



Microalgae in Bioplastic Production: A Comprehensive Review

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Abstract

The current era of industrialization includes a constantly increasing demand for plastic products, but because plastics are rarely recycled and are not biodegradable plastic pollution or “white pollution” has been the result. The consumption of petroleum-based plastics will be 20% of global annual oil by 2050, and thus there is an inevitable need to find an innovative solution to reduce plastic pollution. The biodegradable and environmentally benign bioplastics are suitable alternative to fossil-based plastics in the market due to sustainability, less carbon footprint, lower toxicity and high degradability rate. Microalgal species is an innovative approach to be explored and improved for bioplastic production. Microalgae are generally present in abundant quantity in our ecosystem, and polysaccharide in the algae can be processed and utilized to make biopolymers. Also, these species have a high growth rate and can be easily cultivated in wastewater streams. The review aims to determine the recent status of bioplastic production techniques from microalgal species and also reveal optimization opportunities involved in the process. Several strategies for bioplastic production from algal biomass are being discussed nowadays, and the most prominent are “with blending” (blending of algal biomass with bioplastics and starch) and “without blending” (microalgae as a feedstock for polyhydroxyalkanoates production). The advanced research on modern bioengineering techniques and well-established genetic tools like CRISPR–Cas9 should be encouraged to develop recombinant microalgae strains with elevated levels of PHA/PHB inside the cell.

Keywords Bio-based plastic · Microalgae · Degradable plastics · Bioeconomy · CRISPR–Cas9 (clustered regularly interspaced short palindromic repeats) · LCA (life cycle assessment)

1 Introduction

The increased global demand for plastic production doubled from 245 to 359 million tonnes (2008–2018) [1] and is expected to increase threefold by 2060 [2]. With the rise in the global population, there is a surplus rise in the demand for plastic production. Approximately 40% of the plastic shares are occupied by the packaging sector followed by building and construction sector (20%), automobiles (10%), electronics (6%), households and sports (4%, and others (20%). Almost 80% of all plastics types, polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), are used for

the majority of applications for consumer goods [3]. This increase is a continuous threat to the environment worldwide, and its consequences are not only limited to pollution, but also cause harm to human and marine life [1]. According to the reports investigated by the U.S. Environmental protection agency (2020), about 34 million metric tons of plastics leaks into the aquatic environment every year due to poor waste management techniques, and is expected to increase threefold by the year 2040 if proper waste management treatment remedies were not considered [4]. These plastics end up in landfills (72%) or incinerated, and the rest leak into the environment. According to the European Commission, global plastic produced of about 4% leaks into oceans annually. In Europe, out of 25.8 million tonnes of plastic produced annually, only 30% of the plastic gets recycled, and the rest leaks into the environment. However, according to reports from 2016, a 0.3% increase in the recycling rate was observed (30–31.3%) [5, 6]. As per 2018 reports, only 25% of plastic gets recycled and 22% incinerated, and the rest ends up in landfills. During the COVID-19 pandemic, a

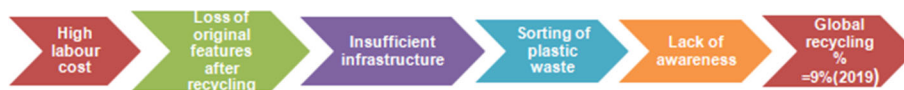
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Fig. 1 Challenges in plastic recycling



sudden surge in plastic usage (plastic used in medical kits, packaging, and face masks) was observed, which accelerated plastic production. In the USA only, out of 35.68 million tons of plastic, merely 8.5% of plastic gets recycled, and the rest is incinerated/landfilled. With the current inferior waste management practices, about 12 billion metric tons of plastic waste will end up in the environment by 2050 [7, 8]. Also, plastics harm human health in such a way that, when ingested or inhaled, they cause respiratory issues, skin diseases, eye damage, cancer, allergies, and significantly more fatal health issues [9]. The overwhelming majority of such plastics are due to the lack of efficient disposal system and poor recycling rate. Therefore, to reduce the plastic load on the environment, recycling practices should be employed as waste management strategies. Recycling provides opportunities to reduce oil usage, carbon dioxide emissions and the quantities of waste requiring disposal. But recycling practices are sometimes associated with significant issues/challenges that generally involve high labor costs and insufficient infrastructure for recycling mixed plastic waste. As a further matter, all types of plastic face issues while recycling due to the lack of separate recycling streams, and hence, there comes the challenge of sorting out the desired plastic waste for proper recycling. Besides, hazardous materials present in plastics make them difficult to recycle. Another concern with recycling is getting inferior plastic quality (in terms of chemical and physical properties) compared to the original one, limiting its future use and shelf life [10, 11]. Figure 1 depicts different challenges related to plastic recycling [2].

