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A review on upcycling waste cooking oil into polyhydroxyalkanoates (bioplastic): A pathway for sustainable material

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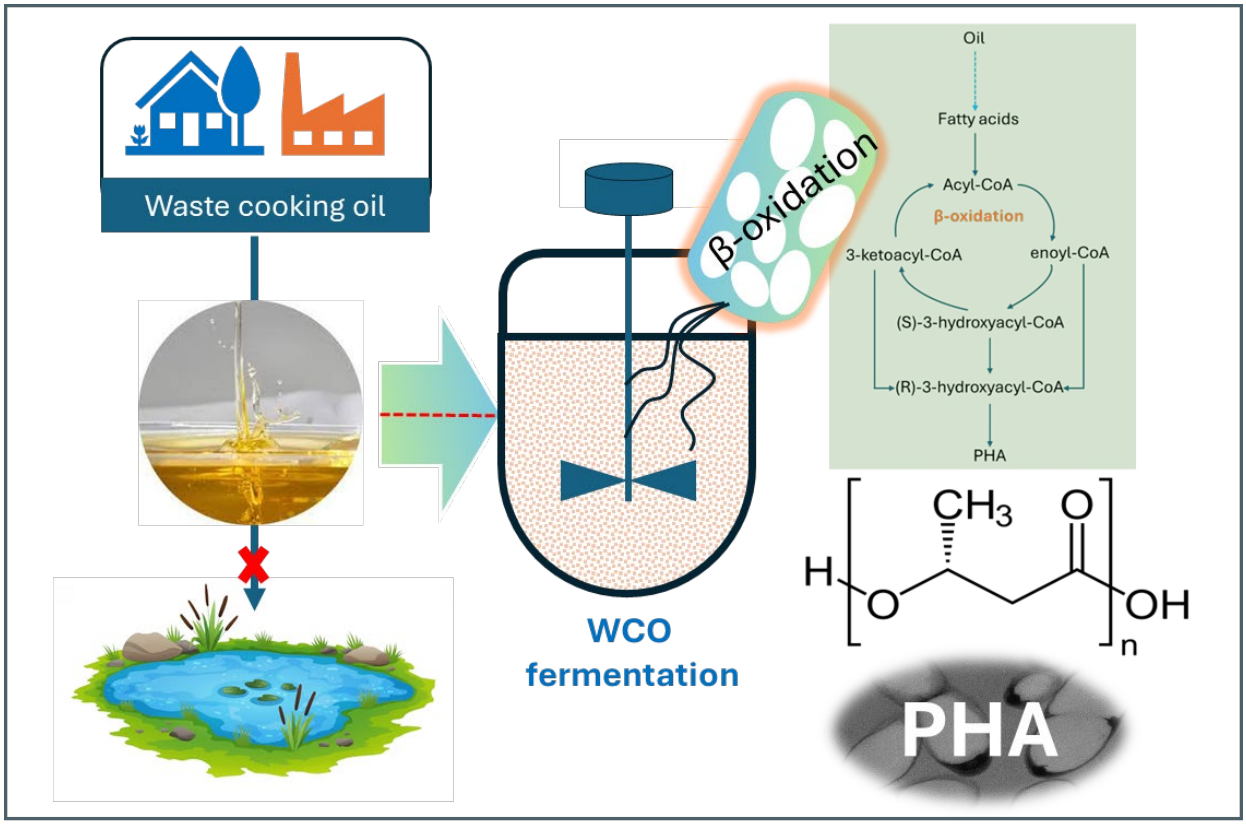
Graphical abstract



Abstract

Waste cooking oil (WCO) improper disposal leads to water pollution, ecosystem disruption, and human health hazards. Various upcycling strategies have been explored, including conversion to biodiesel, surfactants, and biodegradable polymers. Converting WCO into polyhydroxyalkanoates (PHAs), biodegradable and biocompatible bioplastics, offers a sustainable solution aligned with circular economy principles. WCO usually requires minimal or no pretreatment and can be effectively used as a carbon source for microbial fermentation. Free fatty acids (FFAs) from WCO are readily metabolized by PHA producing bacteria such as *Cupriavidus necator* and *Pseudomonas* spp., enabling PHA accumulation ranging from 27% to 96% (w/w). Depending on the microbial strain and fermentation strategy, both short chain length (*scl*-PHA) and medium chain length (*mcl*-PHA) polymers with varied properties can be synthesized. The coproduction of other products, such as carotenoids and surfactants, may further improve the process economics. However, variability in the composition of various oils can cause inconsistent productivity and monomer distribution, highlighting the need for thorough feedstock characterization. Insights from recent studies highlight that oils rich in long chain unsaturated fatty acids (LCFA), such as rapeseed or canola oil, enable the highest biomass and PHA yields, while oils dominated by medium chain saturated fatty acids (MCFA) favor flexible *mcl*-PHAs but with lower productivity. Integrating artificial intelligence (AI) and machine learning could further improve predictive analysis, process control, and strain selection. This review emphasizes the importance of aligning feedstock composition, microbial selection, coproduction, and improved fermentation strategies to advance sustainable PHA production from WCO.

Keywords: Biodegradable; polyhydroxyalkanoates; upcycling; waste cooking oil; circular economy; artificial intelligence

1. Introduction

Waste cooking oil (WCO) or used cooking oil (UCO) generally refers to oil that has been used for the frying process and is no longer suitable for consumption. Vegetable oil is composed of triacylglycerols (TGA) and, during deep frying undergoes hydrolysis, oxidation, cyclization, and polymerization, and decomposes into products (aldehyde, alkanes, and polyunsaturated fatty acids), making it unfit for consumption [1]. It is mainly sourced from domestic and commercial food establishments and represents a significant waste that poses considerable challenges in its management. The annual global production of WCO is estimated at 20-32% of the total 41-52 Mt oil consumed and is supposed to increase by 25% by 2025 [2]. Only 2.5% of WCO oil is recycled, which becomes a challenge in its management [3]. Waste cooking oil has a toxic effect on humans when consumed and a hazardous effect on the environment when disposed of openly. Open discharge causes blockage of drainage, increases the organic load of water, reduces dissolved oxygen, and disturbs the ecosystem [4]. Various methods of WCO upcycling, recycling, conversion, and utilisation as feedstock for microbial fermentation have been suggested for its management [5, 6]. A variety of products have been produced using WCO, like biodiesel, plasticizer, surfactant, and detergent [7-9]. Microbes are also able to utilize WCO and production of various products such as carotenoids, lipase, itaconic acids, polyhydroxyalkanoates (PHA), etc. [10, 11]. *Pseudomonas* and *Bacillus* are the best reported microbes for biosurfactant production, and *Yarrowia* is more suitable for the conversion of WCO into lipids [12, 13].

With industrialization and population growth, the use of petrol-based plastic is increasing, and its annual global yield is recorded at 367 million tons by 2020, which is supposed to increase by 29% till 2028 [14]. About 9% of plastic is recycled, and the rest of the plastic is disposed of in the environment and ends up in the ocean, causing pollution. There is a need to find alternative materials that are biodegradable and have properties similar to plastic.

Polyhydroxyalkanoate is a polyester considered a suitable alternative material as it is biodegradable and biocompatible [15-17]. It is produced by various microbes such as *Cupriavidus necator, Pseudomonas, Halomonas, Burkholderia* under stress conditions and in the presence of an excess of a carbon source [18-21]. A variety of feedstocks have been utilised for PHA production, such as pure sugar, dairy whey, wastewater, lignocellulosic biomass, WCO, etc. [22-24]. In spite of improvements in the fermentation process, the engineering of microbes for increased carbon utilisation and the ability to synthesise various copolymers, still PHA production cost (>$4/kg) is high compared to plastic ($1-2/kg) [25, 26].

WCO holds immense potential as a feedstock for microbial fermentation and PHA production, as it possesses a high lipid content. By upcycling WCO into PHA, the challenge related to the environmental impact of waste oil disposal can be overcome. *C. necator* and *Pseudomonas* spp. are widely studied microbes for oil utilization and short-chain length (*scl*-PHA) and medium-chain length (*mcl*-PHA) production, respectively. Santolin and colleagues were able to achieve the highest PHA production (106.7 g/L) using *C. necator* strain Re2058/pCB113 from two-stage process using rapeseed oil as a carbon source [27]. From the literature survey, it is clear that the researcher's interest increased in WCO utilization as feedstock for various products such as rhamnolipid, carotene, lipids, biodiesel, etc. during the last decades [10, 28, 29].

Keeping in view the importance of waste WCO management and its potential as a valuable feedstock for various products, this review article was planned to cover the recent updates and trends in WCO upcycling into PHA by microbial fermentation. This article provides information about best-reported microbes for PHA production from WCO, the strategy followed for oil utilisation improvements, engineering to produce copolymers, and coproduction of various products, artificial intelligence (AI) in process optimisation, techno-

economic analysis to demonstrate the feasibility of the process at a large scale. Through a thorough examination of the current state-of-the-art in WCO derived PHA production, this review seeks to identify key challenges, explore innovative solutions, and direct the way for a more sustainable and resilient future.

2. Literature search and keyword analysis

This review article presents a comprehensive and structured analysis of the current literature on the upcycling of WCO into PHA. A literature review survey of scientific publications was conducted from academic databases, including Scopus, PubMed, ScienceDirect, Web of Science, and Google Scholar. Among these, Scopus was frequently favored due to its broad keyword coverage and accessibility to diverse sources appropriate to this review. To refine our search and identify the most relevant research, we employed a variety of keywords like polyhydroxyalkanoates, waste cooking oil, poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate), bioplastic, biodegradable, waste cooking oil to polyhydroxyalkanoates, waste cooking oil to surfactant, waste cooking oil to lipids etc., and others. Using these keywords, we identified and selected most of the appropriate articles, thereby designing the backbone of this comprehensive review. The utilization of WCO has attracted attention in the circular economy for various product production, and there are about 3915 (3705 research, 209 reviews) articles that have been published from 1 January 2015 to 31 May 2024. The top 10 countries involved in WCO utilization related research and published a high number of articles include India (657), China (629), Malaysia (380), Egypt (349), Iran (199), the United States (183), Indonesia (152), United Kingdom (124), Brazil (114), Turkey (104) (Fig. 1a, 1b). Keyword search analysis using VOSviewer shows that most work is focused on technology development for the conversion of WCO into biodiesel. Recently upcycling of oil into PHA has also received attention as 511 articles published from 1 January 2015 to 31 May 2024, and Malaysia (92) and Japan (60) are on top, followed by India (46) and

China (43). South Korea is also making remarkable progress in this area and has published 29 articles. Scopus database was preferred for keyword analysis due to its extensive coverage and availability of information required for VOSviewer (bibliometric software). A keyword co-occurrence analysis was performed to identify dominant research themes related to the upcycling of WCO into PHA. A minimum threshold of 10 keyword occurrences was applied to include keywords in the network. The clustering of keywords was performed using the default resolution settings in VOSviewer, which generated distinct clusters representing major thematic areas in the literature. The resulting network visualization map provides a graphical representation of keyword relationships and research trends (Fig. 1c).

