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Review Paper

Third-generation biomass for bioplastics: a comprehensive review of microalgae-driven polyhydroxyalkanoate production

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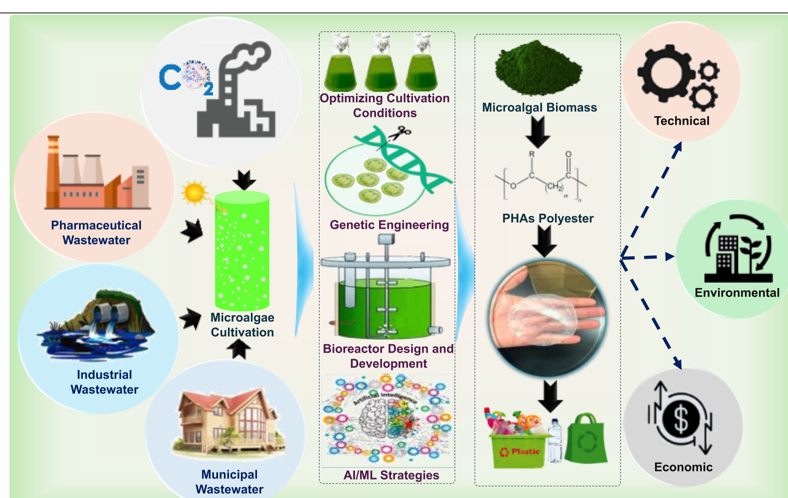
HIGHLIGHTS

- ➢Polyhydroxyalkanoates (PHAs) represent sustainable, valuable, and biodegradable plastics.
- ➢This research suggests aspects of third-generation biomass resources (microalgae) for bioplastic synthesis.
- ➢Integrating wastewater treatment with carbon capture presents a promising approach to PHA production.
- ➢Nutrient regimes, genetic manipulation, and bioreactor design are crucial for advancing PHA production.
- ➢The role of advanced artificial intelligence and machine learning in microalgae PHA production is discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Bio-based plastics, primarily polyhydroxyalkanoates (PHAs), offer a hopeful alternative to petroleum-derived plastics. Third-generation (3G; microalgae/cyanobacteria) biomass has gained significant importance due to its rapid biomass productivity and metabolic versatility. Microalgae can produce PHAs by utilizing CO_2 and wastewater, establishing them as highly promising and eco-friendly systems for bioplastic production. This comprehensive review presents comprehensive insights into microalgae-PHA production, from optimization of physicochemical and cultural conditions to effective PHA purification processes. The critical review also examines the latest advancements in cultivation strategies, metabolic engineering, and bioreactor developments, which may lead to more sustainable and progressive microalgal-based bioplastic accumulation. The effectiveness of algae biomass generation for PHA accumulation through integrated wastewater treatment has been addressed. This review examines the role of mathematical modeling and emerging artificial intelligence in advancing algae-based PHA production processes. Finally, the review concludes with a discussion of the economic and social challenges, life cycle analysis, and prospects for research and development of advanced microalgal-derived bioplastics production and predictions of potential solutions for economically feasible and sustainable microalgae-based PHA production at the industrial scale.

Contents

Abbreviations

3HV	3- hydroxyvalerate	LCA	Life cycle assessment
3HB	3-hydroxybutyryl-coa	ML	Machine learning
3-PGA	3-phosphoglycerate	PGAM	Phosphoglycerate mutase
AI	Artificial intelligence	PHB	Poly(3-hydroxybutyrate)
ANN	Artificial neural networks	PHV	Poly(hydroxyvalerate)
CRISPR	Clustered regularly interspaced short palindromic repeats	PBAT	Polybutylene adipate terephthalate
CRISPR-Cas9	CRISPR-associated protein 9	PBS	Polybutylene succinate
DCW	Dry cell biomass weight	PHA	Polyhydroxyalkanoates
GA	Genetic algorithms	PLA	Polylactic acid

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1. Introduction

Human life upgradation has boosted the dependency on fossil fuel-based plastics over the past few years due to their superior flexibility, convenience, cost-effectiveness, and remarkable thermal properties. Petroleum-based plastic production has surpassed nine billion tonnes, with a compound annual growth rate (CAGR) of about 5.1% (Chia et al., 2020; Baranwal et al., 2022). Of this, only 9% is recycled, while the remainder is discarded in landfills, oceans, and lakes. The accumulation of plastic waste has led to increased carbon footprints, adversely impacting natural ecosystems, global warming, and human health. However, it was projected that plastic waste would double by the year 2030 (Patrício Silva et al., 2021; Ali et al., 2023). Furthermore, micro- or nano-plastics released from petrochemical plastics elude recycling processes, threatening marine ecosystems and potentially entering the food chain. These particles can accumulate in tissues, adversely affecting biological and metabolic processes and posing toxic effects on human health (Shen et al., 2020).

While an outright ban on plastics is not a feasible solution due to their essential role in various sectors, including food packaging, healthcare, and energy (Saratale et al., 2021), this circumstance has stimulated research and development efforts to create sustainable alternatives to fossil fuel-based plastics from renewable sources. These alternatives aim to mitigate and remediate the adverse effects of petrochemical plastics and their products. Moreover, they hold the capability to counteract the enduring negative effects of plastic waste on ecological systems. Within this context, bioplastics derived from biological or other renewable sources are regarded as an excellent substitute for fossil-based plastics, as they possess comparable physicochemical properties (Noreen et al., 2016).

Bioplastics are typically categorized into three main classes: i) biodegradable and bio-based (e.g., cellulose, polylactic acid (PLA), starch, polyhydroxyalkanoates (PHA), polybutylene succinate (PBS)); ii) bio-based and non-biodegradable (e.g., polyethylene (PE), polyethylene terephthalate (PET), polytrimethylene terephthalate (PTT)); and iii) petroleum-based and biodegradable (e.g., polybutylene adipate terephthalate (PBAT), polycaprolactone (PCL)). The advantages and disadvantages of bioplastics compared to petroleum-derived plastics are illustrated in **Figure 1**. This rise in bioplastics is primarily driven by increased production of bio-based PHAs, PBATs, and PBS, where PHAs are produced by bacteria, and PBAT and PBS are synthesized chemically using biologically derived monomers (Ali et al., 2023).

In this context, photosynthetic microalgae serve as a reliable alternative to overcome these bottlenecks due to their rapid biomass production and substantial metabolic flexibility. Worldwide, various companies, including Algenol Biotech LLC (USA), TerraVia Inc. (USA), Veramaris (Netherlands), Sophie's Bionutrients (Singapore), Greentech Saint-Beauzire (France), and Alver World SA (Switzerland), produce biofuels (biodiesel, bioethanol, and jet fuel) and value-added compounds necessary for cosmetic, pharmaceutical, and nutraceutical fields on a commercial scale. Companies like Sapphire Energy Inc. (USA), Algatech (Israel), and Algae. Tec (Australia) have also showcased promising industrial-scale solutions for applications in food, feed, and agriculture (Banu et al., 2020).

Microalgae can autotrophically produce PHAs by actively consuming inorganic carbon sources (CO_2 or HCO^-) and sunlight without requiring aeration, thus offering the dual benefits of bioplastic production and carbon sequestration. This process aids in lowering costs and mitigating the effects of global warming (Devadas et al., 2021; Prasad et al., 2021). Furthermore, algal biomass production is feasible on non-arable land and can utilize wastewater for growth, pollutant removal, and high-value compound synthesis in the form of PHAs. In contrast, heterotrophic and microalgae-based PHA production has not yet been thoroughly investigated. Although PHA accumulation in microalgae biomass with dry cell weight is generally low, enhancing PHA yield and reducing production costs remain crucial objectives (Kamravamanesh et al., 2019; Arias et al., 2020). **Table 1** highlights the diverse perspectives and recent innovations in microalgal-based PHA production as discussed in this review compared to the recent review articles (2019–2024).

To the best of the authors' knowledge, this review article succinctly summarizes recent advances and sustainable efforts in microalgae-based PHA production, extraction, and purification. It addresses various strategies, including cultivation conditions, genetic engineering, bioreactor design, modeling approaches, and integrated wastewater biorefinery methods to enhance PHA accumulation in microalgal biomass. The review also provides insightful information on the application of advanced artificial intelligence (AI) methods for the sustainable production of microalgae-

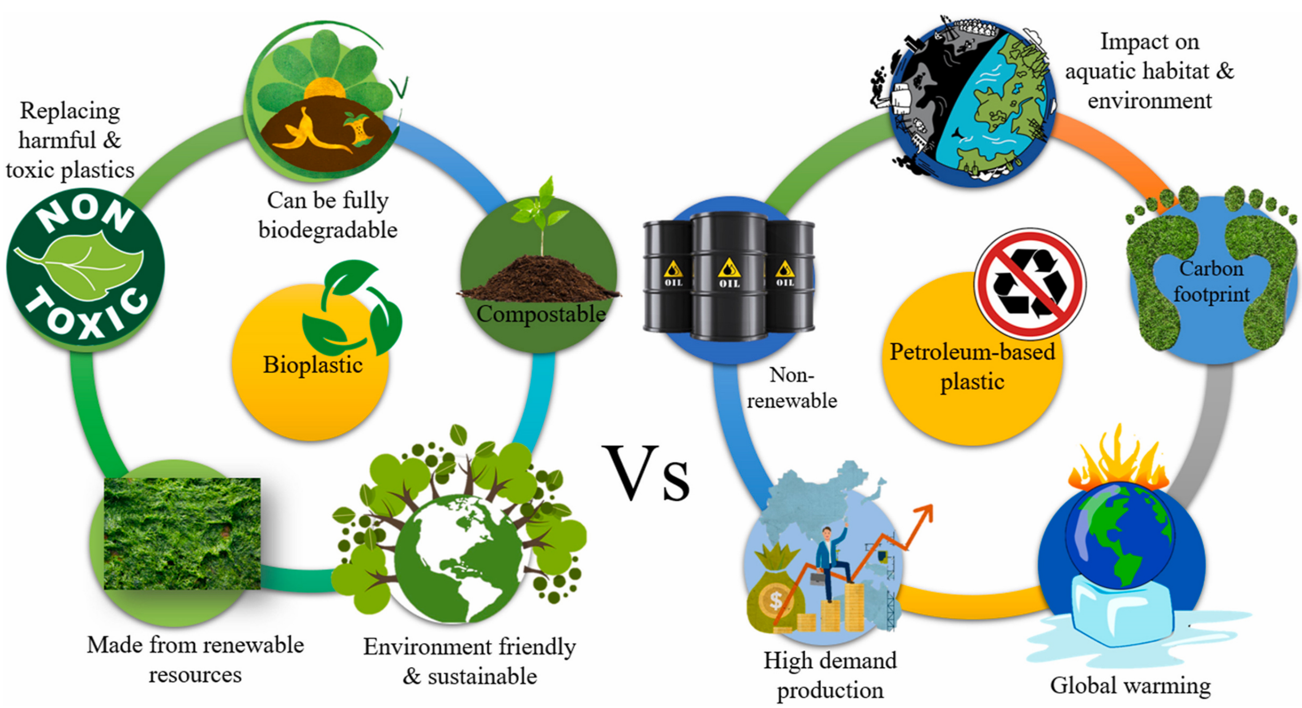

Fig. 1. Comparative assessment of advantages and disadvantages between bioplastics and petroleum-derived plastics (Reproduced from Roy Chong et al. (2022) © Elsevier with permission. License Number: 5832561470192).

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Table 1.
Comparison of the proposed review article with previously published review papers (2019–2024) on microalgae-based PHA production.

Microalgae PHA Producers	PHA Physicochemical Properties	PHA Synthesis Mechanisms	Biotic and Abiotic Factors Influencing PHA Production	Synthetic Biology and Genetic Engineering Approaches to Enhance PHA Production	PHA Production Integrated with Wastewater Treatment	Bioreactor Developments to Enhance PHA Production	AI and ML Technologies to Develop Algae-PHA Production	Techno-economic Challenges and Future Perspectives	Reference
✓*	✓	×	×	✓	×	×	×	✓	Diankristanti et al. (2024)
✓	✓	×	×	×	×	×	✓	✓	Bin Abu Sofian et al. (2024)
✓	✓	✓	✓	×	×	×	×	✓	Ray et al. (2023)
✓	×	×	×	×	✓	×	✓	✓	Oruganti et al. (2023)
✓	×	✓	×	×	×	×	×	✓	Cheah et al. (2023)
✓	×	×	×	✓	✓	×	×	✓	López-Pacheco et al. (2022)
✓	✓	✓	✓	×	✓	×	×	✓	Mastropetros et al. (2022)
✓	✓	✓	✓	✓	×	×	×	✓	Mal et al. (2022)
✓	✓	✓	✓	✓	×	✓	×	✓	Yashvanth et al. (2021)
✓	✓	×	×	✓	×	×	×	✓	Madadi et al. (2021)
✓	×	✓	×	✓	×	✓	×	✓	Sirohi et al. (2021)
✓	✓	✓	×	✓	✓	×	×	✓	Afreen et al. (2021)
✓	✓	×	×	✓	×	×	×	✓	Chia et al. (2020)
✓	✓	✓	✓	✓	×	✓	×	✓	Price et al. (2020)
✓	✓	✓	✓	✓	×	×	×	✓	Costa et al. (2019)
✓	✓	✓	✓	✓	✓	✓	✓	✓	**Present Review**

* ✓: Included, ×: Not included.

based PHAs. Finally, it highlights the techno-economic constraints, LCA analysis, and recommendations for future developments in biopolymer production using microalgae, offering a novel direction for scientists involved in microalgae-based PHA biorefinery processes.

2. Polyhydroxyalkanoate physicochemical properties and advantages

PHAs serve as carbon storage granules and act as terminal electron acceptors for bacteria under stress conditions. They are typically composed of linear repeating units of (R)-3-hydroxy fatty acid units linked by ester bonds (Fig. 2) and classified into short chains (C3-C5) and medium chains (C6-C14) based on their carbon chain length (Zheng et al., 2020; Saratale et al., 2020). In comparison to petroleum-based plastics, poly(3-hydroxybutyrate) (PHB) (e.g., scl-PHAs) and poly(hydroxyvalerate) (PHV) exhibit comparable physical properties that make them suitable for various applications.

