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Dual-Target Bioprocessing Using Oleaginous Microorganisms: Converting Food Waste into Lipids and Biopolymers

Running title: SCO and PHA Bioproduction

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SUMMARY

The increasing demand for sustainable alternatives to fossil-derived fuels and plastics has intensified research into microbial platforms that can convert abundant waste resources into valuable products. This review focuses on the emerging field of dual-target bioprocessing using oleaginous microorganisms to produce single-cell oils (SCOs) and polyhydroxyalkanoates (PHAs) from food waste. We discuss key microbial strains, alongside their metabolic pathways, co-production capabilities, and substrate preferences. Emphasis is placed on utilizing food waste as a low-cost and carbon-rich feedstock, thereby enhancing both economic feasibility and environmental sustainability. We also analyzed some integrated bioprocess strategies devised to overcome existing challenges,

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such as yield optimization and metabolic bottlenecks. This dual-production platform addresses the principles of circular economy, facilitating the conversion of waste into high-value bioproducts.

Keywords: oleaginous microorganisms; single cell oils (SCOs); polyhydroxyalkanoates (PHAs); food waste conversion; integrated bioprocessing

INTRODUCTION

The escalating global demand for sustainable food resources, non-fossil-based fuels, and biodegradable packaging materials underscores the necessity for environmentally friendly and renewable alternatives. With growing population pressures, worsening environmental degradation, and the finite availability of fossil resources, current production and consumption systems are facing increasing challenges (*1*). Traditional lipid sources, such as crops and animal fats, necessitate expansive agricultural production, contributing to deforestation, habitat loss, and biodiversity loss. The projected rise in edible oil demand to USD 307 billion by 2029 may require an additional 317 million hectares of cropland by 2050 (*2*). Meanwhile, depletion of fossil fuels and increasing pollution from plastic waste further exacerbate environmental concerns (*3-5*).

Oleaginous microorganisms, including certain yeasts, fungi, and bacteria, represent a promising alternative for producing high value product like single-cell oils (SCOs) and poly-hydroxyalkanoates (PHAs) from low value substrate such as food waste (*6*). This dual-target not only addresses the urgent need for sustainable biofuels and biodegradable bioplastics but also aligns with principles of the circular bioeconomy by enhancing efficiency and reducing environmental impacts (*7,8*). Leveraging food waste can significantly decrease production costs, potentially by up to 75 %, improving the commercial viability of microbial oil production (*9*).

The scope of this review encompasses the characterization of microbial strains, metabolic pathways, fermentation strategies, downstream processing, and techno-economic and environmental advantages of adopting dual-target bioprocessing oleaginous microorganisms.

OLEAGINOUS MICROORGANISMSAND THEIR STORAGE METABOLITES

Oleaginous microorganisms are defined by their ability to synthesize intracellular lipidic and polymeric storage materials with a non-polar nature (oleochemicals) through varied substrates, including carbon dioxide, sugars, and organic acids (*10*). Among the accumulated metabolites, the two of most interest are SCOs and PHAs. Both are produced via distinct metabolic pathways, and they are often produced in similar environmental triggers, such as nutrient imbalance and carbon

excess (*10,11*). While most of the PHA-producing microorganisms belong to prokaryotes, SCO-producing ones often belong to eukaryotes. Notable oleaginous genera with high production include *Cutaneotrichosporon oleaginosus* and *Lipomyces starkeyi*, which exhibited the highest single-cell oil (SCO) concentrations, reaching approx. 16.77 and 32.7 g/L, respectively, on glucose under nitrogen-limited fed-batch conditions (*11*). Moderate producers such as *Rhodosporidium toruloides* (10–16 g/L) and *Pichia cactophila* (7.1 g/L) displayed favorable profiles enriched in mono- and polyunsaturated fatty acids, while *Yarrowia lipolytica*, though a lower native producer (2.5–3.1 g/L) offers strong potential for yield improvement through metabolic engineering (*11*), alongside certain bacteria such as *Cupriavidus necator, Bacillus subtilis* and *Pseudomonas* spp. (*12*). These microorganisms can accumulate significant amounts of storage compounds under optimized growth conditions (*13-16*).

DIVERSITY AND BIOCHEMISTRY OF MICROBIAL OILS (SCOs)

A key advantage of oleaginous microbes as lipid-producing platforms lies in their unique ability to biosynthesize specialized fatty acids (omega-3 and omega-6 groups) that are rare or absent in plants and animals. While fish oil has been the conventional source, concerns over marine resource depletion and quality variability have intensified research into microbial production. These offer a renewable, sustainable and scalable platform for producing specific polyunsaturated fatty acids (PUFAs) under controlled conditions, presenting an eco-friendly alternative to traditional sources. These microorganisms can generate lipids enriched with PUFAs of high nutritional and pharmaceutical relevance, such as γ-linolenic acid (GLA), dihomo-γ-linolenic acid, arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), compounds that are essential for human health and widely used in functional foods and medical formulations (*9,17*). These profiles of SCOs can vary significantly, reflecting both strain-specific characteristics and substrate types (*18*). Certain microbial strains have been shown to produce significant amounts of valuable PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are essential for human nutrition (*19,20*). For example, the marine oleaginous thraustochytrid *Aurantiochytrium* sp. T66 (ATCC-PRA-276) has been reported to efficiently produce polyunsaturated fatty acids, particularly DHA, from volatile fatty acids, reaching up to 42.6 % of total lipids and demonstrating the potential of non-yeast oleaginous microorganisms for sustainable PUFA production (*21*).