3. Waste cooking oil: A neglected resource

Waste or used cooking oil (WCO/UCO) is produced during the deep frying process of various plant oils (canola oil, sunflower oil, soybean oil, palm oil, olive oil, rapeseed oil, etc.) and animal fats (butter, ghee, fish oil, etc.). During the cooking process, oil undergoes various physical and chemical reactions, including hydrolysis, oxidation, degradation, and polymerization, leading to the production of free fatty acids responsible for foul smell and corrosion of metals [30]. The amount of WCO oil is increasing around the globe due to industrialization and escalated food production to meet the demand of the growing population. The worldwide WCO production is around 29 million tonnes [31]. Observer research foundation analyzed that per capita utilization of cooking oil has doubled in India during the last decade so on WCO amount [31]. Almost 60% of the used cooking oil in India makes its way back to food. Repeated use of used cooking oil is linked to increased chances of cancer, heart disease, and organ failure. The collection of used cooking oil has not reached its full potential, as in the EU, only 45% of oil is collected from restaurants and only 16% from private households. In the UK, it is a legal requirement that WCO should be collected by only

authorized collectors for its proper management [32]. The disposal of WCO becomes a challenge as it causes socioeconomic and environmental hazards. Due to a lack of public awareness and availability of proper disposal facilities, it is directly disposed of in sewers and the ground. WCO disposal in the open may coat animals and plants, causing depletion of oxygen and affecting their growth. Open disposal of waste oil causes blockage of drainage and increases operating costs of waste treatment plants, as it slows down the degradation of other organic pollutants. To reduce its hazardous effects on the environment and health, the implementation of regulations is necessary. A majority of WCO is used for biodiesel production in China and Korea [33, 34]. The global used cooking oil market size was valued at around $ 6.2 billion in 2022 and is expected to increase up to $ 11.5 billion by 2032 with a CAGR of 6.4% [35]. Europe holds the largest share of the global used cooking oil market owing to a high level of awareness about sustainable and ecofriendly sources and the implementation of various regulations and initiatives to encourage used cooking oil recycling. Asia-pacific region's WCO market is expected to increase at a faster rate as countries like China and India follow rapid industrialization.

4. Used cooking oil as feedstock for microbial fermentation

Microbes have the ability to utilize oil as a carbon source, involving intricate metabolic pathways within microbial cells. Waste cooking oil primarily consists of triglycerides, which are large molecules composed of glycerol and fatty acids. Oil metabolism involves steps like hydrolysis, transport, and intracellular metabolism to produce precursors and products. Microorganisms employ extracellular lipases or esterases to hydrolyze triglycerides into glycerol and free fatty acids. Once released, the free fatty acids are transported into microbial cells, where they undergo intracellular metabolism. Fatty acids are activated through the addition of coenzyme A (CoA) to form acyl-CoA derivatives. These derivatives enter β-

oxidation or other metabolic pathways to generate acetyl-CoA units, which are further oxidized in the citric acid cycle (TCA cycle) to produce energy in the form of ATP and reducing equivalents such as NADH and $FADH_2$. Microbes can produce diverse products like biosurfactants, enzymes, carotenoids, lipids, etc. utilizing oil (Table. 1). Biosurfactants are amphiphilic molecules with hydrophilic and hydrophobic regions, enabling them to reduce surface tension and enhance the solubility of hydrophobic compounds [36].

In the cytoplasm of microbial cells, acetyl-CoA is utilized in the mevalonate pathway or the methylerythritol phosphate (MEP) pathway to generate isoprenoid precursors, which are further transformed into carotenoids. Nanou et al., able to convert WCO into carotene (2021 mg/L) using *Blakeslea trispora* under submerged fermentation [37]. Under specific growth conditions, certain microorganisms exhibit lipid accumulation, leading to the synthesis of intracellular lipids or triacylglycerols (TAGs). Waste cooking oil can serve as a favorable substrate for lipid production in oleaginous microorganisms. Many other microbes like *Yarrowia, Rhodosporidium, Rodococcus,* have been reported with lipids accumulation potential [38, 39]. Microbial fermentation of WCO can also result in the production of single-cell proteins (SCP), which are microbial biomass rich in proteins, amino acids, and other nutrients. During fermentation, microorganisms assimilate carbon and nitrogen from the oil substrate, synthesizing biomass through protein biosynthesis pathways. SCP derived from WCO fermentation can be used as a sustainable protein source in animal feed, aquaculture, or food applications. Various products produced using oils are discussed in the table. 1. All these studies show the potential of WCO as a feedstock for microbial fermentation and valuable product production.

5. Upcycling of WCO into PHA

The use of WCO has also been reported for PHA production through fermentation. Microbes utilize and metabolize WCO through the *β*-oxidation pathway and produce various types of PHA.

5.1 PHA biosynthesis and types

A variety of microbes can accumulate PHA under adverse conditions. Different pathways are involved in PHA production from different carbon sources (Fig. 2). When oil is used as a substrate, PHA synthesis starts from acetyl-CoA produced by the *β*-oxidation of oil. The *β*-ketothiolase (PhaA) enzyme initiates the PHA synthesis process by catalyzing the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, which is further converted into (R)-3-hydroxybutyryl-CoA by acetoacetyl-CoA reductase (PhaB) through reduction with NADPH as a cofactor. Finally, the PHA synthase enzyme (PhaC), polymerizes hydroxyacyl-CoA monomers into PHA polymers by forming ester bonds between them in the cytoplasm of microbial cells [46]. PHA is classified as short-chain (*scl*-PHA (C3-C5)), medium-chain length (*mcl*-PHA (C6-C14)), and mixed (*scl-mcl* PHA) based on carbon chain length. Short-chain length polymers have a thermoplastic nature, while *mcl*-PHA is elastic and considered a more suitable material for cosmetic and tissue engineering applications [47]. There are around 150 types of monomer units that have been reported for the synthesis of various copolymers. Generally, microbes accumulate poly(3-hydroxybutyrate) (PHB), while the synthesis of other copolymers is achieved by precursor feedings and an engineering approach. The most commonly produced *scl*-PHA are poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)], poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] using precursor feeding approach [48-50]. Short chain length PHA are poor in properties, and researchers have produced other types of copolymers such as poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)], poly(3-hydroxyhexanoate-*co*-3-hydroxyoctanoate) [P(3HHx-*co*-3HO)],

poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HV-*co*-3HHx)] [51-53].

Various analytical techniques are essential in PHA research to ensure comprehensive material characterization (Table 2). Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) aid in identifying monomer composition, nuclear magnetic resonance (NMR) offers structural insights at the molecular level, fourier-transform infrared spectroscopy (FTIR) confirms functional groups in the polymer, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) assess thermal properties such as stability and crystallinity [54, 55]. Scanning electron microscopy (SEM) reveals surface morphology, transmission electron microscopy (TEM) is used for internal structure (PHA granule), gel permeation chromatography (GPC) is used to determine molecular weight distribution, which influences the polymer's mechanical and processing behavior [56]. Together, these methods enable a detailed understanding of PHA properties for both research and application development.

Most of commercial-scale PHA productions are based on pure carbon sources and account for almost 40% of the production cost. Researchers have explored a variety of feedstocks (lignocellulosic biomass, whey, sludge waste, syngas, etc.) to make the PHA production process more economical (Table. 3). There are different challenges associated with different feedstocks like lignocellulosic biomass requires pretreatment to release the free sugars which require additional steps and chemicals [57]. Further methods like dilute acidic pretreatment result in the production of various side products like acetic acid, formic acid, furfural, and hydroxymethylfurfural (HMF), which affect microbial growth and PHA accumulation [58]. Biomass hydrolysate further requires detoxification (activated carbon and ion exchange) to remove these byproducts before subjecting it to fermentation [59, 60]. Hydrolysate contains mixed sugars (glucose, xylose, and arabinose), but many microbes can

utilise only glucose and can not metabolize pentoses efficiently [61]. Whey has an acidic pH, and most microbes are not able to utilize lactose and lactic acid, the main components present in it. Similarly, other wastes like orange peel possess limonene, which is toxic to microbes and inhibits their growth [62]. The use of WCO as a feedstock may be advantageous as it will not require any pretreatment and provide precursors for *mcl*-PHA.

5.2 Strategies for WCO conversion into PHA

Microbial fermentation is an environmentally friendly and promising method for upcycling WCO into PHA (Fig. 3a, b). WCO oil requires filtration when used for biodiesel and soap production, while it can be directly used as feedstock for PHA production. *Cupriavidus necator* (previously known as *Ralstonia eutropha*) was used by Verlinden et al., for waste frying oil (rapeseed oil) utilization and PHA production [72]. Batch fermentation was performed at 20 g/L oil concentration, and the medium was sonicated to achieve a homogenized medium. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) is the other most widely studied copolymer due to its improved properties, and high HV content is desired for industrial applications. Kokpinar et al., cultivated *C. necator* H16 at 5 L scale using WCO and investigated the effect of various factors like nitrogen, phosphorous, and oxygen limitation on PHBV production. Under optimised conditions with propionic acid feeding the process leads to 121 g/L PHBV with 13.9% HV content under oxygen limitation conditions. Nitrogen limitation reduces the cell biomass and PHBV production, while phosphorus limitation increases the HV fraction up to 19.8% [73].

Ruiz et al., developed a high cell density fermentation method for *P. putida* KT2440. Waste cooking oil was chemically hydrolyzed to free fatty acids, and a feed strategy was developed to delay the stationary phase. The fermentation process resulted in PHA accumulation composed of 3-hydroxyhexanoic acid (3HHx), 3-hydroxyoctanoic acid (3HO), 3-hydroxydecanoic acid (3HD), 3-hydroxydodecanoic acid (3HDD), 3-hydroxyundodecanoic

acid (3HUDD) [74]. Pan et al., used *P. alcaligenes* with waste frying oil as a carbon source and reported PHA production composed of 3HO, 3HD, and 3HDD [75]. Wastewater produced during the process was further used for methane production. Sharma et al., isolated *Pseudomonas chlororaphis* strain PA2363 from soybean roots and studied it for PHA production using free fatty acids and vegetable oil as a carbon source [76]. Cultivation of strain with even chain fatty acid (octanoic acid) resulted in a polymer with 90% 3HO, while with odd chain fatty acid (nonanoic acid) polymer composed of 40% 3-hydroxyheptanoate and 60% 3-hydroxynonanoate was produced. With waste oil as a feedstock produced polymer composed of HHx (10.79%), HO(54.58, HD (36.63%), HDD (4.03%), HTD (3.2%) was produced [76].