PHA-based plastics exhibit key characteristics such as low permeability to water and gases (CO_2 and O_2), making them advantageous for food packaging (Table 2). Their biodegradability, flexibility, and biocompatibility are particularly valuable in the medical sector for applications such as soft tissue regeneration through scaffold construction and targeted drug delivery methods (Elmowafy et al., 2019; Hanna et al.,

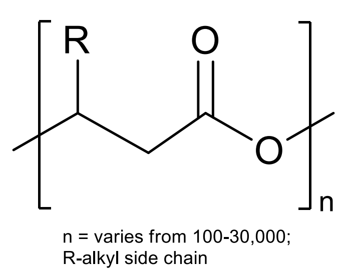

Fig. 2. Chemical structure of polyhydroxyalkanoate.

2023). Widely used in agriculture, PHA-based degradable mulch films help to reduce labor and plastic waste pollution, leading to more sustainable farming practices. Additionally, the capacity of PHAs to degrade under various environmental conditions and produce CO_2 and other nontoxic by-products positions them as ideal for manufacturing biodegradable fishing nets to combat marine plastic pollution (Pandey et al., 2022).

PHAs are versatile and have extensive uses across various industries, such as cosmetics, medicine, packaging materials, disposable goods, and

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Table 2.
Physicochemical properties, production process, application, and pricing of various widely used conventional polymers and biopolymers.

Type of Polymer	Manufacturing Process	Tg (°C)	Tm (°C)	Tensile Strength (MPa)	Tensile Module (MPa)	Elongation Break (%)	Biodegradable Properties	Price ($USD·kg^{-1}$)	Possible Applications
Polypropylene (PP)	Petrochemical	-10	171	33	1800	400	No	0.97-1.2	Packaging, consumer goods, automobiles, electronics
Polyethylene Terephthalate (PET)	Petrochemical/ fermentation + polymerization	73-80	245-265	48-72	2800-4100	30-300	No	1.51-2.12	Packaging; textiles automobile
Low-density polyethylene (LDPE)	Petrochemical/ fermentation + polymerization	-120	98-115	8-20	300-500	100-1000	No	0.95-1.18	Packaging; consumer goods; construction
High-density polyethylene (HDPE)	Petrochemical/ fermentation + polymerization	-120	129	39	600-1500	650	No	0.82-1.01	Consumer goods; construction
Polybutylene adipate terephthalate (PBAT)	Petrochemical	-30	110-115	34-40	--	500-800	Yes	2.5	Packaging; agriculture; transport; coating and adhesives
Polycaprolactone (PCL)	Petrochemical	-60	59-64	4-28	390-470	700-1000	Yes	--	Packaging; biomedical
Polylactic acid (PLA)	Fermentation + polymerization	40-70	130-180	48-53	3500	30-240	Yes	3.5	Packaging; textiles; consumer goods; agriculture; coating and adhesives; construction; electronics
Starch blends	Agro-polymers	--	110-115	35-80	600-850	580-820	Yes	2.4-4.8	Packaging; textiles; consumer goods; agriculture; automobile; coating and adhesives
Cellulose-based	Agro-polymers	--	--	55-120	3000-5000	18-55	Yes	2.5-3.5	Packaging; textiles; consumer goods; construction; biomedical
Polyhydroxybutyrate (PHB)	Fermentation (Photosynthetic microorganisms)	0.9	175	30.2	3.8	4.9	Yes	7.7	Packaging; consumer goods; agriculture; electronics; biomedical
Polyhydroxybutyrate-valerate (P(3HB-3HV)	Fermentation (Heterotrophic microorganisms)	0-30	100-190	25-30	600-1000	7-15	Yes	7.0-20.0	Packaging; consumer goods; agriculture; automobile; electronics; biomedical

containers (Elmowafy et al., 2019; Kostas et al., 2021). Major companies worldwide, including Biomer (Germany), Bio-On (Italy), CJ Bio (South Korea), Danimer Scientific (USA), TianAn Biologic Materials Co. (China), and RWDC Industries Ltd. (Singapore), have demonstrated industrial-scale production of PHAs, ranging from 1000 to 10000 tonnes annually, with significant applications in the agriculture, food, marine, and medical sectors (Mukherjee and Koller, 2022). The global commercial-scale PHA production, along with details about production capacities, properties, and applications, is showcased in **Table 3**. However, the commercial utilization of PHAs faces limitations due to high production costs, the lack of efficient downstream processing for polymer extraction and purification, and stiff competition from conventional fossil fuel-based plastics. Enhancements in substrate types, supply methods, cultivation conditions, advanced AI, and genetic alterations could lead to enhanced characteristics and reduced production costs.

3. Potential of algae for polyhydroxyalkanoate production

Worldwide efforts have focused on increasing bioplastic production using renewable biomass resources characterized by their biodegradability and biocompatibility (Liu et al., 2022). Microalgae/cyanobacteria, distinct groups of photoautotrophic microorganisms, excel at utilizing carbon dioxide for biomass production, require minimal energy for growth, and do not demand aeration for oxygenation. Microalgae are globally recognized for their crucial role in adapting to harsh environments and their biotechnological applications (Saratale et al., 2018). Moreover, industrial microalgae bioprocesses do not compete for arable land needed for agricultural farming and generate a high amount of biomass compared to terrestrial plants (Lutzu et al., 2021; Al Azad et al., 2024).

Microalgae can thrive in various harsh environmental conditions and produce PHAs using sunlight and inorganic carbon sources. Microalgae also exhibit a remarkable ability to remediate and thrive in various types of wastewater, thereby serving as an efficient and versatile candidate for wastewater treatment. This process proves to be more profitable and energy-efficient while also reducing chemical usage and greenhouse gas emissions (GHG) (Salam, 2019; Abdelfattah et al., 2022). The microalgae species, particularly *Chlorella* sp., *Scenedesmus* sp., *Desmodesmus* sp., *Botryococcus* sp., and *Selenastrum* sp., are widely studied in the wastewater treatment process (Abudaqqa et al., 2024).

Garcia et al. (2021) thoroughly investigated various operational and nutrient conditions in freshwater microalgae species, *Scenedesmus*, and their impact on PHA production. They found that PHA accumulation ranged from 0.82% to 29.92% (w/w) of their dry cell biomass weight (DCW). Other studies involving *Chlorella fusca* and *Chlamydomonas reinhardtii* explored the influence of different light and nutrient-limited conditions to boost PHA accumulation (Cassuriaga et al., 2020). Furthermore, microalgae can photosynthetically synthesize PHA and its co-polymers by incorporating various organic carbon sources. **Table 4** summarizes the literature on microalgae capable of producing PHAs. Therefore, algae-derived PHAs offer a promising and sustainable alternative to petroleum-based plastics and can mitigate their negative environmental impacts. However, limitations exist, including 1) lower PHA yield and output compared to heterotrophic bacteria; 2) the need for specific cultivation conditions such as light, temperature, nutrients, and CO_2 supply, which complicate and increase maintenance costs on a large scale; 3) the economic viability of progressing PHA production using microalgae, including cultivation, harvesting, and extraction processes, which renders it less competitive than other bioplastic -producing methods; and 4) the development of photobioreactors as a strategic approach since open systems for microalgae cultivation are susceptible to contamination by undesirable microorganisms, potentially competing with microalgae and reducing PHA production efficiency. The preceding sections of this review have comprehensively discussed current research and the development of the physiological roles

Table 3.
Global manufacturers of PHAs at a commercial scale.

Manufacturer	PHA Trademark	Type of PHA Biopolymer	Strains Used	Wild or Genetically Modified	Production Capacity (Tonne·Yr^{-1})	Applications	Properties
Biomer, Germany	Biomer	PHB*	*Azohydromonas australica*	W	900	Biomedical, Packaging	Biodegradable and compostable
Bio-On, Italy	MINERV-PHA	PHB	*Cupravidus necator*	W	2000	Electronics, packaging, automotive	Biodegradable
CJ Bio, South Korea	Biopol	P(3HB-*co*-4HB)	*Escherichia coli*	GM	5000	Food packaging, cosmetics, mulching bag	Biodegradable
Danimer Scientific, USA	Nodax	P(3HB-co-3HHx)	*Ralstonia eutropha*	GM	10,000	Medical, compostable bags, Paper coatings	Biodegradable and compostable
Shenzhen Ecomann Biotechnology Co., Ltd. China	Ecomann®	P(3HB-*co*-4HB)	ND	GM	75,000	Films, packaging, paper coatings, cosmetics, personal care, bottles	Biodegradable and compostable
PhaBuilder, China	PhaBuilder	P3HB, P34HB, P34HBHV	*Halomonas* sp. TD40	W	1000 to 10,000	Medical, cosmetics, fiber and textiles, degradable materials	Biodegradable
Kaneka, Japan	AONILEX®	P(3HB-*co*-3HHx)	*Cupravidus necator*	GM	5000	Films, composite bags, automobiles, electronics	Biodegradable
Newlight Technologies LLC, USA	Air Carbon	PHB	"Newlight´s 9X biocatalyst"	NA	--	Food packaging	Biodegradable and compostable
TianAn Biologic Materials Co., China	ENMAT™	P(3HB-*co*-3HV)	*Cupravidus necator*	W	2000	Thermoplastics, fiber, and nonwovens, fine chemicals	Biodegradable and compostable
RWDC Industries Ltd, Singapore	Solon™	P(3HB-co-3HHx)	*Cupriavidus necator*	GM	4000	NA	NA
Metabolix USA	Mirel®	P(3HB-*co*-3HV)	*Cupriavidus necator*	W	50,000	Cosmetics, coatings for paper and cardboard, plasticizers	NA
Biocycle Brazil	Biocycle®	P(3HB) P(3HB-co-3HV)	*Cupriavidus necator* *Paraburkholderia sacchari*	W	10,000	Plastic sheets, films and coatings, veterinary applications	Compostable
Tianjin GreenBio China	Sogreen™	P(3HB,4HB)	*Escherichia coli*	GM	10,000	Packaging	NA

* *Abbreviations:* PHB: Polyhydroxybutyrate, PHA: Polyhydroxyalkanoate, PHBHHx: Poly-hydroxybutyrate-hydroxyhexanoate, PHBV: Poly-hydroxybutyrate-hydroxyvalerate; NA- Not available; ND- Not disclosed; W- Wild strain; GM: Genetically modified strain.

of PHAs in microalgae, along with strategies for enhancing PHA accumulation and reduce production costs.

4. Polyhydroxyalkanoate production metabolic pathways in microalgae

The synthesis of PHAs in microalgae comprises numerous metabolic pathways and regulators that have not yet been fully identified. Generally, microalgae/cyanobacteria are capable of producing two key correlated carbon storage molecules, primarily glycogen and PHA in PHB form. The synthesis of PHB commences with the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA, facilitated by the enzyme β-ketothiolase. Subsequently, the reduction of acetoacetyl-CoA to 3-hydroxybutyryl-CoA (3HB) by acetoacetyl-CoA reductase requires one mole of NADPH. The polymerization of 3HB to PHB is then catalyzed by heterodimeric PHA synthases (Costa et al., 2019). Four distinct classes of PHA synthases have been identified, with classes I, III, and IV capable of synthesizing scl monomers, whereas class II synthesizes mcl monomers (Madadi et al., 2021; Tan et al., 2022). PHB biosynthesis is closely associated with glycogen synthesis, with both molecules participating in the metabolic intermediate 3-phosphoglycerate (3-PGA). During CO_2 fixation in microalgae, the 3-PGA produced in the Calvin cycle is typically directed toward glycogen production rather than PHB. It is also hypothesized that the PHA granule is a critical structural element for cell survival under conditions of nutrient stress, osmotic shock, and harsh environments (Sirohi et al., 2021).

One mole of PHB synthesis requires one mole of NADPH, suggesting that PHB is synthesized when high levels of reduction equivalents (NADPH and $FADH_2$) are present in the cultivation conditions. Consequently, PHB granules help to recover the reducing power necessary for cell metabolism without utilizing storage carbon in the form of glycogen (Hauf et al., 2015; Mal et al., 2022). Costa et al. (2018) investigated PHA production using *Spirulina* sp. LEB-18 and *Synechococcus subsalsus.* A nitrogen deficiency in the culture medium leads to an increase in fatty acid composition. These findings indicate that saturated and unsaturated fatty acids initiate the production of PHA monomers. Hu et al. (2008) determined that saturated fatty acids are initially synthesized under nitrogen-depleted conditions, followed by the insertion of double bonds through the enzyme stearoyl-acyl desaturase, forming PHAs. Numerous other studies have also highlighted the synthesis of PHA metabolic pathways *via* the production of fatty acids, though the complete mechanism remains elusive (Costa et al., 2018; Cheah et al., 2023).

Synechococcus elongatus was cultured in artificial seawater nutrient medium-III with 1% glucose under conditions deficient in nitrogen and phosphorus, resulting in an increase in DCW from 7.02% to 17.15% (w/w) in PHA accumulation (Mendhulkar and Shetye, 2017). Additionally, an increase in PHA accumulation was observed in *Botryococcus braunii* when cultured in BG-11 medium under nitrogen-limited conditions (Kavitha et al., 2016). Under nitrogen and phosphorus limitations, microalgae preferentially produce PHAs as an alternative carbon-storage polymer alongside glycogen. However, another study reported that *Synechocystis salina* produced PHA in the range of 5.5 to 6.6% (w/w) DCW in BG-11 medium without nutritional limitations (Kovalcik et al., 2017). It has been suggested that although PHAs are not utilized as a carbon source in chlorosis recovery, they serve as structural elements in nucleotides and stimulate cell proliferation (Koch et al., 2020). The exterior surface of PHB is coated with unique proteins known as phasins, which are closely associated with PHB metabolism. These phasins regulate transcription mechanisms, PHB hydrolysis, and granule formation. Phasin (*PhaP*) in *Synechocystis* sp. PCC 6803 influences cell size and granule quantity by

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controlling PHB synthetase activity (Hauf et al., 2015).

