a substrate, a PHA yield of 83% and 6.8 g/L of CDW was observed (Taniguchi et al. 2003). *C. necator* H16 utilized waste frying rapeseed oil that led to the production of 67.9% PHA with 10.8 g/L of CDW (Obruca et al. 2014). *C. necator* H16 produced copolymer P(3HB-co-3HV) with a yield of 4.7–8.3 g/L by utilizing jatropha oil with sodium valerate as a substrate, with a PHA yield varying from 6.7 to 9.2 g/L (Ng et al. 2010). *C. necator* KCTC 2662 produced PHB homopolymer from soybean oil, where CDW was produced up to 4.5–8.3 g/L and PHA yield was found up to 0.23–0.45 g/g (Park and Kim 2011). *C. necator* DSM 428 utilized UCO and spent coffee grounds oil that produced PHB up to 63-78.40% and CDW of 11.6–16.7 g/L (Cruz et al. 2014, 2015).

PHA biosynthesis from various vegetable oils have also been widely evaluated using recombinant *C. necator* (Wong et al. 2012; Jiang et al. 2025). *C. necator* H16 Re 2058/pCB113 utilized date seed oil with date molasses as a substrate and produced copolymer P (3HB-co3-HHx) within a range of 2–28%, where PHA yield varied from 0.38 to 0.62 g/g and 7.6 CDW (Purama et al. 2018). Crude palm kernel oil and coconut oil were used as a substrate and displayed the maximum 3HHx monomer content, recorded at 56% and 62%, respectively. The increased content of 3-hydroxyhexanoate (3HHx) monomers was correlated with the integration of the PhaJ gene and deletion of the native PhaB gene within the recombinant *C. necator* Re2160/Pcb113 strain. In a recent study, an unbiased genetic approach was explored such that the developed strain can efficiently utilize waste cooking oil and food-grade palm oil. At 150 m^3 production scale, the engineered *C. necator* H16 reached the highest 264 g/L PHA yield with 100 g/g conversion rate of palm oil within 60-h fed-batch culture (Jiang et al. 2025). It was revealed that the two-component system (*H16_A3043/H16_A3044*) regulates biogenesis and stress and its deletion removed the constraint leading to increased oil consumption. Further engineering of lipase confers elevated PHA production and robust process that can be scaled up to 150 m^3 (Jiang et al. 2025).

The production of mcl-PHA by another Gram-negative bacteria belonging to *Pseudomonas* species using different WCOs has also been explored. *Pseudomonas* species utilized corn oil and produced PHA up to 35.63% and 12.53 g/L of CDW (Chaudhry et al. 2011). The *Pseudomonas* species are known to produce lipases and surfactants such as rhamnolipids, the former helps in oil degradation while the latter facilitates the accessibility of substrate. In another study, *P. aeruginosa* IFO3924 produced mcl-PHA by utilizing palm oil as a substrate that led to the production of 36.0% PHA and 2.2 g/L of CDW (Marsudi et al. 2008). *P. aeruginosa* L2-1 utilized cassava wastewater supplemented with WCO and produced mcl-PHA up to 39% of PHA and 4.2 g/L CDW (Costa et al. 2009). In addition, *P. resinovorans* utilized olive oil deodorizer distillate (OOD) and produced mcl-co-polymer of 12.1 mol% (3HHx), 31.6 mol% (3HD), 48.3 mol% (3HO), and 8.0 mol% (3HDd) with 12.7 g/L CDW (Cruz et al. 2016a). *P. resinovorans* and *P. citronellelis* both utilized OOD, biodiesel fatty acid byproducts, and UCO as carbon sources and produced P (3-HO-co-3HD) with 36–43 mol% 3HO and 28–40 mol% 3HD, with CDW varying from 2.6 to 7.1 g/L (Cruz et al. 2016b). *P. putida* KT2440 produced mcl-PHA from hydrolysed WCO, and produced 36.4 g/L of CDW and PHA yield up to 0.76 g/g (Ruiz et al. 2019a). In a recent study, *P. mendocina* PSU was observed to efficiently use 2% v/v WCO and co-produce scl-mcl PHA to the tune of 2.58 g./L and 8.85 g/L alginate (as a coproduct). The PHA polymer was rich in P(3HB) with variable fraction of 3HHx, 3HO, 3HDD and 3HD having a polydispersity index, Tg and Tm of 1.58, 157 °C, and − 20 °C, respectively (Chanasit et al. 2025).Others organisms are also known to produce PHA from WCO. *Paracoccus* sp. LL1 that produced PHA from WCO with 3.24 g/L CDW and 30.89% as PHA yield (Table 3) (Kumar and Kim 2019). *Wautersia eutropha* ACTC 17,699 utilized canola oil supplemented with fructose as a carbon source and produced a copolymer of 3HB, 3HV with mcl-PHA of 3HO and 3HD, having 18.27 g/L CDW and 0.68 g/g PHA yield (López-Cuellar et al. 2011). In one study, rubber seed oil having a high content of unsaturated fatty acids, especially oleic acid (23.16%) and linoleic acid (36.96%) displayed PHA yield better than when saturated fatty acids were used as substrate by Kynadi and Suchithra (2017).