C. necator is generally able to produce PHB and is not suitable for industrial applications. Budde et al., hypothesized that a high level of HB-CoA in *C. necator* limits the incorporation of other monomer units, even the PHA synthase able to polymerize HB-CoA and HHx-CoA [77]. They engineered a strain by deleting acetoacetyl-CoA reductase, and overexpressing *phaJ* and *phaC,* and were able to produce P(HB-*co*-HHx) with >12% HHx content [77]. The same engineered strain was further explored for high cell density cultivation using oil as a carbon source, and the process resulted in P(3HB-*co*-3HHx) containing 19 mol% HHx [78]. Kamilah et al., engineered *C. necator* by introducing *phaC* synthase gene of *Aeromonas caviae,* and were able to produce P(3HB-*co*-3HHx) up to 85% w/w from used oil [79]. In another study, Qing-Fook et al., engineered *C. necator* by overexpressing *phaC* from uncultured microbes, and the resulting strain was able to produce P(3HB-*co*-3HHx) where mealworms were utilised for PHA recovery, and physicochemical properties of the extracted PHA were similar to chemically extracted PHA [80]. Pernicova et al., used *Halomonas hydrothemalis* for PHA production from waste frying oil supplemented with valerate and reported P(3HB-*co*-3HV) production with 50.16 mol% HV fraction [81]. *E. coli* is considered as a suitable host for engineering and controlled copolymer production as precursor availability

can be assured only by introducing recombinant enzymes. Vastano et al., engineered *E. coli* by overexpressing PHAs biosynthetic operon from *Bacillus cerus* 6E/2, having high specificity for incorporation of HHx monomers. Using the engineered *E. coli* they were able to produce P(3HB-*co*-3HHx) with 99.7% mole fraction using WCO [82].

The oil's fatty acid composition, combined with the microbial strain's metabolic capabilities, critically governs PHA yield, monomer composition, and polymer properties. From the analysis of data of various oil compositions (table 4) and PHA productivity from various oils (table 5), a clear pattern linking SFA/UFA content and chain length with PHA production efficiency, productivity, and properties is observed (Fig. 4). Oils with long-chain unsaturated fatty acids (UFAs), especially those rich in oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, enabled exceptional biomass and PHA productivity, particularly with robust strains like *C. necator* Re2058/pCB113. For instance, rapeseed oil (94.03% UFA, dominated by C18:1–C18:3) supported the highest yields (124 g/L DCW, 106.7 g/L PHA, 86.1% accumulation), illustrating that high-UFA oils with long-chain (C16–C22) FAs maximize biomass and PHA accumulation [27]. Similarly, canola oil (90.74% UFA) consistently achieved strong performance across various reports with engineered *C. necator* strains (up to 13 g/L DCW and 88% PHA accumulation), producing copolymers like P(3HB-*co*-3HV-*co*-3HHx) with tunable properties and balanced flexibility [52]. The use of sunflower (95.47% UFA) and soybean oil (83.99% UFA) further reinforces that high UFA, long chain oils consistently drive superior productivity and enable various copolymer production [83-85]. Conversely, oils high in medium chain saturated fatty acids (MC-SFAs) notably coconut oil (100% SFA) and palm kernel oil (82.1% SFA) produced moderate biomass (5–8 g/L DCW) and lower PHA productivity, though they favored *mcl*-PHA copolymers like P(3HB-*co*-3HHx) [50, 86]. This reflects the ability of microbes like *C. necator* to incorporate MC-SFAs directly into *mcl* monomers, but at the expense of total yield due to slower cell growth on saturated

substrates. Balanced oils such as palm oil (~50% SFA, 50% UFA) led to high PHA accumulation (up to 80%), but PHAs dominated by rigid 3HB (>99%) [87]. In conclusion, long-chain oils with >80% UFA content, especially those rich in oleic and linoleic acids (e.g., rapeseed, canola), are optimal feedstocks for maximizing biomass, PHA productivity, and accumulation. Their fatty acid profile aligns perfectly with *C. necator*'s metabolic pathways, enabling both high yields and diverse, flexible copolymers.

6. Strategies to improve oil valorisation

6.1 Improved oil solubility and utilization

Oils are hydrophobic, having poor solubility in water with a lack of uniform dispersion in aqueous medium. Further oil sticks to the bioreactor walls and makes the correct analysis of the process challenging. Microbes show different growth behavior and PHA accumulation with oil and its hydrolyzed products. Oil first needs to be hydrolyzed into glycerol and fatty acids and then utilized further through the *β*-oxidation pathway. Different methods like hydrolysis, saponification, and sonication have been reported to increase oil solubility and utilization. Walsh et al., studied different *Pseudomonas* strains for oil and PHA accumulation. Among the four tested strains, only *P. chlororaphis* 555 was able to grow well on oil and free fatty acids and produce PHA, while some strains were able to grow on free fatty acids only [100]. To perform high cell density cultivation Ruiz et al., hydrolyzed WCO with 6 M NaOH at 60 ºC for 90 min to produce free fatty acids. In another study, Mozejko et al., used 2% ethanolic potassium hydroxide solution for the saponification of oil and used it as a carbon source for *Pseudomonas* sp. G10 and produced *mcl*-PHA 43% w/w [101]. Surfactants can also be used to increase oil solubility and uniform dispersion. Kumar and colleagues used various surfactants (Tween-80, sodium dodecyl sulfate (SDS), and gum acacia) and performed a comparative analysis, and 0.1% of Tween-80 was found as the optimum concentration for *Paracoccus* sp.

LL1 growth and PHA accumulation (24 %w/w) and gum acacia have no effect while SDS showed a toxic effect [102].

6.2 Coproduction of products

High capital and operational costs related to fermentation control, separation, and purification limit commercial scalability. Coproduction offers a realistic path forward to overcome these barriers. By sharing cultivation and operational costs across multiple products, the process becomes more financially sustainable. Furthermore, certain microbes naturally produce both intracellular and extracellular products, allowing simpler physical separation techniques. For instance, PHA granules are harvested from biomass, while rhamnolipids or carotenoids remain in the culture medium or cell membranes, reducing overlap in recovery steps. Technological innovations like surfactant assisted oil uptake, optimized fermentation strategies, and metabolic pathway engineering further enhance productivity. Biosurfactants are one of such compounds, amphiphilic in nature, and have emulsifying, antioxidant, and antimicrobial activity. Kourmentza and colleagues studied *B. thailandensis* for the coproduction of PHA and rhamnolipids using WCO as a carbon source, and were able to coproduce 7.5 g/L PHA and 2.2 g/L rhamnolipids [98]. Marsudi et al., explored *P. aeruginosa* IFO3924 for palm oil utilization and coproduction of PHA and rhamnolipid. *P. aeruginosa* IFO3924 secretes lipase and hydrolyzes oil into fatty acids and glycerol, which are further used for PHA and rhamnolipids production. PHA and rhamnolipid production start when the nitrogen source is exhausted, and PHA synthesis continues till all fatty acids are exhausted, while rhamnolipid synthesis still goes on until all glycerol is consumed [101]. Still, there is no study to understand the extent of carbon metabolic flux favors the production of one product over the other. Kumar and colleagues explored *Paracoccus* sp. LL1 for the coproduction of PHA and carotenoids production using used cooking oil as a carbon source, and able to achieve 1.0 g/L PHA with 0.89 mg/L astaxanthin [102]. Rodrigues et al., cocultured *C. necator* and *Xanthomonas campestris* using

palm oil and reported coproduction of PHA (3.39 g/L) and xanthan gum (1.77 g/L) [103]. The ability to integrate waste valorization, biopolymer production, and coproduct generation into a single streamlined process supports the broader goals of circular bioeconomy and resource efficient manufacturing. These systems not only mitigate waste disposal issues but also provide an avenue for generating multiple high value products from a single, low cost input, positioning WCO as a strategic resource in sustainable biotechnology.

6.3 Engineering of microbes

Microbes can be engineered to improve their capability to utilize oil and produce PHA by expressing enzymes from various sources. The acyl-CoA dehydrogenase (FadE) and enoyl-CoA hydratase (PhaJ) are related to *β*-oxidation pathway where FadE converts fatty acids into enoyl-CoA, a substrate required by PhaJ to produce PHA. Flores-Sanchez et al., overexpressed *fadE* and *phaJ* in *C. necator* H16 and used it for canola oil valorization into PHA (4.17 g/L) [94]. To improve the HHx in copolymer Harada et al., improved *phaC* of *Aeromonas caviae* using site directed mutagenesis, and the mutated gene was overexpressed in *C. neacator*. Engineered strain able to produce P(3HB-*co*-3HHx) with increased HHx (13 mol%) fraction without affecting PHA content [104]. To improve the oil utilization Wong et al., heterologously expressed *lipAB* in *Cupriavidus malaysiensis* USMAA2-4, and the engineered strain showed 40 fold increased lipase activity compared to the wild strain. Engineered strain cultured with palm olefin and 1-pentanol as a precursor able to accumulate P(3HB-*co*-3HV) up to 68 %w/w [51]. Along with precursor availability, synthesis of various copolymers depends on PHA synthase substrate specificity. Valdes et al., engineered *C. necator* by heterologous expression of *phaC2* from *P. putida* CA-3 and native expression of *phaC1* and were able to produce tetrapolymer containing 3HB, 3HV, 3HHx, 3HO from canola oil [95].