Substrate choice plays a crucial role in both lipid yield and fatty acid composition. Using diverse carbon sources from simple sugars to agricultural byproducts and even lignocellulosic biomass can significantly influence the balance of saturated, monounsaturated, and polyunsaturated fatty acids produced. Simple sugars provide predictable growth and lipid profiles, while food waste

valorization offers a sustainable, low-cost alternative. This highlights the versatility of oleaginous microbes for industrial single-cell oil (SCO) production. Strains such as *Yarrowia lipolytica*, known for their ability to utilize a broad array of substrates, present promising options for large-scale production of lipids enriched in specific fatty acids with important industrial and nutritional applications. This microorganism has been subjected to metabolic engineering to achieve high yields of SCO production and improved product specificity. The ω-3 fatty acids produced by *Yarrowia lipolytica* mainly consist of DHA, EPA, and α-linolenic acid (ALA) (*22*).

Table 1 represents microbial PUFAs and their producers and related companies that have successfully commercialized microbial oils rich in polyunsaturated fatty acids (PUFAs). Martek Biosciences, later acquired by DSM, pioneered large-scale production of DHA and ARA for infant nutrition using *Crypthecodinium cohnii* and *Mortierella alpina*. DuPont engineered *Yarrowia lipolytica* to produce EPA-rich oils, achieving GRAS status for food applications. These microbial platforms have proven economically viable in the nutraceutical market, especially for high-value lipid products, though their use as biodiesel feedstocks remains limited due to high production costs and scalability challenges (*23*). Table 1 summarizes the most commercially relevant microbial producers of long-chain polyunsaturated fatty acids (PUFAs), including ω-3 (DHA, EPA) and ω-6 (ARA, GLA) families (*24*). These compounds are primarily derived from oleaginous microalgae and filamentous fungi cultivated by leading biotechnology companies such as DSM Firmenich, Corbion, Veramaris, and CABIO.

TABLE 1

POLYHYDROXYALKANOATE (PHAs) MICROBIAL PRODUCTION

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polymers synthesized by various bacteria and some archaea under limited nutritional conditions as intracellular carbon and energy storage compounds (*24,25*). These biopolymers have attracted considerable interest due to their biodegradability, biocompatibility, and potential to replace petrochemical plastics in diverse applications (*25*). The synthesis of PHAs is highly dependent on the availability and type of carbon source; lipid-based substrates and fatty acids can serve as precursors for PHAs monomer synthesis, linking lipid metabolism and polymer production (*7,26*). Key enzymes, including β-ketothiolase, acetoacetyl-CoA reductase, and PHA synthase, can be modulated to enhance PHA accumulation under food-waste-derived fermentation conditions (*8*). Liu *et al.* (*8*) comprehensively reviewed microbial strategies for converting food-derived substrates, such as volatile fatty acids (VFAs) and

hydrolysates, into PHAs, emphasizing the influence of nutrient limitation and carbon source optimization on polymer yield (*8*).

PHA VS. SCO: DUAL POTENTIAL OF OLEAGINOUS MICROORGANISMS

Both SCOs and polyhydroxyalkanoates (PHAs) are intracellular carbon storage compounds mostly produced by oleaginous eukaryotes (yeasts and filamentous fungi) and prokaryotes respectively, but they differ significantly in chemistry and physical properties. SCOs predominantly comprise triacylglycerols (TAGs) that mimic plant or animal lipids in structure and functionality, which have a glycerol backbone esterified with three long-chain fatty acids (C_{14} to C_{24}), rendering them suitable for a spectrum of applications, including biodiesel production, cosmetics, and functional foods (*18,19*). These fatty acids can be saturated or unsaturated (*e.g*. oleic, linoleic, DHA), with unsaturation lowering intermolecular packing and keeping SCOs liquid at ambient temperature (*17,23*). In contrast, PHAs are biodegradable polyesters, stored as granules in microbial cytoplasm formed by enzymatic polymerization of (R)-3-hydroxy fatty acid monomers, such as 3-hydroxybutyrate or 3-hydroxyvalerate (*7,25,27*). Unlike TAGs in SCOs, which serve as short-term energy reserves in oleaginous yeasts, fungi, and algae, PHAs are high-molecular-mass polymers synthesized mainly by bacteria under nutrient-limited conditions, serving as long-term carbon and energy storage (*13*).

Structurally, TAGs are hydrophobic lipids suited for nutrition and biofuel applications, while PHAs, due to their polyester nature, are biodegradable and biocompatible, making them ideal for bioplastics and medical uses. The fatty acid composition in SCOs influences properties like oxidative stability and melting point, whereas the monomer makeup in PHAs affects polymer flexibility, crystallinity, and mechanical strength (*17,23*).

Metabolically, SCO accumulation occurs via de novo fatty acid synthesis, regulated by enzymes, such as ATP citrate lyase (ACL) and malic enzyme (ME), especially under nitrogen limitation, which redirects carbon flux from biomass to lipid synthesis (*23*). Similarly, PHA synthesis is governed by PHA synthase enzymes using substrates like acetyl-CoA or propionyl-CoA under nutrient-limited conditions (*7,13,25*). Similar metabolic shifts under nitrogen limitation also trigger PHA accumulation in food-waste-fed bacteria, suggesting overlapping regulatory signals for both lipid and polymer biosynthesis (*8*). Several oleaginous microorganisms (specified as the border zone of producers) have both metabolic pathways of PHAs and SCOs production and are capable of producing both SCOs and PHAs, making them promising candidates for integrated bioprocesses (*28*). These microorganisms, which belong to actinobacteria, include *Rhodococcus* spp., *Streptomyces* spp. or some species of *Bacillus,* such as *Bacillus subtilis* (*12,29-33*).

STRATEGIES FOR LOW-COST INTEGRATED BIOPROCESS DEVELOPMENT USING FOOD WASTE VALORIZATION

Oleaginous microorganisms can produce single-cell oils SCOs and PHAs from diverse substrates, including sugars, agricultural byproducts, and food waste. In particular, Liu *et al.* (*8*) demonstrated that optimized fed-batch and nutrient-controlled strategies using food waste hydrolysates increased PHA productivity while lowering operational costs, confirming the economic feasibility of waste-derived systems (*8*).