PHA purification methods and associated challenges

Solvent extraction

Solvent extraction is the most widely used approach for recovering PHA from cell biomass and is commonly applied in laboratories due to its simplicity and efficiency. The process mainly involves two steps: first, altering the permeability of the cell membrane, which facilitates the release and solubilization of PHA. This step is subsequently followed by non-solvent precipitation (Jacquel et al. 2008). Commonly, PHA is extracted using solvents such as chlorinated hydrocarbons (e.g., chloroform and 1,2-dichloroethane) or cyclic carbonates like ethylene carbonate and 1,2-propylene carbonate (Lafferty and Heinzle 1979; Ramsay et al. 1994). Among the lower-chain ketones, acetone is the most widely used solvent, particularly for extracting mcl-PHA (Jiang et al. 2006). Precipitation is generally carried out using non-solvents such as methanol or ethanol (Ramsay et al. 1994). Compared to other PHA recovery techniques, solvent extraction offers clear advantages in terms of efficiency. This approach can also eliminate bacterial endotoxins while causing minimal degradation of the polymers, thereby enabling the recovery of pure PHA with high molecular weight (Jacquel et al. 2008). However, its large-scale use is often considered environmentally unsustainable. Moreover, the use of solvents is limited by factors such as high capital investment and operating expenses. An additional challenge arises from the high viscosity of the polymer solution when the P(3HB) concentration surpasses 5% (w/v). The increased viscosity hinders the removal of cell debris, leading to a prolonged separation process. Furthermore, solvent extraction may alter the native structure of P(3HB) granules, which could be valuable for specific applications. Accidental release of large quantities of highly toxic and volatile solvents into the environment also poses a serious risk (Tamer et al. 1998; Yu and Chen 2006; Yu 2009). To address these concerns, 1,2-propylene carbonate has been suggested as a safer alternative to halogenated solvents for PHA recovery (Fiorese et al. 2009; McChalicher et al. 2010). With a high boiling point of 240 °C, 1,2-propylene carbonate resists evaporation at lower temperatures and can be reused for multiple purification cycles. This feature lowers overall solvent consumption, making the process more cost-effective. In addition, 1,2-propylene carbonate is regarded as safe because of its low toxicity and is extensively used in various fields, including cosmetics (McChalicher et al. 2010). A maximum PHA recovery of 95% with 84% purity from *C. necator* cells at 130 °C for 30 min without any pretreatment was obtained, which is comparable the extraction yield obtained using chloroform (94% yield and 98% purity) (Fiorese et al. 2009).

Digestion methods

Solvent extraction techniques dissolve the PHA granules, whereas digestion methods break down the cellular materials surrounding these granules. Digestion methods, developed as an alternative to solvent extraction, are well-established and can be categorized into chemical or enzymatic approaches. Due to the easy availability of chemicals with well-characterized properties, chemical digestion methods have been more extensively explored than enzymatic digestion (Jacquel et al. 2008).

Chemical digestion

Several chemical digestion methods have been investigated for recovering PHA from cellular biomass. These methods work by solubilizing the non-PHA cellular mass (NPCM) and primarily employ agents such as sodium hypochlorite or surfactants. The key characteristics of sodium hypochlorite, including its strong oxidizing power and non-selectivity, can be utilized to digest NPCM and promote PHA recovery (Yu and Chen 2006). Various surfactants, such as palmitoyl carnitine, betaine, sodium dodecyl sulfate (SDS), and Triton X-100, have been tested, with SDS demonstrating the most effective performance. However, PHA recovered using either surfactants or sodium hypochlorite alone was of insufficient quality. Consequently, a combination of surfactant and sodium hypochlorite was employed (Yu 2009). While surfactant digestion yielded PHA with a slightly higher molecular weight, the purity was lower compared to that obtained with sodium hypochlorite digestion. In contrast, sodium hypochlorite yielded PHA of higher purity, but led to significant degradation of molecular weight, up to 50% (Ramsay et al. 1990). Sequential treatment with surfactant and hypochlorite enabled faster and more efficient PHA recovery, while also reducing overall costs by approximately 50% compared to solvent extraction (Ramsay et al. 1990; Dong and Sun 2000). Although this process offers low operating costs and technical simplicity, these advantages are offset by the challenges associated with surfactant disposal in wastewater treatment and the comparatively high cost of chemical agents such as SDS and sodium hypochlorite (Yu 2009).

Enzymatic digestion

The recovery of PHA via enzymatic digestion involves a relatively complex procedure. The solubilization of non-PHA cell components generally includes heat treatment, enzymatic hydrolysis, and surfactant washing (Holmes and Lim 1990). Various enzymes, particularly proteases, have been investigated for their effectiveness in inducing cell lysis (Yasotha et al. 2006). *Microbispora* sp. culture, known to secrete protease, was used in the fermented broth of *Sinorhizobium meliloti* containing 50% PHA to facilitate hydrolysis (Lakshman and Shamala 2006). The culture was introduced into heat-inactivated (80 °C for 10 min) biomass of *S. meliloti* and incubated for 72 h, during which the protease lysed the cells, leading to the release of intracellular contents along with the PHA granules. Subsequent filtration and extraction using chloroform resulted in PHA of 94% purity. In comparison, chloroform extraction from undigested cells yielded PHA with only 66% purity. A mixture of *Microbispora* sp. culture with *B. flexus*, followed by separation using an aqueous two-phase system (ATPS), yielded PHA with 92% purity (Divyashree et al. 2009). The enzymatic approach is considered advantageous due to its mild operational conditions (Middelberg 1995; Kapritchkoff et al. 2006). Since enzymes exhibit high specificity toward the reactions they catalyze, the recovery of PHA with superior quality can be anticipated. However, the elevated cost of enzymes and the complexity of the recovery procedure often limit their practical advantages.

Mechanical disruption

Mechanical disruption is a common method for releasing intracellular proteins, and this approach has also been explored for the recovery of PHA from bacterial cells (Harrison 1991). Among different mechanical disruption techniques, bead milling and high-pressure homogenization are the most widely employed for large-scale cell disruption in the pharmaceutical and biotechnology industries (Bury et al. 2001). In contrast to other recovery methods, mechanical disruption is preferred primarily for its cost-effectiveness and its ability to cause only minimal damage to the products. Mechanical cell disruption does not require chemicals, thereby reducing environmental pollution and lowering the risk of product contamination. Overall, the limitations of mechanical disruption include high capital investment, extended processing time, and challenges in scaling up (Park et al. 2007; Balasundaram and Harrison 2008).