6.4 Artificial intelligence in PHA production

Researchers are turning to AI to overcome complex process challenges that traditional trial-and-error methods struggle to resolve. One key area is bioprocess optimization, where instead of manually adjusting culture conditions to improve yield, scientists are using artificial neural networks (ANNs) and genetic algorithms (GAs) to predict and fine-tune factors such as substrate concentration, nitrogen limitation, pH, and temperature (Fig. 3b). Zafar et al., used a hybrid ANN–GA model to optimize PHA production from cane molasses and volatile fatty acids using *Azohydromonas lata* and were able to achieve 6.70 g/L (3HB-*co*-3HV) with 16.35 mol% HV fraction that outperformed conventional statistical tools like response surface methodology (RSM) [105]. In another study, Laurence et al., used a double layer ANN model to optimize sunflower oil based PHA production, allowing accurate prediction of biopolymer yield under varying conditions. Optimisation by ANN model resulted in 10.41 g/L PHA from the sucrose/oil ratio of 1.7 (w/v) with an inoculum dose of 0.85 g/L [106]. The benefit of using AI is that once the model is trained, these models can simulate many scenarios rapidly, saving time and cost while also guiding the operator toward ideal operating zones. In continuous production systems, where conditions constantly change, hybrid models combining mechanistic equations with machine learning provide even deeper process insights. These models can adapt in real time to shifts in nutrient levels or microbial behavior and can be used successfully to simulate dual nutrient limited growth in *P. putida*, which is critical for steady PHA synthesis [107]. Beyond just fermentation, AI is also influencing strain development. Rational engineering, aided by model driven approaches, allows researchers to modify metabolic pathways more precisely, optimizing carbon fluxes and redox balances to push more carbon toward PHA synthesis instead of cell maintenance or byproducts. With genome scale metabolic models supported by AI prediction tools, scientists can simulate the impact of deleting or overexpressing certain genes before doing any lab work. This saves time and

resources while enhancing the likelihood of success. Lastly, AI helps in extraction and quality control. Techniques like AI powered spectroscopy can be integrated for real time monitoring of PHA accumulation and purity without needing labor intensive chemical assays. Taken together, the role of AI in PHA production is no longer theoretical, and it is becoming central to scaling these sustainable materials. From selecting the right waste oil, predicting fermentation outcomes, and designing better microbes, to recovering the product efficiently, AI is helping researchers unlock higher yields, lower costs, and more consistent biopolymer quality. These advances are vital in making WCO to PHA conversion competitive with fossil based plastics.

7. Factors that affect oil upcycling into PHA

Upcycling of WCO into PHA is influenced by various fermentation process parameters and oil composition (Fig. 5). The use of virgin oil and used oil results in different PHA productivity as explored by Martino et al., for *C. necator* where virgin rapeseed oil resulted in 20% PHA, while used rapeseed oil 40% PHA due to the high content of FFA in the latter [108]. Used cooking oil composition and storage affect microbial growth and PHA accumulation. During the deep frying of oil, peroxides and free fatty acids are produced. With the increase of storage time free acid content increases. Kongpeng et al., used oil stored for 4 weeks and 10 weeks for PHA production and reported low content of PHA with oil stored for a longer time due to higher content of FFA [109]. Oh et al., evaluated lauric acid and myristic acid effects on engineered *C. necator* H16 and reported 1.5% and 2% as optimum concentrations for growth and PHA production, and above this concentration, a decrease in growth and PHA production was noticed [18]. At lower concentrations, FFA can serve as effective carbon sources for PHA producing microorganisms; however, at higher concentrations, they exhibit inhibitory effects on microbial growth and PHA accumulation. This concentration dependent behavior is largely due to the amphipathic nature of FFAs, which at elevated levels can disrupt cell membrane

integrity, interfere with DNA/RNA replication, and induce oxidative stress through the formation of peroxides and reactive oxygen species [110]. Fatty acid types (chain length, even, and odd number) also affect microbial growth, PHA accumulation, and monomeric composition. Blunt et al., studied *mcl*-PHA production in *P. putida* LS46 using medium chain (octanoic acid) and long-chain fatty acids under microaerophilic conditions. They observed that *P. putida* LS46 growth ceased in the octanoic case when the oxygen uptake rate was limited, and cells were able to accumulate 61.9% PHA, while in the case of long chain fatty acids cell biomass continued to increase even under oxygen limitation but PHA accumulation was low (31%). Cofeeding of both substrates resulted in improved PHA titers compared to individual substrates, and the study demonstrates that long chain fatty acids improve growth even in oxygen limited environments [111]. The mole fraction of various monomers in the copolymer can be controlled by using different fatty acids. Oh et al., explored fatty acids from C6 to C18 as carbon sources for *C. necator* H16 containing $phaC2_{Ra}$-$phaA_{Cn}$-$phaJ1_{Pa}$ and reported that with lauric acid (C12) the HHx mole fraction increased by 26.5 % [18]. Along with oil composition, other operating parameters such as fermentation strategy, C/N ratio, oxygen availability, etc., also affect PHA productivity. Two-stage fermentation is considered an ideal approach for PHA production as it can achieve high cell density and avoid substrate inhibition. Masood et al., performed two-stage fermentation with *Bacillus cereus* FA11, where in the first step, glucose was used as a carbon source, and in the second stage, olive oil was used as a carbon source, and the process resulted in ter-copolymer production with 60.31% w/w [112]. In another study, Mozejko et al., cultivated *Pseudomonas* sp. Gl01 with rapeseed oil using different feeding strategies, and able to achieve high PHA accumulation with pulsed feeding (44%) compared to continuous feeding (24.6 %) [113]. Along with the effect on polymer composition, oil sources also affect molecular weight. Zhila et al., used palm oil and sunflower seed oil and reported PHA of different molecular weights $682x10^3$ Da and $479x10^3$

Da, respectively [53]. C/N ratio is also an important factor, Santolin et al. studied the effect of C/N (5-90) on *R. eutropha* Re2058/pCB113 with canola oil as a carbon source and found that at lower C/N (5) there is little biomass and PHA accumulation and increase was noticed till C/N 22, beyond this biomass starts to decrease and a slight increase in PHA accumulation was noticed [52]. Kokpiar et al., studied the effect of nitrogen, phosphorus oxygen limitations on *C. necator* H16 ability to produce P(3HB-*co*-3HV) from WCO and found that nitrogen limitation reduces biomass while phosphorus and oxygen limitation increase PHA accumulation and HV fraction [73]. Halophilic microbes require NaCl for their growth, and its concentration affects biomass, PHA accumulation as well as the molecular weight of the polymer. Pernicova et al. studied the effect of NaCl on *H. hydrothermalis* cultured on waste frying oil and reported 4% is the optimum concentration with 3.64 g/L dcw, 2.26 PHA g/L, 61.98% and reduced production at 10% NaCl i.e. 1.90 g/L dcw, 1.2 PHA g/L, 48.54%. The molecular weight of PHA also reduced from 382.77x10^3 Da to 245.15 10^3 Da at 10% NaCl [81]. From all these studies its clear that oil composition and operating parameters have a direct effect on PHA production and its composition.

8. Technoeconomic and life cycle assessment of PHA production

Technoeconomic analysis (TEA) results show that PHA price directly depends on the process and feedstocks used. Most PHA production processes are based on pure carbon sources like glucose or sucrose and account for 40% of the production cost. For instance, the cost of PHA from various feedstocks such as glucose ($8.6/kg), sucrose ($3.7-11.9/kg), dairy whey ($5.1-$7.9), methane ($4.1–$6.8/kg), and waste glycerol from biodiesel ($2.0-2.6 /kg [25, 114] has been analysed. These high costs often result in expensive end products, making it difficult to compete with conventional plastics that typically cost $1-2 per kilogram. The use of waste like food waste, offers a cost-effective alternative due to its abundance and low price. However, the conversion process requires complex and costly pretreatment and hydrolysis steps. Rajendran

et al., performed a TEA of the integrated process of PHA and biofuel production and concluded that it is possible to reduce the minimal selling price of PHA to $2.41/kg [115]. Waste cooking oil presents an attractive option due to its low cost and abundant availability, often costing less than $100 per ton. Utilizing WCO as feedstock for microbial fermentation, PHA production can be reduced further as it doesn't require any pretreatment, and high productivity can be achieved. There is no study related to TEA analysis of PHA production from WCO.

Life cycle assessment (LCA) of PHA production evaluates the environmental impacts from raw material procurement to end-of-life disposal. Yu et al., performed a cradle-to-factory gate life cycle assessment related to greenhouse gas emission and energy requirement for bioplastic production and concluded that 1kg PHA production emits only 0.49 kg $CO_{2\text{-}e}$ compared to 2-3 kg $CO_{2\text{-}e}$ of the petrochemical counterpart. Further energy requirement for PHA is 44 MJ, much less compared to petrobased plastics (77-88 MJ) [116]. Nitkiewicz et al., evaluated different processes, i.e, i) production of *mcl*-PHA.P(3HB) from crude vegetable oil, (ii) production of PHB from used vegetable oil, and iii) *mcl*-PHA.P(3HB) with biodiesel byproducts. They found that PHB production is lower than that of *mcl*-PHA and has a greater environmental impact, and it can be greatly reduced by using used oil as a feedstock rather than virgin oil [117]. Energy use in the production process, including pretreatment and fermentation, is a critical factor, with lignocellulosic biomass requiring significant energy input for pretreatment, estimated at 2-3 MJ per kg of biomass processed, while waste cooking oil processes are less energy-intensive. Overall, the LCA indicates that utilizing waste WCO and lignocellulosic biomass for PHA production is more environmentally friendly compared to using pure carbon sources, supporting sustainable bioplastic production with reduced greenhouse gas emissions and lower resource consumption.

9. Challenges and future direction

The conversion of WCO into PHA offers an environmentally friendly alternative to conventional plastics, but several technical and economic hurdles stand in the way of making it commercially viable. Collecting and transporting WCO from scattered sources like households and restaurants adds logistical expenses, especially in areas without organized collection systems. Not all microorganisms can hydrolyze triacylglycerols because they lack lipase production, which limits the range of microbes available for efficient PHA production from oil. Additionally, WCO often contains impurities such as free fatty acids, mono- and di-glycerides, water, and food particles, which can affect microbial growth and fermentation efficiency. Using oils in an aqueous medium also poses challenges because oils are not soluble and can solidify at low temperatures, necessitating the addition of surfactants to increase solubility. However, these surfactants can adversely affect microbial growth and PHA synthesis. Employing microbial cocultures where one partner is able to produce lipase can be a suitable option, as these microbes can hydrolyze triacylglycerols and provide free fatty acids to PHA-producing microbes. Additionally, using microbes that produce biosurfactants can also be beneficial, as these can increase the solubility of oils in the medium. The use of halophilic microbes should also be explored for PHA production as they are robust and cultured without contamination [118]. Strain goods in oil utilization and lack of PHA production can also be engineered by expressing PHA synthesizing genes to get better productivity. Other approaches, like cell engineering to increase cell size and accumulate higher PHA content, and production of high-value copolymers with better properties, can also help to make the process economic. WCO varies in composition, and microbes have different preferences for oil. Identifying and utilizing novel microbial strains with inherent capabilities to degrade and assimilate a wide range of oil substrates can also expand the potential for efficient PHA production from waste

oils, contributing to sustainable biotechnological processes for the production of biodegradable materials.