A study from the University of Anbar (Iraq) demonstrated that *Bacillus subtilis* isolates can efficiently produce SCO using local soil and environmental wastes as carbon sources (*33*). Palm fronds were identified as the most effective substrate, yielding oils rich in linoleic (46 %) and palmitoleic (16 %) acids. The results highlight the potential of locally sourced, low-cost bacterial systems for sustainable SCO production and food applications.

In the review by Nguyen *et al.* (*11*), various agro-industrial and food waste streams were investigated as alternative substrates for SCO production by oleaginous yeasts. Reported wastes included molasses, sugarcane bagasse hydrolysate, wheat straw, dried sweet sorghum stalks, corn stover hydrolysate, cassava peel waste, apple pomace, vegetable residues, distillery effluents, and waste cooking oil. Compared to synthetic media such as glucose- or xylose-based formulations, these waste-derived substrates substantially reduce raw material costs and enhance the overall sustainability of the process by utilizing renewable organic residues. The choice of carbon source strongly influences lipid accumulation and fatty acid composition in oleaginous yeasts. Strains such as *Cutaneotrichosporon oleaginosus* and *Lipomyces starkeyi* achieved the highest SCO yields (up to 30–33 g/L) when cultivated on glucose- or xylose-based media, while the use of agro-industrial residues and crude glycerol offered a more sustainable and cost-effective alternative, still supporting yields in the range of 10–25 g/L. *Yarrowia lipolytica* and *Rhodosporidium toruloides* performed efficiently on low-cost substrates such as molasses, crude glycerol, and lignocellulosic hydrolysates, producing oils enriched in mono- and polyunsaturated fatty acids. Although food waste hydrolysates resulted in lower lipid titers (4–10 g/L), they represent an eco-friendly route for circular bioeconomy valorization, emphasizing that substrate selection not only determines lipid yield but also tailors the biochemical profile of SCOs, enabling targeted production for biofuel, food, and nutraceutical applications (*11*). Some recent reports have highlighted the valorization of food-derived wastes for microbial lipid production. Småros *et al.* (*34*) achieved 26.1 % lipid accumulation by *Apiotrichum brassicae* grown on dairy side streams, yielding fatty acids comparable to cocoa butter. Likewise, Vemparala *et al.* (*35*) used canteen food waste hydrolysate as substrate for *Candida neerlandica*,

obtaining 0.415 g/L lipids with profiles rich in palmitic, oleic, and linoleic acids, confirming the potential of food waste-based media as sustainable alternatives to synthetic substrates (*34,35*). Recent findings by Dimitriadis (*36*) demonstrated that municipal and food waste can serve as cost-effective feedstocks for oleaginous microorganisms such as *Yarrowia lipolytica* and *Rhodococcus opacus*, enabling high lipid accumulation comparable to sugar-based systems. The study emphasized that integrating microbial lipid production with waste management significantly reduces process costs and supports the circular bioeconomy. Moreover, recovered single-cell oils were proposed as dual-purpose intermediates, suitable both for biodiesel synthesis and as substrates for polyhydroxyalkanoates (PHAs) production, reinforcing the potential of dual-target bioprocessing from waste-derived carbon sources (*36*).

Some researchers are exploring new pathways to reduce the downstream processing costs in food waste valorization. Ma *et al.* (*37*) comprehensively analyzed various food waste streams as substrates for microbial lipid production via volatile fatty acid (VFA) pathways. The study included fruit and vegetable residues (such as orange, apple, and banana peels), kitchen leftovers, bakery waste, dairy effluents, meat processing residues, and waste cooking oil. These VFA-rich wastes enabled oleaginous microorganisms like *Yarrowia lipolytica* and *Cutaneotrichosporon oleaginosus* to accumulate lipids with yields ranging from 0.5 to 1.4 g/L (comparable to those obtained from glucose-based systems). In contrast to sugar-rich wastes requiring enzymatic hydrolysis, VFA-based substrates could be directly assimilated, significantly lowering process costs and leaving almost zero solid residue. This highlights the superior environmental performance and circular bioeconomy potential of acidogenic food waste valorization for microbial lipid production (*37*).

Co-production of PHAs and SCOs

In nature, the production of PHAs and SCOs typically occurs separately: oleaginous yeasts, fungi, and microalgae are well-known for accumulating triacylglycerols (SCOs), while bacteria, such as *Cupriavidus necator* are classic producers of poly-(3-hydroxybutyrate) (a type of PHAs). However, several oleaginous and metabolically versatile microorganisms have been reported to produce either SCOs or polyhydroxyalkanoates (PHAs), and in some cases, both, either naturally or through metabolic engineering. Notable examples include *Yarrowia lipolytica* and *Rhodosporidium toruloides*, which are well-known lipid-accumulating yeasts capable of synthesizing triacylglycerols (TAGs) and have been engineered to express PHA biosynthetic pathways (*38-40*). Similarly, members of the genus *Rhodococcus* are also recognized for their dual capacity to accumulate both TAGs and PHAs as intracellular carbon and energy reserves (*41-43*). Also, in a study by Kumar *et al.* (*44*), efficient co-

production of polyhydroxyalkanoates (PHAs) and carotenoids was achieved by *Paracoccus* sp. strain LL1 using glycerol as the sole carbon source. Under optimized fermentation conditions, the strain produced up to 9.52 g/L of PHA and 7.14 mg/L of carotenoids, demonstrating an integrated bioprocess that enhances the overall economic feasibility of PHA production.