What are needed the materials that are inexpensive, resourceful, and convenient for sustainable development. Petroleum-based plastics are low-weight polymers, resistant to various degradable chemicals and microbes (water, chemicals, sunlight, and bacteria), and are economical. Though conventional plastics have a wide range of benefits in terms of physical properties like durability, moldability, and robust mechanical properties still, they come with enormous environmental costs. For instance, let's consider polystyrene (PS), a widely used plastic, is cheap, easy to process, and has strong mechanical properties but has a poor biodegradable rate. It can persist in the environment for decades without decaying [1]. As a result, plastic debris fragmented into smaller particles starting with macroplastics (< 25 nm), then microplastics (< 5 mm), and further fragmentation gives rise to smaller nanoplastics (< 100 nm), which reside in the environment untouched for many years. These microplastics are known to be emerging pollutants that cause a threat to the environment [8, 12]. Several reports have suggested

that the time taken for degradation by plastic bags is around 20 years or even 1000 years, while some plastic bottles can take up to 450 years to degrade, and some can merely degrade [13]. Degradation time is entirely dependent on the type of polymer and environmental conditions. Table 1 enumerates plastic types and their biodegradation conditions.

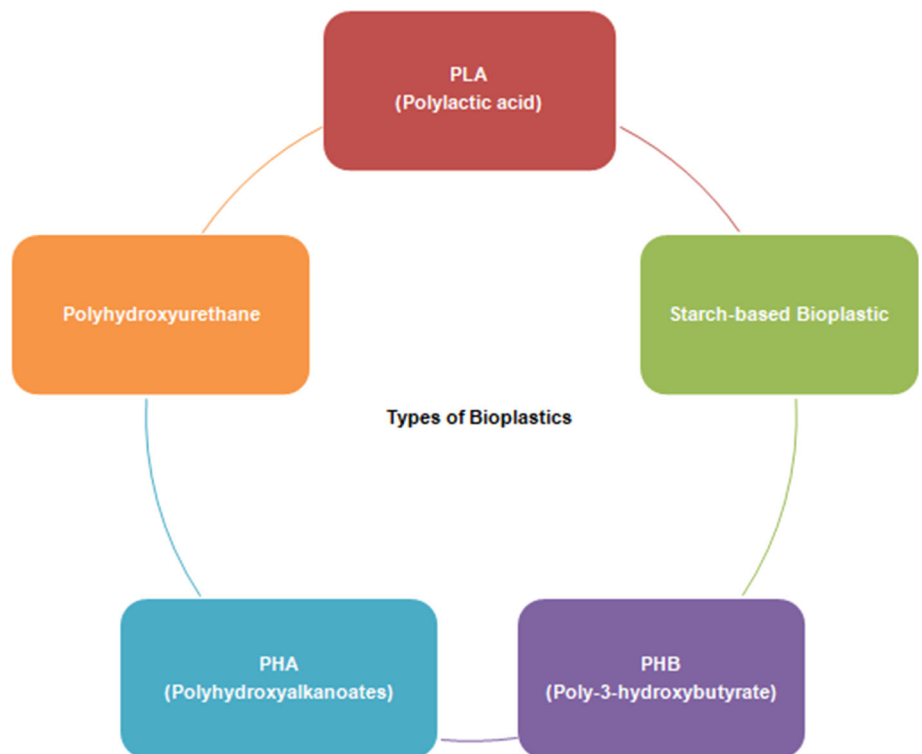
Furthermore, synthetic plastic production requires raw materials derived from natural resources like natural gas or refined crude oil. Hence, this 'White Pollution' caused by plastic production and its supply chain not only causes health issues but also depletes our Earth's natural resources [1, 27]. Consequently, the need for sustainable alternative solutions is the need of the hour. Nowadays, bioplastics derived from numerous bio-based sources to reduce dependence on petroleum-based materials is a thrust area of research. According to European Bioplastics (2021), the global market for bioplastics is expected to increase from 2.4 million tonnes to 7.6 million tonnes by 2026 [28]. Bioplastics have become commercially available or are in the pre-marketing phase. Examples of biopolymers include starch blends, polyhydroxybutyrates, polylactic acid, cellulose, casein, and chitin polymers [30]. Despite the growing demand of bioplastics market, practically it still represents only 1% of all the plastics produced annually (367 million tonnes/year) across the globe. Thus, extensive research should be focused on the synthesis of bioplastics. There are generally three main categories into which bioplastics are divided (Fig. 2):

1. Bio-based non-degradable plastics: PE (polyethylene), PP (polypropylene), PET (polyethylene terephthalate). These are called bio-based non-degradable plastics because they resemble structurally petroleum-based plastics and are made fully or partially from the natural bio-based source. For instance, let's take PET as an example, which builds from 32% of monoethylene glycol (bio-based source) and the rest 68% of terephthalic acid (petroleum-based source). Therefore, due to their resemblance with petroleum-based sources, they are not biodegradable [5, 6]. As of 2021 data, they hold up to 36% of total global bioplastic production, and the production is expected to decrease by 6% (30%) in the next 4 years [28].
2. Bio-based biodegradable plastics: PLA, PHA, PHB (polyhydroxybutyrate), starch, and cellulose. These types of bioplastics are in high demand as they are manufactured from bio-based sources (plants, microbes) and are biodegradable. Out of all the sources mentioned, the most commonly used is starch due to its abundance,