The fermentation process itself tends to have low productivity, demanding larger bioreactors and longer cultivation times, which increases both capital and operational costs. Specialized bacterial strains and controlled fermentation setups are often necessary to efficiently convert WCO into PHA, but these require investment in R&D and infrastructure that many producers can't afford. Fed-batch and pulse-feeding processes are commonly employed to control substrate concentration and mitigate the inhibitory effects of free fatty acids. Maintaining an optimal carbon-to-nitrogen (C/N) ratio and dissolved oxygen levels is also essential, as these factors significantly influence microbial growth and PHA yield. High oil concentrations can lead to oxygen transfer limitations due to the oil's hydrophobic nature, necessitating careful monitoring and adjustment of aeration and agitation rates in bioreactors. Additionally, the extraction and purification of PHA from oil-based fermentation processes can be complicated by the presence of residual oils and impurities, requiring efficient downstream processing techniques to obtain high-purity PHA. Most PHA extraction processes are solvent-based and not eco-friendly. Therefore, developing extraction processes based on biological methods and using less toxic solvents is desirable to make industrial PHA production more sustainable.

Artificial intelligence offers targeted solutions to many of these hurdles. Machine learning models can analyze WCO composition data and predict fermentation outcomes, helping preselect suitable WCO batches or recommend necessary pretreatments. AI-integrated sensors and control systems can dynamically adjust feeding rates, aeration, and emulsifier dosage in real time to maintain optimal growth conditions. In hybrid modeling approaches that combine mechanistic and data driven models, AI can bridge gaps in metabolic understanding and provide reliable predictions even when data is noisy or incomplete. AI can also play a role

in microbial strain improvement. Genome scale metabolic models, integrated with AI tools, can predict gene targets for knockouts or overexpression, guiding metabolic engineering toward better substrate utilization and PHA yields. For downstream processing, AI can assist in automating separation techniques by monitoring emulsion stability and phase transitions, predicting the optimal timing for recovery steps, and reducing the need for harsh chemicals. Advanced analytics can also track impurities and assist in quality control of the final biopolymer. Ultimately, integrating AI into WCO to PHA systems transforms the process from a rigid, static setup into a flexible, intelligent biomanufacturing platform capable of handling the variability inherent to waste based substrates and meeting industrial scalability demands.

Meanwhile, WCO is already in demand for biodiesel production, creating competition that can push up feedstock prices. Even if production hurdles are overcome, PHA still faces limited market demand due to its higher price compared to conventional plastics, lack of consumer awareness, and underdeveloped supply chains. Regulatory approvals and certifications, especially for food contact or medical applications, can also be costly and time consuming. To improve inter laboratory comparability and reproducibility in PHA production from WCO, standardisation across metrological, analytical, and procedural dimensions is essential. Establishing uniform protocols for WCO characterization, such as measuring free fatty acid content, total lipid composition, and oxidation indices would ensure consistency in substrate quality. Similarly, standardising microbial fermentation conditions (e.g., pH, temperature, inoculum size, C/N ratio, and fermentation time) and PHA quantification methods (e.g., gravimetric analysis, gas chromatography, and NMR) would allow for reliable comparison across strains and studies. To facilitate global collaboration, a centralized, open access database compiling WCO composition profiles, microbial strains used, culture conditions, and resulting PHA yields would be highly beneficial. Such a resource could serve

as a benchmark to guide future research and decision-making for WCO to PHA valorization processes.

Finally, the lack of large scale processing infrastructure and investor hesitation due to uncertain returns makes it difficult to scale up. Together, these factors create a tough economic landscape for WCO to PHA conversion, despite its clear environmental advantages. More efforts and research are needed to make to WCO to PHA concept a reality for its industrial production and commercialization.

10. Conclusion

Waste cooking oil may affect the environment and health, and requires urgent action for its management. It is readily available at low prices and doesn't require any processing before using as a feedstock. Further, WCO utilization is advantageous due to the presence of free fatty acids, making it easily consumable for *mcl*-PHA productions with better productivity. The two-step fermentation is a preferred process to achieve high PHA production. The coproduction of various products may reduce production costs, but still development of an efficient downstream process is needed. Future research should focus on standardizing feedstock characterization, analyzing impurities impact on biomass and PHA accumulation, improving strain robustness, and scaling up ecoefficient production systems. Artificial intelligence (AI) and machine learning tools hold the potential for optimizing fermentation parameters, predicting microbial behavior, and facilitating real time monitoring, thus accelerating the development of reliable and reproducible WCO to PHA production platforms. In conclusion, upcycling WCO seems a more ecofriendly and economical approach for PHA production.

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Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Shashi Kant Bhatia: Writing – review & editing, Writing – original draft, Visualization, Software, Conceptualization. **Anil Kumar Patel**: Writing – original draft, **Ganesh Dattatraya Saratale**: Writing – review & editing. **Vinod Kumar:** Writing – review & editing. **Yung-Hun Yang**: Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization.

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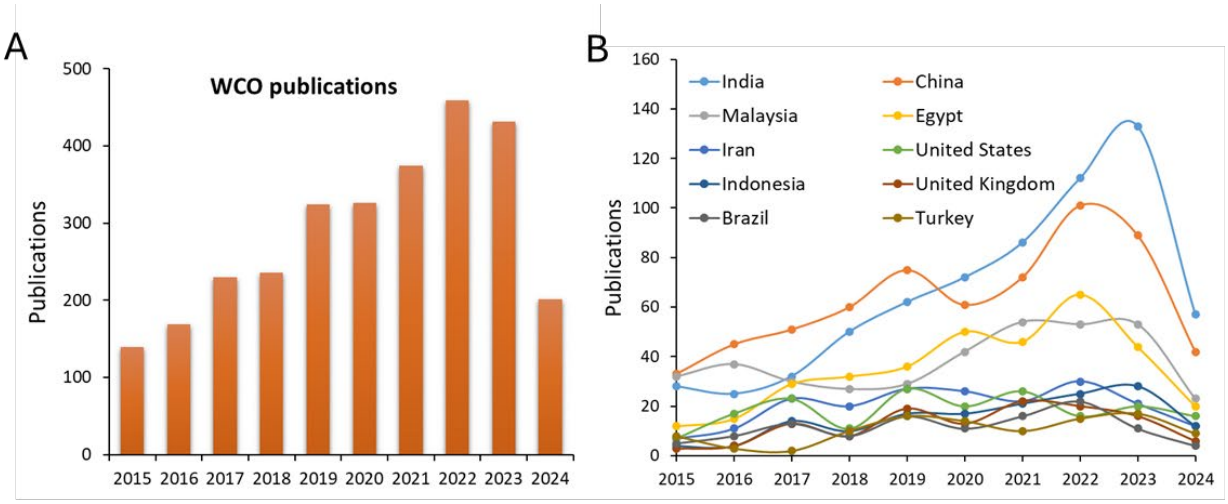
Fig. 1. Waste cooking oil (WCO) related publication data from Scopus (1 January 2015–31 May 2024) (a) research articles published (b) top ten countries publication data (c) keyword map of articles published (minimum number of occurrence 10).



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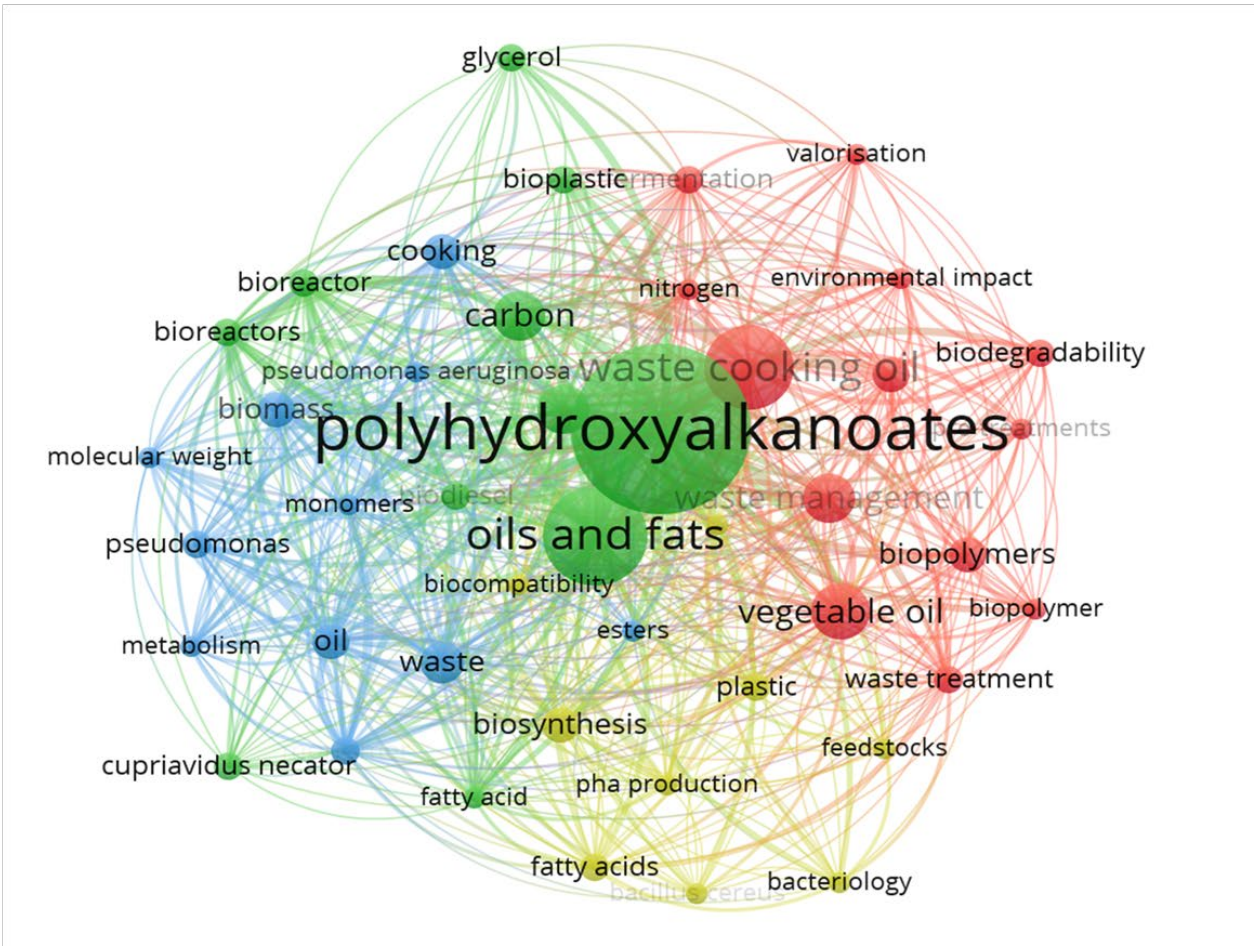

Fig. 2 Pathways involved in polyhydroxyalkanoates production from various wastes.