Understanding the metabolic regulation and genetic determinants governing PHA biosynthesis alongside lipid accumulation enables targeted strain improvement and process optimization. Such dual-production systems hold potential for maximizing the economic viability and sustainability of microbial fermentation (*25,28,45*). Balancing the metabolic pathways between lipid accumulation and polymer biosynthesis is challenging and requires precise control over culture conditions and substrate feeding strategies (*7*). To address this, metabolic engineering and synthetic biology are increasingly used to optimize these pathways, allowing tailored production of desired compounds and improving yields (*13,23*). Such engineered biocatalysts hold great potential for sustainable and economically viable industrial biotechnology.

Table 2 summarizes species capable of sequentially producing both PHAs and SCOs in dual-target bioprocessing aimed at the simultaneous production of both compounds (*31*,*32*,*46-50*).

TABLE 2

Both PHA and TAG biosynthetic pathways rely on common precursor molecules, such as acetyl-CoA, fatty acids, or reducing equivalents like NADPH. When both pathways are active, they compete for these limited resources, which can limit the yield of one product if the other dominates. The biosynthesis of lipids and biopolymers involves redox reactions that require a balanced supply of NADH and NADPH. Imbalances in redox cofactors can disrupt metabolic fluxes, leading to inefficient production or accumulation of undesired intermediates. The ratio of carbon to nitrogen in the medium is a key regulator of both TAG and PHA synthesis. Nitrogen limitation often triggers storage polymer accumulation. However, simultaneous production of both compounds requires careful tuning of the C/N ratio to ensure neither pathway is suppressed while maintaining sufficient cell growth (*7,13,23*).

An alternative strategy to enhance bioprocess efficiency and reduce overall costs is the use of two oleaginous microbial species, each contributing to SCO production within an integrated biocatalytic framework. In such systems, the SCOs produced by one organism can serve as carbon-rich feedstock for a second strain engineered or selected for polyhydroxyalkanoate (PHA) biosynthesis (*51*).

Notably, PHAs are a family of biodegradable and biocompatible polyesters with growing applications in food packaging and biomedical sectors (*52*). This integration of microbial lipid and biopolymer production represents a dual-target bioprocessing strategy, particularly when derived from low-cost or waste-based substrates, and aligns well with the principles of industrial sustainability (*7*). The overall success of this approach depends heavily on factors such as strain selection and compatibility, the nature of the carbon source, and the cultivation mode (*e.g.* batch, fed-batch, or continuous), all of which significantly affect lipid yields and fatty acid composition (*53*).

ANALYTICAL METHODS TO DISTINGUISH PHA AND SCO

In dual-target bioprocesses where both SCOs and polyhydroxyalkanoates (PHAs) are produced by oleaginous microorganisms, accurately distinguishing between these two intracellular storage compounds is essential. This distinction is critical not only because of their structural and functional differences, but also due to their distinct downstream processing, extraction methods, and industrial applications. While both are typically synthesized under nutrient-limited, carbon-rich conditions, PHAs are high-molecular-mass polyesters and solid at room temperature, whereas SCOs consist mainly of triacylglycerols and remain liquid (*54*).

Several analytical techniques have been developed to differentiate PHAs from SCOs. These include fluorescent staining methods, solubility assays, Fourier-transform infrared (FTIR) spectroscopy, gas chromatography–mass spectrometry (GC-MS), and transmission electron microscopy (TEM) (*44,54-56*). Among staining techniques, Nile Blue A specifically binds to PHB and fluoresces pink under UV light, whereas Nile Red preferentially stains neutral lipids with yellow/orange fluorescence (*43,54,55*). Solubility tests also provide reliable differentiation: SCOs are soluble in cold acetone and chloroform, while PHB dissolves only in hot chloroform and precipitates in cold methanol. FTIR spectroscopy offers a rapid and non-destructive tool to chemically distinguish these compounds. PHB typically exhibits a strong ester carbonyl peak around 1720 /cm, while SCOs show additional peaks at ~1740 and ~2920 cm corresponding to triglyceride esters and aliphatic chains GC-MS analysis further supports compound identification; after acid-catalyzed methanolysis, PHB degrades to crotonic acid methyl ester (m/z=86), while SCOs yield fatty acid methyl esters (FAMEs), indicative of lipid composition (*44,56*).

Overall, combining multiple complementary techniques, such as staining, spectroscopy, solubility, and chromatography, not only ensures accurate compound identification but also enhances process monitoring, particularly in integrated biorefineries aiming for the simultaneous production of lipids and biopolymers from renewable substrates.

CHALLENGES AND OPPORTUNITIES IN DUAL-TARGET BIOPROCESSING

While the potential for dual-target bioprocessing is significant, several challenges must be addressed to optimize yield and efficiency. Key obstacles include substrate variability, metabolic bottlenecks, and the need for coordinated regulatory mechanisms in co-production pathways. Optimizing growth conditions, such as nutrient ratios and fermentation parameters, is crucial for enhancing product yields. Moreover, integrating novel bioprocess strategies, including co-culturing techniques, genetic engineering, and innovative fermentation technologies, can facilitate improved co-production of SCOs and PHAs. Research into techno-economic analyses can help delineate the feasibility of industrial-scale applications, exploring not only financial but also environmental sustainability (*57-60*).

Inconsistent nutrient composition of food waste and limited downstream purification efficiency remain the main challenges for stable and scalable production (*20*). Furthermore, improving product recovery efficiency is crucial for achieving economically viable dual-product bioprocesses, particularly when both lipid and polymeric compounds are targeted. As highlighted by Nguyen *et al.* (*11*), one of the major challenges in single-cell oil production is the limited recovery yield caused by inadequate cell disruption and inefficient downstream processing. To overcome these limitations, a combination of physical and chemical treatments, such as sonication, thermal or alkaline pretreatments, and bead-assisted homogenization, can be integrated to enhance intracellular lipid release. Similarly, process optimization strategies including adaptive evolution, nutrient feeding control, and metabolic engineering can increase both biomass productivity and product yield. Such integrated approaches not only improve lipid recovery but can also be adapted for the co-extraction of other intracellular metabolites, thereby maximizing the overall process efficiency in dual-production systems (*11*). Evidence from recent studies confirms that microbial valorization of food waste into PHAs offers a viable route toward resource-efficient circular bioeconomy models (*8*).

