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Unveiling the potential of microalgae for bioplastic production from wastewater – current trends, innovations, and future prospects

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Abstract

Bioplastics derived from polyhydroxyalkanoates (PHAs) are among the promising substitutes for unsustainable petroleum-based polymers. PHA-based polymers demonstrate superior chemical and physical properties, such as hydrophobicity, insolubility in water, iso-tacticity, UV resistance, hydrolysis resistance, and absolute biodegradability. Compared with conventional plastics, bioplastics are more beneficial due to their reduced carbon footprint, energy efficiency, biodegradability, and biocompatibility, and have hence revolutionized the polymer industry. However, further research is needed to explore novel strategies to overcome their limitations, such as decreasing water absorption and brittleness, while increasing the crystallization ability and increasing the thermal degradation temperature. These constraints can be addressed by supplementing the bioplastic synthesis process with reinforcements and plasticizers. The development and adoption of biopolymers as an environmentally friendly and economically viable substitutes for synthetic plastics is imperative, considering the degree of the subsequent exhaustion of petrochemical supplies and the worldwide environmental contamination instigated by the industrial production of synthetic plastics. The goal of this appraisal is to provide an in-depth account of the most recent advancements in the generation of bioplastics derived from various wastewater streams via the use of microalgae, and subsequent harvesting technologies. Bioplastics from microalgae are of higher quality and are made of polymeric biomolecules and include polymers based on cellulose, starch, proteins, PHA, polyhydroxybutyrate (PHB), polyethylene (PE), polylactic acid (PLA), and poly vinyl chloride (PVC). Various types of bioplastic manufacturing methodologies have also been highlighted for researchers and capitalists alike to investigate ways to harness these renewable resources for the development of sustainable bioplastics. Additionally, various innovations, challenges, potential possibilities for the future, and life cycle evaluations of bioplastics are addressed.

Keywords Bioplastics, Polyhydroxyalkanoates, Bioconversion, Environmental sustainability, Biocompatibility, Life cycle assessment

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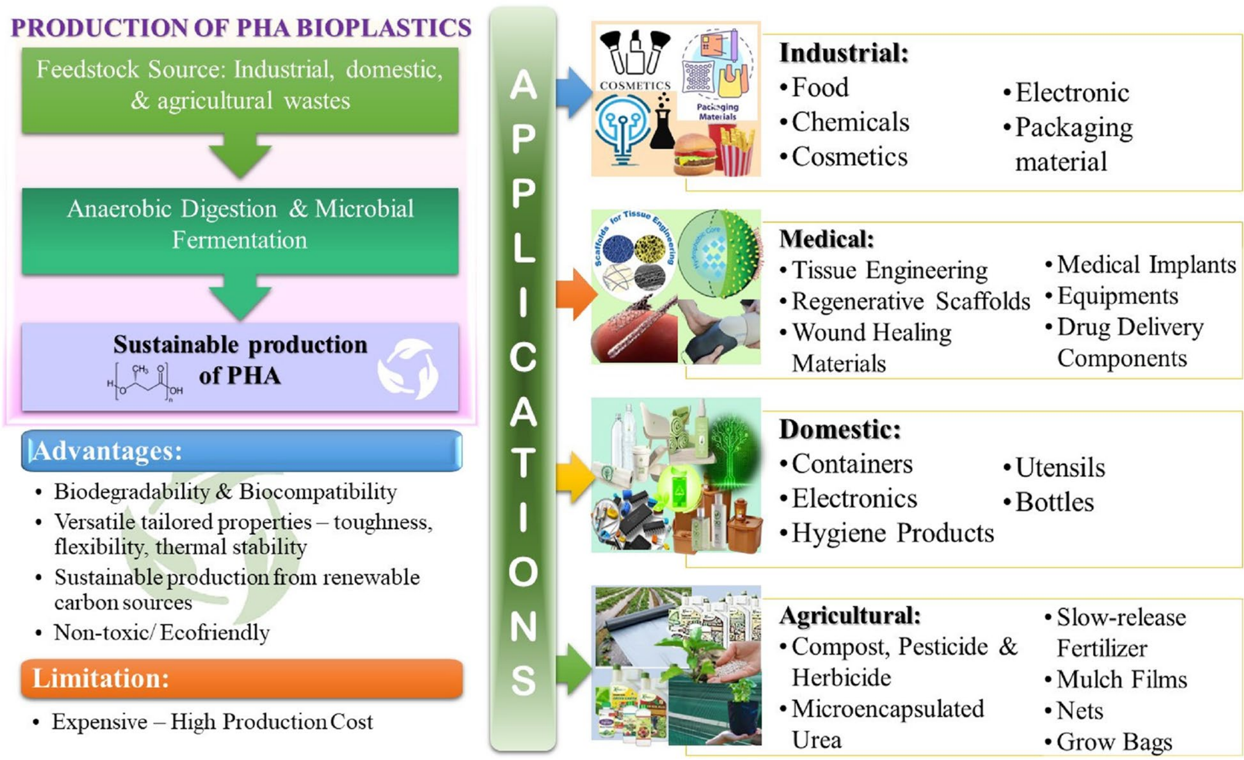
Graphical Abstract



Introduction

The global worth of plastics and polymer-based products in the market surpassed 580 billion US dollars approximately in 2020, showing ~ 15.5% growth from the previous five years' market size. Considering the ~ 37% increase in the production of plastics globally within the last 10 years, it is estimated that by 2028, the market size will increase by 3.4% compounded annually from 2021 to 2028, amounting to ~ 750 billion US dollars [1]. Traditionally, plastics are produced from fossil sources and possess the advantages of a long life-span and resistance to environmental degradation. These polymers also have the properties of high strength and toughness in addition to being lightweight and having low processing and production costs, making them convenient choices for use in various industries. However, the extensive use of fossil-sourced polymers that are hydrophobic and resistant to biodegradation, leads to deleterious environmental changes such as fossil fuel depletion, global warming, and pollution.

The economic and environmental management of waste generated postproduction and the use of plastics by consumers is one of the greatest challenges for economists, scientists, environmentalists and healthcare practitioners worlwide. The United Nations' sustainable development goals (SDGs) are also aimed at the management and use of plastics for providing better health and well-being (SDG3), clean water and sanitation (SDG6), sustainable cities and communities (SDG11), responsible consumption and production (SDG12) of plastics, climate action (SDG13), protection of seas and oceans (SDG14), and the repair of ecosystems and biodiversity (SDG15) [2].

In the quest to find alternatives to conventional plastics, the development of biodegradable, eco-friendly materials called bioplastics has become a keen interest of researchers. Bioplastics are macromolecular biopolymers that are produced biologically or are biodegradable or both. The bioplastics of the new-age circular economy include polymers made from renewable resources. Common examples of bioplastics include biopolyamide (Bio-PA), biopolyethene (Bio-PE), biopolyethene terephthalate (Bio-PET), biopolypropene (Bio-PP), bio-polytrimethene terephthalate (Bio-PTT), cellulose acetate, polybutylene adipate-co-terephthalate (PBAT), polybutylene succinate (PBS), polyethene furanoate (PEF), polyhydroxyalkanoates (PHA), polylactic acid (PLA), poly-ε-caprolactone (PCL), and starch [3]. Considering their melting point, brittleness, toughness, blending capability with other polymers and production-processing costs, PHAs have emerged as the prominent choice of biologically derived aliphatic polyesters. Owing to their biodegradability under both aerobic and anaerobic conditions, PHAs are the most promising remedies for the significant ecological issues caused by conventional plastics, and are preferred alternatives to traditional petroleum-based plastics. They are made even better by the fact that they are derived from plant sugars, which are renewable and help reduce heavy reliance on fossil fuels. PHAs possess biodegradability and biocompatibility and are nontoxic and water-insoluble; however, they may be soluble in chloroform or chlorinated solvents [4].

PHAs are categorized based on the length of their side chains. They may be short chain (< 5 C-atoms, scl-PHAs), medium chain (5 to 14 C-atoms, mcl-PHAs), or long chain (> 14 C-atoms, lcl-PHAs) [5]. They have glass transition temperatures ranging from -50 to 4 °C and melting temperatures ranging from 40 to 180 °C, depending on their chemical constitution and C-chain length [4]. The mechanical and thermal attributes of these materials can be modified by altering the chemical constitution of these polymers to possibly substitute for traditional plastics. The nature and biological function of PHAs were not well understood when they were first detected in 1888. Beijerinck, discovered intracellular PHA inclusions in 1888 and characterized them as lipids. Thirty years after the experiment started, in 1923, Maurice Lemoigne extracted PHB from *Bacillus megaterium* under anaerobic starving conditions using hot chloroform. Lemoigne developed the empirical formula $(C_4H_6O_2)_n$ and suggested that PHB functiones as a storage molecule [5]. PHAs are credited to Lemoigne as their creator because of his extensive study between 1923 and 1951. Lemoigne's theory was disproved in 1958 when Macrae and Wilkinson observed that PHB accumulated under high C:N ratio conditions and decomposed in the absenteeism of a carbon source. These observations supported the metabolic function of PHB as a storage molecule. Since 1959, PHAs have been sold commercially.

PHAs are polymers produced in vivo as polyoxoesters of hydroxyalkanoates, a polymerization catalyzed by microorganisms naturally to store carbon and energy. This synthesis occurs under conditions of carbon abundance coupled with a limitation in essential nutrients such as nitrogen, phosphorous, magnesium, or sulfur [6]. Microbiological processes are usually used to generate PHAs for sale. However, to better regulate the physicochemical qualities of the product, both enzymatic and chemical methods have been extensively studied. PHA synthases are known to recognize > 150 monomeric building units as likely substrates, although the majority of them have just been utilized in laboratory environments [7].

PHA bioplastics are versatile, and are useda in the medical field for drug delivery, tissue engineering, and medical devices, as well as in agriculture for seed encapsulation and as eco-friendly pesticide carriers. The biocompatibility and biodegradability of these materials make them ideal for applications such as surgical sutures and food packaging. Derived from renewable materials, PHAs support sustainable practices across various industries, contributing to a circular economy and reducing plastic waste [8, 9]. Due to the significant benefits and diverse applications of PHA bioplastics, many research efforts have been dedicated to improving microbial production methods to increase PHA yield. These biopolymers are synthesized in substantial fermenters by many bacteria such as *Pseudomonas* species, *Ralstonia eutropha* H16, *Micrococcus*, *Microlunatus*, *Rhodococcus*, *Parapedobacter*, and genetically modified *Escherichia coli* [10]. In PHA production through bacterial fermentation, the use of significant organic carbon sources, such as sucrose, sugar corn, vegetable oil and mineral salts, accounts for the higher production cost and competition for substrates with the surrounding food sector, thus limiting its industrial application as a polymer compared to some commonly used petroleum-derived plastics. A different method for PHA production involves the use of microalgae, the microorganisms as a part of the phytoplankton community, generate biomass by utilizing light and atmospheric CO2 as their sole energy sources [11]. Microalgae, which encompass eukaryotic algae and cyanobacteria, are currently being employed as potential sources of PHA due to their rapid growth rate, significant biomass production, cheap substrate utilization and non-interference with food and feed stock. These can be utilized either directly as biomass in bioplastic production or indirectly through the extraction of starch and PHBs from their cells. Among the various microalgae species, *Chlorella* and *Spirulina* are the most studied because they typically have small cell sizes (˃50 µm), making these microalgal biomasses ideal for packaging and coating applications where fine particle size is a crucial requirement [12].

PHA production by microalgae faces significant challenges, particularly a low production rate compared to that of their bacterial counterparts [13, 14]. Therefore, optimizing autotrophic PHA production is crucial for increasing the intracellular concentration of PHA and reducing production costs, making it a competitive alternative. In this context, utilizing wastewater effluents as a nutrient source is a cost-effective and eco-friendly option for producing microalgal biomass [12]. Microalgae are prevalent in our environment and contain polysaccharides that can be used to produce biopolymers. These fast-growing organisms are found in wastewater streams. The synthesis of PHA can be achieved by hydrolyzing wastewater microalgal biomass [15]. In the biological treatment of wastewater, activated sludge efficiently converts degradable substances into PHA, which serves as a granular internal storage material for microorganisms. Microorganisms may adapt to various external environmental conditions, such as high temperatures, dryness, H_2O_2, UV light, and osmotic pressure due to the internal carbon and energy sources provided by PHA. Various techniques of treating wastewater and complex community patterns in activated sludge systems lead to different anabolic processes in PHA. Both the concentration of activated sludge and the community's substrate preferences impact the amount and constitution of PHA in the sludge [16].

Recent works have quoted the use of various strains of microalgae, feedstocks, production processes and conversion technologies in PHA synthesis and yet, the ideal bioplastic is far from achieved that proves as a 100% replacement for the conventional fossil-based polymers with economic sustainability. Hence, the comprehension of existing research, that may fuel further studies providing a focused approach toward the production of novel biopolymers with wider industrial applications is needed. Thus, the present review provides an in-depth understanding of the production processes and applications of PHA based bioplastics produced by microalgae, and the integration of microalgae-based PHA production with wastewater treatment processes and further summarizes the present as well as future possible innovative technologies to improve bioplastic production in terms of efficiency and sustainability. This study also explains the limitations and environmental implications of PHA bioplastics in terms of life cycle assessments of their environmental footprint.