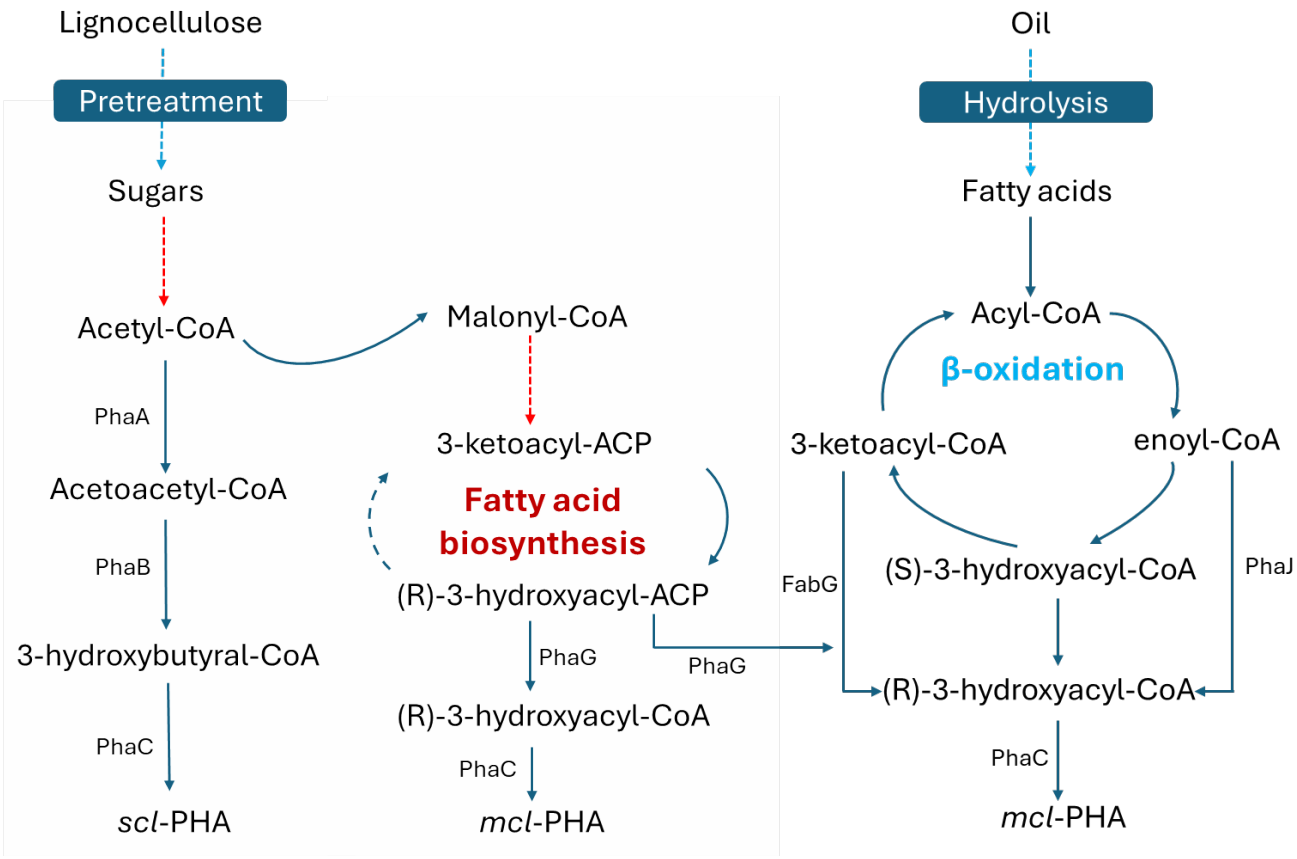

Fig. 3 WCO upcycling into polyhydroxyalkanoates (a) process and strategy for PHA production (b) Artificial neural network (ANN) for process optimization.

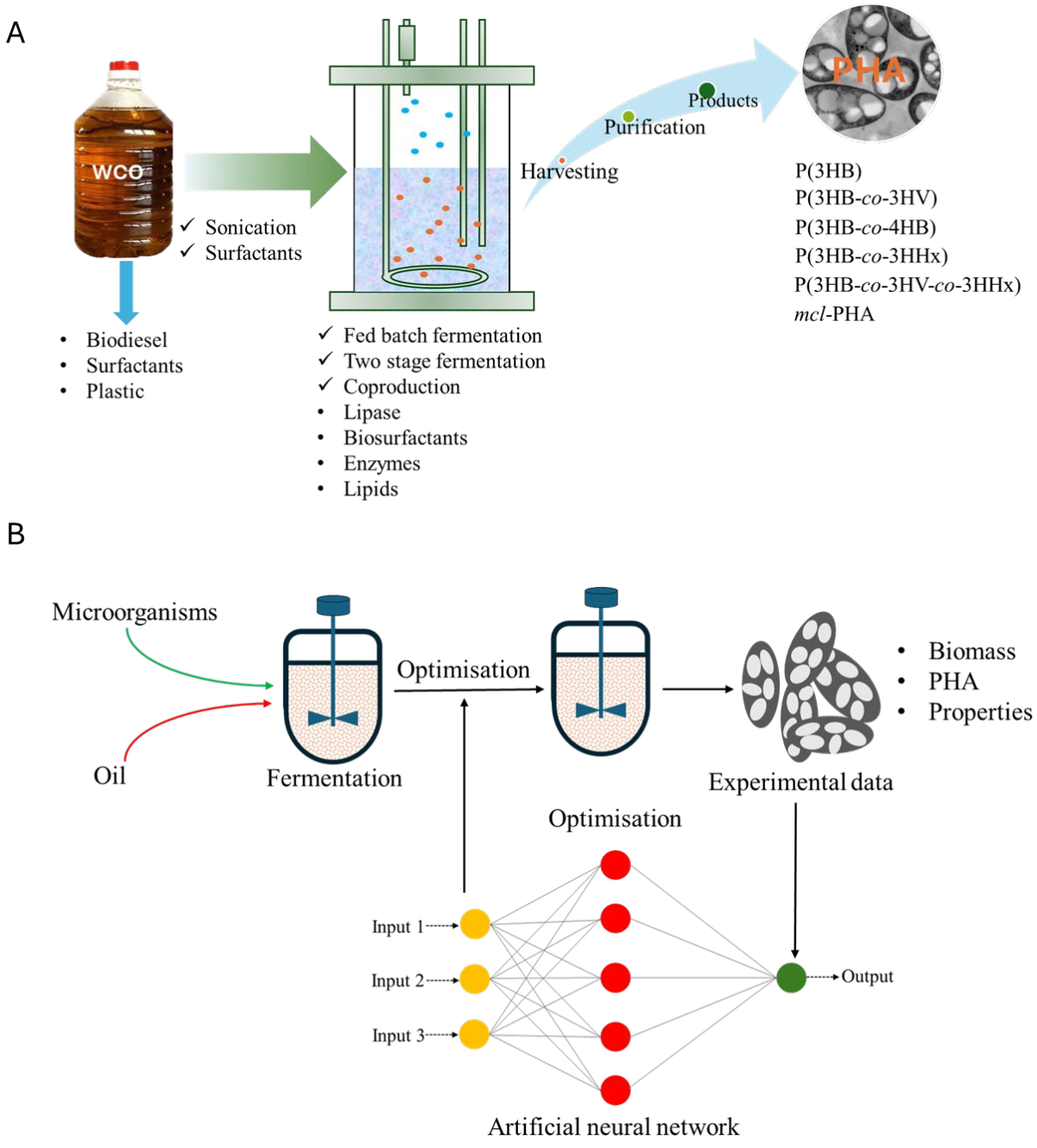

Fig. 4 Decision-making flow chart for feedstock (WCO) and microbes selection for PHA production.

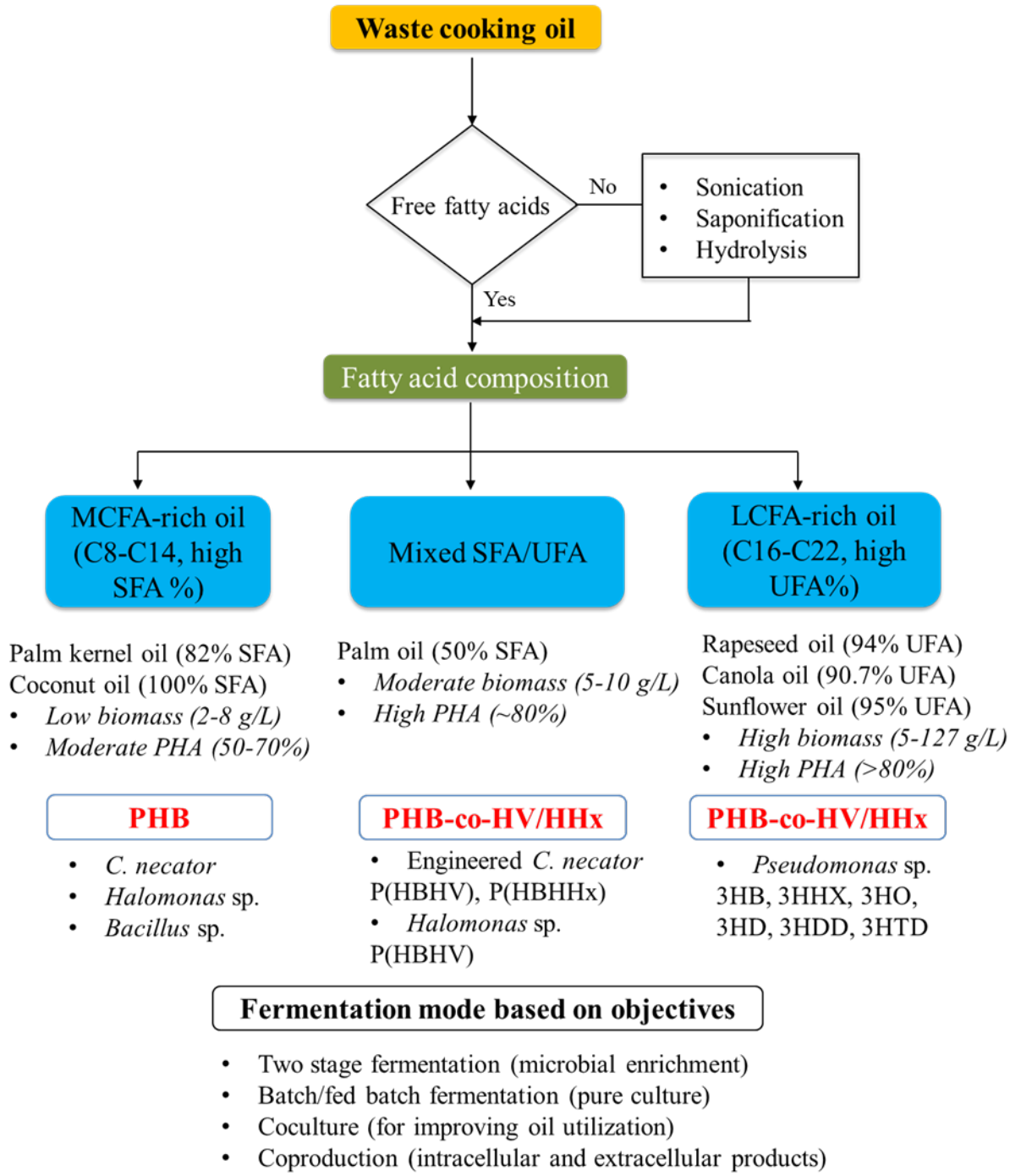

Fig. 5 Various factors and challenges associated with WCO upcycling into PHA.