Versatile applications of PHAs

PHAs have several applications in various sectors. The following paragraphs describe various applications of PHAs. PHAs provide a significant opportunity for the plastics industry due to their remarkable biodegradable properties, wide range of processing capabilities, biocompatible properties, and potential to serve as an alternative to fossil fuel-based synthetic polymers (Akaraonye et al. 2010; Medeiros Garcia Alcântara et al. 2020). The key characteristics of PHAs include their insolubility in water and resistance to ultraviolet rays, as well as their low susceptibility to hydrolysis. They are degradable by acids and bases and exhibit solubility in chlorinated chloroform and chloroform solvents (Mahato et al. 2023). PHAs are molded into various shapes and used for packaging films, fabrics, agricultural films, components, coatings, adhesives and biomedical applications (Rastogi and Samyn 2015; Basumatary et al. 2022). Indeed, 3HAME – the chmical degradation product of PHA produced from 4.0% (w/v) of WCO under batch fermentation was found to have properties similar to Thailand's fuel standards and ASTM (Sangkharak et al. 2021). This reflects a unique aspect of PHA polymers that can be converted to biofuels after their first use as plastic alternative.

Bioplastics have diverse industrial applications. PHA, in particular, is biodegradable, highly flexible, heat resistance, and has good resistance to hydrolysis, providing a balance between toughness and stiffness (Kumar et al. 2025). PHA exhibits similarity with linear low-density polyethylene (LLDPE) in many aspects, making it versatile enough to be used in a variety of molded products, films, and fibers (Medeiros Garcia Alcântara et al. 2020). Manufacturers of durable consumer products recognized the potential of PHA for a variety of applications due to its versatility and similarity to LLDPE. In addition to its use in sports equipment, furniture, and appliances, PHA can also be used in molded components of home appliances, such as fasteners, covers, housings, clips, and filters (Mahato et al. 2023). Bioplastics are used in connectors, cables, and casings for consumer electronics. There is a growing trend among electronics manufacturers such as Fujitsu, Samsung, Sony, Nokia, and NEC, to incorporate bioplastics into their products. Consumers are demonstrating increasing interest and support in eco-friendly product materials, even if it comes at a high premium price, particularly in packaging materials for cosmetics and personal care items. Products are promoted by highlighting their eco-friendly features. The flexibility of PHA makes it an ideal bioplastic for blister packs, lids, bottles, and a variety of containers used in the consumer goods sector. It can also be utilized for packaging purposes in the food industry, including items like flower pots, bottles, laminated films, fishnets, disposable cups, fast food containers, agricultural films, sanitary products, and fibers used in textiles. The characteristics of different copolymers and block polymers, along with their ability to be processed, broaden their potential applications like PET and PVC, PHA demonstrates excellent barrier properties, making it suitable for use in the packaging industry as a bioplastic and helping to address issues related to environmental pollution. Because of these characteristics, PHB is a strong alternative to replace PE, PET, and even PP. Resins based on poly(vinyl acetate) improve the physical properties that contain PHB, greatly facilitating the processing. Additionally, the excellent heat resistance of PHB enables its potential use in applications like hot filling (Poltronieri and Kumar 2017). Compounders discovered that PHA has significant potential as a modifier for PVC, enhancing toughness and plasticization without compromising transparency or UV stability (Basumatary et al. 2022). Due to its high compatibility with PVC, PHA is easy to handle and process under the same conditions as PVC. In general, PHA is a highly promising polymer for various applications. For example, it provides better barrier properties and mechanical strength than more commonly used bioplastics such as polylactic acid (PLA). Despite its inherent brittleness, significant progress has been achieved by formulating PHA with optimized additives and blends, resulting in enhanced mechanical properties and improved processability for extrusion or injection molding. This versatility allows PHAs to be made into a variety of textiles, molded products, textiles, and biofilms. Two major industrial items that have taken advantage of the easy processing of PHA polymers into multiple shapes are blow-molded bottles and injection-molded hair caps (Mahato et al. 2023). These advancements will enhance its ability to penetrate markets such as packaging foils used for food product storage.

PHA is a highly adaptable polymer that is suitable for a variety of applications. It has excellent barrier properties, a favorable oxygen transmission rate, a good water vapor transmission rate, and strong mechanical strength compared to other bioplastics such as PLA. Despite its brittleness, significant progress has been achieved by developing PHB with optimized additives and blends, resulting in enhanced mechanical properties and processability through extrusion or injection molding. These advances will enhance its ability to reach markets and discover new industrial applications. Additionally, further developments such as new block polymers, changing the monomer ratio in copolymers, and grafted polymers may enable the creation of even more flexible or transparent grades of PHA by controlling their crystallization (Poltronieri and Kumar 2017). The automotive sector is leading advancements in components for cars and buildings by focusing on the recycling of plastic parts. The EU End-of-Life Vehicle Directive states that, starting January 1, 2015, passenger vehicles and light commercial vehicles must have a reuse and recycling content rate of 85% (Kanari et al. 2003). As a result, car manufacturers are increasingly incorporating bioplastics and other bio-based materials into their products.

Currently, Hyundai and Toyota are integrating bioplastics into their vehicles. Due to its physical characteristics, PHA has the potential to be a more eco-friendly alternative to automotive tubing, seat materials, interior panels, and trim components. If PHA becomes more competitive and affordable in terms of cost, making it comparable to cheaper non-degradable plastics and similar in price to petrochemical-based polymers (which are expected to become less available over time), it is anticipated that the packaging industry will experience a rise in demand for PHA-based films, molded forms, fiber-reinforced materials for textiles, and bioplastic composites for the aerospace and automotive sectors. The sustainability of materials is an essential factor that must be considered, as it needs to comply with current and future regulatory standards in various markets. Biobased products are expected to gain a larger portion of the consumer market (Poltronieri and Kumar 2017).