Considering all these observations, it can be concluded that the regulation of PHA synthesis is achievable through gene activation related to PHA synthesis under nutrient-deprived conditions and by promoting PHA synthase activities through definite cellular components or metabolic intermediates. Additionally, inhibiting metabolic pathways that compete with PHA synthesis and enhancing the cultivation conditions with metabolic intermediates can further improve the PHA synthesis mechanism (Costa et al., 2019; Cheah et al., 2023). Precise control over PHB metabolism and its physiological functions is still not well understood. Nevertheless, further advanced research is needed to fully understand the PHA synthesis mechanism and its regulation, aiming to achieve higher yields of PHAs and develop new modified strains.

5. Optimization of cultivation conditions for better algae-based polyhydroxyalkanoate production

Several biophysical factors influence growth, biomass productivity, and microalgal biochemical composition. These include cultivation conditions and ecological factors such as light period and intensity, carbon source, temperature, salinity, pH, and nutrient composition in the culture medium, all of which significantly impact PHA accumulation. Therefore, different cultivation strategies must be carefully regulated to optimize microalgal biomass productivity and PHA accumulation (Chen et al., 2017).

5.1. Robust microalgae strain selection

A variety of potential microalgal strains have been identified, with strain selection playing a critical role in PHA production, directly impacting both product yield and process efficiency. The selection of strains for PHA production largely depends on rapid growth, the ability to thrive in wastewater and CO_2, effective biomass productivity, high yield of target products (PHAs), and tolerance to stress variations in diverse environmental conditions (Banu et al., 2020; Kadri et al., 2023). Over 100 strains of microalgae/cyanobacteria have demonstrated the capacity to produce PHAs photoautotrophically, with yields ranging from 0.5% to 80% (*w/w*) DCW (Mastropetros et al., 2022). The species of *Chlorella, Spirulina* algae have been researched for bioplastics production. Additionally, other algae species such as *Nannocloropsis gaditana, Phaeodactylum tricornutum, Scenedesmus almeriensis, Neochloris oleoabundans*, and *Calothrix scytonemicola* have been extensively explored for bioplastics production.

Furthermore, cyanobacteria *Synechocystis* sp., *Nostoc* sp., and *Oscillatoria* sp. have demonstrated the capability to produce PHA, while *Gloeothece* spp., *Calothrix scytonemicola, Aphanothece* spp., and *Synechococcus* spp. have been extensively investigated for PHA production using CO_2 as a carbon source (Madadi et al., 2021). Microalgae are highly sensitive to various operational parameters such as temperature, pH, salinity, light intensity, and CO_2 concentration, which can directly influence biomass productivity and desired metabolite production. Thus, selecting an effective microalgae strain is crucial for enhancing the output of desired metabolites, their concentration, and physicochemical properties using a non-genetically modified approach. Roja et al. (2019) explored the growth and PHA production capabilities of four *Chlorella sp., Leptolyngbya valderiana, Synechococcus elongatus*, and *Oscillatoria salina* microalgae species under nitrogen and phosphorus-deprived conditions. These studies revealed differences in growth and PHA production between various microalgae species. The thermal properties of PHA produced by all four strains were assessed; notably, *L. valderiana* exhibited significant thermal resistance (Roja et al., 2019). In another study, *N. muscorum* microalgae was examined for P(3HB-co-3HV) copolymer films and PHB homopolymer production. The resulting biopolymer exhibited superior mechanical and thermal stability relative to those produced using bacteria (Bhati and Mallick, 2015). Details of algae species and their PHA accumulation efficiency are presented in **Table 4.** Additionally, microalgae should exhibit resilience to contamination while growing in an open pond system. Presently, polycultures and consortia of microalgae and bacteria are increasingly prevalent for enhancing adaptability, biomass productivity, and desired product yield.

5.2. Influence of inorganic carbon sources

Several microalgae species have demonstrated the ability to grow photoautotrophically using inorganic carbon sources, primarily carbon dioxide (CO_2) and bicarbonates (HCO_3^-). Typically, microalgae utilize CO_2 or HCO_3^- as carbon sources in moderate quantities (below 5% CO_2 or 150 mg·L^{-1} as $NaHCO_3$) for their growth. Microalgae exhibit a CO_2 fixation capability that is significantly higher (10–15 times) than that of terrestrial plants, leading to efficient biomass productivity and substantial PHA accumulation, thus making the process both eco-friendly and cost-effective. PHA accumulation using CO_2 as a carbon source has been documented in *Synechocystis* sp. PCC 6714, achieving 16.4% (w/w) of dry cell weight (DCW) under 2% CO_2, and in *Thermosynechococcus elongatus*, reaching 14.5% (w/w) DCW under 5% CO_2 (Kamravamanesh et al., 2017; Ray et al., 2023). In both studies, elevated CO_2 concentrations were shown to impact PHA production negatively. This reduction is likely due to higher CO_2 concentrations in the culture medium, leading to reduced pH levels, which directly inhibit metabolic activity or cause culture loss due to uncontrolled pH changes.

In a related study, *Synechocystis* sp. was analyzed for PHA production using $NaHCO_3$ across varying levels of dissolved inorganic carbon (DIC). PHA production increased (up to 14% (*w/w*) DCW) when higher DIC levels (2000 mg·L^{-1}) were coupled with overexpressed genes involved in the synthesis of glycogen and PHB (**Fig. 3**) (Rueda et al., 2022). Similarly, this research also showed that the feast-famine fermentation strategy with DIC enhanced the conversion of glycogen to PHB. These findings indicate that maintaining optimal levels of CO_2 and DIC is crucial for effective PHA production (Rueda et al., 2022).

5.3. Influence of organic carbon sources

Several microalgae species can utilize organic carbon (C) sources, including fructose, glucose, and volatile fatty acids (VFAs; butyric acid, acetic acid, valeric acid, and propionic acid), with or without light for growth in heterotrophic cultivation. It is anticipated that adding organic C sources and VFAs would increase acetyl-CoA concentrations, leading to enhanced PHA synthesis. Typically, incorporating large amounts of organic carbon results in elevated PHA accumulation levels. However, this varies depending on the strain, cultivation conditions, and other unspecified factors. The addition of co-substrates may be beneficial for increased PHA copolymer accumulation. *Synechocytis* PCC6803 demonstrated a significant PHB production increase to about 38% (*w/w*) DCW in the presence of acetate and fructose as co-substrates under phosphorus-limited conditions (Panda and Malick, 2007).

The type of carbon source used also influences the quantity and composition of PHAs produced. Polyhydroxybutyrate is the initial PHA polymer; however, its highly brittle and crystalline nature is prominent. Introducing additional monomers, particularly 3- hydroxyvalerate(3HV), enhances PHB crystallinity and thermal properties (Saratale et al., 2021). Adding valerate and propionate to the culture medium containing CO_2 with diazotrophic cyanobacterium *Nostoc muscorum* resulted in increased P(3HB-co-3HV) accumulation (Bhati Malick, 2015). In another study, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) was successfully synthesized by culturing *Synechocystis* sp. PCC 6803 with γ-butyrolactone (Tanweer and Panda, 2020). The potential quantity of each copolymer can be varied by adjusting the concentrations of each organic C source. While adding organic carbon sources is an effective strategy for increasing PHA content, it can significantly raise production costs and elevate the risk of bacterial contamination, particularly when scaling up to unsterile outdoor environments. Thus, the supplementation of organic C in the culture medium should be minimalized and carefully deliberated to achieve efficient productivity. Further research is crucial to determine the optimal balance of organic carbon utilization, cost-efficiency, and contamination risks (Mastropetros et al., 2022).

5.4. Light (quality and quantity)

Light is a vital factor for efficient and productive photosynthesis in microalgae. The type of light (solar or artificial), the method of light delivery, and light intensities are significant factors that directly affect microalgae growth, their biochemical compositions, and the production of

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Table 4.
Summary of strategies for enhancing microalgae-based PHA accumulation through various cultivation methods.

Strain	Culture Conditions (Light, Temperature, pH Stress Conditions)	Operation Time (d)	PHA Content %DCW (*w/w*)	Type of PHA	Extraction Procedure	Reference
Nutrient limitation						
Synechocystis sp. PCC 6803	50 μmol·m^2·s, 28 °C, 7-8, N and P deficient condition	13	17	PHB	Sulfuric acid extraction	Koch et al. (2020)
Oscillatoria okeni TISTR 8549	75 μmol·m^2·s, 32 °C, 7.5, N deficient condition N deficient condition + dark condition N deficient condition + dark condition+ 0.4% acetate	28	14.4 42.8 42.0	P(3HB-CO-3HV)	Solvent extraction	Taepucharoen et al. (2017)
Calothrix scytonemicola TISTR8095	50 μmol photon·m^2·s, 28 °C, 7.5, CO_2 as C source; N deficient condition	30	25.4	PHB	Solvent extraction	Kaewbai-ngam et al. (2016)
Synechococcus elongates	Light:dark (14:10 h), 24 °C, --, 1% sucrose, N deficient condition	15	17.15	PHB	Solvent extraction	Mendhulkar and Shetye (2017)
Synechococcus elongates	Light:dark (14:10 h), 24 °C, --, 1% sucrose, P deficient condition	15	7.02	PHB	Solvent extraction	Mendhulkar and Shetye (2017)
Carbon (inorganic and organic) source						
Coelastrella sp., *Ettlia texensis*	3000 lx, 27 °C, Moderated BG11 medium + Galactose (10 g·L^{-1}) or Sucrose (10 g·L^{-1})	3	15.18 and 13.55 15.08 and 13.46	PHB	Lyophilized biomass + 2 ml of acidic methanol and digested at 100°C for 3.5 h, followed by GC	Samadhiya et al. (2022)
Chlorogloeopsis fritschii PCC 9212	50 μmol·m^2·s, 26 °C, BG-11 medium CO_2 and acetate deficient condition 1% (*v/v*) CO_2 10 mM acetate	18	5.0 15.0	PHB	Solvent extraction	Zhang and Bryant (2015)
Nostoc muscorum	25 °C, 8.0; P deficient condition + 1% (*w/w*) glucose +1% acetate + CO_2	23	21.5	PHB	Sulfuric acid extraction	Haase et al. (2012)
Spirulina sp. LEB. 18	3200 lx Light:dark (12:12 h), 30 °C, 7.0, $NaHCO_3$ 8.4 g·L^{-1}, $NaNO_3$ 0.25 g·L^{-1}	15	44.19	PHB	Solvent extraction	Martins et al. (2014)
Nostoc muscorum	75 μmol·m^2·s, 25 °C, pH 8.1, BG11 medium + 0.2% acetate and 0.4% propionate	16	31.4	P(3HB-co-3 HV)	Solvent extraction	Mallick et al. (2007)
Nostoc muscorum Agardh	Deficiency of Aetate and phosphate and acetate + valerate (each 0.4%)	21	58-60	P(3HB-co-3 HV)	Hot chloroform extraction-precipitation with cold diethyl ether	Bhati and Mallick (2012)
Light intensity and period						
Chlorella sorokiniana SVMIICT8	200 μmol·m^2·s, Light:dark (12:12 h), 25 °C 7.0, Bold's medium + sodium acetate	16	29.5	PHB	Hot chloroform extraction-precipitation with chilled acetone	Kumari et al. (2022)
Stigeoclonium sp. B23	10–30 μmol·m^2·s, 25 °C, pH7, Z8 medium N deficient condition	45	12.16	PHB	Solvent extraction	Mourão et al. (2021)
Chlorella fusca LEB 111	BG-11 medium, 28 °C, N deficient condition (Light:dark (18:06 h), with 28 μmol·m^2·s of light intensity +arabinose Light:dark (18:06 h), with 28 μmol·m^2·s of light intensity +xylose Light:dark (06:18 h), with 9 μmol·m^2·s of light intensity +xylose	10	14.0 16.2 17.4	PHB	Sulfuric acid extraction	Cassuriaga et al. (2018)
Synechococcus subsalsus *Spirulina* sp. LEB-18	41.6 μmol·m^2·s, 28 °C, Light:dark (12:12 h), N deficient condition	15	16 12	PHA	Hot chloroform extraction-precipitation with cold methanol	Costa et al. (2018)
Synechocystis sp. B12	40 μmol·m^2·s, Light:dark (24:0 h), 22 °C, BG-11 medium $^+$ 4 g·L^{-1} acetate	5	31.5	PHB	Sulfuric acid extraction + GC	Gracioso et al. (2021)
Scytonema geitleri	95 μmol·m^2·s, 30 °C, pH 8.5, Light:dark (14:10 h), 30 mM acetate	28	7.12	PHB	Hot chloroform extraction-precipitation with cold methanol	Singh et al. (2019)
Salinity stress						
Scenedesmus sp. UTEX 1589	100 μmol·m^2·s, 20 °C, 8.2, BG-11 medium N and P deficient condition NaCl (0.5–2.0 g·L^{-1}) + Glucose 1-4 g·L^{-1})	14	17.14 to 29.92	PHB	Solvent extraction	García et al. (2021)
Nostoc muscorum NCCU- 442	25 μmol·m^2·s Light:dark (24:0 h), 28 °C, 1g NaCl·L^{-1}	21	8.15	PHB	Sulfuric acid extraction	Ansari and Fatma (2016)
Gas exchange limitation						
Aulosira fertilissima	Gas exchange limitation with 0.5% acetate	14	49.0%	PHB	Hot chloroform extraction-Precipitation with cold methanol	Samantaray and Mallick (2015)
Synechocystis sp. PCC6803	Gas exchange limitation + fructose and acetate as C source, P deficient condition	14	38	PHB	Hot chloroform extraction-Precipitation with cold diethyl ether	Panda et al. (2007)

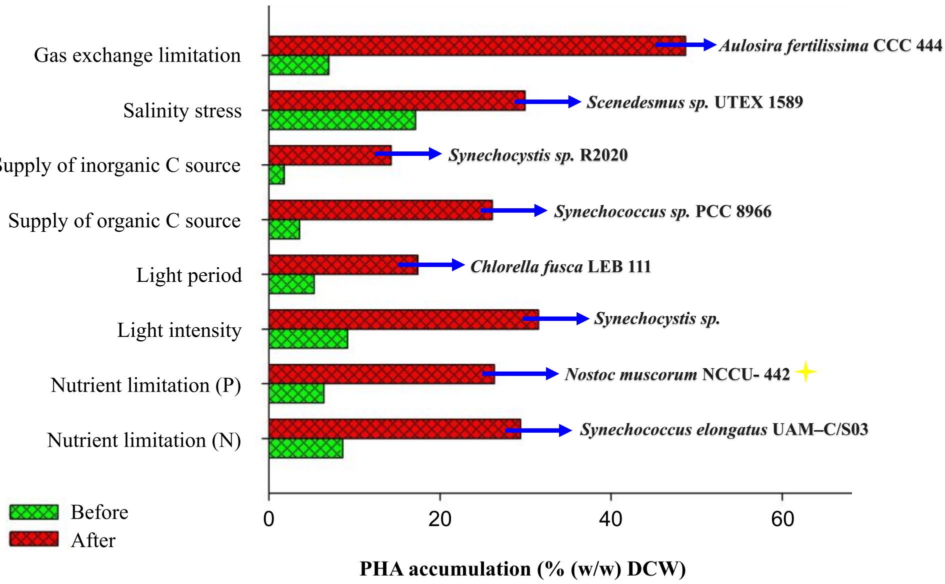

Fig. 3. Effects of various cultivation parameters on enhancing microalgae PHA production and their corresponding results (adapted from Samantaray and Mallick, 2015; Ansari and Fatima, 2016; Cassuriaga et al., 2018; Garcia et al., 2021; González-Resendiz et al., 2021; Graciosa et al., 2021; Rueda et al., 2020 and 2022). ✦ Phosphorus (P) limitation accelerates PHA accumulation, reducing the time required from 21 to 7 d.