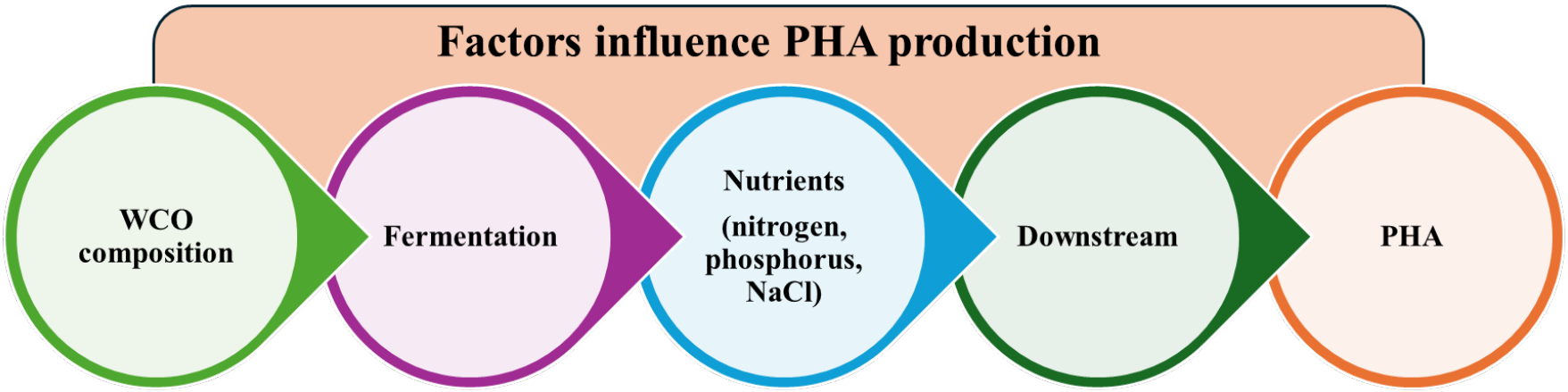

Table. 1 Microbes-mediated upcycling of oil into valuable products

Product	Microorganism	Productivity	Key point	Reference
Biosurfactant	*Pseudomonas syringae pv tabaci*	2.7 g/L syringopeptin was produced.	Reduce the water surface tension down to 38 mN/m and have emulsified effectively 67 %.	[12]
Biosurfactant	*Bacillus pseudomycoides* BS6	56 mg/L cyclic lipopeptide was produced.	Able to reduce the surface tension of water from 71.6 mN/m to 30.2 mN/m and have an emulsification index 62.8-94.2%.	[40]
Biosurfactant	*Pseudozyma aphidis* ZJUDM34	61.5 g/L mannosylerythritol lipid.	Biotransformation method was used. Water surface tension reduced up to 32.83 mN/m.	[41]
Biosurfactant	*Bacillus* sp. HIP3	Lipopeptide.	Shows metal removal capacity for copper (13.57%), lead (12.71%), zinc (2.91%), chromium (1.68%), and cadmium (0.7%).	[13]
Biosurfactant	*Pseudomonas aeruginosa* MTCC7815	11.2 g/L rhamnolipid.	Water surface tension reduced up to 26.2 mN/m.	[28]
Biosurfactant	*Pseudomonas* SWP-4	13.93 g/L rhamnolipid.	Water surface tension reduced up to 24.1 mN/m.	[42]
Carotene	*Blakeslea trispora*	2.021 g/L carotene.	The oxidative stress in *B. trispora* induced by hydroperoxides of WCO increased the carotene production.	[37]

Carotene	*Saccharomyces cerevisiae*	0.47 g/L carotene.	Strain was engineered by expressing lipase from *Y. lipolytica* and carotene synthesis genes from *Xanthophyllomyces dendrorhous.*	[10]
Lipids	*Cryptococcus curvatus*	20.34 g/L lipids	Lipids production was higher with ultrasound treated oil compared to unsonicated one (12.21 g/L).	[43]
Lipids	*Rhodotorula glutinis*	11.85 g/L lipids	Glycerol was used as an additional carbon source.	[29]
Lipids	*Y. lipolytica*	41-64 % lipids	Extracted lipids suitable for biodiesel production.	[39]
Lipids	*Rhodococcus* sp. YHY01	2.39 g/L lipids, 70% w/w	Lipid was suitable for biodiesel production.	[44]
Lipase	*Y. lipolytica*	1.164 U/mL lipase	Lipase suitable for biodiesel production.	[11]
Itaconic acid	*Y. lipolytica*	54.55 g/L itaconic acid	The strain was engineered by expressing 10 genes related to acetyl-CoA pathway with knockout of competitive pathway genes.	[45]

Table. 2 Analytical techniques for PHA characterisation.

Technique	Application	Advantages	Disadvantages
GC	Quantification of PHA monomer composition after methanolysis.	High sensitivity and accuracy, and well-established method.	Requires derivatization, destructive, and time-consuming.
GC-MS	Identification and quantification of monomer units.	Provides both qualitative and quantitative data with high specificity.	Expensive equipment and need sample preparation.
NMR	Structural elucidation of PHA polymers and determines monomer sequence.	Non-destructive and detailed structural insight.	Requires pure samples, and needs an expert for interpretation.
FTIR	Functional group analysis and confirmation of PHA structure.	Fast and non-destructive and require minimal sample preparation.	Limited quantitative analysis and interpretation can be complex.
TGA	Determines thermal stability and degradation temperature.	Simple, fast, and requires a small sample.	No information on chemical composition.

DSC	Measures thermal transitions (e.g., Tg, Tm, Tc).	Reveals crystallinity and thermal behavior.	Overlapping transitions can complicate interpretation.
SEM	Surface morphology of PHA films and polymer granules.	High-resolution imaging of microstructures.	Requires conductive coating, vacuum required, and non-quantitative
TEM	Internal structural analysis and visualization of PHA granules within microbial cells.	Ultra-high resolution, allows observation of internal ultrastructures.	Sample preparation is complex, requires thin slicing, is expensive and time consuming.
GPC	Determines molecular weight and polydispersity index.	Accurate molecular weight distribution.	Requires solvents, calibration, and an expensive setup.

Table. 3 Polyhydroxyalkanoates production from various wastes.

Waste type	Microorganism	Strategy applied	Polymer type	PHA (g/L)	Reference
Brewers spent grain	*C. necator* (pH: 7, T: 30 °C, Time 72 h)	BSG was pretreated with dilute acid and inhibitors were removed using enzymatic hydrolysis and detoxification method.	PHB	1.13 g/L	[48]
Cheese whey	*Paracoccus homiensis* (pH: 7.6, T: 28 °C, Time 72 h)	Centrifugation and filtration were used to remove the large protein aggregates and pH was udjusted at 7.	P(3HB-*co*-3HV)	1.1 g/L	[63]
Cheese whey	*Azohydromonas lata* (pH: 7.6, T: 30 °C, Time 18 h)	Cheese whey was hydrolyzed with dilute acid.	PHB	1.33 g/L (41%)	[64]
Food waste	*Halomonas bluephagenesis* (pH: 8.5, T: 37 °C, Time 48 h)	PHA synthesis operon *phaCAB$_{C}$n* (cloned from *C. necator*) controlled by the essential gene *ompW.*	PHB	56 g/L	[65]
Dairy waste	*Halomonas alkaliantarctica* (pH: -, T: 28 °C, Time 72 h)	Without use of any precursor able to produce copolymer.	P(3HB-*co*-3HV)	0.42 g/L	[49]
Food waste	*Bacillus mycoides* ICRI89	Ultrasonication combined with enzymatic	PHB	2.11 g/L (59%)	[66]

	(pH: 7.0, T: 35 °C, Time 120 h)	hydrolysis enhances sugar release.			
Pineapple peel	*Bacillus megaterium* PP-10 (pH: -, T: 35 °C, Time 12 h)	Biomass was treated using 1% H_2SO_4.	PHB	1.98 g/L (54.5%)	[67]
Slaughter house waste (swine)	*C. necator* DSM 545 (pH: -, T: 30 °C, Time 72 h)	Lipase *lipC* and *lipH* of *Pseudomonas stutzeri* BT3 overexpressed.	PHB	2.9 g/L (65.5%)	[68]
Orange peel waste	*Escherichia coli* (pH: -, T: 37 °C, Time 96 h)	*phaCAB* from *R. eutropha* H16 overexpressed. Developed oxidation system that release sugar and eliminate limonene.	PHB	0.13-0.39 g/L	[62]
Spent coffee ground	*Halomonas halophila* (pH: 7, T: 30 °C, Time 72 h)	Waste was pretreated using dilute acid treatment and inhibitors were removed using styrene-divinylbenzene based resins.	PHB	0.95 g/L (97%)	[69]
Spent coffee ground	*Pseudomonas resinovorans* (pH: 6.8, T: 30 °C, Time 72 h)	Extracted oil was used for PHA production and reaming biomass was used for	*mcl*-PHA	1.6 g/L (29.5%)	[70]

		hydrogen production.				
Coffee oil	*C. necator* DSM 428 (pH: -, T: 30 °C, Time 72 h)	Oil was extracted using supercritical carbon dioxide.	PHB	13 (78.4)	g/L	[71]

Table 4: Fatty acid composition of different oils.