PHA as a coating agent

Barrier coatings are used to protect meals in a way that either keeps moisture and oils in the container or keeps oxygen as well as moisture out (Montazer and Harifi 2017). PHAs, which are biodegradable materials, are commonly used as films and foils. They possess similar properties to polypropylene and are suitable for food wrapping purposes (Basumatary et al. 2022). They are useful for packing items like sanitary towels, razors, diapers, shampoo bottles, milk cartons, and disposable feminine hygiene products because of their waterproof nature (Mahato et al. 2023). These materials were synthesized by the dip-coating technique and showed potential for inhibiting biofilm formation and cell proliferation. An increasing number of firms are producing foodstuff packing materials containing nanoparticles that deliver both antibacterial properties and increased mechanical performance. Castro-Mayorga and colleagues developed an antimicrobial PHA containing silver nanoparticles that effectively hindered the growth of *Salmonella enterica*. This alternative approach was found helpful in food industries (Castro-Mayorga et al. 2017). Biopolymers are also employed by the paper industry, replacing synthetic polymers. PHAs are water-insoluble and resistant to hydrolytic breakdown due to their hydrophobic nature. In addition, PHA’s thin film-forming capabilities produce a shield against oxygen and UV radiation (Rastogi and Samyn 2015). Recently, mcl-co-lcl PHA was produced by *Enterobacter* TS1L using waste oil as substrate of fed-batch fermentation. The film obtained post extraction was casted along with lime oil show potent antibacterial activity against *Staphylococcus aureus* and *E. coli* (Petpheng et al. 2024).

PHAs in therapeutics and biomedical applications

The biocompatible characteristics of PHAs are highly desirable for medical applications. Previously, synthetic polymers used in medical applications were known to be responsible for cancer development (Titz et al. 2012). To address the side effects caused by artificial polymers in the human body, biopolymers should be used as substitutes. PHB and PHBV, when used in antibiotic-loaded titanium implants, exhibit antimicrobial effects that prevent implant-related infections through controlled release of gentamicin. As a result, PHA can be used as an effective substitute for synthetic polymers in therapeutic uses. PHA produced by *C. necator* H16 and its transformants on palm-oil based raw material have been demonstrated for novel cosmetic and wastewater treatment applications (Sudesh et al. 2011). Three types of PHAs (P(3HB), P(3HB-co-3HV) and P(3HB-co-3HHx) cast films show better absorption of sebum without the need of lipophilic additives (commonly used in facial oil). The absorbed oil was about 80% in the case of P(3HB-co-3HHx) cast films. Interestingly, the absorbtion efficiency remained unaffected after washing with detergents indicating the reusability of such PHA films (Sudesh et al. 2011).

PHA in tissue engineering

Biomaterials that can naturally degrade over time are a practical choice for tissue engineering due to their ability to provide physical support to the body. They promote cell adhesion, offer a suitable surface for cellular growth, degrade without causing harm, and consist of porous materials (Zinn et al. 2001). PHAs exhibit compatibility with various cell types, such as chondrocytes, bone cells, and epithelial cells. A bone substitute material was developed by using PHB, which demonstrated positive responses without any signs of infection after 15 days (Huong et al. 2017). Furthermore, when combined with hydroxyapatite (HA), the composite exhibited improved mechanical strength, measuring at 62 MPa, making it suitable for treating bone fractures (Chen and Wu 2005). Similarly in one study, scientists observed extensive bone growth within a span of five months when utilizing a combination of HA and the copolymer PHBV. In addition, scaffolds made of poly (hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx) and copolymer PHB are used in cartilage fabrication (Miu et al. 2022).

Scaffolds are vital in tissue engineering, serving as 3D frameworks that support cell attachment, ECM formation, and localized delivery of bioactive factors (Diniz et al. 2023). Biomaterial choice is crucial, influencing scaffold function and biological outcomes (Jafari et al. 2017). In bone tissue engineering, scaffolds mimic natural ECM to enhance cell adhesion, growth, and differentiation. Ideal scaffolds are biodegradable, biocompatible, osteoconductive, osteoinductive, and bioactive (Qu et al. 2019). In cardiovascular applications, they aid in developing implants and drug-testing models (Scott et al. 2021). Scaffolds also support regeneration in poorly healing tissues like tendons, cartilage, cornea, urinary tract, and airways and are extensively utilized in scaffold fabrication owing to their adjustable degradation rates and versatile surface properties (Mafi et al. 2012). PHAs, either alone or in combination with other polymers, have been explored for diverse biomedical uses such as sutures, wound patches, and dressings, exemplified by PHA synthesized from *B. cereus* MCCB 281 (Mohandas et al. 2018; Soleymani Eil Bakhtiari et al. 2021). Electrospinning has enabled the fabrication of fibrous scaffolds from biopolymers such as poly(3-hydroxybutyrate) [P(3HB)] and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)], which exhibit favorable morphologies for cell attachment and growth (Esmail et al. 2021). Studies have shown that PHA-based films and scaffolds promote human cell proliferation without exerting toxic effects on HEK 293 or embryonic renal cells (Romo-Uribe et al. 2017). Moreover, these materials withstand common sterilization methods like autoclaving and ultraviolet (UV) irradiation. Importantly, fibrous PHA scaffolds degrade within 18 days under culture conditions, aligning with the principle that a scaffold should gradually disappear as the regenerating tissue develops.