Table 1 Biodegradation conditions for different types of plastics

Types	Biodegradation conditions	Microorganism	Enzyme	References
Bioplastic-PHB	Natural conditions of soil-biodegradability rate of 64% within 180 days Sea water-achieve 80% biodegradability rate within 14 days	<i>Streptomyces bangladeshensis</i> , <i>Pseudomonas lemoignei</i> , <i>Penicillium sp.</i> , <i>Aspergillus oryzae</i> , <i>Candida rugosa</i>	Proteinase K cutinase Lipase	[14–19]
Polystyrene (PS)	Stable structure and high molecular weight of PS hinders enzymatic degradation Low biodegradation rate in natural conditions and in presence of sunlight	<i>Lentinus tigrinus</i>	Esterase, styrene	[13, 20, 21]
Polyethylene (PE)	Inert nature of PE Slow biodegradation rate in natural conditions Resistant to anaerobic conditions (no biodegradation in high temperatures of about 350 °C)	<i>Phanerochaete chrysosporium</i> , <i>Trametes versicolor</i> , <i>pseudomonas sp.</i> , <i>Aspergillus flavus</i>	Hydroxylases, laccases, peroxidases reductases	[13, 21, 22]
Polypropylene (PP)	Slow biodegradable rate (below 1%) in natural environment of soil Biodegradability rate can increase upon pretreatment with high temperatures	–	–	[13, 23, 24]
Polyvinyl chloride (PVC)	Non-biodegradable (no degradation when buried in soil even after 30 years) Slow rate under UVB radiations	<i>Pseudomonas citronellolis</i> , <i>Bacillus flexus</i>	–	[23, 25, 26]

Fig. 2 Types of bioplastics



easy availability, low cost, good mechanical properties, and biodegradability. Starch-based bioplastics hold a good global plastic production capacity of (16.4%) as of 2021 reports. However, as most starch comes from plant-based sources, its use will decrease soon [28, 43, 44]. According to European Bioplastics 2021, these bio-based biodegradable plastics hold the maximum share in global plastic production of around 60% (2021) and are expected to increase to 70% by 2026 [28].

3. Fossil-based biodegradable plastics: This group of polymeric materials represents the fossil-based biodegradable plastics that are employed mainly to enhance the physicochemical properties of bio-based biodegradable plastics. The degradability of this polymer is achieved with the incorporation of hydrolytically unstable bonds into the amide, ether or ester bonds of the polymer (ether, amide, or ester bonds), which are generally more susceptible to hydrolytic attack. The most common polymers are polybutylene adipate terephthalate (PBAT) and polycaprolactone (PCL) [45, 46]. These plastics are biodegradable, and their production from biomass may be possible in the future.

The different types of bioplastics such as biodegradable bio-based bioplastics that hold up to 70% and 30% belong to other non-biodegradable bio-based plastics. The most commonly used biodegradable bioplastics are PLA, PHA, PBS and starch blends. According to 2021 data, PLA-based biopolymers (Polylactic acid) and PHA-based biopolymers (Polyhydroxyalkanoates) have a market share of 18.9% and 1.8% (expected to increase by 2026). On the contrary, Starch blends hold up to 16.4%, and PBS (Polybutylene Succinate) up to 3.5%, which will increase by 2026 to 16% [28]. These bioplastics are widely used in the packaging industry followed by other applications which include agriculture, electronics and consumer goods [28]. Bioplastics are better than synthetic plastics in every possible way concerning sustainable development. They provide benefits such as bio-based raw materials instead of using natural resources, being environmentally friendly, harmless to health compared to petroleum-derived plastics, and many more [29]. Moreover, they save the environment by reducing greenhouse gas emissions. For example, when PLA is substituted with PET (polyethylene terephthalate), it reduces greenhouse gas emissions by 60%. Similarly, when replaced with PE, it saves 35% of harmful gas emissions [31].

Nowadays, bacteria and food crops like sugarcane, corn, potatoes, and wheat are used commonly for bioplastic production. The plants are used as a feedstock from which starch, carbohydrates, and enzymes are extracted, which ultimately are used for bioplastic production by blending them with plasticizers like glycerol, sorbitol, and other chemicals [32, 33]. Though plants produce bioplastic with good

mechanical and chemical properties, they cause a threat to the sustainable future resulting in high feedstock competition for human consumption [34]. Moreover, the methods of extracting bioplastic from plants are complicated and possess issues such as poor mechanical properties and water resistance [35]. Therefore, with all these rising concerns about plant-based sources, there rises a need for a better alternative of biodegradable plastic polymer source that is economical and valuable to our sustainable future [36]. Algae, especially microalgae, are gaining enormous attention these days due to their broad range of applications (nanotechnology, biofuel production, bio-filters, biofertilizers, pharmaceutical industry), and researchers over the past few years are now focusing on working on synthesizing bioplastic from algae [1, 35, 37, 38].