PHAs. Microalgae can be classified into photoautotrophic (light + inorganic C sources), heterotrophic (dark + organic C source), or mixotrophic (light + organic C sources) energy sources (Madadi et al., 2021). Lighting is the primary basis of microalgae cultivation and, thus, requires special consideration. Recent research has evaluated the effects of light intensity and day-night cycle durations on PHA production. Modulating light intensity can act as a stimulus to enhance PHA accumulation in various algae species. It was predicted that higher light intensity would improve algae growth rates and nutrient utilization, leading to enhanced nutrient depletion and thereby promoting PHA accumulation. Therefore, determining the optimal light intensity, which depends on the species, necessitates further research.

Instead of continuous illumination, alternating light/dark cycles at different microalgal growth phases has been found to be effective in promoting growth and PHA synthesis (Arias et al., 2018; Koch et al., 2020). The necessity for dark cycles is linked to the ensuing low oxygen or anaerobic conditions, which enhance glycogen catabolism (glycolysis) and lead to ATP production. Under dark conditions, carbon fixation activity ceases, and glycogen synthesis is halted. The energy needed to survive in these dark conditions is obtained through the catabolism of glycogen accumulated during the light phase. The minimal energy required for glycolysis can be recuperated by transforming pyruvate into PHA. In this process, carbon is converted into PHA rather than being utilized by other fermentative procedures (Costa et al., 2018; Troschl et al., 2018; Khan et al., 2021). The impact of dark periods on PHB production, using intracellular glycogen stores in *Synechocystis* sp. PCC 6803 has been explored (Mal et al., 2022). Furthermore, some studies indicate that dissolved oxygen during agitation in the culture medium negatively affects glycogen by consuming it during respiration and hindering its conversion into PHAs (Madadi et al., 2021). The results detailing the effects of various light intensities and light/dark cycles on microalgal PHA production are presented in **Table 4**.

5.5. Temperature and pH

Temperature and pH are both vital factors that directly affect physiological changes in microalgal cells and biomass production. These factors influence the performance of any microalgae strain for the desired product (PHAs) yield since they encompass the metabolic process and the rate of biological reaction. Generally, microalgae exhibit an optimum temperature in the range of 25–30 °C. Numerous studies have confirmed that thermal stress increases PHAs and lipid accumulation in microalgae (Madadi et al., 2021; Mal et al., 2022). Moreover, microalgae species can thrive in a culture medium with a pH range of 7 to 9. Some studies have shown that pH directly affects algae growth and PHA productivity; for instance, *Nostoc muscorum* exhibited negative impacts on growth and PHB production in acidic conditions, alongside nitrogen sources and constant light illumination (Sharma and Mallick, 2005). In another study using *Spirulina* sp., the alkaline pH condition combined with phosphate and constant light illumination had a negative effect on PHA production (Koller and Marsalek, 2015).

5.6. Salinity

The salinity of the culture medium might negatively impact biochemical and physiological characteristics, as well as the growth of microalgae. Changes in salinity induce osmotic stress, ionic stress (salt), and alterations in membrane permeability by activating or inactivating the transport of ions to regulate water content within the cell (Xia et al., 2014). Generally, under saline stress in microalgae, an increase in carotenoids and lipid production is observed, but the direct relationship with PHA production is still less studied (Madadi et al., 2021). It was hypothesized that an increase in PHA accumulation in microalgae might be an adaptation to osmotic stress. Under saline stress, by adding NaCl to 1 $g \cdot L^{-1}$ in the culture medium, *Nostoc muscorum* NCCU-442 exhibited an increase in PHA content DCW from 7.6% to 8.15% (*w/w*) (Ansari and Fatma, 2016).

Meixner et al. (2022) discovered that in the halotolerant cyanobacterium *Synechocystis* cf salina CCALA 192, PHB content increased from 3.2% (*w/w*) DCW to 6.9% (*w/w*) DCW when exposed to 40 g $NaCl \cdot L^{-1}$. The study demonstrated that saline stress varies depending on the strain. These findings also suggest that PHA production is enhanced by a sudden increase in salinity, rather than by the mere addition of NaCl. Consequently, higher salinity levels are necessary to stimulate PHA production. Cultivation of microalgae under salinity stress offers the benefit of limiting contamination from non-target organisms and competing microbes. Elevated salinity concentrations may also inhibit cell growth, induce physiological changes in microalgal cells, and affect polymer characteristics. Thus, it is crucial to maintain an optimal NaCl concentration range, specifically for selected microalgae strains, to achieve substantial PHA accumulation. Illustrations of the impact of saline stress on microalgae and PHA production is shown in **Table 4**.

5.7. Concentration of nutrients in the culture media

Nutrient stress and its effects on enhancing PHA production in various microalgae species have been extensively studied. Research has shown that inducing nitrogen and phosphorus scarcity in the culture media favors PHA accumulation in microalgae as a carbon-rich storage granule. Nitrogen (N) and phosphorus (P) limitations in the cultivation medium resulted in approximately a 20% increase in PHA and other intracellular components (Arias et al., 2018; Calijuri et al., 2022). A lack of N in the culture medium reduces the concentration of nitrogenous compounds such as proteins, enzymes, and chlorophyll, whereas a phosphorus-deficient medium directly impacts the synthesis of nucleic acids (DNA and RNA), lipids, intermediate carbohydrate compounds, and ATP (Costa et al., 2019; Singhon et al., 2021). Under nitrogen-deprived conditions, *Synechocystis* sp. PCC 6803 exhibited a significant increase in PHB accumulation, reaching 53.5% (w/w) DCW (Wu et al., 2002). On the other hand, *Oscillatoria okeni* TISTR 8549, under nitrogen-sufficient conditions with added sodium acetate, showed an enhanced heterotrophic accumulation of PHV at about 42% (w/w) DCW (Taepucharoen et al., 2017).

Thus, N- and P-deficient conditions positively triggered PHA formation in microalgae. However, some studies also observed the necessity for proper nutrient concentrations to mitigate otherwise negative effects (Sirohi et al., 2021). Under nutrient-deprivation, microalgal cells enter a new physiological stage termed chlorosis. During sustained chlorosis, microalgae initially store glycogen, which is progressively catabolized and converted into PHAs (Madadi et al., 2021). The effects of cultivation condition and nutrient stress on microalgae PHA production are summarized in **Figure 3**.

6. Genetic engineering approach to enhance algae-based bioplastic production

Current research activities and developments on the physiological function of PHAs in microalgae and strategies to increase PHA accumulation while reducing production expenses are discussed in detail in the subsequent sections (**Fig. 4**). Genetic engineering is a promising method to advance microalgae-driven biotechnology for bioplastic production. This approach utilizes genetic modifications in microalgae and cyanobacteria or the insertion of specific genes from heterotrophic bacteria, employing various genetic tools to lower production costs and enhance the synthesis of the target product, primarily PHAs (Chia et al., 2020; López-Pacheco et al., 2022). Chaogang et al. (2010) used *Ralstonia eutropha* to examine the effective insertion of the *phbB* and *phbC* genes, which encode PHB synthase in *Chlamydomonas reinhardtii*, to induce intracellular PHA production. In this study, significant amounts of PHB, about 6 $mg \cdot g^{-1}$ DCW, were produced by transgenic *C. reinhardtii* compared with the wild-type strain, which exhibited no PHB production. This result underscored the research focus on enhancing PHB production in the transgenic algae and synthesizing PHB in the chloroplast.

Recently, genetically engineered *Synechocystis* sp. exhibited a 14.0% (*w/w*) DCW in PHA when cultured in BG-11 media with 2–3% CO_2 after 7 days of incubation. However, after the addition of acetate and restricted gaseous exchange, DCW showed an increased PHA accumulation of 41% (*w/w*). RNA sequencing analysis suggested that this increased PHA production was due to decreased expression of PHA biosynthetic genes. This finding indicates that enzyme regulation alone is not the sole determinant of PHA production (Lau et al., 2014). In another study, the enhancement of the sugar catabolism sigma factor, named *sigE* in *Synechocystis* sp. 6803 strain GOX50, significantly boosted PHA synthesis (Osanai et al., 2013). Recently, Koch et al. (2020) found that *pirC* inhibits phosphoglycerate mutase (PGAM), leading to decreased carbon allocation to glycolysis. PGAM primarily facilitates the conversion of 3-phosphoglycerate (3-PGA) to 2-phosphoglycerate (2-PGA).

Orthwein et al. (2021) demonstrated that the deletion of *pir*C in *Synechocystis* sp. PCC 6803 led to overexpressed PGAM activity, reducing the carbon flow toward lower glycolysis via PGAM. Deletion of *PirC* resulted in increased carbon flow to 2-PGA, ultimately yielding higher PHB levels of up to 49% (*w/w*) DCW during prolonged nitrogen deprivation (Orthwein et al., 2021). Other examples of genetic engineering approaches used in microalgae to enhance PHA production are listed in **Table 5**.

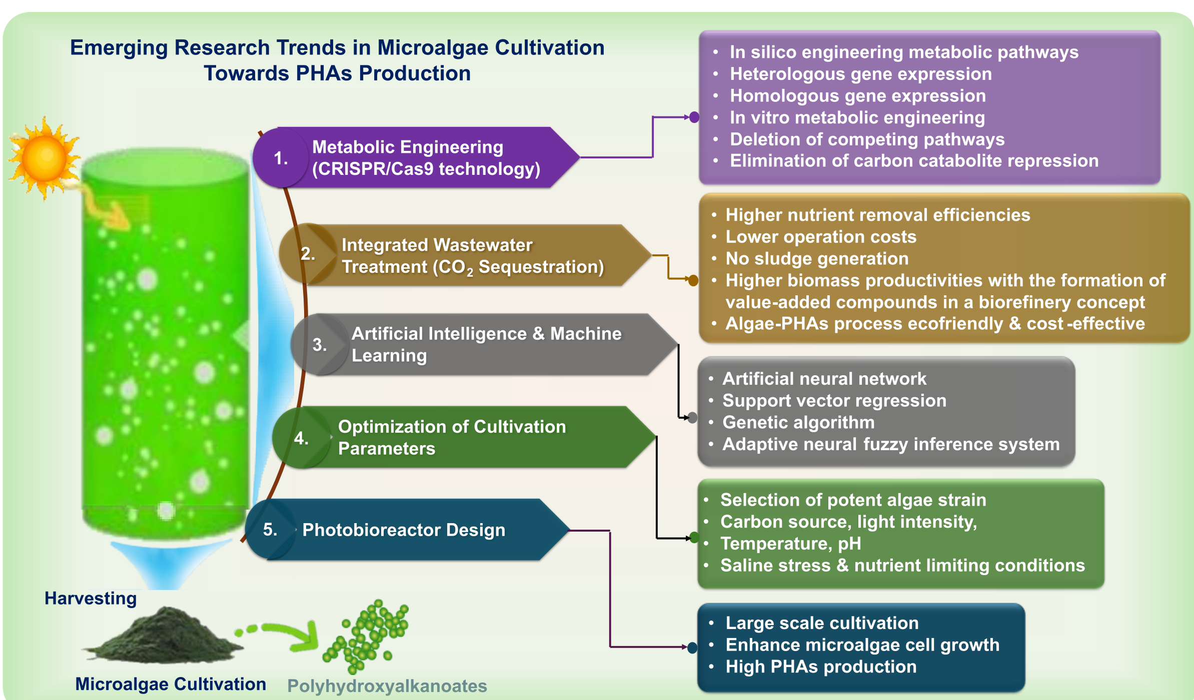

Fig. 4. Various modernistic approaches to improve microalgae-based PHA production.

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Table 5.
Examples of genetic engineering approaches used in microalgae-based PHA production.