The efficiency of PHAs can be further improved by blending with other polymers. For instance, bacterial cellulose (BC), known for its biodegradability and compatibility, has been combined with PHB, yielding composites that enhance osteoblast differentiation and stimulate bone formation while maintaining excellent cytocompatibility (Codreanu et al. 2020). Similarly, hybrid scaffolds incorporating PHB, gelatin, and metronidazole (MTZ) an antimicrobial agent have been reported to promote fibroblast proliferation, suppress bacterial growth (*Escherichia coli* and *S. aureus*). The addition of MTZ allowed increased compatibility with the L-929 fibroblast lineage, also providing good thermal and mechanical properties. Among various formulations, a 7:3 PHB/gelatin matrix with 10% MTZ showed optimal results for skin wound healing by regulating inflammation and accelerating tissue repair (El-Shanshory et al. 2022). Another modification involves PHB combined with gelatin and Fe_3O_4 nanoparticles to generate magnetically active hybrid scaffolds, which show potential for drug delivery, wound dressings, and tissue repair, while remaining non-toxic to cells (Pryadko et al. 2022). Promising outcomes have also been observed when mcl-PHAs, such as poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate) [P(3HO-co-3HHX)], are combined with hydroxyapatite, producing scaffolds with strong biocompatibility and osteoconductivity for bone regeneration (Ansari et al. 2016). Scaffolds developed by integrating PHA with graphene-coated silver nanoparticles (GAg) have shown effective bactericidal properties, making them suitable for managing chronic wounds and sterilization purposes (Mukheem et al. 2018). Likewise, blending PHAs with bioactive glass has supported neuronal growth and differentiation (Lizarraga-Valderrama et al. 2020).

Advanced fabrication technologies further expand scaffold applications. 3D printing has enabled PHA-based scaffolds to be designed as drug carriers, with osteogenic peptides incorporated to accelerate cell differentiation (Saska et al. 2018). Polymeric microspheres represent a type of scaffold that provides a large surface area for cell attachment, supported by their 3D porous structure, and can remain suspended. This interconnected porosity also facilitates nutrient and metabolite exchange, thereby promoting cell proliferation and differentiation. For example, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate-co-3-hydroxyvalerate) (PHBVHHx) porous microspheres not only supported cell attachment and growth but also reduced apoptosis, highlighting their potential in both scaffold fabrication and drug delivery systems (Wei et al. 2018).

PHA in drug delivery

In recent decades, there has been significant interest in biodegradable drug carriers. Polymers possessing biodegradable properties are chosen to encapsulate drugs and control their release (Zhao et al. 2003). PHAs have been employed in various forms, such as microspheres, matrices, and micro tablets, to develop diverse medications (Lenz and Marchessault 2005). Progesterone and steroid hormones, as well as vaccines, have all been successfully delivered via PHA microspheres in the treatment of chronic osteomyelitis. These microspheres have a high drug release rate of 90% within 24 h. The loading capacity of rifampicin in PHB microspheres was remarkably high at approximately 407.6 mg/g PHB. Drug release from these microspheres was shown to be effectively regulated by their size and drug content. The release rate of PHB microspheres was increased by adding ethyl esters of fatty acids (Shrivastav et al. 2013). For the treatment of chronic osteomyelitis, PHB was also used as an antibiotic carrier, and after 30 days, complete recovery was observed. Tetracycline, an antibiotic used to treat periodontal disease, is released under controlled conditions using the copolymer PHBV. PHB shows high release rates as a drug carrier, which may not always be desirable. Considering the rising demand for drugs and the effective drug delivery capabilities of

PHA, it may be further investigated for targeted drug delivery, particularly in the case of tumors.

Application of PHA in agriculture

In recent times, PHA materials are rapidly gaining prominence and are set to become essential in the agriculture sector in the near future due to their eco-friendly nature. The excessive or unregulated use of fertilizers causes significant economic resource losses and serves as a primary contributor to environmental pollution (Chaudhary and Yeshpal 2023). Bio-plastic fertilizers (BpF) have been used for many years in agricultural sectors. It is considered a sophisticated method in the category of slow-release fertilizers (SRF), which is important to supply nutrients to plants while regulating the durability of the product. The use of nano-composite bio-plastics derived from PHA-rich bacterial biomass in agriculture includes the production of slow-release fertilizers. The process involves a bioplastic coating, in which fertilizers are embedded within PHAs granules, films, or bars (Pakalapati et al. 2018).

In agricultural plastic products, including mulch films, various chemicals called "additives" are frequently incorporated to improve properties such as flexibility, durability, stability, and stretchability of the polymer films. These additives also help extend the material's lifespan by providing resistance to sunlight, heat, and, in some cases, exposure to high levels of agrochemicals (Hahladakis et al. 2018; Campanale et al. 2020a). Compounds such as plasticizers, dyes, antioxidants, and ultraviolet (UV) stabilizers are incorporated into the polymer matrix of both conventional and biodegradable mulch films to achieve specific product characteristics (Yu et al. 2021; Campanale et al. 2022; Savino et al. 2022). However, certain plasticizers, like phthalic acid esters (PAEs) used to enhance impact resistance in the film, can pose risks to human health and the environment (Campanale et al. 2020b). Antioxidants are crucial chemicals that safeguard plastics from photo-oxidation and thermal oxidation caused by oxygen and sunlight, which are the primary factors leading to the degradation of hydrocarbon polymers under natural weathering. Common types of antioxidants include phenols (e.g., Irganox® and Evernox®), amines (e.g., Aminox® and Dusantox®), phosphites (e.g., Weston®), and thioesters, and they are used in both conventional synthetic plastics and cellulosic polymers (Ambrogi et al. 2017). UV stabilizers functions by absorbing harmful ultraviolet radiation before it can interact with the photoactive chromophoric groups in the polymer, thereby dispersing the energy safely. Common stabilizers used as plastic additives include benzophenones, benzotriazoles, and hydroxyphenyl triazines. Many of these additives can leach from plastic mulches into the soil during use. They often exhibit high partition coefficients, low biodegradability, and a tendency to bioaccumulate, posing risks of contaminating soil, water, and food systems (Serrano-Ruiz et al. 2021). The faster breakdown and decomposition of biodegradable plastic mulches can lead to a better and quicker release of additives and impurities into the environment compared to conventional plastics (Fan et al. 2022; Sridharan et al. 2022).