According to previous reports, the maximum concentration (80%) of PHB is produced by *Chlorella vulgaris* [39]. A number of cyanobacteria species (*Arthrospira*, *Nostoc muscorum*, *Synechocystis* PCC6803 and *Synechococcus* sp. MA19.) are also reported for PHA production [40]. Algae is emerging as a potential source for synthesizing biodegradable polymers as it is economical, omits the use of a bioreactor and additional nutrients unlike bacteria, holds high biomass production, and is simply available throughout the year [41]. Also, algae do not compete with food sources, can survive the harsh environment, can restore water quality through wastewater treatment, and aid the removal of carbon dioxide from the surroundings by utilizing it as a nutrient source for biomass production [1, 42].

Instead of fossil-based plastics, bio-based biodegradable plastics are preferred more these days due to sustainability (based on source) and biodegradability rate. Bio-based biodegradable plastics sources are cellulose, starch, PHA/PHB, and microbial biomass. PHA and PHB are produced naturally by bacterial fermentation of sugars and lipids or through algal biomass. Both polymers are widely accepted and used to make bio-based biodegradable plastics due to their sustainability and biodegradable rate, which is why they are widely used in the packaging, medical, and pharmaceutical industries. Algae are now emerging as a better source of bioplastic production (sustainable, biodegradable, cheap, easy to availability throughout the year, and less harmful than fossil-based plastics). Researchers all over the world are using several strategies to produce algal bioplastics such as

- Blending microalgal biomass with starch
- Biopolymers like PHBs
- Starch cultivated intracellularly
- Microalgae biomass as a feedstock for polyhydroxyalkanoates production
- Biorefinery approach to producing PHAs [36].



Despite having so many benefits of bioplastics, still, it requires extensive research for better modifications to eliminate its shortcomings. Modern bioengineering tools like synthetic biology, CRISPR-cas9, and metabolic engineering are at the preliminary research stage that requires lots of input. The researchers face many challenges in sustainable bioplastic production—selecting suitable polymers from the algae and omitting the emission of harmful gases from bioplastic. The factors that need to be well thought out for the selection of suitable polymers are biodegradability, sustainability, good mechanical and thermal properties, consumer acceptability, size, molecular weight, and moisture content.

Bioplastics are relatively more costly than synthetic plastics resulting in no market demand for them. This issue requires further insight, and thus government should take active measures by providing more funding for this research and impose strict banning on harmful synthetic plastics. Regardless of the urgent need for bioplastic, many challenges lie ahead for researchers to eliminate so that the day will come when bioplastic will become smoothly available in the market, economical, and pollution-free.

2 Methodology

A gap analysis of the literature was performed using 124 articles published till 2022 which contains the latest facts and information about bioplastics. A Web of Science search showed that the bioplastics production from algal strains is not extensively exploited and there is very limited literature on bioplastics production by different algal strains, especially microalgae. A well-defined systematic search of internet sources, research and review literature articles were followed to understand different mechanisms associated with algal bioplastic production. Information on bioplastics was also collected from press releases, media, and newsletters. Search keywords were either closely related terms of algal bioplastics such as bio-based plastics, degradable plastics, life cycle assessment, microalgae, types of bioplastics, and policies to eradicate plastic pollution were used in this study. We performed Google and PubMed search by all keywords and it was observed that after approximately 150–200 articles, the searches were either unrelated or duplicative. We also searched for different policies laid down government of different countries for manufacturing and consumption of plastic associated products. The fundamental gaps related to the life cycle assessment (LCA), the need for modern bioengineering techniques for scale-up production, bioplastic market size, issues faced by up-scaling of algae and the need for more research in specific areas were performed through this article. There are limited data on LCAs of every type of bioplastics available which will ultimately help to choose the most sustainable bioplastic for public use. This

review describes detailed LCA to access the potential impacts of products on the ecosystem throughout a product's life cycle. Also, detailed and important analysis of bioengineering techniques like CRISPR/Cas9, metabolic engineering, and synthetic biology for bioplastic production is also mentioned which directs more research in these critical areas. The review connects different important points of bioplastics and joints them to formulate a beautiful picture.

3 History and Present Scenario of Bioplastics

The first man-made bio-based plastic named Parkesine (1862) is derived from cellulose. Later, several attempts to develop synthetic plastics started, which serve a wide variety of applications. Plastics such as celluloid (1868) and Bakelite (1897) are derived from formaldehyde resins [47, 48]. However, increased environmental damage, including waste disposal issues, greenhouse gas emissions, depletion of natural resources, and poor biodegradability, has forced scientists to find the best alternative. Biodegradable polymers were needed because of the serious environmental concerns and diminishing available landfill areas.