Integration of microalgae-based PHA production with wastewater treatment processes

In recent decades, growing concerns over climate change and pollution from human activities have propelled microalgae into the spotlight as a promising solution. Microalgae offer a unique biological platform to mitigate carbon dioxide emissions while also producing valuable compounds for various industries, including pharma, food-feed, bioenergy and biopolymers. Despite this potential, the large-scale industrialization of microalgae cultivation and utilization faces significant challenges, particularly in terms of economic feasibility and productivity. Efforts to enhance resource efficiency are crucial for overcoming these challenges, as they can reduce manufacturing costs and improve the long-term sustainability of microalgae-based products. One effective strategy is the use of wastewater as a nutrient source, which not only lowers production expenses but also addresses the pressing issue of global water shortage. Wastewater, with its high salt and organic content, is a valuable resource with multiple uses [17, 18]. Microalgae cultures have emerged as a sustainable solution for treating wastewater, offering the capacity to break down complex contaminants in tertiary and quaternary treatment processes and promotes the concept of circular economy in a sustainable manner [19]. The use of microalgae in wastewater treatment systems provides a sustainable solution by effectively removing pollutants, lowering operational costs, and generating eco-friendly by-products, all while contributing to carbon capture efforts (Fig. 1).

Recently, various wastewater types have been employed to cultivate algal biomass for phycoremediation. Wastewater is categorized by its origin: municipal (households), agricultural (farming and livestock), and industrial (manufacturing processes) with its composition varying according to its source. The presence of significant volumes of both organic and inorganic nutrients in these wastewaters can lead to ecological imbalances because of their high levels of chemical and biological oxygen demand (COD) and BOD [19]. One of the most challenging environmental issues in the world is water eutrophication, which is caused by an excess of nutrients, especially phosphorus (P) and nitrogen (N). The composition of wastewater significantly impacts the growth of microalgae, the rate of pollutant remediation and the production of biopolymer molecules. The ability of microalgae to remove pollutants and survive depends on a number of factors, including the carbon source, organic or inorganic carbon, macronutrients, nitrogen, phosphorus, micronutrients, vitamins, and trace elements in wastewater [20, 21]

Various studies have examined the buildup of bioplastics from microalgae cultured in municipal wastewater [22–27]. Kavitha et al., [28] investigated the utilization of sewage wastewater to cultivate a microalga *Botryococcus braunii* for polyhydroxybutyrate (PHB) production, highlighting its potential for eco-friendly plastic synthesis. The study showed that pH, temperature, and substrate concentration notably influence PHB yields, with a 60% wastewater concentration enhancing PHB production thus demonstrates the efficient integration of algae-based PHB synthesis with wastewater treatment processes.

The process of bioplastic production from municipal wastewater involves two main steps: first, converting sludge from wastewater treatment into volatile fatty acids (VFAs) via anaerobic digestion, with primary sludge

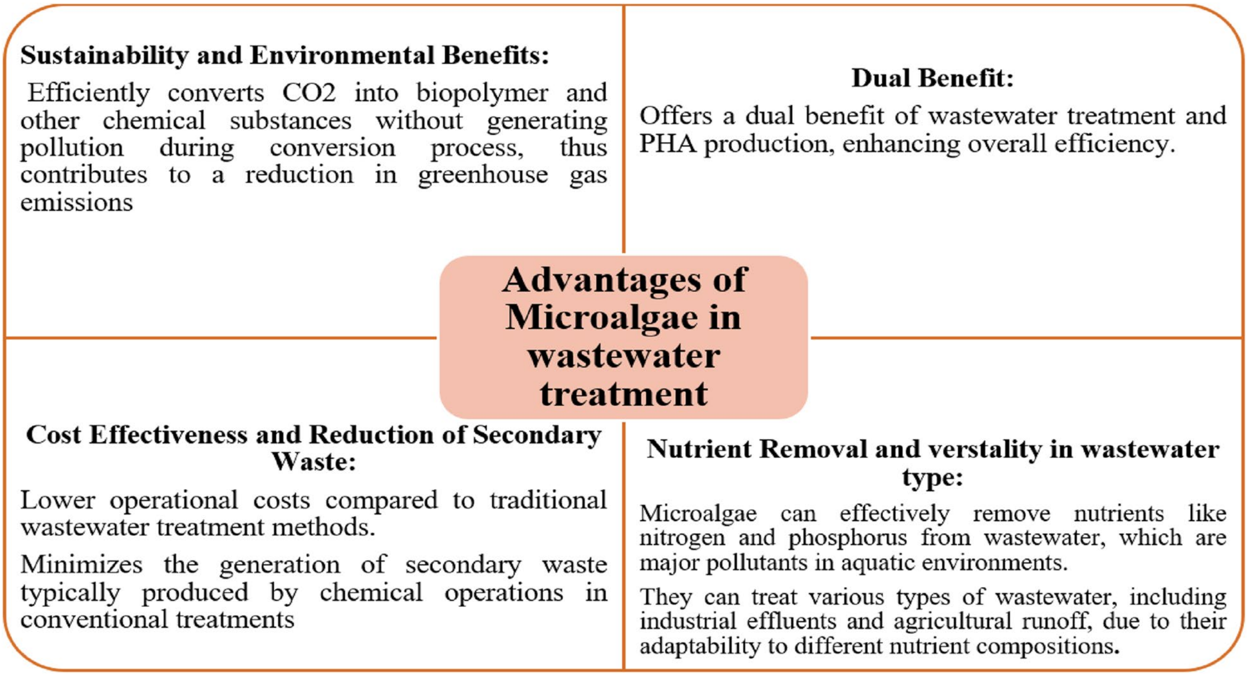


Fig. 1 Benefits of harnessing microalgae in wastewater treatment process [19]

yielding up to 40% COD conversion to VFAs. Second, VFAs are converted into biopolymers (PHA) through aerobic fermentation by pure or mixed microbial cultures. Pilot tests have achieved a maximum PHA concentration of 15% of the dry cell mass, under different environmental conditions (pH, temperature, nutrient limitations) [28]. Mahapatra et al., [29] investigated the efficacy of a large-scale algae-based sewage treatment plant (STP) in India, highlighting the traditional use of lagoons for decentralized sewage treatment. The STP, operating without external energy inputs, demonstrated moderate performance with notable removal efficiencies for COD (60–50%) and BOD (82–70%).

Researchers have also investigated the production of valuable bioproducts, including PHA-bioplastics from microalgal biomass grown in urban wastewater [25, 30, 31]. One crucial aspect of microalgae treatment is the nutritional makeup of various urban wastewaters. To achieve wastewater discharge criteria, different categories of wastewater should be mixed to balance the ratio and concentration of nutrients. It is important to consider how newly discovered contaminants may affect how well microalgae treatments work [21]. Table 1 summarizes the sources of various wastewaters, composition, and microalgal treatments of these wastewaters.

High-rate algal ponds (HRAPs) are a type of photosynthetic system treating wastewater using microalgal-bacterial consortia. They are widely used around the world for two purposes: either directly treating wastewater before it is discharged into surface waters, or pretreating wastewater in conventional wastewater treatment plants. They are typically operated in open environments with sunlight shining on them, liquid heights between 0.3 and 0.6 m and a variety of mixing systems, such as paddle wheels, submersible jet mixers, or low energy algae reactor mixers [54, 55]. Almeida et al., [56] explored the production of PHA using phototrophic mixed cultures (PMCs) enriched in phototrophic purple bacteria (PPB) within HRAPs for wastewater treatment. Operational strategies like phosphate cycling and switching from low to high organic loading rates improve PHA accumulation. The transition from selection under low organic loading rate to high organic loading rate resulted in improved PHA production. PMC has potential for PHA production from complex wastewater feedstocks under outdoor conditions, leveraging sunlight as an energy source. This highlights the importance of operational strategies and feeding methods in optimizing PHA productivity and wastewater treatment efficiency [56].

Wastewater-grown algal biomass for PHA bioplastic production offers numerous advantages (Fig. 1). However, the process can be complex to manage due to the need for specific conditions to optimize both the bioplastic yield and wastewater treatment. Additionally, the variability in wastewater composition can affect the consistency and quality of PHA production. Managing

Table 1 Sources, composition, and microalgal treatment of various wastewater types

Wastewater category	Subcategory	Composition	Challenges in treatment	Microalgal strain	Wastewater treatment	Reference
Municipal wastewater: wastewater generated from household activities	Raw sewage (wastewater before primary settling), primary sewage (wastewater recovered after primary settling), secondary sewage (wasterwater generated after activated sludge treatment in aeration tank, centrate (wastewater generated after sludge treatment contain abundant nutrients	15–90 mgL^{-1} N, 5–20 mgL^{-1} P and a low COD concentration of 300 mgL^{-1} [21]	Wastewater's chromaticity and emerging pollutant concentration hinder microalgae growth and photosynthetic efficiency [21]	*Chlorella vulgaris, Neochloris oleoabundans*, and the mixed microalgal strains	Microalgae growth varied as per the type of wastewater and the mixed microalgal strains showed higher waste remediation	[32]
				Parachlorella kessleri	> 70% removal of ammonia nitrogen and total nitrogen, > 65% of total phosphorus, and complete reduction of COD from simulated domestic wastewater after 10-day treatment period	[33]
				Scenedesmus obliquus	N (99.8%) and P (83.1%) removal from primary and secondary waste settling tanks	[34]
				Plectonema terebrans BERC10	Removal of 80–90% of total phosphorous, 90–99% of total nitrogen, with significant decrease in COD, BOD, and TDS content of urban wastewater along with production of a variety of products including 53 mgg^{-1} of valuable pigments and high-quality biodiesel with the descending processing of the algal biomass	[31]
				Chlorococcum humicola, *Scenedesmus vacuolatus* and *Tetradesmus* sp.	Removal of 83% nitrate nitrogen, 70% phosphorus, and heavy metals (31 to 74%) from municipal wastewater	[35]

Table 1 (continued)

Wastewater category	Subcategory	Composition	Challenges in treatment	Microalgal strain	Wastewater treatment	Reference
Agricultural Wastewater: wastewater released during the production of agricultural products, raising livestock, and growing crops	Palm oil mill effluent, animal manure and farmland drainage wastewater, starch processing wastewater, potato processing wastewater etc. [21]	Total nitrogen level of 800–2300 mgL^{-1}, and total phosphorus levels of 50–230 mgL^{-1}. [36] For many microalgae species, excessive levels of turbidity, ammonia, and nitrate nitrogen in agricultural wastewater hinder their growth [37]	High turbidity and elevated ammonia levels block light and inhibit the growth of algae used for the treatment A few algae species are capable of thriving in such wastewater Needs dilution beforehand to reduce turbidity and nutrient concentrations, which adds complexity and cost to the treatment process [37]	*Chlorella vulgaris*	After treating wastewater for 3 to 5 days using the two processes, the removal efficiencies for 92% COD, 83–94% ammonia nitrogen, and 91–94% total phosphorus from undiluted cattle farm wastewater after 3–5 days of incubation	[38]
				Synechococcus sp	Removal of 84% ammonia nitrogen, 86% total nitrogen, 85% phosphorus and 60% of COD from cattle farm wastewater	[39]
				Chlorella sorokiniana strain AK1	Removal of 78% total nitrogen, 98% total phosphorus 90% of COD from diluted swine wastewater supplemented with BG11 medium after 15 days of incubation	[40]
				Chlorella sp. strain S5	Removal of 95% nitrate nitrogen and 91% phosphate from agricultural wastewater	[41]
Industrial Wastewater: contain effluent generated from various industries		Contaminants including antibiotics, oil and grease, various heavy metals, and other chemical substances Significant concentrations of SS (180 mgL^{-1}), chloride (314 mgL^{-1}), fluoride (1.88 mgL^{-1}), calcium (124.8 mgL^{-1}), TDS (3725 mgL^{-1}), TS (3905 mgL^{-1}), BOD (3480 mgL^{-1}) and COD (7880 mgL^{-1}) as found in sugar mill effluent, an industrial wastewater type [42, 43]	High levels of total nitrogen, phosphorus, BOD, COD, and toxic heavy metals make industrial wastewater challenging to treat Dilution and anaerobic pretreatment are necessary to make the wastewater suitable for microalgae-based treatment, but increase both cost and complexity of the process [44]	*Cholorella* and *Scenedesmus acuminatus*	Removal of approx. 97% of phosphate and 99% of ammonia nitrogen from pulp and paper mill biosludge digestate	[45]
				Chlorella sp.	Removal of ammonia nitrogen and COD by 84% and more than 60% respectively in raw textile wastewater	[46]

Table 1 (continued)

Wastewater category	Subcategory	Composition	Challenges in treatment	Microalgal strain	Wastewater treatment	Reference
				Chlorella sorokiniana CY-1	62.07% of total nitrogen, 47.09% of COD, and 30.77% of total phosphorus were removed from acid-heat pretreated (30% v/v) palm oil mill effluent	[47]
				Chlorella sp.	Removal of heavy metals; Cr, Ni, Pb, Cd, Co, Zn and Cu in 81.36 μgL^{-1}, 82.15 μgL^{-1}, 70.53 μgL^{-1}, 163.29 μgL^{-1}, 58.92 μgL^{-1}, 83.43 μgL^{-1} and 64.83 μgL^{-1}, concentrations respectively from tannery waste water	[48]
				Chlorella vulgaris	Removal of 64% for nitrogen and 70% for phosphorus from cheese whey	[49]
				microalgae-bacterial consortium	Removal of approx 59% nitrate nitrogen, 86% phosphate, 91.5% COD and 42% color removal in real textile wastewater	[50]
				Scenedesmus sp.	Removal of 35 – 96% of chromium (IV), cadmium (II) and copper (II) from petroleum wastewater cultured with microalgae for 15 days at 27 ± 2 °C	[51]
				Chlorella sorokiniana TU5	Removal of V(III), a heavy metal discharged from industries like steel and semiconductors, achieving a significant removal rate of 20.38 mgL^{-1}	[52]
				Chlorella vulgaris, Nannochloropsis oculata, and Scenedesmus sp.	Removal of phosphate, total Kjeldahl nitrogen, ammonium nitrogen, COD and total organic carbon from wastewater mixture comprising of brewery wastewater, cheese-whey and expired orange juice with removal efficacies ranging between 80–90%	[53]

the cocultivation of microalgae and bacteria for efficient treatment and PHA synthesis is also challenging, requires careful control of environmental parameters. Further research is needed to optimize the process, address safety concerns associated with using different categories of wastewater, assess the technoeconomic landscape—particularly focusing on PHAs—and explore new strains for implementing the integrated biorefinery concept. These strategies could enhance profitability in this area and drive industrial transformation for microalgae-mediated biopolymer extraction from wastewater effluents [57].