A recent study reported that chemicals leached from the incubation of Microplastics (MPs) and Micro-bioplastic (MBPs) triggered a toxicological response in the microalga *Chlorella vulgaris*. The study compared the toxic effects of MBPs (PLA, PBS) and conventional MPs (PE, PA) on marine microalgae, finding that PLA exerted the strongest inhibitory impact on algal growth. This effect was most likely attributed to particle physicochemical characteristics (such as surface roughness), additive release, and elevated oxidative stress (Su et al. 2022). Furthermore, incorporating residual biodegradable mulch films into the soil through tilling and ploughing at the end of the crop cycle remains a largely unexplored area in terms of potential environmental impacts.

Economic insights and life cycle assessment

Life Cycle Assessment (LCA) is essential for conducting an economic evaluation and environmental impact assessment of the entire PHA production process in order to assess the overall impact of bio-based plastics and products. This is crucial for assessing the feasibility of substituting current petroleum-based plastics with PHA. The economic potential should be evaluated considering the extraction protocol, including the cost of extraction and the recyclability of the solvents and reagents used. The extraction protocols are assessed based on three key aspects: material costs (including expenses for digestion agents, extraction solvents, purification reagents, and anti-solvents), processing costs (such as the energy demands of the extraction processes), and environmental considerations (the associated carbon footprint, potential hazards and risks encompassing waste management) (Koller et al. 2013; Requiso et al. 2021).

In terms of economic and environmental considerations, processes involving solvents or antisolvents are considered less advantageous due to environmental concerns, particularly issues related to wastewater management (De Souza Reis et al. 2020). An LCA-based assessment of three manufacturing methods to produce PHB from wastewater (dichloromethane solvent, surfactant hypochlorite, and alkali-surfactant) revealed that the solvent method had the highest cost and environmental impact. PHB costs 1.95 €/kg, emits 4.26 kg CO_2-eq/kg, and has an MJ value of 156. According to the evaluation, digestion recovery is preferable to solvent recovery in terms of both environmental and economic benefits. However, for digestion recovery to be practical, purity and yield increases are required, as solvent recovery revealed better PHA yields and quality equivalent to commercial polymers. PHA's purity influences its availability in many applications (Fernández-Dacosta et al. 2015). PHA with a purity above 90% is adequate for commercial uses (Werker et al. 2016). However, biomedical applications require a purity greater than 99.9% and endotoxin requirements (Sevastianov et al. 2003). This review found that solvent recovery can attain 95% purity without further purification, whereas digestive recovery requires extra purification owing to its purity of 88%. Solvent re-dissolution is commonly utilized in purification to attain high purity, making solvent recovery a more cost-effective option than digestive recovery for purity levels over 90%. In contrast, digestive recovery usually achieves higher yields than solvent recovery, making it economically viable as long as the purification method minimally impacts the yield.

For biomedical applications, achieving high purity ($>$ 99.9%) and meeting strict endotoxin standards ($<$ 0.5 or $<$ 0.06 endotoxin unit/mL) is essential (Sevastianov et al. 2003). Since PHAs are produced by microorganisms, they may contain lipopolysaccharides and other compounds that can trigger toxic reactions in the human body, requiring an extensive purification process. PHB is a promising biopharmaceutical due to its degradation product, 3HB, which has anti-inflammatory, anticarcinogenic, and antioxidant properties, emphasizing the need for high purity and minimal endotoxin levels. However, increasing the number of separation and purification processes may improve purity but reduce yield. Additional purification operations can be expensive, increasing product costs. The mechanism of PHA synthesis is not well known, and there is no clear approach to reducing manufacturing process expense. However, ongoing research into process development, along with local and government initiatives to regulate the use of petroleum-based plastics and promote biodegradable alternatives, is critical to addressing the increasing demand for these materials.

Industrial production of PHA using WCO substrates

Large-scale utilization of WCO for PHA production has been explored by a few companies operating globally. For instance, RWDC Industries Limited, a company based in Singapore and the US, produces Solon™ PHA through a process of upcycling WCO https://www.rwdc-industries.com/. Using a recombinant *C. necator* strain that produces P(3HB-co-3HHx) upto 80% of cell biomass, it is expected to replace about 1.4 trillion drinking straws by 2025 (https://www.techinasia.com/133m-bet-biodegradable-plastic-hold-water). Currently, there are two operations site (Athens and US) having annual production capacity of 25–50 kilotonnes (kt) and 4,000 tonnes annually. The production is projected to increase upto 350 kt of Solon™ by 2025, which would be more than ten times the current global PHA production capacity. In order to compete in the market with petroleum-based plastics, it has been suggested that Solon™ PHA must be priced below USD 4 per kg. In addition to drinking straws, the polymer product may be marketed for coffee cups, lids, cutlery, lightweight shopping bags, food containers, and textile fibers.