At present, three types of biodegradable plastics are used widely—polymers having susceptible groups that are prone to hydrolysis attack by microbes, naturally occurring PHA, and polymer blends with several additives biodegraded by microbes [49]. The increasing interest in bioplastic production has led the scientific community to evaluate more of its properties, out of which bioplastic biodegradation is one of the most crucial factors for the evaluation to examine its use in terms of sustainable development. Figure 3 illustrates the mechanism of biodegradation of bioplastic by the action of microbes. The mechanism of plastic polymer degradation starts with microbes attaching to the surface of polymers followed by secretion of extracellular enzymes for hydrolysis attack, which induces depolymerization of high molecular weight polymers into simple monomers [50]. Biodegradation by microbes usually happens under two conditions— aerobic and anaerobic digestion. In aerobic digestion, microbes utilize oxygen and release water and carbon dioxide as end products. In anaerobic digestion, biodegradation of polymers occurs in the absence of oxygen (anaerobic and methanogenic microbes) and yields methane, carbon dioxide, water, and other metabolites as end products [51–53].

The PHA, PLA, and PHB derived from crops are used widely because of their easy processing and biodegradability. As these are continuously being used, the time will soon come when there will be a competition for food as the population is rising rapidly. Many factors affect the biodegradability rate of bioplastics like; the type of polymer used and exposure to temperature, pH, etc. Table 2 enumerates factors affecting

Fig. 3 Mechanism of biodegradation of plastic polymers by microorganisms

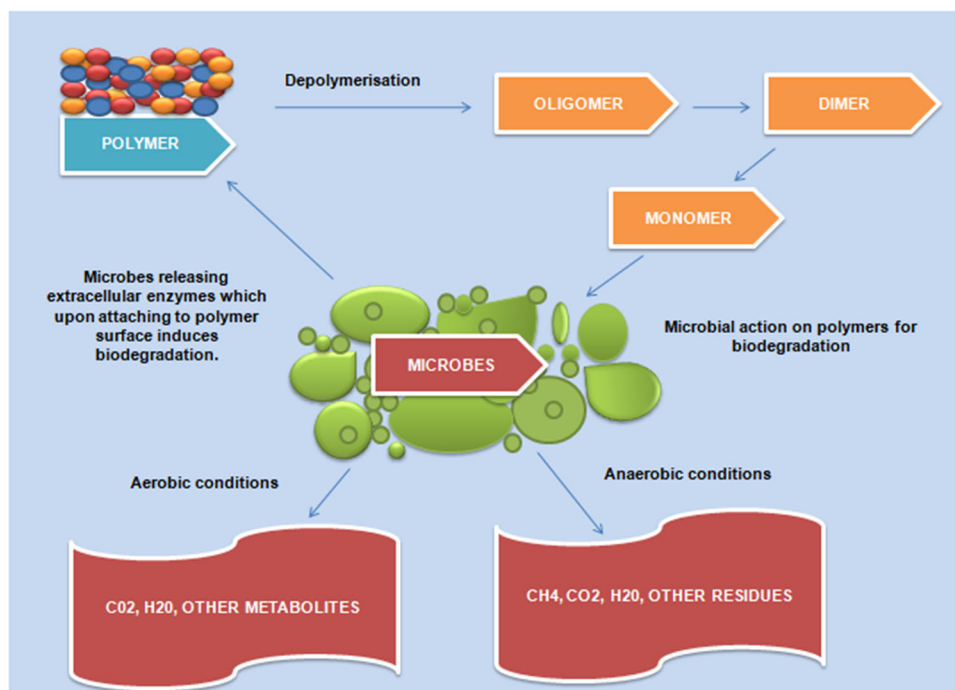


Table 2 Factors affecting biodegradability rate

Different factors	Description	References
1. Based on exposure		
Moisture	Rate of biodegradation of plastics increases upon presence of adequate moisture in soil as moisture enriched environment will facilitate process of hydrolysis	[36, 54]
Temperature and pH	Hydrolysis of polymers can be achieved through change in pH by providing optimum basic and acidic conditions. For example, Optimum pH for PLA hydrolysis is 5 Unfavorable conditions like high temperature will decrease the activity of microbial enzymes, hence decreasing biodegradation rate (depending upon polymer used-exception is PLA having high biodegradability in presence of high temperature)	[36, 54–56]
Enzymatic exposure	Wide polymer biodegradation because of the presence of unique active sites on enzymes. Enzymes cleave the complex polymer into simple monomers through hydrolysis. For example, lipase is responsible for degradation of polylactic acid	[36, 46, 57]
2. Based on polymer characteristics		
Molecular weight	Molecular weight has inverse relationship with rate of biodegradability. Lower the molecular weight of plastic polymer, higher will be biodegradability rate as they can be easily depolymerized by microbial enzyme action	[36]
Size and shape	Polymers with large surface area and lower complexity in shape can be degraded faster compared to polymers having smaller surface area and 3D structures	[36, 54, 58]
Biosurfactants	Addition of biosurfactants will increase rate of biodegradation, hence will enable depolymerization of polymers under extreme conditions as well due to presence of specific functional groups in biosurfactants	[36]

the biodegradability rates of plastics. Bioplastics are used globally for food packaging, crockery production, and other industrial applications like clothes, cosmetics, toys, sports, medical kits, household products, constructions, electronics, and transport [28]. The use of bioplastics will thus continue to rise in the future if we improve its major drawbacks (discussed later in the paper).