FUTURE DIRECTIONS AND PERSPECTIVE

The convergence of microbial lipid and biopolymer production marks a crucial advance in sustainable industrial biotechnology, especially when using low-cost feedstocks like food waste. Dual-target bioprocessing, enabling simultaneous production of SCOs and polyhydroxy-alkanoates (PHAs), offers significant benefits in resource efficiency, cost reduction, and environmental sustainability. Future research should focus on optimizing metabolic fluxes in native or engineered oleaginous strains for concurrent synthesis. It suggested that combining microbial consortia with

metabolic rewiring approaches can enhance carbon flux partitioning toward PHA biosynthesis from food waste, paving the way for next-generation integrated bioprocesses (*8*).

Synthetic biology and metabolic engineering will be key in precisely tuning pathways, balancing cofactors, and dynamically regulating processes to maximize yields without sacrificing growth or stability. Integrating advanced analytical tools with bioreactor automation and in situ sensing will improve product monitoring, quality control, and scalability.

Emerging biorefinery concepts emphasize full biomass valorization through cascade utilization, directing lipids to biofuels or nutraceuticals and using residual biomass for PHA or microbial protein production. Multi-stage or co-cultivation systems with tailored microbial consortia can further enhance substrate use and product diversity. While biotechnological valorization focuses on microbial conversion of food wastes into biopolymers and lipids, green extraction approaches such as microwave- and ultrasound-assisted methods have also been developed to recover bioactive compounds and essential oils from food processing byproducts, expanding the circular bioeconomy (*61*).

Commercial success depends on supportive policies, circular bioeconomy incentives, and life cycle assessments confirming environmental and economic advantages over fossil-based alternatives. Overall, the evolution of dual-target microbial bioprocesses aligns with sustainable development, waste valorization, and industrial decarbonization, positioning this field as a cornerstone of future bio-manufacturing through interdisciplinary innovation.

CONCLUSIONS

The use of oleaginous microorganisms for the dual-target bioprocessing of food waste into SCOs and PHAs presents a valuable opportunity to mitigate resource depletion and pollution while addressing the growing demand for sustainable bio-based products. By aligning this approach with circular bioeconomy principles, we can better utilize low-cost substrates while reducing the overall environmental footprint of production processes. Continued research is necessary to overcome existing challenges and optimize integrated bioprocessing strategies, thereby fostering a transition towards a more sustainable and resource-efficient bioeconomy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Zahra Montazer: methodology, validation: analysis, investigation, and data curation writing-review and editing, Kianoush Khosravi-Darani: conceptualization, methodology, validation: analysis, investigation, and data curation, writing original draft preparation, writing-review and visualization, supervision, project administration.

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REFERENCES

1. Zhou W, Bergsma S, Colpa DI, Euverink GW, Krooneman J. Polyhydroxyalkanoates (PHAs) synthesis and degradation by microbes and applications towards a circular economy. J Environ Manage. 2023;341:118033. https://doi.org/10.1016/j.jenvman.2023.118033
2. Tilman D, Clark M. Global diets link environmental sustainability and human health. Nature. 2014;515(7528):518-22. https://doi.org/10.1038/nature13959
3. MarketsandMarkets. Edible Oil Market Forecast Pune (India): MarketsandMarkets; 2024–2029 [Available from: https://www.marketsandmarkets.com.
4. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv. 2017;3(7):e1700782. https://doi.org/10.1126/sciadv.1700782
5. Wright SL, Kelly FJ. Plastic and human health: A micro issue? Environ Sci Technol. 2017;51(12):6634-47. https://doi.org/10.1021/acs.est.7b00423
6. Koller M, Salerno A, Dias MMdS, Reiterer A, Braunegg G. Modern botechnological polymer synthesis: A review. Food Technol Biotechnol. 2010;48:255-69.

7. Chen GQ, Patel MK. Plastics derived from biological sources: present and future: a technical and environmental review. Chem Rev. 2012;112(4):2082-99. https://doi.org/10.1021/cr200162d
8. Liu X, Wang Z, Zhang Y, Shah AA, Gong C. Microbial strategies on polyhydroxyalkanoates production from food waste to support the circular bioeconomy. Int J Biol Macromol. 2025;320:146023. https://doi.org/10.1016/j.ijbiomac.2025.146023
9. Shah AM, Yang W, Mohamed H, Zhang Y, Song Y. Microbes: A hidden treasure of polyunsaturated fatty acids. Front Nutr. 2022;9:827837. https://doi.org/10.3389/fnut.2022.827837
10. Nunes DD, Pillay VL, Van Rensburg E, Pott RWM. Oleaginous microorganisms as a sustainable oil source with a focus on downstream processing and cost-lowering production strategies: A review. Bioresour Technol Reports. 2024;26:101871. https://doi.org/10.1016/j.biteb.2024.101871
11. Nguyen QD, Nguyen T-V-L, Tran TTV, Khatri Y, Chandrapala J, Truong T. Single cell oils from oleaginous yeasts and metabolic engineering for potent cultivated lipids: A review with food application perspectives. Future Foods. 2025;11:100658. https://doi.org/10.1016/j.fufo.2025.100658
12. Vilchez A, Guajardo G, Sepúlveda M, Seeger M, Acevedo F, Navia R. Analyses of substrates and bacterial genera in biological polyhydroxyalkanoates production performance: A review. Bioresour Technol Reports. 2025;31:102224. https://doi.org/10.1016/j.biteb.2025.102224
13. Sitepu IR, Garay LA, Sestric R, Levin D, Block DE, German JB, *et al*. Oleaginous yeasts for biodiesel: current and future trends in biology and production. Biotechnol Adv. 2014;32(7):1336-60. https://doi.org/10.1016/j.biotechadv.2014.08.003
14. Kosa M, Ragauskas AJ. Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol. 2011;29(2):53-61. https://doi.org/10.1016/j.tibtech.2010.11.002
15. Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie. 2004;86(11):807-15. https://doi.org/10.1016/j.biochi.2004.09.017