Overview of PHA Bioplastic production (pathways)

PHAs are produced by microalgae through complex metabolic processes influenced by environmental factors and nutrient levels. When nutrients such as nitrogen or phosphorus are limited, microalgae generate PHAs as energy storage molecules by altering their metabolic pathways. When nutrients are abundant, the focus shifts to protein synthesis for cell growth, leading to a reversible change in metabolism. Different types of microalgae accumulate PHAs under various growth conditions [58].

Biological synthesis of PHA from microalgae

Microalgal PHA generation involves a complex series of biochemical pathways. Many studies have proposed that microalgal PHA production occurs under nutrient-deficient conditions, such as limited nitrogen and/or phosphorus. When microalgae face these nutrient limitations, their basal metabolism is redirected to create carbon-rich compounds, including PHAs, that are used for energy storage. Microalgae utilize different metabolic pathways, such as fatty acid metabolism, to produce the necessary precursor molecules for PHA synthesis (Fig. 2).

The main precursor for PHA biosynthesis is acetyl-coenzyme A (acetyl-CoA), a molecule derived from the breakdown of carbohydrates or fatty acids through various metabolic pathways [59]. The synthesis of PHAs involves a series of enzymatic reactions. The key enzymes involved include the following:

(i) β-ketothiolase (encoded by the phaA gene): catalyzes the condensation of two molecules of acetyl-CoA forming acetoacetyl-CoA.
(ii) NADPH-dependent acetoacetyl-CoA reductase (encoded by the phaB gene): converts acetoacetyl-CoA to D-3-hydroxybutyryl-CoA.
(iii) PHA synthase (encoded by the phaC gene): catalyzing the binding of D-3-hydroxybutyryl-CoA to an existing PHA molecule, forming the polymer chain via an ester linkage.

In addition to the traditional method, there are numerous other biosynthetic pathways for PHA synthesis. These pathways differ depending on the substrates, enzymes, and microorganisms involved. Because it can polymerize 3-hydroxyalkanoate (3HA) units via many pathways, including the pathways for fatty acid β-oxidation, the methylmalonyl-CoA, and the de novo fatty acid synthesis pathway, the enzyme PHA synthase is essential for PHA synthesis [60, 61]. PHA synthases are classified into four groups according to the substrate specificity and subunit makeup of each class [62]. The class I PHA synthases,

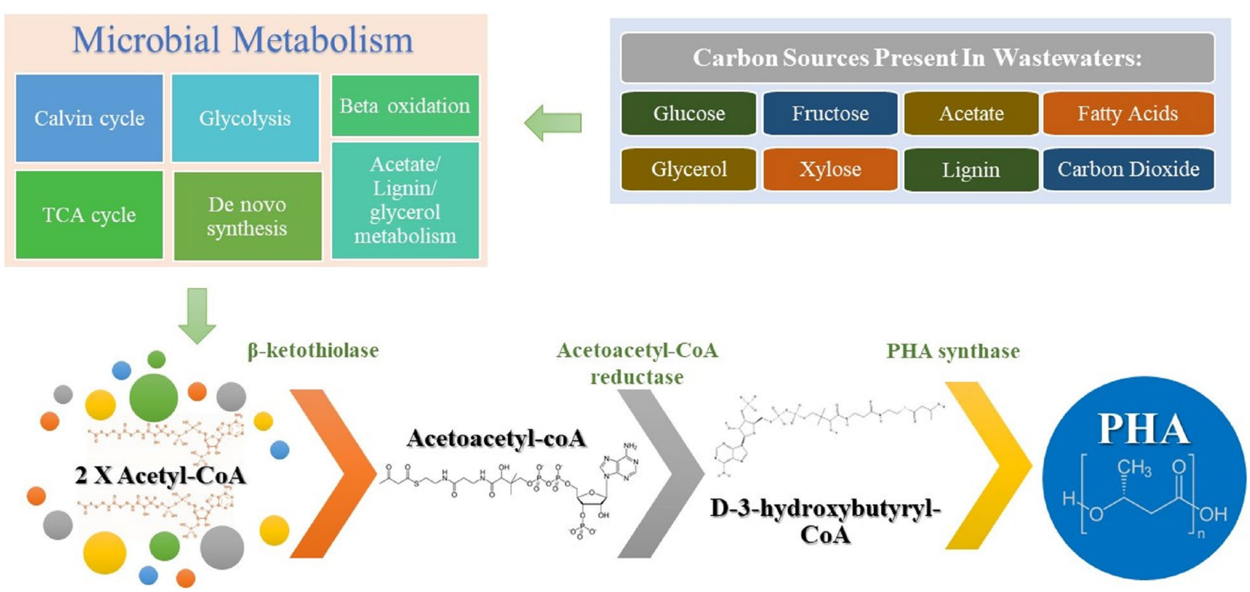


Fig. 2 Synthesis of PHAs in Microalgae

composed of a single subunit (phaC) with molecular weight of 64 kDa, polymerize short-chain-length-3HA (C3-C5) units. Class II PHA synthases are also composed of a single subunit (PhaC) with a comparable molecular weight of approximately 63 kDa, polymerizes medium-chain-length-3HA (C6-C14) units. Class III and Class IV PHA synthases are heteromeric with Class III being composed of two subunits (PhaC, PhaE) with molecular weights of approx. 40 kDa each and polymerizing short-chain-length-3HA units. Class IV, needs additional subunits (composed of either PhaC, E or Pha C, R) to function and polymerize short-chain-length-3HA to medium-chain-length-3HA and short-chain-length-3HA alone, respectively [63].

Acetyl-CoA, a precursor metabolite for the PHB synthesis pathway is derived from the Krebs cycle. This pathway is an important metabolic hub in cells that uses oxygen to produce energy. PHAs are also produced during lipid metabolism. During β-oxidation of fatty acids, different HAs are produced through bioconversion of alkanes, alkenes, and alkanoates. Acetyl-CoA intermediates of the β-oxidation pathway are channeled to R-hydroxyacyl-CoA by the enzyme enoyl-CoA hydratase. PHA synthase then polymerizes R-hydroxyacyl-CoA into PHAs. Glycolic precursors and fatty acid biosynthesis intermediates are also converted into 3-hydroxyacyl-CoA and then polymerized into PHAs by PHA synthase [64].

Polyhydroxyalkanoates are naturally found in microalgae, but their concentration is typically quite low, often below ten percent of the cell's dry weight, which is considerably less than what is found in other microbes such as bacteria [65]. The literature has revealed that under standard growth conditions, PHA producing microalagal strains have natural PHA levels between 3.5% of their dry weight [66]. Other research has documented PHA levels in microalgae to be under 9% of their dry weight. However, these concentrations can notably increase when the culture conditions are optimized [65, 67]. Optimizing culture conditions such as ample light and carbon sources, optimal pH and salinity, and the appropriate concentration of nutrients in the growth medium are crucial for enhancing PHA production [68]. Altamira-Algarra et al. [69] optimized nutrients, temperature, and light to enrich a cyanobacteria-dominated microbiome for enhanced polyhydroxybutyrate (PHB) production. Over 197 days in a 3 L photobioreactor, the microbiome achieved up to 22% PHB dry cell weight, despite the presence of green algae. These results indicate that culture composition is crucial for maximizing PHB yield. Researchers are also investigating the potential of coculturing microalgae with other microorganisms to create a more efficient and sustainable production system. Since microbial mixed cultures can be run in open systems without any concern of maintaining pure culture, and can use inexpensive by-products and waste streams as feed, thus their use in PHB production has the potential to significantly lower operating costs compared to those of pure cultures [69–71].

Once PHAs are synthesized and accumulate within microalgae cells, the biomass needs to be harvested and pretreated followed by extraction of PHA and purification [72]. The extraction processes often involve the use of solvents, and innovative strategies are being explored to make this step more efficient and environmentally friendly. Biomass drying, especially for certain microalgae such as *Spirulina*, can contribute significantly to the overall production cost. Drying methods need to be energy-efficient to enhance the economic feasibility of PHA production [73].

The extraction and purification of PHA from microalgae involve several methods aimed at the efficient recovery and purification of the polymer from microbial cells. Various physical, chemical, and biological approaches are employed for primary cell disruption to release internal components (lipids, proteins, and pigments) and enhance the PHA yield. Physical methods include bead milling, high-pressure homogenization, high-speed homogenization, homogenizer mills, pulse electric field, thermal treatments, and ultra-sonication. These methods efficiently disrupt microbial cells, aiding in the recovery of PHA [74–80]. Combining appropriate chemical methods with mechanical extraction can enhance recovery without altering the polymer's features. Chemical methods of PHA extraction comprise the usage of solvents such as acetone, chloroform, methyl isobutyl ketone, surfactants, and others to interact with cell membranes, alter membrane phospholipids and aid in product recovery [81]. Surfactants such as hexadecyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), and myristyltrimethylammonium bromide (MTAB) are commonly used [82]. Sodium dodecylbenzenesulfonate (SDS) has also been utilized for the cell disruption of *Nannochloropis salina* microalgae [83]. Solvent extraction is the most commonly used chemical method utilizing solvents such as chloroform, hexane, methanol, and ethanol, achieving purity levels greater than 90% [65, 84]. The Soxhlet and Folch methods are commonly used in solvent extraction. The Folch method is a two-step method in which chloroform/methanol with a 2:1 ratio is used. In the Soxhlet method, petroleum ether or hexane are used for the extraction of components via reflux in solvents [85]. The Folch technique of extraction yields a higher product yield [86]. The polyhydroxyalkanoates from *Spirulina* sp. were extracted using methanol and sodium hypochlorite, and a purity of 63.51 to 93.62% was attained [87]. PHA was also extracted from

Cupriavidus necator using nonchlorinated solvents such as cyclohexanone and γ-butyrolactone [88]. PHA could be recovered from a mixed culture of activated sludge with up to 98% purity by using acetone and butanol [89]. However, the use of alkalis such as NaOH or KOH is one of the most popular and least expensive ways to extract PHA [90]. Supercritical fluid extraction (SFE) with CO2 has also shown promise in PHA extraction [91].

Biological lysis methods are used when there is requirement of less energy and process to be operated under mild conditions. Biological lysis involves the use of enzymes to hydrolyze PHA-containing cells under mild conditions with high selectivity resulting in high-quality polymer recovery. Enzymes like lysozymes, cellulases, glucanases, nucleases, and proteases, when used in combination with mechanical and chemical methods, enhance PHA recovery [92]. Longer treatment time, optimal conditions, high levels of product inhibitions are some of the drawbacks that hinder their utilization in industry [92]. However, a combination of mechanical, chemical and enzymatic methods is commonly employed at biorefinery for the highest recovery of the desired product. Table 2 provides a comparative analysis of key PHA extraction methods. These methods utilize different techniques to extract biopolymers from microalgae biomass, offering advantages such as reduced extraction time, environmental friendliness, and improved extraction yields compared to traditional methods.

After extraction, PHA is purified using techniques such as ammonium sulfate precipitation, ultrafiltration, electro membrane filtration, aqueous two-phase separation, and a three-phase partitioning system to obtain high-purity PHA [99–103]. The extracted PHAs undergo purification processes to remove pigments and other impurities without degrading the polymer [64]. These approaches highlight advancements in sustainable bioplastic production processes, leveraging microalgae as a capable feedstock for PHA manufacturing. The integration of optimized extraction and purification methods is crucial for scalable and efficient PHA bioplastic production [104].

Factors influencing PHA production efficiency

The competent industrial generation of PHA and accurate regulation of PHA constitution via fatty acid metabolism are currently under considerable research. Comprehending the β-oxidation cycle and the biology of PHA synthesis in cellular inclusions is crucial for achieving high yields and specific PHA traits. The use of cultivation strategies such as altering nutritional medium, adding acetate and propionate, raising salt levels, restricting gas exchange, and utilizing wastewater is vital for maximizing PHA concentrations. The selection of efficient strains enhances polymer production, resulting in PHA yields between 5.0% and 70% based on dry microalgae cells.

PHA synthesis is affected by elements such as the carbon-to-nitrogen ratio, nutrient availability, fermentation type, and fermenter activities. Nitrogen availability is essential for microbial growth and the production of PHA [105]. The carbon-to-nitrogen (C/N) ratio, phosphate concentration and C/P ratios have a considerable impact on the efficiency of PHA synthesis and the composition of the polymer. A low C/N ratio is beneficial for cell growth since nitrogen is essential for protein synthesis and cell proliferation. Increased C/N ratios encourage the accumulation of PHA, leading to a reduction in microbial protein synthesis and a shift of resources towards PHA production.. Altering the C/N ratio can lead to variations in polymer traits, such as adjustments in hydroxyvalerate (HV) concentration. Khatami et al. [106] reviewed that generation of PHA can be doubled if carbon and nitrogen were individually introduced into the bioreactor, and the polymer thus produced was altered by increasing the HV concentration by 82%, hence proving that the properties of the polymer can be manipulated through altering C/N ratios.