4 Advantages of Bioplastic

4.1 Eco-friendly and Low-Carbon Footprint

Bioplastics often produce fewer amounts of carbon-containing gases (greenhouse gases like carbon dioxide and

methane) and contain no harmful toxins than traditional plastics. There is no net increase in carbon dioxide gas emission upon biodegradation of bioplastics. The amount of CO₂ generated during biodegradation is the same as the amount absorbed by plants for growth [59]. According to experiments conducted by Yu and Chen [60], there was 80% less emission of CO₂ observed in the case of bioplastic when compared with traditional petrochemical plastics. However, the carbon footprint of a bioplastic critically depends on the storing capability of carbon extracted from the atmosphere by the growing plant [61].

While bioplastics are derived from renewable resources like plants and microorganisms, they are considered to be eco-friendly. Moreover, they emit less greenhouse gas emissions and are biodegraded in months, causing less harm to the environment [48, 62].

4.2 Biodegradable

Compared to synthetic plastics which are derived from non-renewable resources (petroleum), Bioplastics are derived from renewable resources (plant and microbial biomass). As the world is now facing a shortage of natural resources (coal, petroleum), the time will soon come when the world stock of natural resources will completely vanish, posing a threat to future generations [63, 64]. During recent years of development, biodegradable polymers developed from renewable resources are gaining significant attention because of their excellent biodegradability rates in natural environments, unlike petroleum-based plastics. For example, PLA-based biopolymers have an 84% biodegradability rate (humidity and aerobic conditions) and take 60 days for biodegradation [62, 65]. Similarly, PHB-based biopolymer achieves biodegradation in > 3 months (industrial-based biodegradation).

4.2.1 Kinetics of Biodegradation

The biodegradation potential of bioplastics is followed by measuring either the oxygen demand or carbon dioxide evolution or is based on the amount of carbon dioxide and methane evolved when the bioplastic aerobically or anaerobically biodegraded. Thus, the biodegradation standard tests generally account for the mineralized carbon and are not dependent on the carbon which is fixed in the form of cell biomass. The mineralization gives us information regarding the actual metabolic ability of microorganisms to convert the polymers into biomass, gasses (CO₂, CH₄), and potentially other metabolites. Thus, it is important to study overall carbon balance analysis which could give us comprehensive information on biodegradation. This carbon balance approach is very important to quantify biodegradation and thus provides us detailed conclusion regarding the fate of

polymer and its potential biodegradation in a given environment. The biodegradation kinetics and carbon fate of several bioplastics have been investigated under both aerobic and anaerobic conditions in aqueous conditions. According to standards, PCL was biodegraded only under aerobic conditions, while the degradation of PHB and PHBV is not dependent on medium conditions. The polymers of lower particle sizes generally favored higher rates of mineralization rates. The combination of carbon balance and kinetics analysis will provide a detailed and improved development in the field of testing of biodegradable components. Therefore, an experimental framework is required to study a detailed understanding of biodegradation pathways and also the correlation between various factors influencing biodegradation [66].

4.3 Energy Efficiency

Production or process of manufacturing bioplastic requires less amount of energy compared to traditional synthetic plastics. It has been experimentally proved that using PLA instead of PET, leads to a reduction of about 40% of energy, whereas substituting Master-Bi bioplastic with PE saves about 27% [31]. Master-Bi bioplastics are produced by mixing starch with PCL (Polycaprolactone), another biodegradable polymer, and are widely used in the packaging industry [67].

Traditional plastics made from petroleum products influence the world's total petroleum consumption as this traditional plastic-like propylene, and styrene-based plastics are manufactured from petroleum oil. It is estimated that up to 99% of plastics come from petroleum and use about 4% of total oil consumption for production. Moreover, if this trend continues in the future, then plastics will use about 20% of the world's total oil consumption by the end of 2050 [68, 69].

4.4 Reduction in Litter

The use of bioplastic will reduce permanent litter in the environment, especially in marine life. Plastic bags used in shopping or packing are the most common examples showing pollution caused by traditional plastics (the majority of the fraction of the litter in the ocean originated from plastic bags only).

4.5 Other Advantages

Greater water vapor permeability, more tactile, good surface quality, clearer and more transparent, and less probability of imparting different taste to the product when stored in a bioplastic container [70, 71].



5 Disadvantages

5.1 High Cost of Manufacturing

Bioplastics are double the cost when compared to traditional plastic due to less market value and less government funding. However, with the rise in bioplastic need and its production, the cost will decrease [51].