16. Watsuntorn W, Chuengcharoenphanich N, Srimongkol P, Alagappan RP, James A, Rene ER, *et al*. Optimizing lipid production in oleaginous yeasts for sustainable bioenergy: A review of process parameters, cultivation strategies, and machine learning integration. Biomass Bioenergy. 2025;197:107810. https://doi.org/10.1016/j.biombioe.2025.107810
17. Donot F, Fontana A, Baccou JC, Strub C, Schorr-Galindo S. Single cell oils (SCOs) from oleaginous yeasts and moulds: Production and genetics. Biomass Bioenergy. 2014;68:135-50. https://doi.org/10.1016/j.biombioe.2014.06.016
18. Antonopoulou I, Spanopoulos A, Matsakas L. Single cell oil and ethanol production by the oleaginous yeast *Trichosporon fermentans* utilizing dried sweet sorghum stalks. Renew Energy. 2020;146:1609-17. https://doi.org/10.1016/j.renene.2019.07.107
19. Jones AD, Boundy-Mills KL, Barla GF, Kumar S, Ubanwa B, Balan V. Microbial lipid alternatives to plant lipids. Methods Mol Biol. 2019;1995:1-32. https://doi.org/10.1007/978-1-4939-9484-7_1
20. Dar RA, Tsui T-H, Zhang L, Tong YW, Sharon S, Shoseyov O, *et al*. Fermentation of organic wastes through oleaginous microorganisms for lipid production - Challenges and opportunities. Renew Sustain Energy Rev. 2024;195:114328. https://doi.org/10.1016/j.rser.2024.114328
21. Patel A, Rova U, Christakopoulos P, Matsakas L. Assessment of fatty acids profile and omega-3 polyunsaturated fatty acid production by the oleaginous marine thraustochytrid *Aurantiochytrium* sp. T66 cultivated on volatile fatty acids. Biomolecules. 2020;10(5):694.
22. Gu Y, Lu X, Liu T, Song Y, Sang E, Ding S, *et al*. Engineering the oleaginous yeast *Yarrowia lipolytica* to produce nutraceuticals: From metabolic design to industrial applications. Food Bioeng. 2023;2(3):187-99. https://doi.org/10.1002/fbe2.12062
23. Ratledge C. Microbial oils: an introductory overview of current status and future prospects. OCL. 2013;20(6):D602.
24. Ochsenreither K, Glück C, Stressler T, Fischer L, Syldatk C. Production strategies and applications of microbial single cell oils. Front Microbiol. 2016;7:1539. https://doi.org/10.3389/fmicb.2016.01539

25. Koller M, Marsalek L. Principles of glycerol-based polyhydroxyalkanoate production. Appl Food Biotechnol. 2015;2(4):3-10. https://doi.org/10.22037/afb.v2i4.8270

26. Koller M, Atlić A, Dias M, Reiterer A, Braunegg G. Microbial PHA production from waste raw materials. In: Chen GG-Q, editor. Plastics from bacteria: Natural functions and applications. Berlin, Heidelberg: Springer Berlin Heidelberg; 2010. pp. 85-119.

27. Koller M. Advances in polyhydroxyalkanoate (PHA) production, Volume 3. Bioengineering. 2022;9(7):328.

28. Kourmentza C, Plácido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN, *et al*. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering. 2017;4(2):55.

29. Koreti D, Kosre A, Jadhav SK, Chandrawanshi NK. A comprehensive review on oleaginous bacteria: an alternative source for biodiesel production. Bioresour Bioprocess. 2022;9(1):47. https://doi.org/10.1186/s40643-022-00527-1

30. Cappelletti M, Presentato A, Piacenza E, Firrincieli A, Turner RJ, Zannoni D. Biotechnology of *Rhodococcus* for the production of valuable compounds. Appl Microbiol Biotechnol. 2020;104(20):8567-94. https://doi.org/10.1007/s00253-020-10861-z

31. Alvarez HM, Kalscheuer R, Steinbüchel A. Accumulation and mobilization of storage lipids by *Rhodococcus opacus* PD630 and *Rhodococcus ruber* NCIMB 40126. Appl Microbiol Biotechnol. 2000;54(2):218-23. https://doi.org/10.1007/s002530000395

32. Hernández MA, Mohn WW, Martínez E, Rost E, Alvarez AF, Alvarez HM. Biosynthesis of storage compounds by *Rhodococcus jostii* RHA1 and global identification of genes involved in their metabolism. BMC Genomics. 2008;9(1):600. https://doi.org/10.1186/1471-2164-9-600

33. Al-Obeidi WDM, Al-Rawi DF, Ali LH. Production of single-cell oil from a local isolate *Bacillus subtilis* using palm fronds. Int J Biomater. 2023;2023:8882842. https://doi.org/10.1155/2023/8882842

34. Småros F, Vidgren V, Rondou K, Riihinen K, Mohammadi P, Dewettinck K, *et al*. Microbial production of food lipids using the oleaginous yeast *Apiotrichum brassicae*. Food Res Int. 2025;200:115481. https://doi.org/10.1016/j.foodres.2024.115481