Microalgae accumulate PHAs when exposed to nutritional deficiency or environmental stress conditions for upto 80% of their cell weight. For instance, N-deficiency triggered PHA generation of 5.8 mg/g cell weight in *Arthrospira platensis*, P-deficiency caused PHA accumulation of 29% w/w in *Scenedesmus* sp., and medium supplementation with sugar hydrolysates increased PHB generation in *Chlorococcum* sp. [107]. Adetunji and Erasmus [107] have further summarised how the modulation of PHA production by microalgae is possible through altering various conditions like nitrogen limitation, phosphorus deprivation, dark conditions, media supplementation (acetate, citrate, dipotassium phosphate, D-xylose, propionate, sodium bicarbonate, valerate or 3-hydroxyvalerate), aeration, and saline conditions.

Altering the substrates also affects the constitution of the microbial communities, which in turn modulates the first step in PHA production, i.e. acidogenic VFA generation. Lagoa-Costa et al. [105] used a PHA producing acidogenic bioreactor for treating wastewater form cheese whey and breweries, to study the changes in the microbial consortia in response to alteration in the constitution of feedstock. They observed that as the ratio of wastewater increased in proportion to the organic content the bacterial consortia changed drastically in their constitution, with emergence of even unidentified strains, and acidification decreased simultaneously. Although all ratios between wastewater and organic load led to similar PHA production, but the monomeric constituents were slightly different.

Table 2 Evaluation of various PHA extraction techniques

Microbial strain	Extraction and Purification method	Production of PHA/PHA precursor	Process impact	Reference
Chlorella vulgaris	Supercritical CO2 (sCO2) fluid extraction	Lipid yield of 46.74 wt% (a precursor for bioplastic) achieved at 60 °C temperature, 35 MPa pressure, and a flow rate of 4 ml/min	Lower the amount of organic waste used in the extraction process by using CO2 solvent, which can be recycled back into the extraction systems without losing its extractive qualities	[91]
Chlorococcum sp.	Thermochemical pre-treatment of algal biomass with 2% HCl or 1% H_2SO_4. Pre-treated microalgal hydrolysate used as a feedstock for PHB producing bacteria *Halomonas halophila*	PHB production upto 27% of dry cell weight with 0.27 ± 0.05 g PHB/ g DW	Maximal sugar solubilization after acid pre-treatment resulted in high PHB production, However, large-scale acid handling is expensive and labor-intensive	[93]
Chlorogloea fritschii TISTR 8527	Solvent extraction using hypochlorite-chloroform mix	PHB extraction with 95.51 ± 3.16% of the recovery along with pigments removal from microalgae	Solvents make it easier to recover PHA with higher purity but also, harm the environment and make large-scale industrial application difficult due to high cost	[94]
Chlorogloea fritschii TISTR 8527	Solvent extraction using green solvent dimethyl carbonate (DMC) with prior pretreatment with chilled methanol for pigment removal	Recoverable PHB with a yield of 83.34 ± 0.76% and 75% purity	Reusable due to low evaporative loss; environmentally friendly due to low vapor pressure preventing the release of any harmful vapors in the environment	[94]
Mixed microbial culture	Surfactant: Pre-treatment with sodium hypochlorite followed by treatment with NH_4-laurate	Recoverable PHB with 74 ± 8% yield and 100 ± 5% purity	Presence of large-scale detergents necessitates further final effluent purification treatment	[95]
Synechocystis salina	A combination of physical and chemical method; cell disruption by bead mill followed by pigment removal by solvent extraction	PHB: 988 kg mol-1	PHB production along with pigment removal and use of residual algal biomass reduces the process's environmental impact	[96]
Cupriavidus necator	Biological method: Feeding freeze-dried *C. necator* cells containing PHA to mealworm (*Tenebrio molitor*)	Excretion of PHA granules in the mealworm feces. Further purification using detergent and heat treatment produces 100% pure PHA granules without any reduction in molecular weight	A sustainable approach that ensures no harm to the environment	[97]
Bacillus flexus	A combination of biological and chemical method; *Bacillus flexus* cell lysis by using *Microbiospora* sp. culture filtrate containing protease for enzymatic hydrolysis followed by lysate incubation in aqueous two-phase extraction (ATPE) system [polyethylene glycol (12%, w/v) and potassium phosphate (9.7%, pH 8.0)] at 28 ºC for 30 min	PHA yield of 49.3 ± 2.50% of cell's dry biomass and 95% purity	Salts like phosphatases and sulfatases used in ATPE-salt system could disturb the ecosystem by increasing the concentration in effluent streams	[98]

pH plays a significant role in the production of PHA, as mentioned by Montiel-Jarillo [108], who reported that a wider pH range during the accumulation stage of activated sludge fed acetate led to increased polymer content in the biomass, whereas alkaline pH values led to decreased PHA levels. Monshupanee et al. [109] discovered that maximizing PHB accumulation during production may be accomplished by adjusting the acetate supply, light and nutritional conditions, and heterotrophy in the dark. The composition of the feedstock also contributes to the overall production of PHA. In 2016, Cui et al. examined how acetate, glucose, and starch affected three halophilic microbial mixed cultures (MMCs) in the enrichment phase. PHA production from the starch-enriched microbial population was 27.3%, while reactors fed glucose and sodium acetate generated 64.7% and 60.5% cell dry weight (CDW) of PHA, respectively [110]. The type and structure of the polymers produced are influenced by the characteristics of the raw materials. studied how different substrate ratios of acetate and propionate mixtures affected the concentration and components of PHA in a mixed microbial culture (Fig. 3). Increasing the 3HV % in the poly(3-hydroxybutyrate-co-3-hydroxyvalerate) polymer might enhance the flexibility and resistance, thereby expanding its range of uses [111]. The amalgamation of feedstock can influence the prevalence of specific genera during the enrichment phase of MMC operations. Research on the use of several low-cost feedstocks for the production of PHA has led to changes in the dominant bacterial groups in the microbial community [112]. Recently, microalgae have become the preferred biofactories providing biomass as a carbon source, feedstock because of the increased production of carbohydrates, absence of lignin, and economic availability of sugars for fermentation when integrating with bacterial PHA production.

Microalgal PHA production is modulated through the use of additives. Polymers and chemicals can be blended with microalgae such as *Chlorella* or *Spirulina* or both, to produce PHAs with enhanced thermochemical and mechanical properties. These additives include PP, PE, PVA, wheat gluten, acetone, sodium sulfite, etc. Choice of additive depends upon the desired quality of bioplastic produces, for instance, PVA is used to increase strength and flexibility, whereas adding maleic anhydride further improves structural stability. Similarly macromolecular plasticizers can be blended to improve processibility, flexibility, extensibility of the bioplastic. Glycerol, a plasticizer, facilitates the degradation of macromolecules during the PHA production process by improving their availability. Although the microalgae can be easily cultivated, they still need basic nutrients (like nitrogen, phosphorus, carbon), light, oxygen (aeration) and carbon dioxide. Further, it also matters whether the microalgae are grown in open ponds or closed photobioreactors [104].

Comparison with traditional plastic production methods

When comparing bioplastics with conventional plastics, assessment of their environmental influence from

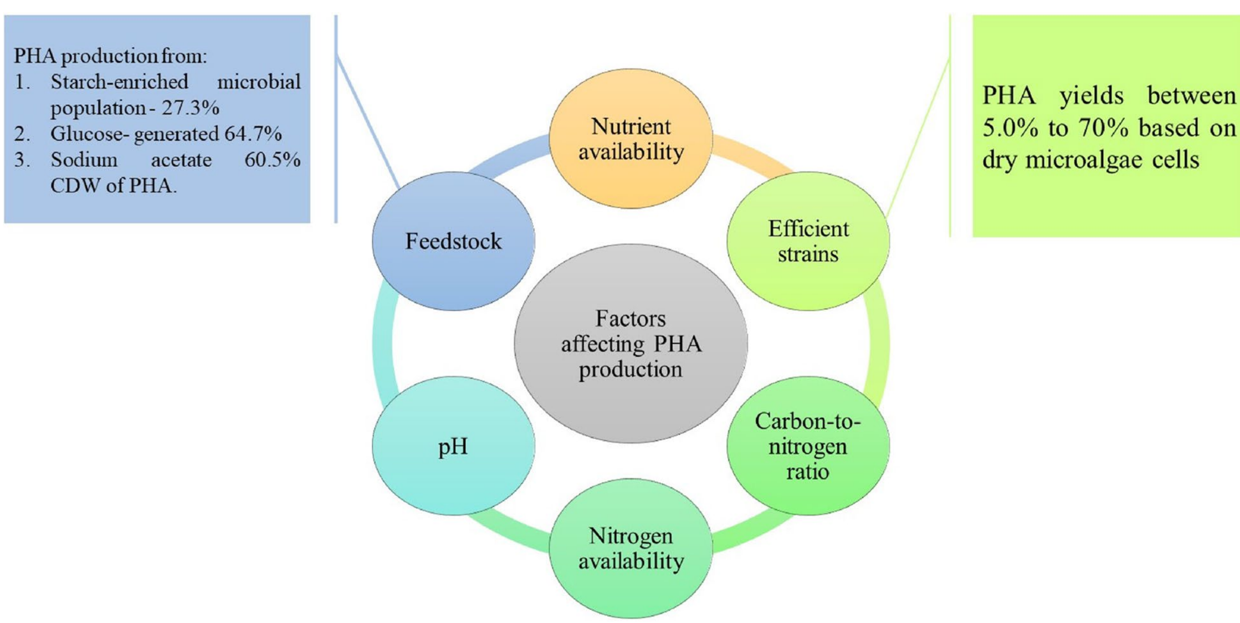


Fig. 3 Factors affecting PHA production and yields

manufacture to discarding becomes vital (Fig. 4). One of the main methods used to evaluate this impact is Life Cycle Assessment (LCA) or cradle-to-grave analysis. Assessing the environmental impacts of a product involves analyzing each stage of its life cycle, initiating with extraction of raw materials to processing, manufacture, distribution, and use [113]. Traditional plastics and bioplastics are manufactured through different processes and using different raw materials, resulting in varying environmental impacts, resource utilization, and sustainability levels. Derived mainly from fossil fuels like crude oil and natural gas, traditional plastics consist of polyethylene, polypropylene, polyvinyl chloride, and other materials. Bioplastics are sourced from renewable materials like plant starch, sugarcane, corn, or another biomass. Some common types are polylactic acid (PLA), PHA, and bio-PE made from sugarcane. Traditional plastics undergo intricate chemical transformations to convert fossil fuels into polymers, typically involving high temperatures and pressures, leading to the release of greenhouse gases. Bioplastic production typically includes fermentation or extraction processes to acquire polymers from natural sources, resulting in a lower environmental influence in relation to energy usage and greenhouse gas discharges [114].

The energy consumption associated with traditional plastics is significant, primarily driven by the refining of crude oil, transportation of raw materials, and energy-intensive chemical processes. Bioplastics strive to reduce energy consumption, particularly when made from agricultural feedstocks that require minimal processing. Traditional plastics have a limited biodegradability, leading to long-term environmental pollution. Recycling rates differ, with a notable amount ultimately going to landfills or becoming litter, which adds to environmental pollution. Bioplastics serve as environmentally friendly alternatives to conventional plastics, known for their lower carbon footprint and lack of toxic components. The environmental benefits include decreased greenhouse gas emissions, quick biodegradability in a matter of months, and reliance on renewable resources like plants and bacteria [115]. PLA-based biopolymers exhibit a biodegradability rate of 84%, and decompose within 60 days, whereas PHB-based counterparts break down in just over three months. When evaluating biodegradation potential, it is important to track oxygen consumption, carbon dioxide production, and analyze gases released during decomposition in different environments [116]. Bioplastics exhibit a notable energy efficiency edge compared to traditional plastics because of their reduced production energy demands. By opting for PLA instead of PET, energy consumption can be reduced by approximately 40%, and using Master-Bi bioplastics instead of PE can result in a 27% decrease [117]. Developing bioplastics with biodegradability or composability is gaining traction as a promising option to reduce environmental harm when they reach the end of their life cycle. There are different types of bioplastics with various end-of-life options, including recycling or composting, that could enhance waste management sustainability. Bioplastics generally offer environmental advantages over traditional plastics, especially when derived from sustainable and renewable sources [118]. Nevertheless, issues like competing with food production for feedstocks and high industrial-scale production expenses must be tackled for wider acceptance. The traditional and bioplastic

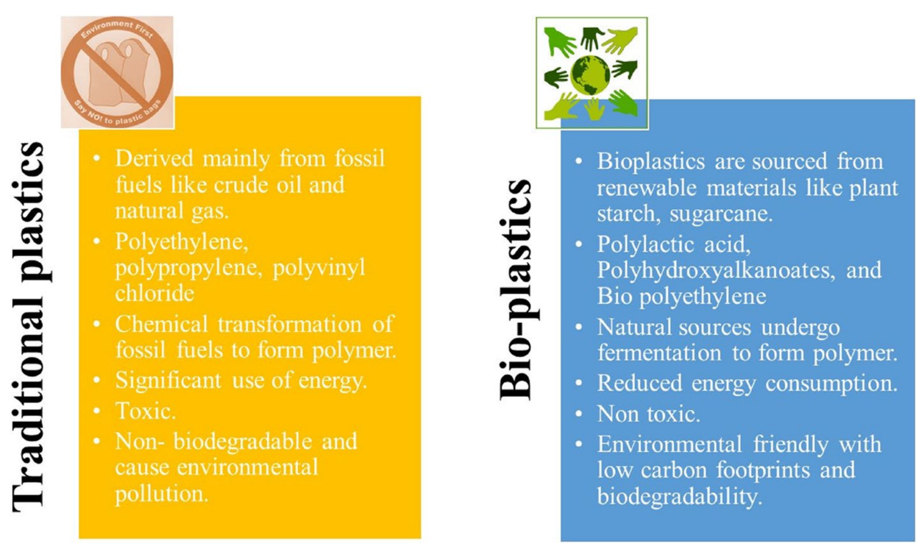


Fig. 4 Comparison of Traditional Plastics with Bioplastics

industries are both advancing, with continuous research and technological progress being key to enhancing the sustainability of plastic materials.