5.2 Increasing Competition for Food

Bioplastic can be made from microbes as well as using plant sources. Plant sources include corn, sugarcane, etc., which upon ample usage during bioplastic production will lead to competition for food. Hence, there is an urgent need for exploring more raw materials which should be cost-effective and economical [72].

5.3 Emission of Greenhouse Gas

Upon decomposition, bioplastic polymers produce methane, which is 20 times more toxic than carbon dioxide [73]. However, it depends on the type of bioplastic and its physical/chemical properties [74]. Also, it depends on the method of disposal of bioplastic. Most of the bioplastic stays inert in landfills and poses little threat but some studies showed that bioplastic produces methane only in anaerobic biodegradation, depending upon the type of bioplastic subjected to the process of biodegradation [74, 75].

6 Microalgae: An Emerging Source for Bioplastic Production

Agricultural sources are used widely for the production of bioplastics. Although they are giving promising results, many challenges which are concerned with depleting sources, competition for food, and poor sustainable development lie ahead. Microalgae are an emerging potential source for bioplastics production, responsible for a sustainable future that does not lead to food competition, unlike plant-based bioplastics. The following are the advantages of using microalgae as a source for bioplastic production [1, 35, 76].

- Widely available throughout the year, they are known to colonize water bodies, even sewage water.
- Less harvesting time.
- It does not lead to food competition for human consumption.
- Can survive harsh conditions like high temperature, humidity, etc.
- High biomass production rate in the form of starch (18–46%), PHB, PHA, lipids (12–48%), and proteins

(18–46%), which is 5–10 times faster if we compare with food crops.

- It does not require any additional nutrient sources to grow and can capture carbon dioxide from the environment (as algae are photosynthetic organisms) and uses it as a carbon source, thus helping in decreasing greenhouse gases (carbon dioxide). Moreover, carbon gas entrapped will not be emitted back into the atmosphere. It is observed that 1 kg of microalgae utilizes 1.83 kg of atmospheric carbon dioxide for growth, thus helping to remove excess CO₂ from the environment [77, 78].
- Algae-based biopolymers have a good biodegradable rate and hence can perform industrial applications like food packaging, the agriculture industry, etc.
- High protein and lipid content in algae serves as a beneficiary factor for generating PHB biopolymer through the thermo-mechanical polymerization process.

There are several approaches to manufacturing bioplastics through microalgae, like directly using microalgal biomass, the Biorefinery approach, and the blending approach (described later in the paper). *Chlorella* (green algae) and *Spirulina* (bluish-green algae) are now becoming the center of attraction for bioplastic production due to crack resistance and hard cell wall, and high biomass production [76]. Other microalgae species include diatoms (*Phaeodactylum tricornutum*) and *Microcystis aeruginosa* from which PHB biopolymer can be extracted for bioplastic production and green algae (*Chlorella sp.*) from which starch granules are produced to act as raw materials for bioplastic processing. Table 3 depicts different algal species used for bioplastic production.

Many studies were conducted for optimizing microalgae culture conditions to enhance PHA/PHB productivity. A report published by Kavitha et al. [84], on *Botryococcus braunii* algae for PHB production by optimizing culture conditions and the results showed maximum production of PHB (247 ± 0.42 mg/L) by using sewage water (60%) as a culture medium [84]. In another study, the production of starch-based bioplastics under sulfur-depleted medium conditions gave excellent starch content [85] for bioplastics production. Also, bioplastics production through leftover algal biomass is also a sustainable approach and this was proved in a study in good amount of PHB content (27%) was produced with leftover algae biomass [86]. The microalgae are also low-cost substrates which are used for PHA accumulation ($7.51 + 0.20$ g/L) inside bacterial strains that will decrease the cost of bioplastic production through bacteria [87].



Table 3 Types of bioplastic products generated by different microalgal species

No.	Algal species	Product type	Bioplastic content (mg mL ⁻¹)	Culture conditions	References
1	<i>C. vulgaris</i>	100% algal-based plastics Hybrid blends PHB	–	Aeration and phosphorus starved media elevated PHB levels	[27, 79]
2	<i>Microcystis aeruginosa</i>	PHB	0.49 ± 0.5	Biomass collected from high-rate algal pond (HRAP) and no additional nutrient required	[80]
3	<i>Haematococcus pluvialis</i>	PHB	0.39 ± 0.42	Biomass collected from (HRAP)	[80]
4	<i>Phaeodactylum tricornutum</i>	PHB	–	Nitrate-rich medium	[81]
5	<i>Botryococcus braunii</i>	PHB	247 ± 0.42	60% concentration of sewage water as culture medium	[82]
6	<i>Nannochloropsis gaditana</i>	Biomass PBAT	–	–	[83]

7 Different Strategies of Bioplastic Production Through Algal Biomass

7.1 Blending Microalgal Biomass with Bioplastics

PLA (derived from lactic acid) is completely bio-based plastic produced from plant sources like corn and sugar beet, which makes them supreme candidates for blending with microalgal biomass as they are biodegradable in nature [88]. Studies conducted on green algae where its biomass is blended with PLA in the low algae-to-plastic ratio (20:80) produce bioplastic with a tensile strength of 45 MPa [89]. Figure 4 compiles different strategies of bioplastic production from biomass.