35. Vemparala G, Chiliveru A, Begum S, Amri MBFA, Anupoju GR. Analyzing and optimizing the lipids production potential of the oleaginous yeast *Candida neerlandica* from synthetic carbon sources and real wastes. Next Res. 2025;2(2):100196. https://doi.org/10.1016/j.nexres.2025.100196

36. Dimitriadis A, Tourlakidis N, Bezergianni S. Microbial lipids from municipal solid wastes to advanced aviation and marine e-fuels via catalytic hydrotreatment. Biomass Bioenergy. 2025;198:107866. https://doi.org/10.1016/j.biombioe.2025.107866

37. Ma Y, Liu S, Cui L, Fei Q, Wang Q. Turning food waste to microbial lipid towards a superb economic and environmental sustainability: An innovative integrated biological route. Environm Res. 2024;255:119125. https://doi.org/10.1016/j.envres.2024.119125

38. Li ZJ, Qiao K, Liu N, Stephanopoulos G. Engineering *Yarrowia lipolytica* for poly-3-hydroxybutyrate production. J Ind Microbiol Biotechnol. 2017;44(4-5):605-12. https://doi.org/10.1007/s10295-016-1864-1

39. Park YK, Nicaud JM, Ledesma-Amaro R. The engineering potential of *Rhodosporidium toruloides* as a workhorse for biotechnological applications. Trends Biotechnol. 2018;36(3):304-17. https://doi.org/10.1016/j.tibtech.2017.10.013

40. Liu J, Yan J, Cui Z, Qi Q. Engineering *Yarrowia lipolytica* as a yeast cell factory for the de novo production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate). Synthetic Syst Biotechnol. 2026;11:152-60. https://doi.org/10.1016/j.synbio.2025.09.002

41. Juarez A, Villa JA, Lanza VF, Lázaro B, de la Cruz F, Alvarez HM, *et al*. Nutrient starvation leading to triglyceride accumulation activates the Entner Doudoroff pathway in *Rhodococcus jostii* RHA1. Microbial Cell Factories. 2017;16(1):35. https://doi.org/10.1186/s12934-017-0651-7

42. Pérez R, Díaz-Moreno N, Rodríguez S, Lebrero R. Valorization of toluene from waste gas streams: assessing PHA production by *Rhodococcus opacus*. J Hazard Mater Adv. 2025;19:100823. https://doi.org/10.1016/j.hazadv.2025.100823

43. Tan HT, Lei Y, Chek MF, He M, Pow KC, Gong S, *et al*. Engineering chimeric polyhydroxyalkanoate synthases for enhanced copolymerization of poly(3-hydroxybutyrate-

co-3-hydroxyhexanoate): A promising biotechnological approach. Bioresour Technol. 2026;439:133307. https://doi.org/10.1016/j.biortech.2025.133307

44. Kumar P, Jun HB, Kim BS. Co-production of polyhydroxyalkanoates and carotenoids through bioconversion of glycerol by *Paracoccus* sp. strain LL1. Int J Biol Macromol. 2018;107(Pt B):2552-58. https://doi.org/10.1016/j.ijbiomac.2017.10.147
45. Kourmentza C, Plácido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN, *et al*. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering (Basel). 2017;4(2). https://doi.org/10.3390/bioengineering4020055
46. Hori K, Abe M, Unno H. Production of triacylglycerol and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by the toluene-degrading bacterium *Rhodococcus aetherivorans* IAR1. J Biosci Bioeng. 2009;108(4):319-24. https://doi.org/10.1016/j.jbiosc.2009.04.020
47. Gao C, Qi Q, Madzak C, Lin CS. Exploring medium-chain-length polyhydroxyalkanoates production in the engineered yeast *Yarrowia lipolytica*. J Ind Microbiol Biotechnol. 2015;42(9):1255-62. https://doi.org/10.1007/s10295-015-1649-y
48. Rigouin C, Lajus S, Ocando C, Borsenberger V, Nicaud JM, Marty A, *et al*. Production and characterization of two medium-chain-length polydroxyalkanoates by engineered strains of *Yarrowia lipolytica*. Microb Cell Fact. 2019;18(1):99. https://doi.org/10.1186/s12934-019-1140-y
49. Tourang M, Xiong X, Sarkhosh S, Chen S. Polyhydroxybutyrate (PHB) Biosynthesis by an engineered *Yarrowia lipolytica* strain using co-substrate strategy. Fermentation. 2023;9(12):1003.
50. Kang Z, Du L, Kang J, Wang Y, Wang Q, Liang Q, *et al*. Production of succinate and polyhydroxyalkanoate from substrate mixture by metabolically engineered *Escherichia coli*. Bioresour Technol. 2011;102(11):6600-04. https://doi.org/10.1016/j.biortech.2011.03.070
51. Jovanovic S, Dietrich D, Becker J, Kohlstedt M, Wittmann C. Microbial production of polyunsaturated fatty acids — high-value ingredients for aquafeed, superfoods, and pharmaceuticals. Curr Opin Biotechnol. 2021;69:199-211.