Strain	Type of Genetic Alteration and Results	Culture Conditions	PHA Content (%)	Reference
Synechococcus elongatus UTEX 2973	Insertion of heterologous phaABC operon of *C. necator* into *S. elongatus.* Upon expression, the engineered strain produced 2.4-fold higher PHB productivity relative to the initial strain	Photoautotrophic	16.7	Roh et al. (2021)
Synechococus elongatus PCC 7942	Insertion of ATP-hydrolysis created a driving force module into the *S. elongatus*. Upon expression, the engineered strain produced a significant PHB titer relative to the initial strain.	Photoautotrophic	1.2 $g \cdot L^{-1}$	Ku and Lan (2018)
Synechocystis sp. PCC 6803 (Δgdc mutant)	Inactivation of glutamate decarboxylase gene (gdc) of *Synechocystis sp.* The engineered strain exhibited a 2.5-fold higher PHB relative to the initial strain.	Photoautotrophic	5.5	Monshupanee et al. (2019)
Synechocystis sp. PCC6803	The engineered *Synechocystis* PCC6803 comprised the inactivation of the competing pathway by deleting slr1829 and slr1830 (encoding PHB polymerase). The engineered strain exhibited significant production of PHB in the presence of sunlight and CO_2	Photoautotrophic	533.4 $mg \cdot L^{-1}$	Wang et al. (2013)
Synechocystis sp. PCC6803	Overexpression of phosphoketolase (*XfpK*) from *Bifidobacterium breve* in *Synechocystis* sp. showed a rise in acetyl-CoA levels. The engineered strain displayed a greater PHB accumulation using CO_2 as the only carbon source	Photoautotrophic	12.4	Carpine et al. (2018)
Synechocystis sp. PCC 6803 (GOX50)	Overexpression of RNA polymerase sigma factor (SigE) in *Synechocystis sp.* PHB accumulation is improved by sigE overexpression under N-deprived conditions	Photoautotrophic	1.4 $mg \cdot 100\ mg^{-1}$	Osanai et al. (2013)
Synechocystis sp. PCC 6714 (mutant MT_a24)	Random UV mutagenesis in phosphate-specific transport system integral membrane protein A (*PstA*) of *Synechocystis* sp. The mutated strain displayed an increase in cell growth, CO_2 consumption, and PHB accumulation, which is higher than the wild strain.	Photoautotrophic	37	Kamravamanesh et al. (2018)

6.1. Mutagenesis

Mutagenesis is recognized as an alternative genetic engineering approach commonly used to modify bacterial strains and enhance desired productivity. UV irradiance, with specific exposures, directly affects the growth and metabolic activities of microalgae. Recent studies have shown that UV mutagenesis applied to the mutated strain MT_a24 of *Synechocystis* sp. PCC 6714 resulted in a substantial increase in PHB production. In this study, mutation led to increased cell growth and significant CO_2 consumption (1140 mmol) and PHB accumulation (37%; w/w) in DCW, which are four and two and a half times higher than those of the wild strain, respectively (Kamravamanesh et al., 2018). UV mutagenesis induced mutations in the *pstA* gene, a membrane protein involved in phosphate (P) transport. This mutation altered cell metabolism by decreasing the responsiveness of the *sphU* gene, a regulator of P transport, thus halting further phosphate assimilation by deactivating the transporter when a sufficient amount of P is present in the culture medium. The mutation also triggered the overexpression of genes linked to carbon uptake, polyphosphate conversion to ATP, and glycogen catabolism, which ultimately enhanced PHA accumulation. The results are promising and suggest that the UV irradiance mutation strategy could be beneficial for improving microalgae cell growth, carbon sequestration, and PHA production.

In another study, transposon-targeted mutagenesis was used to develop a mutant library of *Synechocystis* PCC6803 and to select strains with higher PHA production. The authors investigated whether mutations in the *sll0461* and *sll0565* genes, known as γ-glutamyl phosphate reductase, were responsible for enhanced PHA accumulation. γ-Glutamyl phosphate reductase, a critical component of proline biosynthesis, undergoes disturbance in these genes, leading to stressful conditions for the host and resulting in increased PHA accumulation. In this study, mutant strains displayed greater potential than the wild strain, demonstrating higher PHA accumulation both in the presence and absence of acetate (Tyo et al., 2009). Krasaesueb et al. (2019) developed a mutant of *Synechocystis* sp. PCC 6803 by removing phosphate (P) transport regulation genes (*ΔSphU)* and culturing it in filtered shrimp wastewater. The engineered strain allowed for P integration within the cell even when P was abundant in the cultivation medium, leading to an increased PHB production level of approximately 32.5% (w/w) DCW after 14 days of cultivation. This elevated P level within the cell triggers acetyl phosphate synthesis, which serves as an intermediate in producing acetyl-CoA and intracellular acetate.

6.2. Role of CRISPR/Cas9 technology in polyhydroxyalkanoate production

Cell and gene editing tools, especially clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR-Cas9), are widely used due to their ability to produce engineered strains with desired properties. The CRISPR/Cas9 technology has facilitated targeted gene editing, enabling the incorporation of genes at specific genome locations, multiple gene targeting, generation of alternative nonfunctional alleles, and specific gene regulation for precise genome engineering (**Fig. 5**). This method has been employed in various applications in a range of organisms, including animals, plants, and microalgae (Jeon et al., 2017; Jeong et al., 2023). With the aid of cutting-edge CRISPR-Cas9, it has become evident that the genes responsible for PHA synthesis in microalgae can be modified (Jiang et al., 2014; Jinkerson and Jonikas, 2015). This results in enhanced quality and quantity of PHA in microalgae species, consequently opening new avenues for the up-scaling of microalgae PHA production (Knott and Doudna, 2018). *Chlamydomonas reinhardtii* has been extensively explored using this advanced technique, and the results are promising, potentially boosting microalgal bioplastics production (Chen et al., 2019; Pickar-Oliver and Gersbach, 2019).

Moreover, *Ostreococcus tauri*, *Nannochloropsis* sp., *Cyanidioschyzon merolae*, *Phaeodactylum tricornutum*, *Chlorella* sp., *Porphyridium purpureum*, and *Tetraselmis* sp. have been studied for their advances in microalgae biotechnology, aimed at producing biofuels and industrially important biomolecules and bioactive compounds. Significant enhancements in lipid accumulation, protein synthesis, and the production of value -added biocompounds, mainly lutein, zeaxanthin, and fucoxanthin, were observed (Dhokane et al., 2023). Recently, Lin et al. (2021) applied the CRISPR/Cas9 system to modify the metabolic pathways of *Haloferax mediterranei* to enhance carbon flux, resulting in a 165% improvement in PHBV accumulation. The CRISPR-Cas9 system has been used to target the omega-3 fatty acid desaturase (*fad*3) gene in *Chlorella vulgaris* FSP-E and knock out the zeaxanthin epoxidase (ZEP) gene in *C. reinhardtii* CC-4349, leading to significant improvements in lipid and zeaxanthin accumulation, respectively (Lin and Ng, 2020; Diankristanti et al., 2024). CRISPRi gene silencing tools have been widely used to enhance PHA production. Targeting genes *sad, sucC, sucD*, sdhA, and *sdhB*, which code for succinate dehydrogenases and succinyl-CoA synthetase in *E. coli*, leads to an increase in the precursor 3-hydroxybutyrate, consequently improving PHB production (Meng and Chen, 2018). In another study, the knockout of target genes by CRISPRi in *Pseudomonas putida* resulted in an eight-fold increase

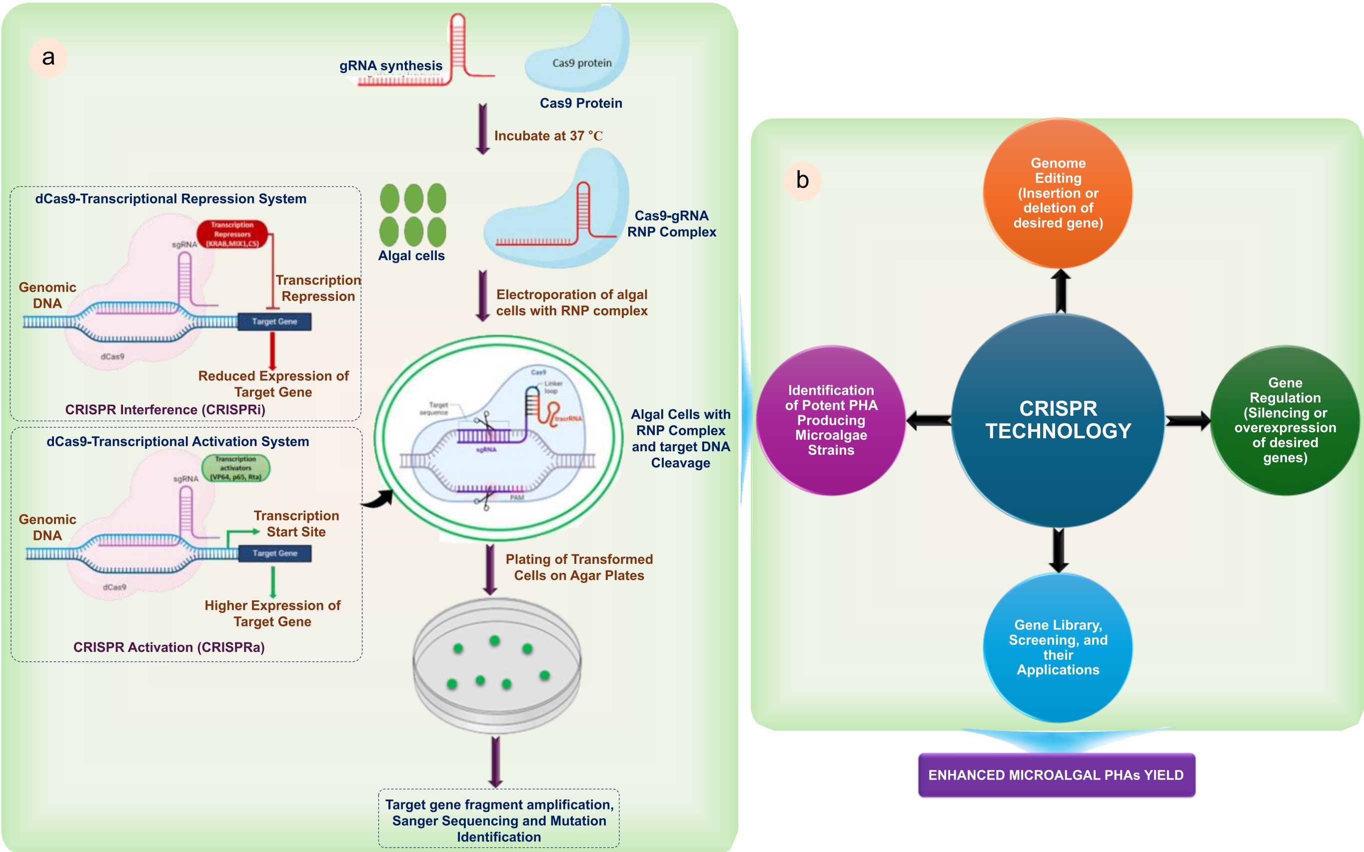

Fig. 5. (a) Schematic representation of CRISPR/Cas9 technology for gene editing (overexpression, repression of genes) in microalgae (adapted and modified from Dhokane et al. (2023)) and **(b)** applications of CRISPR technology for the development of microalgae-based PHA production.

in acetyl-CoA concentration, leading to a substantial improvement in PHB production (Kozaeva et al., 2021).

However, studies utilizing CRISPR-Cas technology to enhance PHA production in microalgae are still in the preliminary stages, with few successful outcomes. The complex structure of microalgae, as well as the appropriate selection and strategy of CRISPR constructs, are critical. Furthermore, species-specific transformation research is essential due to the unique structure of the membranes and cell walls in each taxon. Therefore, addressing the knowledge gap between CRISPR-Cas technology and algae is crucial for ensuring sustainable PHA production. Although CRISPR-based technologies enable selective genome modification across various species, continued investments in research and development are essential for improving editing efficiency and develop effective vector delivery systems, with a particular focus on reducing the production costs of microalgae-PHAs. Additionally, advanced genetic tools, such as Effector Nucleases (TALENs), Transcription Activator-Like (TAL) effectors, and Zinc-Finger Nucleases (ZFNs), have been investigated in *C. reinhardtii* to enhance bioplastic production (Jeong et al., 2023). Metabolic flux analysis and high-throughput omics approaches have also been explored for engineering microalgae for biofuel production (Kadri et al., 2023).

7. Polyhydroxyalkanoate production from microalgae integrated with wastewater treatment

Over the past several years, microalgae have demonstrated remarkable capabilities in removing a wide range of pollutants from wastewater, including nutrients (N and P), heavy metals, colored effluents, and emerging pollutants (antibiotics, antipyretics, hormones, antifungals, plasticizers, and surfactants), which disrupt aquatic ecosystems and lead to eutrophication. Microalgae have shown a high capacity for nutrient uptake (N, P, and COD) from wastewater, contributing to carbon neutrality while enhancing biomass productivity (Bhatia et al., 2021; Devi et al., 2023). Microalgae are efficiently utilized in wastewater treatment due to their advantageous attributes: 1) superior nutrient removal efficiencies, 2) low operational costs, 3) absence of sludge production, 4) extensive surface area with excellent biosorption capabilities, and 5) increased biomass productivity coupled with the production of value-added compounds within a biorefinery framework (Ahmed et al., 2022; Hasan et al., 2023). Microalgae can thrive in diverse wastewater streams, including those originating from municipal, agricultural, and industrial sources, as well as anaerobically digested effluents (Khan et al., 2021; You et al., 2022). Consequently, the microalgae-PHA approach is acknowledged as an economically viable and environmentally sustainable strategy for wastewater treatment while also addressing environmental challenges associated with synthetic plastic pollution (Cheah et al., 2023). A microalgal system integrated with wastewater treatment to produce bioplastic PHAs remains in the early stages of research, with few studies exploring this promising and sustainable approach. For example, *Botryococcus braunii* using sewage wastewater; *Tetradesmus obliquus* and *Desmodesmus* sp. biomass in municipal wastewater; and *Aulosira fertilissima* in aquaculture wastewater have been studied for PHAs production (Kavitha et al., 2016; González-Balderas et al., 2020).