Microalgae as a sustainable feedstock

Advantages of using microalgae in PHA production

The accessibility of cellulose in microalgae, coupled with their rapid growth, makes them attractive as a feedstock for various processes, including the synthesis of PHAs. Microalgae show great potential as a sustainable source for producing bioplastics, with several benefits compared to conventional agricultural sources. They are available year-round and may thrive in many water bodies, including sewage water, unlike plant-based bioplastics. Due to their quick growth and shorter harvesting period, these plants are very efficient, with biomass production rates that can be 5–10 times faster than those of food crops [119]. Microalgae farming is non-competitive with food resources, alleviating worries about resource depletion and supporting sustainable development. These adaptable organisms can withstand extreme circumstances like high temperatures and humidity, and effectively absorb carbon dioxide from the atmosphere, helping to decrease greenhouse emissions. Microalgae-derived biopolymers such as starch, PHB, PHA, lipids, and proteins have exceptional biodegradability, rendering them appropriate for many commercial uses including food packaging and agriculture. Additionally, the significant protein and lipid levels found in microalgae make them highly beneficial for producing biopolymers such as PHB, which helps in promoting a more eco-friendly and enduring future [120]. Microalgae can be utilized either as biomass directly or indirectly by extracting starch and PHBs from their cells [121].

Species of microalgae suitable for bioplastic synthesis

There are approximately one million species of algae, categorized as macroalgae and microalgae. Microalgae, small organisms, use solar energy for making ATP and survive in both freshwater and marine conditions and their products are usually known to contain carbohydrates (5%–23%), lipids (7%–23%), and proteins (6%–52%). *Chlorella*, a spherical single-celled green microalgae, present in freshwater sources, and containing approximately 58% protein, is commonly utilized for commercial purposes in open ponds [104]. Along with rapid proliferation rate *Chlorella* also has high level of saturated and unsaturated C18 fatty acids, which are identical to those in vegetable oils. This substance is commonly used as a dietary supplement and shows promise as a potential alternative to oil. The material demonstrates increased resistance to breakage as it has compact cell wall with excellent thermal endurance. This type is frequently employed in biomass-polymer combinations. *Chlorella vulgaris* is known for its production of high-quality bioplastic. Starch, a biopolymer derived from microalgae biomass of *C. sorokiniana*, is extensively used in the bioplastic, chemical, and culinary, industries because it has higher gelatinization temperature [122]. *Arthrospira* (*Spirulina*) is a type of filamentous cyanobacteria commonly found in salt lakes and freshwaters. *Arthrospira* is recognized for its high protein and vitamin content and can be mass-produced, making it ideal for open pond cultivation. Despite having less lipid, *Arthrospira* biomass can accumulate significant quantities of carbohydrates when exposed to dietary stress, making it a useful feedstock for the production of bacterial PHA [123].

Chlorella fritschii and *Phaeodactylum tricornutum* have the potential to generate PHB levels between 10.6% and 17% of their dry weight. *Calothrix scytonemicola*, *Neochloris oleobundans,* and *Scenedesmus almeriensis* have been identified as suitable candidates for biopolymer production due to their high starch or PHA content [104]. Another microalgae that are commonly present in both freshwater and brackish water habitats is *Nannochloropsis*. It has been used in biopharmaceutical applications for bioactive chemicals and has garnered interest in aquaculture feed and biodiesel generation because to its rapid growth, more lipid content, and ability to withstand various irradiation settings [124]. *Phaeodactylum tricornutum* is a remarkable marine diatom known for its unique characteristics, including the capability to thrive without silicon. This product includes beneficial components such as fucoxanthin, eicosapentaenoic acid, and chrysolaminarin. *Phaeodactylum tricornutum* is regarded as a typical species of non-green algae because of its biomass composition, which is very suitable for many uses [125]. Overall, microalgae are excellent choices for biomass production because of their distinctive characteristics and diversity, which might be valuable in various applications, such as PHA synthesis (Table 3).

Microalgae as a feedstock for microbial fermentation to produce PHA

More than 300 microbial species including bacteria, archaea, microalgae and cyanobacteria have been discovered that produce PHAs as intracellular granular aggregates. Common bacterial species engaged in PHA fermentation include novel and recombinant strains of *Bacillus*, *Cupriavidus necator*, *Escherichia coli*, and *Pseudomonas*. Since the production costs of producing PHAs from bacterial fermentation costs 2–5 times the production costs of conventional plastics, the potential of photoautotrophic microalgae as bio-factories of carbon rich feedstocks have recently been explored to serve

Table 3 Biomass yield, PHA content, and growth conditions for various microalgal strains

Microalgae Strain	Biomass Yield	PHA Content	Optimal Growth Conditions	References
Chlorella vulgaris	High	Moderate-High ~30% from 0.94 mg/L biomass	Temperature: 20–30 °C, pH: 6.5–7.5, Light: Moderate, Nitrogen: Sufficient	[91]
Spirulina platensis	High	Low-Moderate 12 – 30% w/w dry biomass	Temperature: 35–37 °C, pH: 9–11, Light: High, Nitrogen: Limitation	[87]
Scenedesmus sp.	Moderate	Moderate 0.8–30%, w/w dry biomass	Temperature: 20–25 °C, pH: 6.5–7.5, Light: Moderate, Nitrogen: Limitation	[65]
Haematococcus pluvialis	Low-Moderate	Low-Moderate 0.39 ± 0.42 mg/mL	Temperature: 20–25 °C, pH: 6.5–7.5, Light: Moderate, Nitrogen: Limitation	[73, 126]
Nostoc muscorum	High	High 20% DW, 40–60% of cell DW, 110 mg/L/d	Temperature: 20–25 °C, pH: 7.5–8, Light: Moderate, Nitrogen & Phosphorus: Limitation	[127]
Calothrix sp.	High	High 25% DW	Temperature: 18–20 °C, pH: 3.5–6.5, Light: Moderate, Nitrogen & Phosphorus: Limitation	[123, 127]

as biomass sources in PHA bioreactors. Microalgae provide carbohydrate rich and lignin free feedstocks, facilitating economic recovery of fermentable sugars. Afreen et al. [127] proposed a hybrid system consisting of 'two modules' – photoautotrophic microalgae/cyanobacteria and heterotrophic bacteria cultured with different types of regimes to develop the most efficient method of PHA production. The short chain length copolymer PHA poly(3-hydroxybutyrate-co-3-hyroxyvalerate) or P(3HB-co-3HV, is the most commonly produced bacterial PHA using microalgal biomass. Its properties like flexibility, 148–168 °C melting temperatures and − 5.5 to − 2.2 °C transition temperatures, make it an economically profitable ideal candidate to substitute conventional plastics. Recent advances have also utilised wastes from algal biodiesel industries rich in glycerol as carbon sources for bacterial PHA generation [126]. Table 4 enlists various microalgae that are being utilised as biomass feedstocks to produce carbohydrates for bacterial fermentation culminating in production of PHAs.

Direct bioplastic synthesis using microalgae

Recent research has investigated several techniques for manufacturing bioplastics from microalgae, such as hot moulding, compression, melt mixing, injection moulding, and solvent casting [104]. *Chlorella* and *Spirulina* are extensively researched microalgae because of their diminutive cell sizes, which enhance matrix dispersion when combined with polyolefins. An 80% polyethylene mixing ratio results in equivalent modulus readings and more tan delta observations for both *Spirulina* and *Chlorella*. *Spirulina* has improved blending attributes at 50% and 65% polyethylene ratios due to the hydrophobic, nonpolar amino acids present and the enhanced contact between polyethylene and *Spirulina*. *Chlorella* has improved performance at polyethylene concentrations of 20%, 35%, or 80%, behaving more like a filler than a distinct phase [12].

Spirulina has excellent mixing properties because of its unique physicochemical characteristics. Research indicates that *Spirulina platensis* boasts a notable protein content and is well-suited for creating bioplastic blends. When *Chlorella* and *Spirulina* are combined with PE, their unique amino acid compositions result in distinct behaviors and bioplastic capabilities. By incorporating compatibilizers, the properties of *Chlorella*-based bioplastics can be improved. Studies in this field primarily focus on the production of bioplastics using *Chlorella*

Table 4 List of microalgae and bacterial systems for PHA production

Microalgae as biomass➔	Carbohydrate produced ➔	Bacteria utilizing carbohydrate➔	PHA produced
Algal biodiesel waste, *Chlorella* (pretreated), *Corallina mediterranea* *Desmodesums,* *Gelidium amansii,* *Jatropha* biodiesel waste, *Laminaria japonica,* *Sargassum* sp., *Scenedesmus,* *Ulva*	Fermentable reducing sugars Glycerol (biodiesel waste) Monosaccharides—arabinose, glucose, galactose, mannose, rhamnose, xylose Starch Sucrose	*Alcaligenes latus* *Azeobacter vinelandii* *Bacillus megatarium* *Cupriavidus necator* *Haloferax mediterranei* *Halomonas* sp. *Paracoccus* sp. *Saccharophagus degradans* *Hydrogenophaga pseudoflava* *Pseudomonas* sp.	PHB P3HB P(3HB-co-3HV)

and *Spirulina*, with some studies exploring other types of microalgae [128].

Current trends in PHA bioplastic applications

PHAs represent a fascinating category of biodegradable polymers and show alike thermoplastic characteristic as petrochemical plastics. They exhibit a varied range of characteristics making them suitable for various applications as depicted in Fig. 5.

PHA in food Packaging application

PHAs are increasingly being recognized for their potential in the packaging industry, particularly due to their biodegradability and excellent barrier properties. These materials find application in a range of packaging types, including bags, films, and containers. Glass, metal, paper, polymers, and wood are examples of traditional packaging materials that are commonly divided into primary, secondary, and tertiary categories [129]. Primary packaging comes into direct contact with the material itself whereas, secondary and tertiary packaging assist in the transportation and handling of these goods. The transition from conventional plastic materials, such as polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET), to bioplastics like PHAs is seen as a promising solution to address environmental concerns and reduce reliance on fossil fuels [130]. However, PHAs only constituted 1.4% of the global bioplastics production in 2018, largely due to high production costs [131].

PHAs are good water barriers because of their hydrophobic qualities, which are vital for applications involving food packing [132]. Within the PHA family, short-chain-length variants like PHBs are notable and stands out due to their plasticity, ease of processing, and compatibility with common manufacturing techniques such as injection molding and thermoforming. Their biodegradability and the ability to sartor their mechanical properties make them suitable for use in food packaging [133]. Research has also underlined the potential of PHAs as a sustainable alternative to traditional plastics in food packaging [134]. Enhancements through the incorporation of nanoparticles and composite materials have been explored to further enhance these properties [133]. Additionally, recent advancements in PHA-based packaging include active packaging techniques with antioxidant and antimicrobial functions, and use of high-barrier packaging to extend food shelf-life [135].

Further innovations like use of cross-linking agents in starch/PHA blends have shown improvements in thermal stability, elasticity, and compatibility, enhancing the functional properties of PHA films [136]. Despite these advancements, challenges persist such as the economic competitiveness with traditional plastics and aspects of PHA biosynthesis and purification remain significant hurdles [133]. Efforts to improve the cost-effectiveness of PHA production are ongoing, focusing on the use of inexpensive carbon sources and the use of high-yielding strains [137]. While there are still many challenges to overcome, use of PHAs in food packaging has considerable benefits. More research and development is needed to address the current limitations and fully realize the benefits of PHAs as a sustainable and environmentally friendly alternative in the food packaging industry [138].

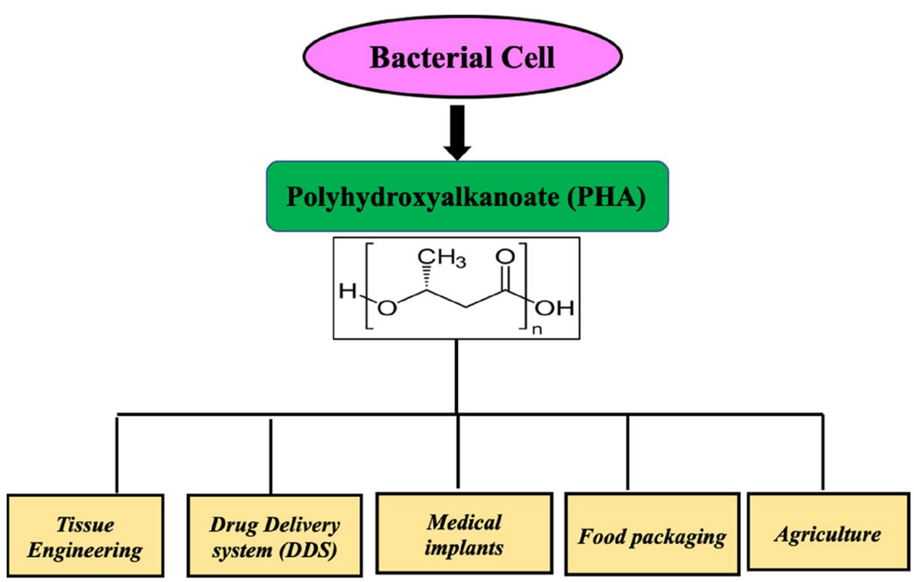


Fig. 5 Applications of polyhydroxyalkanoates

Biomedical applications

PHAs have shown great potential in various medical applications, including drug delivery, tissue engineering, and medical implants [139–141]. Their biocompatibility, biodegradability, and ability to be customized make them suitable for these purposes. In particular, PHA-based scaffolds have been used in tissue engineering for bone graft substitutes, cardiovascular patches, and nerve repair [141], moreover they have the inherent characteristic of biocompatibility achieved via the degradation of molecules naturally existing in the body, like PHBs, that yields R-3-hydroxybutyric acid, a prevalent component in blood. The biodegradable nature of these materials renders them well-suited for making drug carriers and delivery, biocontrol agents and adhesives for dressings [142–145]. Similarly, polylactic acid (PLA) has been used in controlled drug delivery systems and as temporary extracellular matrices in tissue engineering [146].