https://doi.org/10.1016/j.copbio.2021.01.009

52. Koller M, Maršálek L, de Sousa Dias MM, Braunegg G. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. New Biotechnol. 2017;37:24-38. https://doi.org/10.1016/j.nbt.2016.05.001
53. Bellou S, Triantaphyllidou I-E, Aggeli D, Elazzazy AM, Baeshen MN, Aggelis G. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Curr Opin Biotechnol. 2016;37:24-35. https://doi.org/10.1016/j.copbio.2015.09.005
54. Zuriani R, Vigneswari S, Azizan MNM, Majid MIA, Amirul AA. A high throughput Nile red fluorescence method for rapid quantification of intracellular bacterial polyhydroxyalkanoates. Biotechnol Bioprocess Eng. 2013;18(3):472-78. https://doi.org/10.1007/s12257-012-0607-z
55. Spiekermann P, Rehm BH, Kalscheuer R, Baumeister D, Steinbüchel A. A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Arch Microbiol. 1999;171(2):73-80. https://doi.org/10.1007/s002030050681
56. Karr DB, Waters JK, Emerich DW. Analysis of poly-beta-hydroxybutyrate in *Rhizobium japonicum* bacteroids by ion-exclusion high-pressure liquid chromatography and UV detection. Appl Environ Microbiol. 1983;46(6):1339-44. https://doi.org/10.1128/aem.46.6.1339-1344.1983
57. Qian X, Gorte O, Chen L, Zhang W, Dong W, Ma J, *et al*. Co-production of single cell oil and gluconic acid using oleaginous *Cryptococcus podzolicus* DSM 27192. Biotechnol Biofuel. 2019;12:127. https://doi.org/10.1186/s13068-019-1469-9
58. Silva JdMe, Martins LHdS, Moreira DKT, Silva LdP, Barbosa PdPM, Komesu A, *et al*. Microbial lipid based biorefinery concepts: A review of status and prospects. Foods. 2023;12(10):2074.
59. Wang K, Hobby AM, Chen Y, Chio A, Jenkins BM, Zhang R. Techno-economic analysis on an industrial-scale production system of polyhydroxyalkanoates (PHA) from cheese by-products by halophiles. Processes. 2022;10(1):17.

60. Pozo-Morales L, Rosales Martínez A, Baquerizo E, del Valle Agulla G. Simulation Tool for the techno-economic assessment of the integrated production of polyhydroxyalkanoates as value-added byproducts of a wastewater treatment plant. Processes. 2025;13(2):295.

61. Ali EAE, Mohammed DM, El Gawad FA, Orabi MA, Gupta RK, Srivastav PP. Valorization of food processing waste byproducts for essential oil production and their application in food system. Waste Manag Bull. 2025;3(3):100200. https://doi.org/10.1016/j.wmb.2025.100200

Table 1. Most applicable type of microbial PUFAs and their producer companies (*24*)

Company	Country	Microorganism	Fatty acid	Brand name (if any)	Main application
DSM†	Netherlands	*Mortierella alpina*	ARA§	ARASCO™	Infant formula, dietary supplements
Martek Biosciences (part of DSM)	USA	*Crypthecodinium cohnii*	DHA§	DHASCO™	Infant formula, food supplements
Martek Biosciences	USA	*Schizochytrium* sp.	DHA	DHASCO™* (also for general food/feed)	Adult nutrition, animal feed
Suntory	Japan	*Mortierella alpina*	ARA	Not specified in reference	Infant nutrition, health supplements
Wuhan Alking Bioengineering	China	*Mortierella alpina*	ARA	Not specified in reference	Infant formula, supplements
Lion Corporation (historically)	Japan	*Mortierella alpina*	ARA	Not specified (early developer)	Cosmetics, food (initially for chicken flavor)
Various / Historical	-	*Mucor circinelloides*	GLA‡	Oil of Javanicus (discontinued)	Formerly as GLA source

					(replaced by borage oil)

†DSM is the world's leading producer of ARASCO™, under license to Martek, §ARA (arachidonic acid) and DHA (docosahexaenoic acid) are commercially the most successful microbial SCOs, primarily used in infant formula, *DHASCO™ is produced from the microalgae *Crypthecodinium cohnii* and approved for infant nutrition in many countries, ‡GLA production from *Mucor circinelloides* (Oil of Javanicus) was discontinued due to competition with plant-based sources like borage oil. Many infant formula manufacturers (*e.g.* Abbott, Nestle, Mead Johnson) use ARASCO™ and DHASCO™ under license

Table 2. Co-production of PHA and SCO by one microorganism (actinobacteria or bioengineered ones)

Microbial group	Strain / System	Reference	Genetic or cultivation strategy	Key finding
Natural "borderline" actinobacteria	*Rhodococcus ruber* NCIMB 40126 *Rhodococcus ruber* PD 630	(*31*)	Grown on glucose under nitrogen limitation	Sequential accumulation: PHBV in early phase, then TAG; approx. 1:1 ratio at end
	Rhodococcus aetherivorans IAR1	(*46*)	Toluene as C-source, N-limited	Simultaneous PHBV and TAG production before N depletion; TAG continued after carbon was exhausted
	Rhodococcus jostii RHA1	(*32*)	Genome contains 3 distinct pha clusters	Genomic evidence of dual lipid storage pathways (PHA and TAG) active under different conditions
Minimally or Fully Engineered oleaginous yeasts	*Yarrowia lipolytica*	(*47*)	Overexpression of *phaC1* from *P. aeruginosa* in peroxisome	mcl-PHA up to 5 % CDW; yeast still retained strong oil-producing capability

	Engineered *Yarrowia lipolytica*	(*48*)	Multicopy *phaC*, modified β-oxidation	Simultaneous production of mcl-PHA (25–28 % CDW) and natural TAGs
	Yarrowia lipolytica PHB32	(*49*)	Full PHB operon + glucose/acetate co-substrate strategy	12 % PHB with high growth rate; confirmed compatibility of PHB and SCO synthesis from cheap feedstock
Other chassis (less common)	*Saccharomyces cerevisiae* (engineered)	(*48*)	Single-copy *phaC* gene; compared to *Y. lipolytica*	mcl-PHA up to 7 % CDW; oil accumulation lower
	Escherichia coli	(*48,50*)	Fed glycerol+FA	Achieved ~0.5 g/L mcl-PHA (~6 % CDW); partial TAG synthesis observed

“Borderline” refers to microorganisms exhibiting the ability to accumulate both TAG and PHA or switching between the two