In another study, the consortium of *Scenedesmus obliquus*, *Arthrospira platensis*, *Desmodesmus communis*, and *Nannochloropsis gaditana* was evaluated for growth and PHA production using municipal wastewater in combination with glycerol (López Rocha et al., 2020). *Synechocystis salina* accumulated approximately 5.5% (*w/w*) DCW of PHB while cultivating in

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sterilized digestate from the anaerobic digestion of thin stillage over 36 days (Meixner et al., 2016). The same strain in the synthetic medium displayed 4.8% (*w/w*) DCW of PHB at the pilot scale. These results are significant, suggesting the use of wastewater as a promising and viable option for cultivating microalgae and concurrently producing PHAs. Furthermore, other strains, such as *Tetraselmis* sp. and *Chlorella* sp., demonstrated increased lipid accumulation when cultured in wastewater, which also suggests potential for PHA production. Some researchers have proposed an innovative method of using wastewater generated during PHA extraction to cultivate microalgae. During PHA extraction, several chemicals and non-polymeric biomass serve as effective nutrient sources for growth and PHA production (Da Silva et al., 2018). Several patents relating to PHA production integrated into wastewater treatment plants (WWTP) have been reported, including patents (WO2016020884 and WO2014108878) by Veolia Water Solutions and Technologies, France, and polyhydroxybutyrate-co-valerate production (EP3760591 and WO2015181083) by Paques Biomaterials, Netherlands.

Worldwide, numerous research programs and companies have demonstrated microalgae wastewater treatment systems by using open raceway ponds, tubular photobioreactors, or biofilm systems with varying artificial light conditions. In Chiclana de la Frontera, Spain, local municipal wastewater is partially treated using microalgae in four 1 Ha raceway ponds. In this facility, the resultant algal biomass is co-digested with activated sludge to generate sustainable biogas, which is utilized as car fuel. This initiative is recognized as part of the EU project in collaboration with All-gas Aqualia (https://www.all-gas.eu/en/). In another EU project, SABANA (http://www.eu-sabana.eu/), wastewater treatment is accomplished using 5 Ha raceway ponds, and the produced algal biomass is utilized as bio-stimulants, bio-pesticides, bio-fertilizers, feed additives, and aquafeed.

Algal biofilm systems are extensively studied for wastewater treatment. In this system, algae is cultivated on support materials such as polycarbonate membranes, divergent material filters, woven or nonwoven fabrics, and stainless-steel mesh, which can be easily preserved during treatment. Algaewheel, a US-based company, utilized a rotating biofilm reactor system for wastewater treatment, yielding significant results (https://algaewheel.com/). Furthermore, in Fukushima, Japan, a pilot-scale plant with open paddle wheel-driven ponds was developed to treat municipal wastewater. About 10 ponds covering 1 Ha were developed, and initial experiments were conducted using a consortium of indigenous *Scenedesmus* sp. and *Desmodesmus* sp. (Sasongko et al., 2018). Various pilot-scale wastewater-based microalgae valorization details have been thoroughly discussed (Gondi et al., 2022). The literature review suggests that utilizing microalgae grown in wastewater for PHA production (**Table 6**) is a beneficial approach to enhance environmental merits and potentially reduce production costs.

8. Bioreactor development to enhance microalgae-based polyhydroxyalkanoate production at large scale

The development of an algae -based biorefinery process at its maximum capacity requires specific nutritional and operational conditions. Maintaining these conditions at a laboratory scale is comparatively simple, delivering maximal performance. Large-scale microalgae cultivation for PHA production is feasible in both closed (photobioreactor) and open systems (Ray et al., 2023). From economic and commercial perspectives, open systems are preferred due to their operational simplicity and ability to cover large areas. However, these systems present several challenges, including significant contamination risks, difficulty maintaining environmental conditions, evaporation loss, and inefficient light consumption by microalgal cells (Dogaris et al., 2015; Arias et al., 2020). Therefore, selecting or designing an adequate photobioreactor is crucial for enhanced performance on a large scale. The purpose of designing photobioreactors is to create optimal conditions for algae strains and maximize the efficiency of light energy utilization by microalgal cells through an innovative light route strategy (Lutzu et al., 2021).

Various types of bubble column, bag-based, tubular, flat-panel, and stirred tanks with different sizes of closed photobioreactors have been exploited and applied for the mass cultivation of microalgae and PHA production (Samantaray et al., 2011; de Carvalho et al., 2022). Scaling up involves standardization of variables such as temperature, light, fertilizer supply, culture contamination, mixing, algal film production that causes the PBR wall to become opaque, and oxygen accumulation. Moreover, the performance of a photobioreactor also depends on its fluid dynamics, mass transfer capabilities, heat transfer capacity, and geometry. Energie-Versorgung Niederosterreich AG, an Austrian power company, developed a horizontal tubular photobioreactor using microalgae for PHB and biogas production. By utilizing CO_2 as a carbon source, the developed system is capable of producing 115 kg of PHB and up to 320 m^3 of biogas. The system has demonstrated significant potential (Salehizadeh et al., 2020). The development and PHA production capacity of PBR using microalgae have been summarized in **Table 6**. Some researchers suggest that integrating bioreactor systems could offer a viable approach for scaling up PBR for PHA production.

The algal turf scrubber (ATS) in the United States is commercially implemented in various locations (https://hydromentia.com/technologies/algal-turfscrubber/). In this system, a biofilm of microalgae is attached to an inclined surface, over which wastewater flows for treatment. The ATS system offers several advantages, including reduced investment costs and technical requirements; however, it demands extensive surface areas akin to raceway ponds (Adey et al., 2011). In the current scenario, land availability is a crucial factor for the successful implementation of microalgae-based wastewater treatment plants (Roostaei and Zhang, 2017). To address this, researchers have recently explored the use of a space-efficient high-volume V-shaped pond for pilot-scale treatment of dairy wastewater in India. The system's inverted pyramid shape enhances surface area, thereby improving light penetration and fixation (Kumar et al., 2020).

In Ballana, Aswan, Egypt, a full-scale stabilization pond was developed with the capability of handling approximately 32,000 $m^3 d^{-1}$ of wastewater. The system proved effective in removing COD (65%) and BOD (74%) from wastewater after treatment, although seasonal variations caused fluctuations in efficiency. The treated wastewater was subsequently used for irrigation on 32 feddans of forest (Tawfik et al., 2022). Furthermore, nanotechnology significantly enhances light availability in photobioreactors. A photobioreactor coated with silver nanomaterials has improved light availability, thus promoting the growth of microalgae (Safarik et al., 2016). Nevertheless, several challenges persist at the commercial scale for PBRs, including high costs, maintaining robust microalgae cell factories in harsh environments, retaining monocultures, and achieving efficient production.

9. Modeling techniques for enhancing algae-based polyhydroxyalkanoate production

Machine learning (ML), a branch of artificial intelligence and computer science, significantly improves programs using substantial data and algorithms to enhance the productivity of algal processes efficiently (Alagumalai et al., 2023; Li et al., 2023). Typically, ML functionality involves four major steps: data pre-processing, data compilation, investigation, model advancement, and model implementation. ML and AI processes are integral for microalgae biorefineries, especially for screening microalgae by predicting beneficial traits and selecting strains with optimal PHA production capabilities. Effective microalgal productivity requires optimization of cultivation parameters, such as light intensity, temperature, nutrient availability (N and P), pH, aeration, and fluid mechanics.

ML can also assist in predicting microalgae growth, optimizing cultivation parameters, and resource exploitation to maximize biomass productivity and desired product (PHA) yield (Ching et al., 2022). Additionally, ML tools are employed to optimize downstream processing for sustainable biofuel and biomolecule production, reducing energy input and waste (Reimann et al., 2020; Oruganti et al., 2023). The most studied algorithms for developing algae biorefineries primarily include support vector regression (SVR), artificial neural networks (ANN), genetic algorithms (GA), and adaptive neural fuzzy inference systems (ANFIS), which are briefly discussed in the following section (**Fig. 6**).

ANN is widely used for the development of algae biorefineries, particularly for predicting microalgae growth, optimizing growth parameters, species identification, and extraction of biomolecules (Ansari et al., 2021; Otálora et al., 2021). In a continuous mode algal photobioreactor, the Artificial Neural Network–Model Predictive Control (ANN-MPC) model has been assessed for the production of *Spirulina platensis*. It was

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Table 6.
Summary of the various kinds of photobioreactors employed for microalgae-based PHA production using different kinds of waste streams.

Strain	Culture Conditions	Type of Bioreactor	Volume Capacity (L)	Incubation Periods (d)	Biomass Productivity ($mg·L^{-1}$)	PHA Production %DCW (*w/w*)	Type of PHA	Reference
Synechococcus leopoliensis	Aquaculture wastewater and Liquid anaerobic digestate (840 $mg·L^{-1}$ NO_3^-N), N & P deficient condition, Light:dark, 5 to 10 mbar CO_2 during daytime	Open system Thin-layer packed bed reactor (PBR)	200	10	6000	0.9	PHB	Mariotto et al. (2023)
Botryococcus braunii SAG 807-1	Filtered palm oil mill effluent (POME 40%) +BG11 medium (60%), light intensity 70 µmol·m^2·s, Light:dark (12:12 h), salinity 1 PSU, 30 °C, pH 7.5, Acetate, D-glucose, glycerol as organic C source supplementation	Erlenmeyer flask one step batch	0.1	--	49 $mg·d^{-1}$	35	PHB	Nur et al. (2022)
Consortium of two *Synechococcus* sp.	Agricultural runoff COD 80 $mg·L^{-1}$, TN 1.5 $mg·L^{-1}$, TP 0.01 $mg·L^{-1}$, 30 °C pH 8.7, 2.1 klx, Light:dark (15:9 h)	Semi-continuous PBR	2.5	15	500–1200	4.5	PHB	Rueda et al. (2020)
Synechocystis sp. PCC 6803 (SphU)	Shrimp wastewater nitrate (55.28 $mg·L^{-1}$), nitrite (3.71 $mg·L^{-1}$), ammonium (2.77 $mg·L^{-1}$) and phosphate (11.46 $mg·L^{-1}$) 28 °C, pH 8.51, light intensity 40 µmol·m^2·s, N deficient condition	Flat-plate PBR	10	14	500	32.5	PHB	Krasaesueb et al. (2019)
Chroococus sp., *Pseudanabaena* sp.	Secondary urban wastewater and liquid digestate + BG 11 medium, NH_4Cl 49 $mg·L^{-1}$, K_2HPO_4 5.6 $mg·L^{-1}$, 100 mg Na_2CO_3, light intensity of 91 W·m^2 pH 8.2, 27 °C, P deficient condition	Double jacket acrylic sequencing batch reactor (SBR)	2.0	1	–	3.8	PHB	Arias et al. (2018)
Synechocytis sp. CCALA192	Mineral medium BG11 + $NaHCO_3$ (0.5 $g·L^{-1}$) + Na_2CO_3 (0.5 $g·L^{-1}$), 25 °C, pH 9-10, light intensity was 1000 lx, Light:dark (16:8 h)	Tubular PBR	200	75	1000	12.5	PHB	Troschl et al. (2018)
Synechocystis salina	Diluted (1/3) pretreated and centrifuged digestate, COD 200 $mg·L^{-1}$, TP 0.5 $mg·L^{-1}$, 20 °C, pH 7.8, N deficient condition	Tubular PBR	200	30	2070	6.0	PHB	Meixner et al. (2016)
Nostoc muscorum Agardh	Poultry waste TOC 10.5 $mg·L^{-1}$, TN 12 $mg·L^{-1}$, TP 9.5 $mg·L^{-1}$, Light intensity 75 µmol·m^2·s, Light:dark (14:10 h), 25 °C, pH 7.8, 10% (*v/v*) CO_2	Cylindrical glass PBR	5	20	627.1	23	PHB	Bhati and Mallick (2015)
Thermosynechococcus elongatus BP1	BG11medium Light intensity 180 µmol·m^2·s, 50 °C, pH 7.8, CO_2 concentration (5-20%)	Water-jacketed stirred tank reactor	2.7	7	--	14.5	PHB	Eberly and Ely (2012)
Aulosira fertilissima	Discharge of a fishpond COD 218.7 $mg·L^{-1}$ TN 17.5 $mg·L^{-1}$ TP 2.8 $mg·L^{-1}$ pH 7.8, N limitation condition	Fiber-reinforced plastic	--	15	589.8	34.84	PHB	Samantaray et al. (2011)
Mixed microalgae culture	Citrus processing wastewater TSS 4.5 ± 0.23 g TSS·L^{-1}, COD 27,000 mg COD·L^{-1}), 20 °C	Sequencing batch reactor (SBR)	1.5	--	--	0.38 gPHB $gTSS^{-1}$	PHB	Corsino et al. (2021)
Synechocystis sp. PCC 6714	BG11medium, light intensity 40 µmol·m^2·s, 50 °C, pH 8.5, 2% CO_2 (20 mL·min^{-1}), N and P deficient condition	Applikon Bio glass jacketed stirred tank reactor	1.5	15	1800	16.4	PHB	Kamravamanesh et al. (2017)
Rhodopseudomonas palustris sp.	Pretreated olive mill wastewater +acetate, light intensity 74 W·m^2, Light:dark (15:09 h) for one side of the PBR, 30 °C, pH 6.8	Flat glass PBR	0.55	7	–	9	PHB	Padovani et al. (2016)
Synechocystis sp. PCC 6803	Mineral medium BG11, light intensity 150 µmol·m^2·s, 2% (*v/v*) CO_2, N deficient condition	Inclined bubble column PBR	0.8	21	--	1.0	PHB	Carpine et al. (2015)

found that the ANN-MPC effectively understood reactor dynamics and controlled light intensity to enhance microalgal growth (Hu et al., 2008).

Table 7 summarizes the application of machine learning models in the development of microalgae-based biorefinery. Recently, Hossain et al. (2022) utilized SVM in conjunction with appropriate optimization algorithms and a multilayer perceptron artificial neural network (MLP-ANN) to improve the model's accuracy and reliability. The developed SVM-MLP-ANN model predicted the impact of operational parameters such as pH and temperature on the nutrient (N and P) removal efficiencies by *Chlorella kessleri* in municipal wastewater.