Tissue engineering

In the domain of tissue engineering, the integration of biopolymers serves as a strategic approach to circumvent immunogenic reactions induced by conventional biogenic materials due to non-specific host response [147, 148]. The compositional diversity and amenability to functional modifications render biopolymers particularly versatile in this domain [149]. Their array of attributes, ranging from biocompatibility, biodegradability, and hydrophilicity, to non-toxicity and porosity, renders them as attractive candidates across numerous biomedical applications. Notably, the interplay of hydrophobicity with biocompatibility and non-toxicity assumes paramount importance in select applications where hydrophobicity is a defining requirement [150, 151].

Among the various PHAs, Poly-4-hydroxybutyrate (P4HB) is extensively studied for tissue engineering applications. The incorporation of 4HB monomer units increase the degradation rate under in vivo conditions, resulting in superior biocompatibility, non-toxicity, non-carcinogenicity, and non-genotoxicity [152, 153]. Moreover, adjustment of the 4HB monomer ratio renders the material to become more elasticized, expanding its utility in healthcare [154].

P(3HB-co-4HB) electrospun fibers, due to their high elasticity and low crystallinity, significantly accelerated wound healing [155]. Patients treated with cell-loaded fibers displayed a 3.5-fold quicker recovery than those treated with control eschar membranes, achieving complete healing in 14 days, compared to 90% and 70% healing achieved after use of cell-free fibers and control meshes, respectively [155]. Additionally, more success has been achieved with use of PHA biocomposites in tissue engineering and repair [156, 157]. Vigneswari et al. [158] used a biocomposite created by integrating P(3HB-co-4HB) nanofibers with collagen peptides. This enhanced adhesion and growth of murine fibroblast and keratinocyte cells, leading to a higher healing rate of 98% compared to 63% with gauze. Sharifulden et al. [156] used P(3HB-co-4HB) copolymer with addition of sol gel derived bioactive glass and graphene particles for tissue regeneration. This biocomposite speed up the healing process and improve color distinction while lowering the response of inflammatory cells, thus suggesting critical use of PHAs in enhancing cell proliferation and tissue regeneration.

PHA finds extensive application in various biomedical contexts in isolation and in combination with other materials, including sutures, dressings, and patches, as evidenced by the PHA produced *Bacillus cereus* MCCB 281 [159, 160]. Furthermore, the versatility of PHAs extends to scaffold production for regenerative medicine, necessitating ultra-high-purity variants to meet stringent biomedical standards [161, 162].

Drug Delivery system (DDS)

Drug delivery systems (DDS) composed of biodegradable polymers, should leverage their capacity for self-assembly into drug-loaded nanocarriers. PHAs have been identified as ideal candidates for producing nanoparticles (NPs) and serving as scaffolds for drug elution due to their flexibility, durability, biocompatibility, and biodegradability [163]. Literature has reviewed PHA-based therapeutic drug delivery nanosystems, highlighting their potential to increase therapeutic benefits of various pharmaceutical drug [164–166]. Integration of Melittin, a powerful anticancer agent into a PHA microsphere carrier produced Mel-PHA-PhaC-nanostructure. The Mel-PHA-PhaC-nanostructure exhibited greater stability against pancreatic enzymes and enhanced inhibition of cancer cells compared to free Melittin [167]. PHA nanoparticles could also enhance image-guided therapy in drug delivery systems. Tanaya et al. [168] examined the application of fluorescent-pigmented PHA nanoparticles in DDS, a safer and more effective alternative to conventional imaging agents such as fluorescent dyes, quantum dots, and metal nanoparticles those suffer from cytotoxicity, carcinogenicity, and reduced photostability. In such cases, PHA nanoparticles could significantly improve image-guided therapy in DDS. However, research is lacking to validate their safety, biocompatibility and clinical effectiveness for use in human trials. Tanaya et al. [169] evaluated the fluorescent pigmented PHB nanoparticles from *Rhodanobacter* sp. strain KT31 for drug delivery and bio-imaging. The PHB NPs demonstrated non-cytotoxicity, significant cell proliferation, and promising potential for biomedical applications. Additionally, PHA nanoparticles were also employed for breast cancer treatment, further highlighting their versatility in DDS [170–172].

The potential of PHA-based nanocomposites in drug delivery systems is notable due to their unique properties and potential applications [171, 173–177]. To produce materials with improved qualities and functions, these nanocomposites mix natural polymers called biopolymers, with nanoparticles or other materials at the nanoscale [174]. Masood et al. [178] showed that folic acid-grafted poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanocomposites resulted in sustained release of chemotherapeutic drug molecules to selectively kill a high proportion of human breast cancer cell lines. Furthermore, PHA microspheres and copolymers have also been proven effective in antibiotic delivery and infection treatment [179–183].

Medical implants

Biobased polymers are often considered superior materials for two types of implantable medical devices: stents and adhesion barriers. In terms of stiffness and elongation at break, many of these biopolymers fall short compared to metallic stents, despite continuous improvements in polymer production and medical device design [184]. But in terms of biocompatibility, biodegradation, and lowering arterial ruptures, they outperform metal stents.

PHAs, being of biological origin, exhibit inherent biocompatibility, making them suitable for medical devices like surgical sutures and dental implants. Their ability to degrade within the body minimizes long-term side effects, rendering them suitable for applications where permanent devices are not ideal. PHAs offer advantages for medical implants due to their biocompatibility, biodegradability, and customizable physicochemical properties, enabling the production of tailored shapes and sizes for particular applications [185–187]. Further, PHB and its copolymers based medical implants present several advantages, including high biocompatibility, strength, and gradual disintegration, making biopolymers suitable for the production of a broad variety of resorbable medical devices [188, 189].

PHA bioplastic in agricultural and horticultural applications

One of the primary applications of PHAs in agriculture is in the production of mulch film used to cover soil surfaces, retain moisture, control of weed growth, moderating and maintaining soil temperature, and structure [190]. Unlike traditional plastic mulches, PHA-based mulching films degrade naturally after use, eliminating the need for manual removal and disposal [191]. Mulch films crafted from Nodax™ P(3HB-co-3HHx) and Mirel™ PHB have been developed. Further, Havens et al. [192] patented a modified fishing gear incorporating a component composed of PHA, designed to biodegrade in aquatic environments. Moreover, PHA bioplastics are utilized in crop protection applications, including agricultural nets, covers, and barriers, PHA-based irrigation systems, such as drip tapes and micro-sprinklers, and production of biodegradable soil amendment products, such as erosion control blankets, sediment barriers, and biodegradable stakes. These goods support sustainable land management techniques in agriculture, lessen soil erosion, and enhance soil structure [193–197].

In horticulture, PHA bioplastics play a crucial role in facilitating sustainable cultivation practices while minimizing environmental footprint. Greenhouse films made from PHAs offer excellent light transmission, thermal insulation, and durability, creating optimal growing conditions for plants while reducing energy consumption [9]. Moreover, PHA-based pots, trays, and plant containers provide viable alternatives to traditional plastic containers, offering biodegradability and eco-friendliness without compromising functionality. These bioplastic containers can be used for seedling propagation, transplanting, and retail packaging, contributing to the overall sustainability of horticultural operations [193]. Additionally, PHA bioplastics are employed in the production of biodegradable mulch films for use in nurseries, landscaping, and ornamental plant cultivation. These mulch films provide weed suppression, moisture retention, and soil protection benefits while degrading naturally over time, leaving behind no harmful residues [197–199].

Innovations in PHA bioplastic research

Innovations in microalgal bioplastic production revolves around various strategies enhancing both the efficiency and sustainability of the process (Fig. 6). One prominent avenue involves genetic engineering or genome editing to optimize microbial strains for increased bioplastic synthesis [104]. Researchers are manipulating genetic pathways involved in lipid and polymer biosynthesis, aiming to boost the production of biopolymer precursors such as PHA within microalgae [200, 201]. Another innovation towards ecologically sustainable bioeconomy is use of machine learning (ML) along with internet of things (IoT) to generate algal bioplastics and cultivate algae on large scale [202].

Additionally, there's a shift towards utilizing waste streams or waste water for microalgal cultivation, thereby mitigating environmental impact and reducing production costs [203]. Furthermore, researchers are investigating novel extraction and purification methods to isolate bioplastic efficiently from microalgae, aiming for scalable and cost-effective processes [125, 204–206]. Overall,

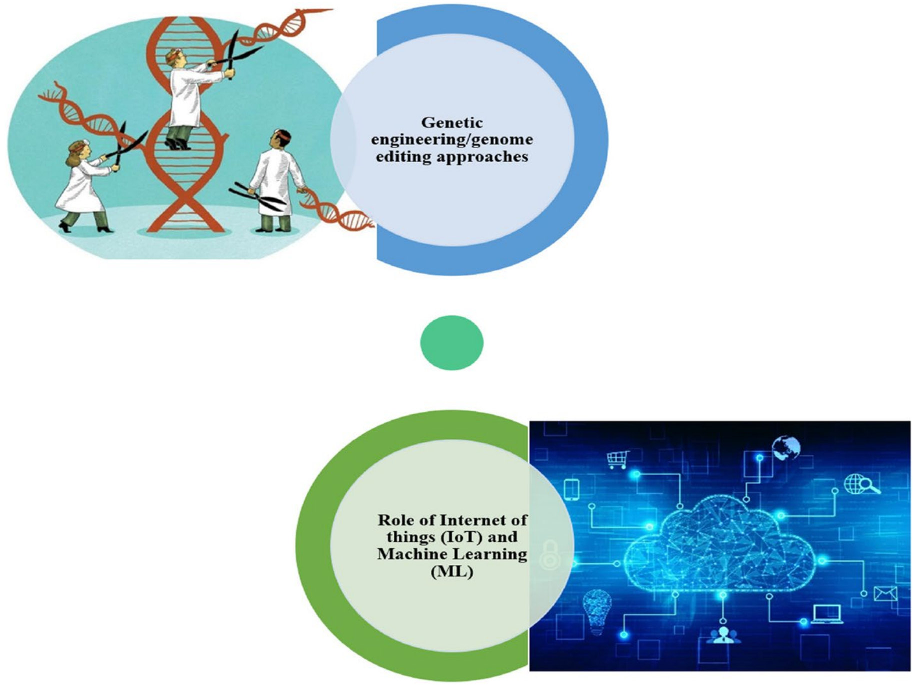


Fig. 6 Innovations in PHA Bioplastic Production

these multidisciplinary efforts aim to establish microalgae as a sustainable and economically viable source for bioplastic production, contributing to the advancement of the circular bioeconomy and reducing reliance on fossil-based plastics.

Genetic engineering approaches and role of Internet of Things (IoT) and machine learning (ML) for enhancing PHA bioplastic production by microalgae

PHAs, made via genetically engineered systems like bacteria, microalgae, and plants, offer a sustainable alternative to petrochemical-derived plastics [207]. With more than 155 unique PHA monomers synthesized by various microorganisms, PHAs exhibit a wide range of modifiable material properties in addition to being environmentally benign [208]. Among PHAs, polyhydroxybutyrates (PHBs) have been extensively studied, and exhibit a high degree of crystallinity because of their linear chain structure and combination of crystalline and amorphous phases. It can be found as a pure polymer or as an ingredient in blends and copolymers [9]. In microorganisms, PHB production involves enzymatic processes catalyzed by three vital enzymes: β-ketothiolase (phaA), acetoacetyl-CoA reductase (phaB), & PHB polymerase (phaC). These enzymes sequentially condense acetyl-CoA molecules to form PHB polymers, which accumulate as amphipathic granules within the cell [209]. Genetic manipulation techniques have enabled the transfer of PHB biosynthesis pathways from bacteria like *Ralstonia eutropha* into microalgae, such as *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* [125, 201]. *Chlamydomonas reinhardtii*, a model organism with a fully sequenced genome, has been extensively studied for its ability to produce PHBs through genetic engineering. Successful incorporation of PHB biosynthesis genes, such as phbB and phbC, from *R. eutropha* inside *C. reinhardtii* has resulted in detectable PHB production [148]. Similarly, *Phaeodactylum tricornutum* has shown promising results with approximately 10.6% PHB achieved after pathway incorporation from *R. eutropha* H16 [125].

Researchers have explored various genetic engineering strategies and microbial species to enhance PHA synthesis. *Synechocystis* sp. PCC 6803 is among the most extensively studied microalga for genetic modification aimed at enhancing its ability to produce PHA. Researchers have thoroughly investigated this strain's growth conditions, metabolic processes, and methods for introducing foreign genetic material. Notably, it was the initial photosynthetic organism to have its entire genome sequenced and analyzed, which included the identification of genes responsible for both PHA production and breakdown enzymes [210]. Table 5 summarizes some key insights from various studies based on *Synechocystis* sp.and other microalgae that showcase genetic engineering methods used and their outcome of PHA production.