Nafigate utilizes the technology developed by the Hydel Consortium in Brno, Czech Republic to produce P(3HB) homopolyesters from waste frying oil, employing a polymer recovery process that uses oils (https://www.nafigate.com/hydal-biotechnology/). This method reduces energy consumption by approximately 50% compared to poly(ethylene) (PE) production. The bioprocess uses wild-type *C. necator* as the producing strain and avoid solvent extraction; instead, they break down microbial cells to produce PHA granules. In 2019, the process reached a technological readiness level of 9, and Nafigate claims it was the first in the world to use 100% waste on an industrial scale for the production of natural PHA biopolymer. The chemically stabilized biopolymer is supplied as granules, mulch films, 3D printing filaments, and also as a main component for microplastic beads in shower milk products, replacing fossil-based plastic beads that are prohibited as primary microplastics in Europe under the Intentionally Added Microplastics Legislation. These product is expected to be used in biomedicine, sustainable packaging, and smart fertilizers. Furthermore, Nafigate is developing formulations with P(3HB) to replace harmful chemicals, such as oxybenzone UV filters in sunscreen creams. These products, marketed under the “Naturetics™” brand, were launched in the Czech Republic in 2021. The large-scale production for packaging purposes expected to begin in 2026. Other companies such as Jiangsu Clean Environmental Technology and Vikas Ecotech have collaborated with Nafigate for industrial scale production and commercialization of P(3HB) formulations.

Another company, Kaneka, based in Minato-ku, Tokyo, is a leading developer and manufacturer of PHA biopolymers. Their PHA biopolymer, PHBH™, is a copolymer of P(3HB-co-3HHx) produced using palm oil. The polymer is similar to Danimer Scientific's product produced using canola oil, differing in the 3HHx content in the final polymer. The U.S. Patent (5,292,860; 1994) by Shiotani and Kobayashi for Kaneka, which outlines the use of *Aeromonas* sp. for the biosynthesis of scl-mcl-PHA, serves as the foundation for industrial production of PHA biopolymers. This patent was later shared with Procter & Gamble. In 2020, they have increased its production capacity to approximately 5,000 tonnes per year, a five-fold increase from its previous capacity https://kanekagreenplanet.com/. In 2019, Kaneka partnered with Shiseido Co., Ltd. (Tokyo, Japan) to develop cosmetic containers and market PHBHTM drinking straws. The product, "AquaGel Lip Palette™," a biodegradable makeup container, became commercially available in 2020. Additionally, numerous global brand owners are exploring various uses for PHBH™, including in cutlery, straws, food containers, plastic bags, and packaging materials.

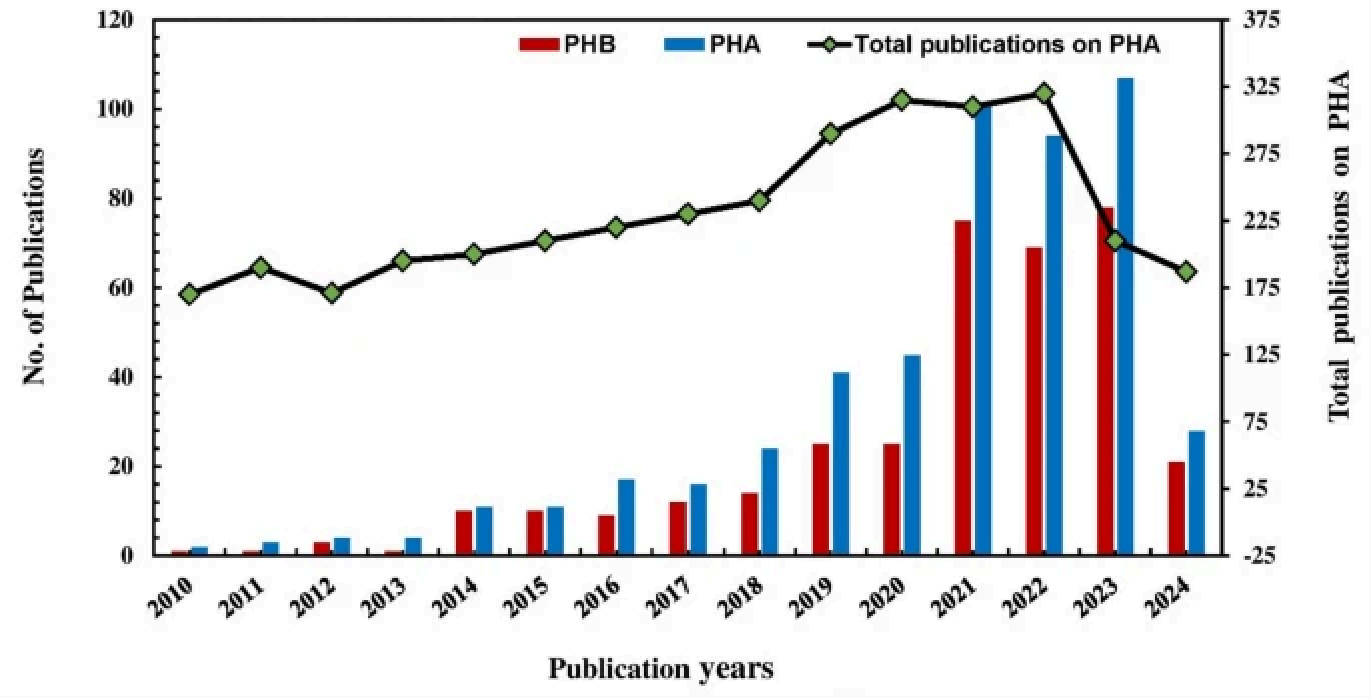
Similarly, China Oil & Foodstuffs Corporation (COFCO), based in Beijing, PR China, is recognized as the largest producer of food and beverages in the People's Republic of China (https://www.cofco.com/en/). Since the past few years, COFCO has commercialized P(3HB) homopolyester through the use of extremophilic production strains, many of which are genetically engineered. This approach, known as Next Generation Industrial Biotechnology (NGIB), utilizes resilient, extremophilic strains like the recombinant *Halomonas bluephagenesis* (formerly *Halomonas* sp. TD01), enabling production under non-sterile conditions with continuous cultivation, all while utilizing low-cost feedstocks and conserving fresh water and energy. The estimated annual production capacity is 1000 tons, downstream processing of COFCO for PHA recovery has been made more efficient as *Halomonas* spp. cells can be easily disrupted by washing with sodium dodecyl sulfate (SDS), allowing the PHA granules to be released into the aqueous phase. The technology was also explored by two other start-ups, Medpha and PhaBuilder. The P(3HB) produced are also having potenital to be used in the manufacturing of textile fibers.