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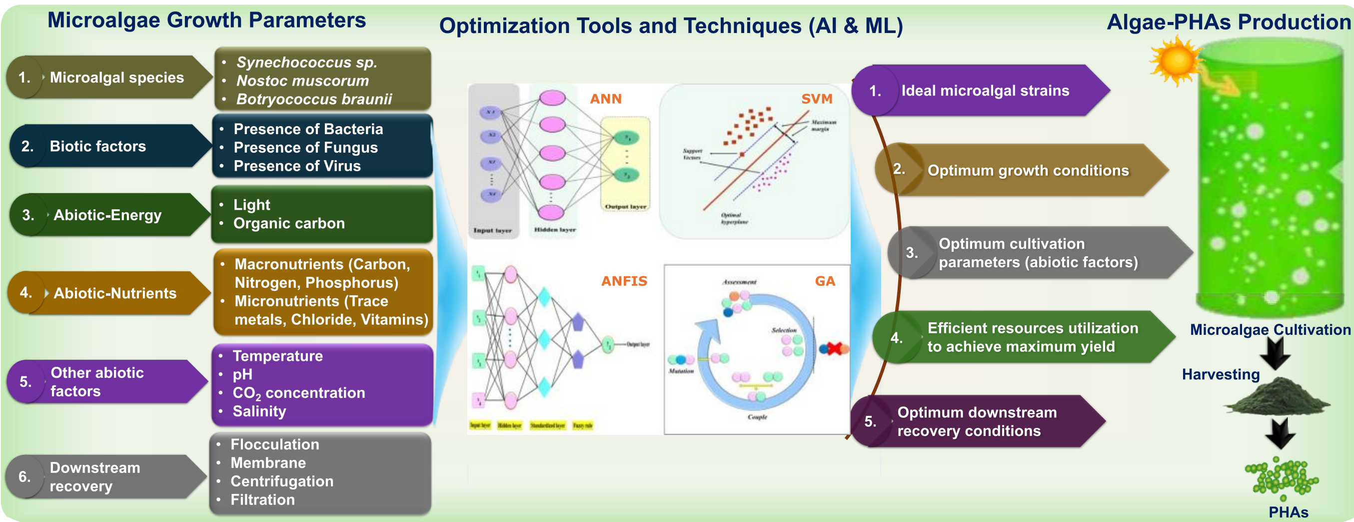

Fig. 6. Schematic representation of AI/ML integrated system to improve microalgae-based PHA production.

Lim et al. (2022) conveyed that the incorporation of IoT and AI offers smart farming practices to improve the efficiency and sustainability of microalgae cultivation, possibly acting as a precursor for advanced control and monitoring through real-time data and predictive modeling. These technologies illustrate a significant step in developing the viability and scalable production of algae-based bioplastics. Thus, they could be integral in developing biobased materials in a way that upholds environmental responsibility and circular economy principles.

10. Techno-economic feasibility of microalgae-based polyhydroxyalkanoate production

Microalgae have demonstrated the ability to grow on waste materials, utilize fewer nutrients, and adapt to diverse environmental conditions. Therefore, microalgae are promoted as an efficient and sustainable third-generation feedstock for bioplastics production compared to food crops and lignocellulosic biomass (Chong et al., 2022). Recent market statistics for European bioplastics indicate that global demand for bioplastics is rising due to their inherent flexibility, biocompatibility, and biodegradability properties, making them extensively employed in the pharmaceutical, healthcare, food, energy, and beverage industries (Boccalon and Gorrasi, 2022). Numerous international brands such as Procter and Gamble, Puma, and Heinz, have already incorporated bioplastic solutions into their products (Chia et al., 2020). Currently, about 1% of all plastics produced globally are bioplastics. The capacity for worldwide bioplastics production is expected to significantly expand from nearly 2.11 million tonnes in 2020 to about 5.3 million tonnes in 2026 (European Bioplastics, 2022).

This surge in bioplastics is primarily due to the increased production of bio-based PHAs, PBATs, and PBS (Afreen et al., 2021; Roy Chong et al., 2022). Heterotrophic microorganisms, exemplified by *Cupriavidus necator*, typically follow this route for PHA production, but their commercialization is impeded by high costs (4.28 USD kg^{-1}) relative to PLA (2.0 USD kg^{-1}). The primary contributor to high costs is the need for organic carbon sources, accounting for about 30~50% of total production expenses (Naser et al., 2021; Rajvanshi et al., 2023). Recently, Walker and Rothman (2020) found that the energy requirements for PHA production (37.9±35.2 MJ kg^{-1} PHA) are less than those for PP (72.4±20.7 MJ kg^{-1} PP) and PLA (42.8±11.7 MJ kg^{-1} PLA), enhancing the potential applicability of PHAs. However, the bioplastic production process involves multiple stages, necessitating overall adjustments to render it more environmentally friendly and cost -effective. Additionally, industrial developments have not yet advanced sufficiently to achieve sustainable and economical biopolymer production (Nanda and Bharadvaja, 2022).

Researchers have conducted a techno-economic analysis on producing algae biomass using various cultivation systems, with costs ranging from 500 to 1200 USD per tonne of biomass. Additionally, converting biomass from a slurry to a powder is estimated to cost between 350 and 760 USD per tonne of dried biomass (Hoffman et al., 2017). Consequently, microalgae-based PHAs are not as cost-effective as those produced by heterotrophic bacteria, which currently generate PHA at a price of 2000 to 16,000 USD per tonne (Price et al., 2020). Microalgae PHA production incurs high costs due to lower biomass productivity, the requisite light, and extensive land surface area. The harvesting and maintenance costs are also considerably higher than those for heterotrophic PHA production processes (Dutta et al., 2016; Troschl et al., 2017). To address these challenges and enhance the sustainability and cost-effectiveness of microalgae-based PHAs, rigorous research is being conducted.

Utilizing wastewater and flue gas-based carbon sequestration capture technology can significantly reduce the cost of microalgal PHA production (Kothari et al., 2021). For example, FCC Aqualia, the largest wastewater treatment plant in Europe, treats approximately 500 Mm^3 of wastewater annually. During this process, it emits about 1000 kilotonnes of carbon dioxide and generates approximately 25 and 5 kilotonnes of N and P, respectively (Acién Fernández et al., 2018). Numerous benefits are associated with using a microalgae-based wastewater treatment system, including the potential to produce up to 500 kilotonnes of microalgae biomass annually, reduce GHG emissions (particularly CO_2), and consume less than half the energy of traditional wastewater treatment facilities. A detailed techno-economic analysis of *Scenedesmus almeriensis* cultivation in tubular photobioreactors was conducted using data from approximately two years. This analysis demonstrated that wastewater and flue gases provide an efficient nutrient resource, lowering the production cost of PHB from 3 €·kg^{-1} to 1.8 €·kg^{-1} (Acién et al., 2012). Microalgae have demonstrated their capability to capture atmospheric carbon (up to 960 kg of CO_2 per tonne) and effectively convert it into biopolymers. *Chlorella vulgaris* was extensively studied for bioplastic production using the maximum amount of CO_2 (Rahman and Miller 2017). Recently, Mohler et al. (2019) conducted a techno-economic study on *Schoenoplectus acutus* cultivated with 9% CO_2-simulated flue gas, converting it into high -value biomolecules. The production cost for microalgae biomass was 492 USD per tonne, while the revenue generated from untreated algal biomass was approximately 1000 USD per tonne. In contrast, distinct components generated carbohydrates (370 USD per tonne), proteins (640 USD per tonne), and lipids (121 USD per tonne). These findings suggest that CO_2 sequestration into biopolymers within a biorefinery framework enhances environmental sustainability.

Moreover, the selection of appropriate PBRs or open pond systems for large-scale microalgal cultivation directly influences the overall economic feasibility of the process. It has been shown that using microalgae-derived

Table 7.
Literature review of the application of machine learning models, ANN, SVM, ANFIS, and GA in the development of microalgae-based biorefinery.

Machine Learning Tools	Application	Microalgae Species Studied	Input Variable Parameters	Remarks	Reference
Artificial neural network (ANN)	Microalgae cultivation	Mixed microalgae culture	• Initial biomass • Acetate and nitrate concentrations • Hydraulic retention time • Harvesting period, • Solar radiation • pH, temperature	• The assembled ANN exhibited optimal conditions for microalgae cultivation in an open raceway pond. • The superior prediction accuracy (R2 > 0.93).	Supriyanto et al. (2018)
	Selection of potent microalgae strains and classification	Six divergent microalgae genera	• Images of microalgae acquired by Flow CAM	• Differentiate six divergent genera of microalgae *Chlorella, Scenedesmus, Haematococcus, Synechococcus, Chlamydopodium,* and *Docystidium*. • The model accuracy of classification thresholds was about 97.27%	Otálora et al. (2023)
	Pretreatment and downstream processing for extraction of biomolecules	Mixed microalgal species Enzymatic pretreatment (Regression)	• Initial biomass concentration • pH • Temperature • Pretreatment time	The assembled model exhibited optimal specifications for the enzymatic hydrolysis of mixed microalgae. Higher accuracy in total reducing sugar yield is about 96.3%.	Shokrkar et al. (2017)
	Sustainable biofuel production and biorefinery	*Chlorella pyrenoidosa* Transesterification using acid catalyst	• Temperature • Reaction time • Acid concentration • Acid catalyst: wet microalgae biomass	• ANN model exhibited better accuracy (R^2 ~ 0.94). • Fatty acid methyl ester yield - 19.90% after transesterification.	Muhammad et al. (2022)
		Chlorella vulgaris acid-facilitated hydrothermal carbonization	• Catalyst:algae feedstock • Temperature	• The superior prediction accuracy (R^2 > 0.99) of the transformation of microalgae to hydrogen. • The obtained yield of hydrogen is 3.04 mol kg^{-1}.	Gruber et al. (2022)
Support vector machine (SVM)	Microalgae screening, classification, and characterization	*Scenedesmus coenobia*	Microscopic images of microalgae	• The SVM-ANN model exhibited 98.63% accuracy for the automatic identification of microalgae.	Giraldo-Zuluaga et al. (2018)
	Microalgae cultivation	*Cyanobacteria* (*Synechococcus elongatus* UTEX 2973)	• Temperature • Light • Initial biomass concentration	• The LDPM (Regression support vector algorithm) showed that in PBR, light is a vital parameter for better growth of *S. elongatus*. • Superior prediction accuracy (R^2 > 0.79–0.85).	Long et al. (2022)
Adaptive-neuro fuzzy inference system (ANFIS)	Microalgae cultivation	*Cyanobacteria* (*Synechococcus elongatus UTEX 2973*)	• Temperature • Light • Initial biomass concentration	• The GRM (Support vector algorithm) exhibited better environmental conditions to attain consistent biomass productivity in semicontinuous reactors. • The model showed superior prediction accuracy (R^2 > 0.992)	Long et al. (2022)
	Pretreatment and extraction of biomolecules	Catalyst-mediated transesterification of Jatropha algal oil blend	• Blending molar proportion • Reaction time • Temperature • Catalyst dose	• The combined RSM-ANFIS model predicted optimal conditions for transesterification of the Jatropha-algal oil blend using a catalyst. • The model showed superior prediction accuracy (R^2 > 0.99).	Kumar et al. (2018)
		Spirulina sp. Extraction of phenolic compounds	• *Spirulina* sp. productivity • Total flavonoids • Extraction yield, • % of flavonoid • % of phenols	• The ANFIS, MLP, and SWLR models were tested for the extraction of total phenolic compounds by using diverse growth mediums. • ANFIS and SWLR showed precise results relative to MLP.	Asnake Metekia et al. (2022)
Genetic algorithm (GA)	Selection of potent microalgae strains and classification	*Chlamydomonas reinhardtii*	• Algal cell concentration • Fluorescence emission spectra, MATLAB	• GA optimized the backpropagation neural network model (GA-BP) and assessed the algal biomass productivity by measuring the records of fluorescence emission spectra.	Liu et al. (2020)
	Microalgae cultivation	*Scenedesmus* sp.	• Coal-fired flue gas • Photoperiod • Light intensity • pH, temperature	• ANN-GA model was applied to determine optimal operational conditions to improve biomass productivity of *Scenedesmus* sp. (57%) in domestic wastewater.	Nayak et al. (2018)

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Table 7.
continued.

Machine Learning Tools	Application	Microalgae Species Studied	Input Variable Parameters	Remarks	References
Genetic algorithm (GA)	Estimating microalgal CO_2 fixation.	Various microalgae strains	• Temperature • pH • CO_2 • Quantity of N and P	• ANFIS-GA model exhibited precise optimal conditions to improve the CO_2 fixation rate. • ANFIS-GA appeared as the better prediction potential relative to the ANFIS model alone	Kushwaha et al. (2022)
	Downstream processing for extraction of biomolecules	*Chlorella* CG12	• Reaction temperature • Reaction time • Methanol:oil molar proportion.	• ANN-GA model displayed optimal conditions for the transformation of algae oil into FAME. • The model showed superior prediction accuracy ($R^2 > 0.99$).	Srivastava et al. (2018)

feedstock in closed PBRs is unsuitable for fuel and bioplastic production due to higher capital and maintenance costs. Closed PBR systems incur costs for algal biomass ranging from 639 USD to 1737 USD per tonne, whereas open ponds usually have lower costs, starting at 494 USD per tonne (Clippinger and Davis, 2019; Behera et al., 2022). Some researchers have explored algal biofilm technology for wastewater cleanup and utilized the collected microalgae biomass to produce bioplastics (Chong et al., 2022).

Another study demonstrated a detailed techno-economic analysis of microalgae-based PHA production. This study achieved a PHB yield of 15% when grown in tubular PBRs and a 60% yield when using a TLS (thin layer system). The influence of climate on PHA production was also considered. After extracting PHA, the waste biomass was used for biogas production, and the digestate was further reused for nutrient recovery. In summary, the PHB resin price is lower (26.4 USD·kg^{-1}), considering a 60% yield using a TLS, while a 15% yield leads to a significant rise in the cost of PHB to 103.5 USD. kg^{-1} (Panuschka et al., 2019).

Moreover, some researchers have suggested that integrated microalgal biorefineries could be a viable strategy to reduce production costs and generate additional revenue. The addition of other operational units alongside PHAs for the recovery of other by-products should also be explored to enhance profitability. Integrating various processes, such as utilizing algae residue after biodiesel production to produce PHA, could help reduce overall costs (Das et al., 2018; Naresh Kumar et al., 2020). However, comparison among processes remains challenging due to varying degrees of optimization. Standardizing LCA studies is essential to evaluate the progress of each process and ensure comparability. Therefore, further investigation is necessary.