Table 5 Genetic engineering-based approaches for making PHA bioplastic

Genetic engineering approach	Microalgae species	Outcome	References
Addition of gene encoding PHA synthase from *R. eutropha*	*Synechocystis* 6803	Two times higher PHA synthase activity in recombinant strain with7 marginal increase in PHB concentration (11 wt% of DCW) over wild type (7 wt% of DCW)	[213]
Gamma-glutamyl phosphate reductase (proA, sll0461) or a putative protein (sll0565) gene disruption	*Synechocystis* 6803 mutants	Significantly higher PHB concentration (7 wt% of DCW) in transposon inserted sll0461 and sll0565 genes mutant strains over wild type strain (7 wt% of DCW) as observed in BG11 medium after 14 days of incubation	[214]
Incorporation of the *R. eutropha* H16 PHB pathway into microalgae	*Phaeodactylum tricornutum*	Increased PHB levels of up to 10.6% of algal dry weight	[125]
Sigma factor sigE overexpression in microalgae	*Synechocystis* sp.	1.4 mg PHA (per 100 mg DCW) recorded in SigE overexpressing strain under nitrogen starved conditions compared to 0.6 mg PHA (per 100 mg DCW) in glucose tolerant strain of *Synechocystis* sp.	[215]
The microalgae were integrated with the genes encoding acetoacetyl-CoA synthase (nphT7ss) from *Streptomyces* sp. CL190, acetoacetyl-CoA reductase (phaABcn) from *C. necator*, and a strong PHA synthase (phaCc5) from *Chromobacterium* sp. USM2	*Synechocystis* sp. PCC 6803	A PHB concentration of 14 wt% of DCW, a maximum production reported in *Synechocystis* sp. (lacking any carbon source) under photoautotrophic conditions	[216]
Overexpression of phaABEC gene cluster	*Synechocystis* sp.	Increased production of PHB in recombinant strain grown in nitrogen-depleted, sugar-free medium, achieving a concentration of 7 wt% of DCW and 12 times higher productivity than the control strain	[217]
Overexpression of phaAB gene operon in nitrogen depleted conditions	*Synechocystis* sp.	PHB content increased 2.6-fold to 26 wt% of DCW in overexpressed phaAB cells after 9 days of incubation, and upon addition of 0.4% (w/v) acetate, PHB production further increased to 35 wt% of DCW	[218]
Metabolic alteration of central carbon of a cyanobacterium to increase the generation of PHA and increase the levels of acetyl-CoA, a key metabolite in PHB (polyhydroxybutyrate) production. Seven genetic altercations made to metabolic pathways, including removing enzyme acetyl-CoA hydrolase (Ach) + adding a gene for a *Bifidobacterium breve* phosphoketolase (XfpK) to boost acetyl-CoA levels. The strain with all three modifications—VfpK overexpression and deletion of Pta and Ach genes—showed remarkably high PHA level	*Synechocystis* sp. PCC6803	Maximum potential for PHA generation, with 232 mg L^{-1} PHA concentrations, around 12% PHA fractions in biomass, and 7.3 mg L^{-1} d^{-1} productivity	[219]
Overexpression of the acetoacetyl-CoA reductase gene	*Synechocystis* sp	The engineered microalgae strain produced an (R)-3HB concentration of 1.84 g L^{-1} after 10 days of cultivation using CO2 and light as the only carbon and energy sources, and a maximum productivity of 263 mg L^{-1} day^{-1}	[220]
Introduction of two genes phaAand phaB from Cupriavidus necator into *Synechocystis* 6803 lacking a regulatory protein PirC. The phaAB genes introduced with control from strong promotor P*psbA2* creating new microalgal strain—PPT1 (ΔpirC-REphaAB)	*Synechocystis* sp. PPT1	Recombinant strain achieved exceptional PHB yield (63 wt% of DCW) produced under nutrient limiting conditions. Further addition of acetate increased PHB content to 81 wt% of DCW	[221]
Introduction of the PHB synthesis mechanism encoding 3 genes: (i) *PhaA* (β-ketothiolase), (ii) *PhaB* (acetoacetyl-CoA reductase), (iii) *PhaC* (PHB synthase) from *Cupriavidus necator* H16, integrated with microalgae *Synechococcus* by conjugation	*Synechococcus elongatus* 2973	Engineered variant produced 420 mg L^{-1} of PHB after 10 days with 2.4-X higher PHB yield than a native PHB producing microalga	[222]

Table 5 (continued)

Genetic engineering approach	Microalgae species	Outcome	References
Genetic modifications in 3 strains of *Synechocystis* sp. PCC 6803: (i) ProC-overexpressing strain (OXP), (ii) adc1 mutant, (iii) OXP strain deficient in adc1 gene (OXP/Δadc1)	*Synechocystis sp.* PCC 6803	The adc1 knockout with proC overexpressing strain (OXP/Δadc1) showed the highest PHB yield (48.9 wt% of DCW) under nutrient stress, with improved glycogen storage and acclimation via altered proline and glutamate levels	[223]
Metabolic pathway engineering in microalgae by heterologously expressing the poly-3-hydroxybutyrate (PHB) pathway from *Ralstonia eutropha*. Environmental conditions were also manipulated to understand how they affect AcCoA flux and integration of the PHB pathway in microalgae strain	*Phaeodactylum tricornutum*	Significant variations in PHB accumulation depending on the environmental conditions. Specifically: Glycerol acted as organic carbon source & facilitated production of PHB and fatty acids	[224]

Genetic engineering techniques have revolutionized PHA production in microorganisms and microalgae, offering sustainable solutions to plastic pollution and resource depletion. Continued research efforts aimed at optimizing biosynthesis pathways and enhancing genetic manipulation tools hold the key to unlocking the full potential of PHAs as eco-friendly alternatives to traditional plastics. Because algae have a simpler genetic makeup than plants and other creatures of complexity, genetic alteration of algae is more straightforward. However, genetic engineering involves screening numerous mutants and isolating desired strains, which is time-consuming and requires specialized equipment and kits. Large-scale cultivation of genetically modified algae faces challenges like varying environmental conditions and contamination risks, as these strains are initially developed in controlled laboratory settings. They are also associated with safety concerns such as potential ecosystem risks, gene migration, and allergic reactions from modified algae [211, 212]. It is evident that despite numerous challenges, genetic engineering offers significant potential for bioplastic production in microorganisms including microalage, thus necessitating rigorous testing under field-like conditions to address real-world challenges.

Furthermore, advancements in genetic engineering technologies, including clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (RNA guided cleaving enzyme) systems, have facilitated targeted gene editing and pathway optimization in microalgae. By using CRISPR-Cas9, researchers can target specific genes responsible for PHA production, such as PHA Synthase, in microorganisms to improve the quality and quantity of PHAs, resulting in the development of high-value bioplastics. Studies demonstrate the potential of CRISPR/Cas9 technology in *C. reinhardtii* for precise gene knockouts and homologous recombination, paving the way for enhanced PHA production [225–229]. Besides *C. reinhardtii,* this gene editing tool has been applied to other microalgal strains like *Nannochloropsis, Phaeodactylum*, and *Thalassiosira*, through methods such as knock-in, knock-out, and knock-down of target genes [230, 231]. However, research on the use of CRISPR-Cas9 for algal genome editing is still in its early phases, and not much progress has been made subsequently.

Role of Internet of Things (IoT) and machine learning (ML)

Multiple studies have explored the part of Internet of Things (IoT) and machine learning (ML) for increased bioplastic making from algae [232, 233]. Specifically, studies have investigated microalgal strain identification, classification, optimizing microalgae cultivation, and developing bioplastics using artificial intelligence (AI), ML algorithms and IoT technologies [234–237]. Integrating ML technologies has shown potential for refining sustainability and competence of bioplastic generation from algae, with applications in strain selection, growth prediction, resource optimization, and decision support systems. Lim et al., [238] demonstrated that ML is highly effective in identifying and classifying microalgae with precision exceeding 90%. This capability enhances bioplastic production by optimizing strain selection based on predictive models of desirable traits. ML also aids in forecasting microalgae growth, enhancing resource allocation, and reducing waste [239]. These algorithms have the potential to improve the quality of the finished product by forecasting its characteristics and functionality. This will increase the products' dependability and acceptance in the market [240]. Many studies have explored this potential: Wong et al. [241] examined the amalgamation of Industry 5.0 along with macroalgae biorefineries, demonstrating specifically potential enhancements in sustainability, cost-effectiveness, and revenue potential. – Otálora et al. [236] showcased the efficacy of AI systems in classifying plastics, contributing to the advancement of microalgae cultivation techniques. These studies collectively emphasize the potential of IoT and ML technologies to revolutionize the bioplastics industry and contribute to a sustainable circular economy.

Although integration of ML and AI into the identification and sorting of microalgae represents significant progress, yet persistent impediments remain. These include challenges such as balancing precision with complexity, substantial capital requirements, and the dependence on methods for preprocessing [202]. Moreover, misclassification may result from overlapping species and inherent variances among microalgal species, and sparse datasets might cause the model to overfit or underfit. In order to drive progress in the development of bioplastics made from algae and machine learning, scientists need to address these obstacles directly and develop novel approaches. This calls for improvements in image capture and processing methods in addition to the creation of more resilient algorithms that can manage big datasets and automatically extract features. In addition, developing adaptability, encouraging data exchange, and improving AI model assessment procedures will be essential to enhance microalgal species identification systems that have widespread and real-world applications. If these challenges are addressed, the manufacturing of algae bioplastics and algal biorefineries using ML and AI has the potential to significantly impact the circular economy and contribute to a more sustainable future.

Environmental impact and sustainability

The concerns over the effects of plastic waste on the environment and greenhouse gas emissions associated with plastic use are also driving the shift to a circular economy. Three guiding concepts underpin the circular economy: slashing waste and pollution, replenishing materials and products, and regenerating the environment. The circular economy can promote social justice, protect the environment, and accelerate economic growth when it is planned meticulously and inclusively. The European Commission developed a strategy on plastic in a circular economy that emphasizes the development of innovative and alternative feedstocks for plastics production [242].

Biodegradability and compostability of PHA bioplastics

Bioplastics, as part of a circular economy, have the potential for more sustainable and profitable life cycles (Fig. 7). Bioplastics are bio-based polymers; the most often utilized form being PHAs [115]. Owing to their biodegradability, high mechanical and tensile strength, and lack of toxins, PHA bioplastics are an excellent alternative to commercial plastics. Microalgae have tremendous potential to be used for making bioplastics due to their quick growth, easy development, and minimal nutrient, and growth requirements [243, 244]. The microalgal- derived bioplastics over the conventional plastics have a low carbon footprint, and have significantly less greenhouse gas (GHG) emission. Additionally, microalgae have no competition with agriculture for freshwater resources or fertile land, and can well be grown in a variety of environments, including wastewater. This makes them an environmentally friendly method of producing bioplastics. The microalgal-derived bioplastics because of their superior strength, pliability, renewability, and durability finds applications in a diverse array of industries, such as food, agriculture, and pharmaceuticals. The environment and the characteristics of biopolymers greatly influence how quickly they break down [245]. The biodegradability of the plastic is primarily dependent upon its chemical composition. There are many mechanisms through which bioplastic undergoes biodegradation such as fouling, hydrolytic, and corrosion [246]. Mixing bioplastics with polymeric substances such as starch and cellulose can improve the mechanical properties and extend the lifespan [115].

Life cycle assessments and environmental footprint

Life-cycle assessment (LCA) is an effective approach for evaluating a process or product's economic and environmental performance. In the case of bioplastic production, LCA helps in the evaluation of the environmental impact of different types of polymers and determines the benefits of using biopolymers before advocating for their widespread use, besides guiding in making decisions processes, and supporting the use of eco-friendly materials [247]. LCA is also an invaluable tool in estimating the profound impact on the product by various environmental factors such as GHG emission, consumption of energy, and other resources [248, 249]. There are four analytical phases of the LCA methodology that are connected by iterative cycles [250]. The phases are comprised of analysis