Table 1 Chemical composition of waste cooking oil

Table 2 Different physical properties of PHA

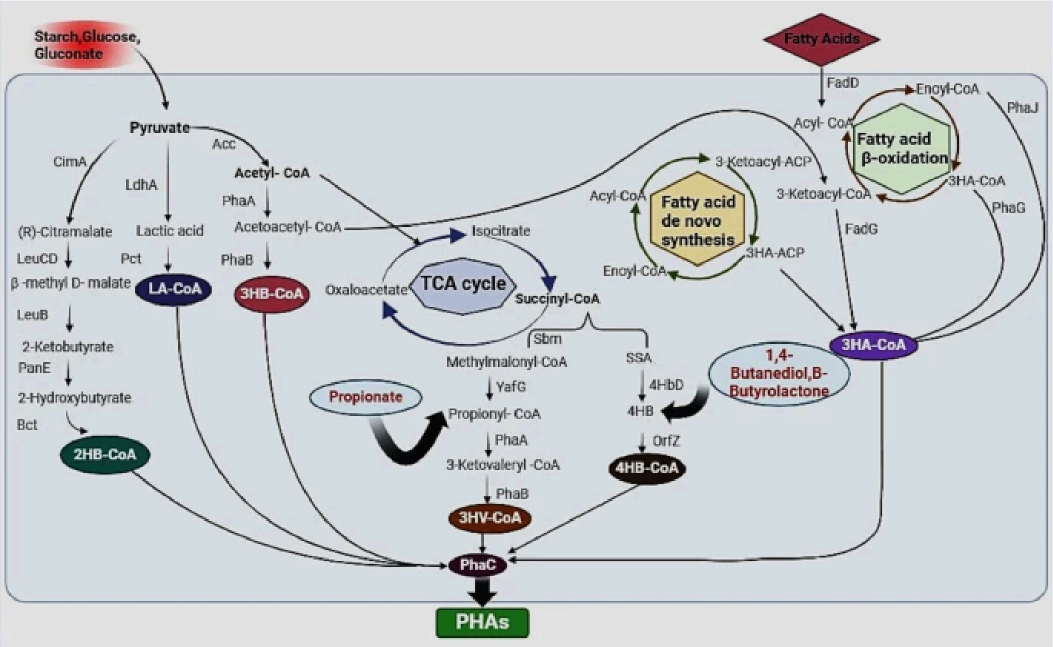
Table 3 Polyhydroxyalkanoates production by several microorganisms using waste cooking oil as feed material

Fig. 1



Number of research articles published on biopolyester using waste cooking oil as substrate from 2010 to 2024 (Scopus data, accessed on April 15, 2024). *PHA* polyhydroxyalka-noate production on WCO (blue bar); *PHB* polyhydroxybutyrate production on WCO (Red bar); total number of publications on polyhydroxyalkanoate in a given year (line; secondary axis)

Fig. 2



Metabolic pathway for PHA synthesis

Conclusions & outlook

WCOs are generated in substantial quantities worldwide, posing a significant challenge for sustainable management. Conventional applications, such as direct use for bioenergy production, are constrained by technological limitations and environmental risks. In contrast, microbial valorization of WCO into high-value bioproducts such as PHAs, biosurfactants, and enzymes offers a sustainable and economically attractive alternative. Despite the presence of impurities, the predominant constituents of WCO, namely TAGs, glycerides, and free fatty acids, provide an excellent carbon source to support microbial growth and metabolite synthesis. To improve process efficiency, advanced strategies such as metabolic engineering of microbial strains, optimization of PHA synthase activity, and pretreatment of waste oils are essential. Furthermore, the integration of complementary waste streams (such as crude glycerol, whey, or wastewater) either individually or as blended feedstocks can enhance nutrient balance and reduce overall process costs. Embedding PHA production within existing industrial infrastructures including wastewater treatment facilities, hydrogen generation systems, and biodiesel plants can further improve operational feasibility and sustainability. In the future, the development of adaptive and resilient bioprocesses capable of accommodating seasonal variations in feedstock quality, together with innovations in strain engineering and downstream processing, will be pivotal. Furthermore, integrating WCO valorization into urban biorefineries within a circular bioeconomy framework has considerable potential to mitigate environmental impacts while generating value-added products. With sustained research, supportive policy frameworks, and industrial collaboration, WCO could serve as a cornerstone feedstock for large-scale, sustainable biopolymer production.

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Author information

Authors and Affiliations

Department of Life Sciences, School of Biosciences and Technology, SSBSR, Sharda University, Greater Noida, 201310, Uttar Pradesh, India
Shivangi Sankhyan, Soumya Pandit & Subhasree Ray

Department of Biotechnology, School of Biosciences and Technology, Sharda University, Greater Noida, 201310, India
Prasun Kumar

Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, 522502, Andhra Pradesh, India
Madan Sonkar

Contributions

Writing – original draft: Shivangi Sankhyan; Writing – original draft, validation and conceptualization: Subhasree Ray; Conceptualization, review and editing: Prasun Kumar; Resources: Soumya Pandit; Validation: Madan Sonkar. All authors have read and agreed to the published version of the manuscript.

Corresponding author

Correspondence to Subhasree Ray.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interests.

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