The Nenu2PHAr project, a large-scale algae-based bioplastics initiative funded by the H2020 European program, commenced as a 5-year project in 2020. It involves numerous industries, small-scale enterprises, and research organizations. Initially, algae were cultivated under specific conditions to accumulate starch molecules. Subsequently, this biomass was used as a carbon feedstock for cultivating bacteria to produce PHA polymer (Geerinck and Schueren 2021; Mogany et al., 2024). The project aims to develop eight different types of PHA-based products to replace fossil fuel-derived conventional plastics effectively. Furthermore, *Botryococcus braunii* was utilized in the pilot project titled SPLASH: Sustainable Polymers from Algae Sugars and Hydrocarbons (2012–2017) (http://eu-splash.eu/) to produce hydrocarbons and exopolysaccharides, which were then converted into sustainable biopolymers. Consequently, the bioplastics market anticipates that commercialization of microalgal-based bioplastics could result in commodity thermoplastics priced between 1540 and 2200 USD per $tonne^{-1}$, biodegradable resins from 2650 to 5500 USD per $tonne^{-1}$, and engineered resins from 1540 to 8800 USD per $tonne^{-1}$ (Research and Markets, 2021). However, substantial investments in research and development, knowledge transfer, and cooperative research efforts are essential to render algal bioplastics economically viable and scalable.

11. Life cycle assessment of microalgal-derived bioplastics

The "life cycle assessment" (LCA) refers to a comprehensive process for evaluating the environmental impacts of products throughout their entire life cycle. LCA is instrumental in identifying environmental "pinch spots," opportunities for cost reduction, and potential modifications in industrial processes (Papadaki et al., 2016). However, LCA studies, especially for microalgae -derived bioplastics, are scant. The United Nations Sustainable Development Goals (UN SDGs) recently emphasized the reduction of traditional plastic use and their replacement with green bioplastic alternatives. Microalgal -derived bioplastics are regarded as eco-friendly and sustainable, aligning with Sustainable Development Goal SDG 12. The production process for algae -based PHAs includes the growth of microalgae, harvesting, and extraction of the biopolymer.

Algal-based bioplastics degrade quickly in natural environments into CO_2, water, and biomass, offering a significant advantage over conventional plastics. This capability aligns with circular economy strategies, reducing plastic waste accumulation in ecosystems and mitigating associated environmental impacts (Costa et al. 2019; Chia et al., 2020). Microalgae exhibit significant carbon fixation capacity, surpassing that of other plants (Ighalo et al., 2022). Typically, 1 kg of algae can fix between 0.73 and 2.22 g·L^{-1}·day^{-1} of atmospheric CO_2, approximately 1.83 kg of CO_2 per kilogram of algae (Yadav et al., 2020). Therefore, algae-based bioplastics demonstrate a lower carbon footprint compared to traditional plastics.

Recently, researchers have shown that algae-based bioplastics reduce ocean acidification, align with the sustainable management of natural resources, and do not compromise biodiversity (Oliveira et al., 2022). However, extensive research focusing on techno-economic analysis and comprehensive life cycle assessments (LCA) of microalgal-based bioplastics is imperative to enhance the processes for advanced applications and investments.

12. Challenges and future perspectives of microalgae-based polyhydroxyalkanoate production

PHAs are highly promising biodegradable plastics within the proposed alternatives to conventional plastics. In subsequent subsections, several critical challenges to achieving environmentally sustainable industrial PHA production using 3G biomass resources are discussed (**Fig. 7**).

12.1. Increased biomass productivity

Various bioreactor configurations have been explored to achieve higher cell densities. However, commercial-scale applications remain underutilized. Significant research into optimizing process parameters for large-scale applications and refined downstream processing for algae biomass harvesting and handling from PBR is crucial. Furthermore, cultures in the form of phototrophic biofilms not only increase biomass concentration in the reactor but also strengthen the process's robustness and simplify the harvesting process. In addition, optimizing photobioreactor designs for scaling up should consider key parameters like effective light transmission, as well as enhanced CO_2 and O_2 exchange rates, to boost microalgal biomass and PHA production. Addressing these factors will be a central focus of future research.

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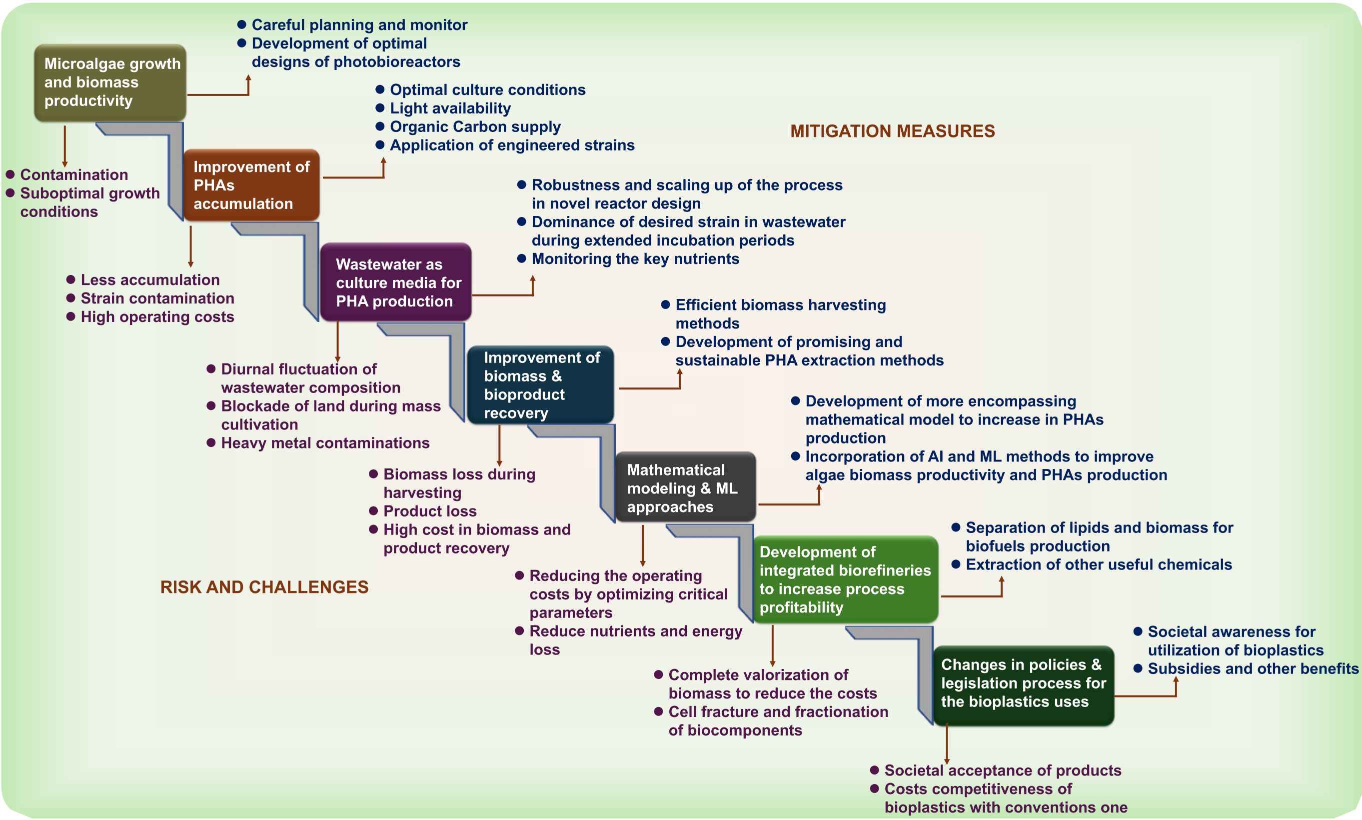

Fig. 7. Techno-economic challenges and future research directions for enhanced microalgae-based PHA production.

12.2. Improvement of polyhydroxyalkanoate accumulation

PHA production is enhanced by optimizing cultivation parameters such as stressing conditions, saline stress, light/dark conditions, and nutritional deficiency; however, the effectiveness depends on the competence of each strain. Light availability appears to improve PHA production, particularly under mixotrophic conditions, though further research is necessary to understand the biochemical changes that lead to enhanced growth by light under these conditions. Organic carbon has also sped up PHA production and improved the properties of co-polymers, but the optimal concentration and source of organic carbon need to be determined to achieve the desired polymer properties for specific applications. Genetic modification of microalgae strains aims to stabilize culture maintenance on a large scale, outdoors, and in non-sterile environments. Yet, several critical issues must be addressed when applying genetically engineered algae strains: i) the robustness and commercial scalability of these strains, given that most research occurs at the laboratory scale; ii) their adaptation and dominance in harsh environments when used for wastewater treatment or in open pond systems; iii) the safety and ethical compliance necessary for the practical applications of these genetically altered strains.

12.3. Improvement of biomass and bioproduct recovery

Harvesting biomass and extracting PHAs pose significant challenges in developing cost-effective production of algae-based PHAs. The major cost factors are dedicated to the recovery and purification of PHAs from biomass, raising environmental concerns. The recovery process includes four main stages: (1) biomass harvesting, (2) pretreatment, (3) PHA extraction, and (4) PHA purification. PHA recovery from microalgae is relatively underdeveloped, and only a few studies have assessed its efficiency. Currently, PHA extraction methods are largely based on those used for heterotrophic bacteria, which are not directly applicable to 3G biomass sources with thicker, more complex cell walls. Therefore, future research should focus on developing environmentally friendly extraction techniques used on heterotrophic bacteria, including new green solvents, mealworms, or enzymatic methods adapted for microalgae biomass.

12.4. Wastewater as culture media

Another innovative approach is the production of PHAs in a microalgae-based biorefinery integrated with wastewater treatment. This novel approach offers dual benefits: reduced production costs and a decreased environmental impact. Although this strategy holds great potential, numerous challenges persist. Due to their significant biosorption capacity during wastewater treatment, heavy metals and other pollutants are readily adsorbed onto the cell surface of microalgae. These pollutants negatively affect PHA production and extraction, thereby impacting the practical and commercial viability of the process. The development of a simple pretreatment is essential for the desorption of pollutants before the extraction of the final product, PHAs, from microalgae biomass. Biological contaminants, such as bacteria, yeast, and other undesirable algae species, also compromise process efficiency, necessitating further research to address these issues (Lutzu et al., 2021). Moreover, important issues, including the robustness and scalability of the process, potential uses for waste commodities, and the dominance of microalgae in wastewater during extended incubation periods (Ruiz et al., 2016; Arias et al., 2020), must be addressed. To counter these drawbacks and maintain microalgae as a dominant culture medium, several recommendations are proposed: i) use of wastewater with low carbon content, ii) maintaining high nitrogen and low phosphorus concentrations, iii) maintaining hydraulic retention time, and iv) applying a feast-famine fermentation strategy to achieve significant PHA accumulation (Morgan-Sagastume et al., 2010; Lutzu et al., 2021). Moreover, microalgae-based PHA production using wastewater faces challenges, including the lack of a robust regulatory framework,

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societal approval of products derived from wastewater, and stakeholder interests.

12.5. Development of mathematical models and machine learning approaches

Mathematical models are essential tools for continually improving and optimizing the design and operation of biotechnological processes. Significant research has been devoted to developing a mathematical model that calculates PHA production using 3G biomass resources. The advancement of accurate models allows for better predictions of algae biomass and PHA accumulation across various conditions. This advancement supports the optimization of system operations and the creation of PHA-enhancing environmental conditions, minimizing the need for additional experimental efforts (Carpine et al., 2020). Future research should focus on developing a more comprehensive model to predict increases in PHA production when cultivation conditions are altered.

The application of novel ML and AI methods has demonstrated significant potential for enhancing algae biorefineries. These methods are effective in identifying potent strains, optimizing microalgae cultivation parameters, and improving CO_2 sequestration efficiency. Furthermore, they have proven beneficial in the production and extraction of bioactive compounds and biofuels. Therefore, integrating AI and ML techniques could significantly boost algae biomass productivity and PHA production. Advancing bioplastics production from microalgae will necessitate a research focus on developing robust algorithms, improving model accuracy, managing complexity, addressing increased investment costs, promoting data sharing, and rigorously evaluating AI models for reliability and effectiveness.

13. Policy and practical implications of the present review

Microalgal bioplastics offer a sustainable end-of-life cycle with a lower carbon footprint, greater biodegradability, reduced ecosystem impact, and prevention of plastic waste accumulation. Yet, technical challenges, commercial viability, and regulatory frameworks remain significant obstacles to the sustainable development of microalgae-based PHA production. Increased research should be directed towards developing economically viable microalgae-based wastewater treatment and bioplastics production.

The development of the blending process with other biobased or fossil fuel-based plastic sources is essential to enhancing the characteristics and feasibility of the process. There is a need to improve public perception of algal-based bioplastics, and a positive approach to sustainability will likely improve their commercial viability (Nanda and Bharadvaja, 2022).

Lastly, advancement in viewing the policy and certification process for bioplastics usage, which is time -consuming and cumbersome, is necessary. Currently, there are no specific policies or obligations concerning bioplastic quality, although numerous certification practices exist for bioplastic decomposition (Chia et al., 2020). Therefore, global policy and legislative reforms regarding bioplastic use and quality assurance practices are essential to drive sustainable adoption and ensure compliance with environmental standards.

14. Conclusions

Third-generation biomass (3G) resources are regarded as a promising, cost-effective, and sustainable option for producing PHAs. This review critically articulates and evaluates recent studies on microalgae-based PHA production. It identifies the effects of cultivation factors such as light, temperature, pH, nutritional limitation, and salinity stress and their optimization to enhance PHA accumulation. New insights into the cultivation strategies of microalgae integrated with wastewater for cost-effective and environmentally friendly PHA production are provided. Modernized approaches in genetic engineering, bioreactor engineering, and machine learning for effective biomass productivity and sustainable microalgae-PHA production are discussed. Based on the compilation of all available information and our understanding, this review presents challenges and future perspectives for the commercial -scale production and validation of microalgae-PHAs.

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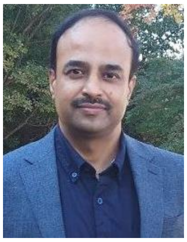

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