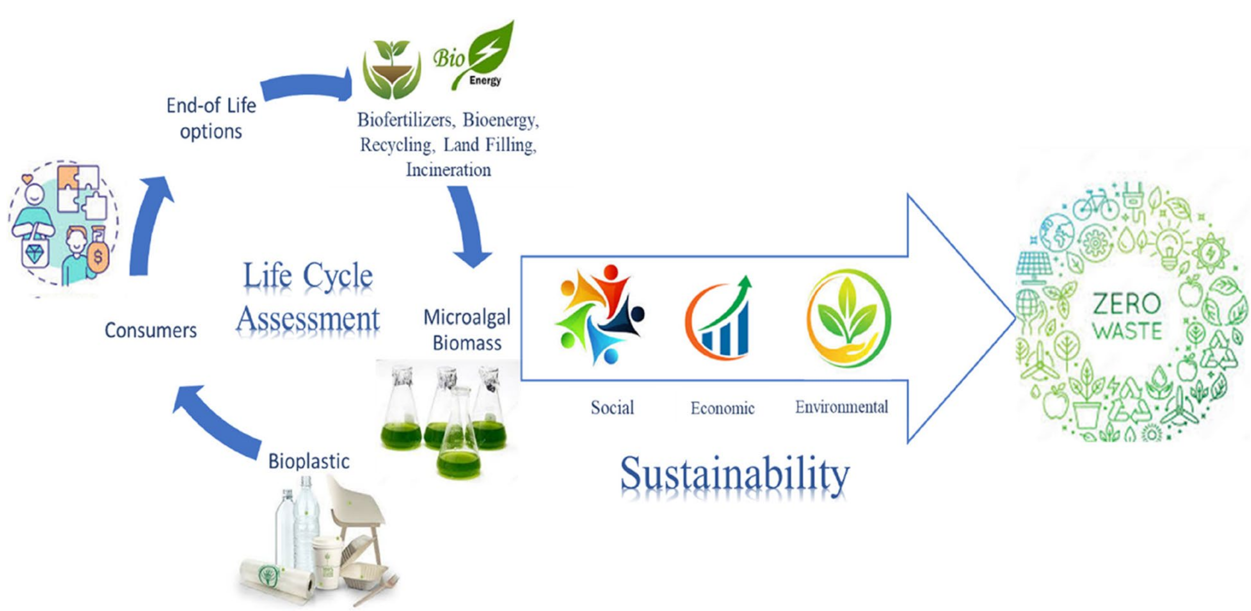


Fig. 7 Bioplastics and the circular economy concept

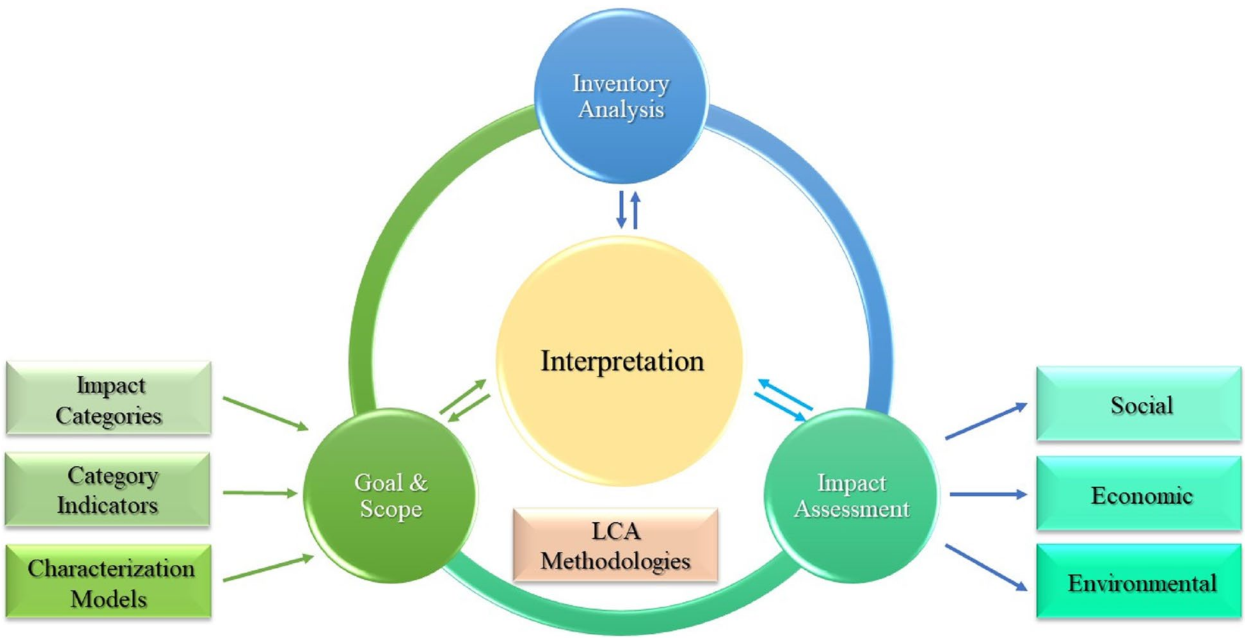


Fig. 8 Life Cycle Assessment (LCA) Methodologies

of the Life cycle inventory, assessment, and interpretation of life cycle impact along with the definition of the assessment's objectives and scope (Fig. 8). The analysis phase complies and quantifies the pertinent inflows and outflows from the processes that help sum up the product's life cycle under study [251]. Every flow that relates to the mass or number of materials, energy, and emission aspects for each life cycle and its related activities is identified, measured, and compiled for the finalization of the Life cycle inventory. The selection of various impact categories as well as the indicators, and characterization models is the first stage of the assessment step. Based on the classification technique, the analysis results are allocated towards different impact categories. After the calculation of the analysis result, based on the assumption of a cause-effect relationship, the characterization model converts the results into common units [252]. Ultimately, the design project is completed through an equitable comparison of competing options that are functionally comparable. LCA software tools have become immensely important in the last few years. There are several software tools currently in use, such as Boustead, GaBi, OpenLCA, SimaPro, and Umberto [253, 254]. For sustainability and LCA evaluation, OpenLCA and SimaPro are used. These programs analyze the results and place them on a map and identify key drivers of the life cycle [255]. For designing, planning, and production GaBi software is used. GaBi software improves the process sustainability by giving users access to both simple as well as extensive LCA [256]. Likewise, Umberto software minimizes carbon emissions and maximizes resource and energy efficiency by evaluating the environmental effects in accordance with ISO 14040 and ISO 14067 [257].

Potential for reducing plastic pollution and greenhouse gas emissions

There are few LCA studies that have been published on microalgal-based bioplastics. Bussa et al. [258] conducted a comparison between PLA production derived from microalgae and plant-derived sources. The results indicated that microalgae can considerably improve the environmental impact in terms of terrestrial ecotoxicity and land use. Beckstrom [259] discovered that cyclic flow photobioreactors had higher impact values than combined systems and open raceway ponds. Medeiros et al. [260] analyzed that microalgal biofuels fared marginally better than fossil fuels in comparison to conventional fuels, moreover, microalgal production systems can significantly reduce GHG emissions [260, 261]. Draaisma et al. [262] state that while the production of food items using microalgae-based manufacturing shows beneficial outcomes with regard to land use, other effect categories—like freshwater demand—show less of an improvement.

The current research focuses primarily on government regulation as a means of combating plastic pollution, but a notable research gap exists in the study and exploration of novel and sustainable materials to build a strong circular economy [263]. Although PHAs have a wide range of applications due to their characteristics, further study is required to maximize extraction and improve purity and yield. The incorporation of Machine learning and the Internet of Things for microalgal-derived bioplastic can go a long way in creating a more sustainable future. Also, combining (ML) with cutting-edge analytical techniques like thermogravimetric analysis (TGA) may improve process efficiency and help create a sustainable circular economy. However, issues including scalability, affordability, and infrastructure development must be resolved if microalgal bioplastics are to reach their full potential [263]. While ML enables predictive modeling and decision-making based on the complied information, IoT precisely controls and monitors the culture condition in real-time which improves energy efficiency, and saves costs [238, 264, 265]. With the help of ML tools, not only the growth of Microalgae can be predicted but also forecasts resource optimization and production. By predicting the ideal input levels for maximum yield and reducing waste and expenses related to excess or shortage of resources, predictive models can further optimize the use of resources [239]. ML algorithms have also significantly advanced the fields of microalgae and bioplastics by identifying, classifying, and cultivating microalgae species [234–236]. Combining ML techniques with LCAs can radically improve environmental decision-making by implementing robust waste management systems [264]. The TGA is a beneficial tool for understanding the pyrolysis of algal biomass and plastics thus advancing the concept of circular economy [265]. For a futuristic approach in microalgal bioplastic production, the focus should drift towards genetic and strain engineering, optimum culture conditions, and the adoption of novel strategies like the mixed integer nonlinear programming model.

Challenges and future directions

Technological hurdles in large-scale PHA production

Bioplastics have become an emerging field of study for scientists worldwide. Microalgae are thought to offer a viable substitute for conventional bioplastic production sources. Numerous conditions and process parameters have been optimized at lab scale to achieve the maximal production of bioplastics from algal sources. Further investigations are also required to achieve its commercial production, which largely focus on factors like environmental friendliness, manufacturing pace, product titre, and cost effectiveness [266]. However, some hurdles need to be resolved in order to shift this technology at large scale in a sustainable manner**.** The limited usage and high production cost in comparison to conventional plastic production, are one of the biggest impediments. Thermal instability, hard and, brittleness and weak water-resistant property of PHA bioplastics constrict their wide application [267, 268].

There are two types of systems: closed bioreactors and open pond systems employed for scaling up of lab-optimized bioplastic production parameters on pilot scale. The production yield of a closed bioreactor is higher than that of an open pond system because it keeps up the sterility, purity, and carried out under controlled conditions. The cost of production and downstreaming makes it impractical for industries to adjust from an economic standpoint [269]. Closed photobioreactors are often used for photosynthetic microorganisms [270, 271]. Open algal ponds, on the other hand, use wastewater as substrate and have cheap operating costs. However, sustaining cultures is a difficult task, particularly when wastewater has a high level of COD. This increases the likelihood of contamination and lowers the production yield [272]. The ability to produce bioplastic is dependent on the strain specific operating conditions and their intrinsic characteristics such as biomass, degradation rate, moisture level, and biopolymer yield [273, 274]. Consequently, in order to develop large-scale bioplastic manufacturing, we cannot rely on a single strain or set of environmental circumstances as not all the species can sustain same conditions for bioplastic production. Rather, a number of trials are required to determine the ideal conditions, which leads to a costly and complex process. Different climatic conditions also one of the barriers in expanding and analyzing its profitable production. The processing of algae harvesting is also an expensive since it requires a lot of energy and water to separate the algae biomass. The blending of polyunsaturated fatty acids and PE bioplastic produces an off-putting smell, which is another barrier to the product's acceptance by consumers and commercialization [275]. Efforts have been carried out to remove this barrier by using the zeolite and activated charcoal during production processes [276]. Without a doubt, genetic engineering works best to produce desired results; yet challenges still need to be addressed, such as genome sequencing, strain maintenance, inability to flourish in pond systems, and potential for random mutations [210]. The release of toxic heavy metal and oxides during extraction process harm the environment [22]. The release of methane and CO_2 upon degradation of bioplastics responsible to increase the greenhouse effect of environment [277].

Economic feasibility and market competitiveness

Globally, the number of industries manufacturing bioplastics is currently smaller than that of petroleum derived plastic. It represents about 0.5 percent of the 400 million tonnes of plastic produced annually. According to the Bioplastic Market (2023), bioplastic production was 2.18 million tonnes in 2023 and is projected to increase to 7.43 million tonnes by 2028. The producing nations are China, Brazil, the US, Italy, and Australia, India and other [278, 279]. Globally, maximal (57%) production comes from Chinese and Asian production units. In addition, the leading producers are Indonesia (27%), South Korea (5%) and the Philippines (4%), and Europe (< 1%) [280]. PHA and biodegradable thermoplastic receiving the majority of attention due to their biological origin and compostable nature. Thus, expected to increase the market size of PHA USD 26.9 million, with a 6.31% CAGR from 2023 to 2028 [281]. However, global utilization rate of bioplastic is not satisfactory might be due to unawareness and acceptance among the customer. In contrast to other bio-based sectors, bioplastics technological development is under preparatory stage not yet ready to hit the market.

Research priorities and emerging opportunities

The demand of environment sustainable bioplastics from algal sources is expected to rise because of having the capacity to mitigate the harmful environmental impacts of traditional plastic production. There are numerous challenges to adopt this technology at industrial-scale manufacturing level and these are, notably the higher production expenses, waste management and market acceptance of this products. Bioplastic production has been limitedly explored on some microalgal species only, new algal species should be found out with respect to their industrial application. Efforts should be progressed on genome sequencing, genetic editing tools and system engineering techniques to develop strains with desired traits, ensuring high polymer productivity and tolerance to wide cultivation conditions relevant to large scale production [282, 283]. To establishing the algal based economy, the utilization of synthetic biology, phenomics, and industry 4.0 should be implemented along with molecular biology in algal manufacturing technology [284]. One of the obstacles impeding the transfer of engineered microbe's technology from the lab to the industrial scale is the lack of means for ensuring stability, degradability and safety when disposing of it. For this reason, emphasis should also be devoted to this area. Focus should also be constrained on mixtrophic cultures which can utilized the extra nutrients present in open system and can be a solution to waste water management. Wider applications and longer shelf-life techniques should be investigated to improve the use of bioplastic and contribute in cost reduction, since its high production costs and unpleasant odor restrict its use [285]. Support from the government, legislators, and business community is essential to the market's acceptance of such products. An additional crucial aspect in boosting the economy is educating students and universities about the Blue Bioeconomy.

Conclusion

To provide a cleaner, healthier, and sustainable environment, the shift from a linear plastic economy to a cyclic bioplastic economy is essential. Bioplastics offer sustainability from biomass feedstock to bioconversion into polymers, biocompatible applications, and eco-friendly biodegradation. However, global industrial applications and investor interest are limited by challenges such as educating end users, adhering to LCA guidelines, and standardizing product labelling. Bioplastics are already used in agriculture, medicine, sports, household products, cosmetics, packaging, electronics, and textiles. Researchers are investigating ways to enhance the durability of these eco-friendly products while maintaining biodegradability, biocompatibility, and economic feasibility. Integrating waste management with bio-product manufacturing and supply-chain management is crucial. Unlike fossil-based synthetic plastics, which harm the environment and human health, green polymers must replace traditional ones to curb increasing plastic production. Addressing industrial challenges, guidelines, labelling, and consumer awareness will help biopolymers become integral to a sustainable circular economy.

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Authors' contributions
All authors contributed to the drafting and revision of the manuscript. R.S. and P.S. led the conceptualization and synthesis of the review, with R.S. primarily responsible for the initial draft writing and formatting, while P.S. coordinated collaborative efforts and contributed extensively to the writing and editing process. M.C., N.G. and P.K. provided substantial input through manuscript writing.

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Competing interests
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