Fatty acids (%)	Bean oil [18]	Tung oil [18]	Coconut oil [18]	Canola oil [88]	Palm oil [89]	Palm Kernel oil [89]	Sunflower oil [84]	Soybean oil [85]	Rapeseed oil [90]	Fish oil [91]
Caproic acid (6:0)	-	-	-	-	-	0.2	-	-	-	-
Caprylic acid (C8:0)	-	-	9.7	-	-	3.3	-	-	-	-
Capric acid (C10:0)	-	-	7.5	-	-	3.5	-	-	-	-
Lauric acid (C12:0)	-	-	42.1	-	0.2	47.8	-	-	-	0.23
Myristic acid (C14:0)	-	-	22.4	0.07	1.1	16.3	-	0.07	0.05	8.64
Palmitic acid (C16:0)	14.69	5.5	18.2	4.29	44.0	8.5	0.09	11.08	4.84	20.89
Palmitoleic acid (C16:1)	-	-	-	-	-	-	6.33	0.09	0.06	13.42
Heptadecanoic acid (C17:0)	-	-	-	-	-	-	-	-	0.14	-
Stearic acid (C18:0)	5.4	-	-	2.59	4.5	2.4	3.45	4.1	0.14	3.01
Oleic acid (C18:1)	26.8	4	-	65.39	39.2	15.4	21.64	21.1	62.73	11.78
Linoleic acid (C18:2)	44.4	8.5	-	16.32	10.1	2.4	67.28	55.07	22.4	-
Linolenic acid (C18:3)	8	82	-	7.54	0.4	-	0.09	7.55	7.50	-
Arachidic acid (C20:0)	-	-	-	0.99	0.1	0.1	0.23	0.30	0.50	-
Eicosenoic acid (C20:1)	-	-	-	-	-	-	0.13	0.18	1.25	-

Eicosapentaenoic acid (C20:5)	-	-	-	-	-	-	-	-	-	18.32
Behenic acid (C22:0)	-	-	-	-	-	-	0.72	0.31	0.30	-
Erucic acid (C22:1)	-	-	-	1.49	-	-	0.11	-	-	-
Docosahexaenoic acid (C22:6)	-	-	-	-	-	-	-	-	-	12.32
SFA (%)	20.09	5.5	100	7.94	49.9	82.1	4.49	15.79	5.67	32.54
UFA (%)	79.2	94.5	0	90.74	49.7	17.8	95.47	83.99	94.03	85.84

Table. 5 Upcycling of various oils into PHA by microbial fermentation and properties of produced PHA.

Oil	Microorganism	Strategy	Key points	PHA	Productivity (dcw g/L, PHA g/L, PHA % w/w)	Weight (M_w kDa)	T_m	T_g	PDI	Reference
Bean oil	*C. necator* H16 (pH: 6.8, T: 30 °C, Time 24 h)	*phaC2Ra-phaACn-phaJ1Pa* overexpressed.	Lauric acid was added, and a controlled HHx fraction 9-31% was achieved.	P(3HB-*co*-3HHx)	7.0, 5.4, 78	165	166.4	-	2.16	[18]
Palm kernel oil	*C. necator* (pH: 6.8, T: 30 °C, Time 48 h)	*Rhodococcus pyridinivorans* BSRT1-1 strain *phaC* was overexpressed in *C. necator.*	Scale up resulted in 1.4 fold increase in productivity.	P(3HB-*co*-2 % 3HHx)	7.7, 3.1, 56	627	160.18	-6.04	1.7	[50]
Sludge Palm oil	*P. putida* S12 (pH: -, T: 30 °C, Time 48 h)	Sludge palm oil is waste from palm oil mill effluent.	Oil contains a high content of palmitic and oleic acid.	3HHX, 3HO, 3HD, 3HDD, 3HTD	1, 0.27, 27	106	-	-0.41	2.33	[92]

Cerbera odollam oil	*P. resinovorans* (pH: 7, T: 30 °C, Time 72 h)	Crude, hydrolyzed, and saponified oils were tested.	Hydroysis and saponification increase the accessibility of oil and diverted metabolic pathways towards biomass.	3HB, 3HHx, 3HO, 3HD	1.32, 0.25, 18.8	-	-	-	-	[93]
Canola oil	*C. necator* H16/pMPJAS03 (pH: -, T: 30 °C, Time 26 h)	*fadE* and *phaJ* were overexpressed.	*FadE* increases the biomass and PHA content while *PhaJ* affects the *mcl* contents.	3HV, 3HHx, 3HO, 3HD	6.32, 4.17, 66.1	-	148	-	-	[94]
Canola oil	*C. necator* H16 (pH: -, T: 30 °C, Time 30 h)	*phaC2* from *Pseudomonas putida* CA-3 and native expression of *phaC1*.	Able to produce tetrapolymer.	94% 3HB, 1% 3HV, 4% 3HHx, 1% HO	6.2, 5.95, 96	-	155-173	-14	-	[95]
Canola oil	*R. eutropha* Re2058/pCB113 (pH: -, T: 30 °C, Time 72 h)	Fructose was used a cos ubstrate	Able to fix HHx fraction 2-17% in a	3HB-*co*-3HHx	9-13, 5.4-11.4, 60-88	-	-	-	-	[52]

			controlled manner.						
Palm oil	*C. necator* Re2160/pHT1-CBP-M-CPF4 (pH: -, T: 30 °C, Time 48 h)	*phaC and phaJ* overexpressed.	$PhaC_{BP\text{-}M\text{-}CPF4}$ displayed much higher preferences towards 3HHx.	(3HB-*co*-13% 3HHx)	4.8, 2.7, 55.8	800	-	-	3.6	[87]
Palm oil	*C. necator* IBP/SFU-1 (pH: 7, T: 30 °C, Time 72 h)	Various carbon sources were evaluated for PHA production.	A change in molecular weight was observed with different carbon sources.	99.80% 3HB, 0.07% 3HV, 0.13% HHx	8.2, 6.56, 80	682	-	-	-	[53]
Palm kernal oil	*C. necator* Re2160/pHT1-CBP-M-CPF4 (pH: -, T: 30 °C, Time 48 h)	*phaC* and *phaJ* overexpressed.	$PhaC_{BP\text{-}M\text{-}CPF4}$ displayed much higher preferences towards 3HHx.	(3HB-*co*-18 % 3HHx)	5.3, 3.4, 63.3	690	-	-	2.2	[87]
Palm olein	*C. malaysiensis* USMAA2-*4ABH16* (pH: -, T: 30 °C, Time 48 h)	*lipAB* heterologously expressed.	Lipase activity increased by 40 folds. 1-pentanol was used as a precursor.	P(3HB-*co*-7%3HV)	5.4, 3.7, 69	-	-	-	-	[51]

Rapeseed oil	*C. necator* strain Re2058/pCB113 (pH: -, T: 30 °C, Time 87 h)	Two stage fed batch cultivation method was used. Fructose was used in the first stage and rapeseed oil in the second stage.	10% of the culture broth was recycled for semi-continuous biomass accumulation and helps in shorten the process.	P(3HB-*co*-16.9 % 3HHx)	124, 86.1	106.7,	-	-	-	-	[27]
Sun flower oil	*C. necator* (pH: -, T: 30 °C, Time 96 h)	Anaerobically digested chicken manure was used as a cosubstrate.	Help on manure management and value added product production.	P(3HB)	10.8, 52.4	5.7,	-	-	-	-	[83]
Sun flower oil	*C. necator* IBP/SFU-1 (pH: 7, T: 30 °C, Time 72 h)	Various carbon sources were evaluated for PHA production.	A change in molecular weight was observed with different carbon sources.	99.77% 3HB, 0.21% 3HV, 0.02% HHx	4.3, 38.7	1.66,	479	-	-	-	[53]
Fish oil	*C. necator B-10646* (pH: -, T: 30 °C, Time 72 h)	Two stage batch culture fermentation was used.	Bacteria utilized only polyenoic acids and	3HB, 3HV, 3HHx	6.5, 4.2, 65		540-760	158-165	-	-	[96]

			monoenoic and saturated acids were not utilised.							
Waste fish oil	*Salinivibrio* sp. M318 (pH: 6.5, T: 30 °C, Time 48 h)	Glycerol was used as a cosubstrate.	Able to utilise various precursors and synthesize copolymers P(3HB-*co*-3HV) and P(3HB-*co*-4HB).	PHB	69.1, 51.5	35.6,	-	-	-	-	[97]
Waste frying oil	*Halomonas hydrothermalis* (pH: -, T: 30 °C, Time 72 h)	Valerate was used as a precursor	Molecular weight can be controlled by NaCl concentration.	P(3HB-*co*-3HV)	2.34, 68.83	1.61,	410	-	-	-	[81]
Waste cooking oil	*Pseudomonas* sp. H3 (pH: 7, T: 25 °C, Time 72 h)	Fed batch fermentation was performed at 5 L scale	Residual ferment was used for anaerobic digestion to produce methane.	PHB	16, 8.6, 54		54.78	34	-20	1.41	[75]

Waste cooking oil	*B. thailandensis* (pH: 7, T: 37 °C, Time 192 h)	Fermentation was performed at 10 L scale	The process also leads to the coproduction of rhamnolipids 2.2 g/L	PHB	12.6, 7.5, 60	511	166.4	-1.5	2.86	[98]
Waste cooking oil	*C. necator* (pH: -, T: 30 °C, Time 48 h)	*phaC* overexpressed	Mealworm was used for PHA extraction.	P(3HB-*co*-3HV)	8.5, 6.5, 75.5	1440	-	-	-	[80]
Tung oil	*R. eutropha* Re2133/pCB81 (pH: -, T: 30 °C, Time 72 h)	Tung oil possess 80% α-eleostearic acid.	Produced PHA have α-eleostearic acid coating and shows antibacterial activity.	P(53% 3HB-*co*-2% 3HV-*co*- 45% 3HHx	1.65, 0.68, 41	280	-	-	1.82	[99]
Coconut oil	*Pseudomonas mendocina* CH50 (pH: 7.2, T: 30 °C, Time 48 h)	Two stage natch fermentation was used.	Highest PHA accumulation noted under nitrogen and oxygen limitation.	P(30.5%3HO-co-48.4%3HD-co-21.2%3HDD)	2.7, 1.56, 58	333	48	-42	2.37	[86]

A review on upcycling waste cooking oil into polyhydroxyalkanoates (bioplastic): a pathway for sustainable material

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2025-09-01

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