7.2 Blending Algal Biomass with Starch

Starch which contains a long chain of amylopectin and amylose has good film-forming properties and is widely used for several industrial-based products including bioplastics [90]. Starch-based bioplastics are often renewable, biodegradable, and cost-effective. Most importantly, starch blend bioplastics are biodegradable at a higher rate by increasing the starch to microalgal biomass ratio [91].

7.3 Microalgae Biomass as a Feedstock for PLA Production

Although PLA is a bio-based biodegradable plastic, it leads to competition for food as they are derived mainly from plant-based sources like sugarcane and corn. So to overcome this barrier, nowadays PLAs are produced by algae which are non-competitive for human food. Generally, it yields through

fermentation of algal biomass aided by bacteria, followed by the polymerization step [92]. PHAs and PHBs are now widely used as they are microbial-driven bioplastics but still need further inputs for finding more economical substrates and raw materials for bioplastic production to reach their full potential. PHBs possess comparable properties to synthetic plastics in having a melting temperature of 179 °C, high tensile strength of 40 MPa, and a young modulus of 3.5 GPa [93].

7.4 Biorefinery Approach to Producing PHAs

The use of the biorefinery approach enables the production of more and more products from biomass utilizing less energy compared to fossil refineries. The biorefinery approach serves a wide range of advantages like producing high-value products with low energy demand, high flexibility of raw materials and products, and producing minimum waste [94]. The biorefinery approach harvests bioproducts from microalgae with enormous economical benefits, viz. cost-effective production [95, 96]. The most common biorefinery approach used is using wastewater microalgae as a feedstock for the production of various products which includes biodiesel, ethanol, and PHB formation using a wet lipid extraction process. Figure 5 illustrates different steps involved in PHB bioplastic production from microalgae through the biorefinery approach [97].

As microalgae biomass generates a wide variety of products like proteins, starch, lipids, vitamins, biopolymers, etc., these products are often used as feedstock for producing high-value products at a time like bioplastics, biofertilizers, biofuels, etc., using biorefinery approach. The use of this



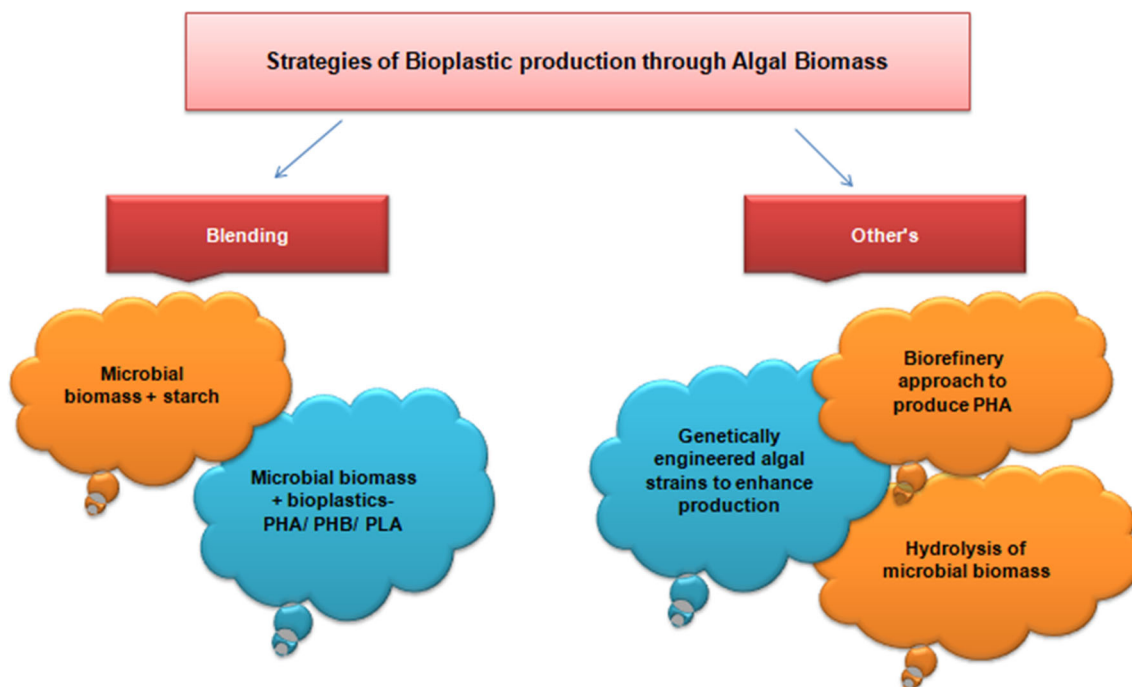
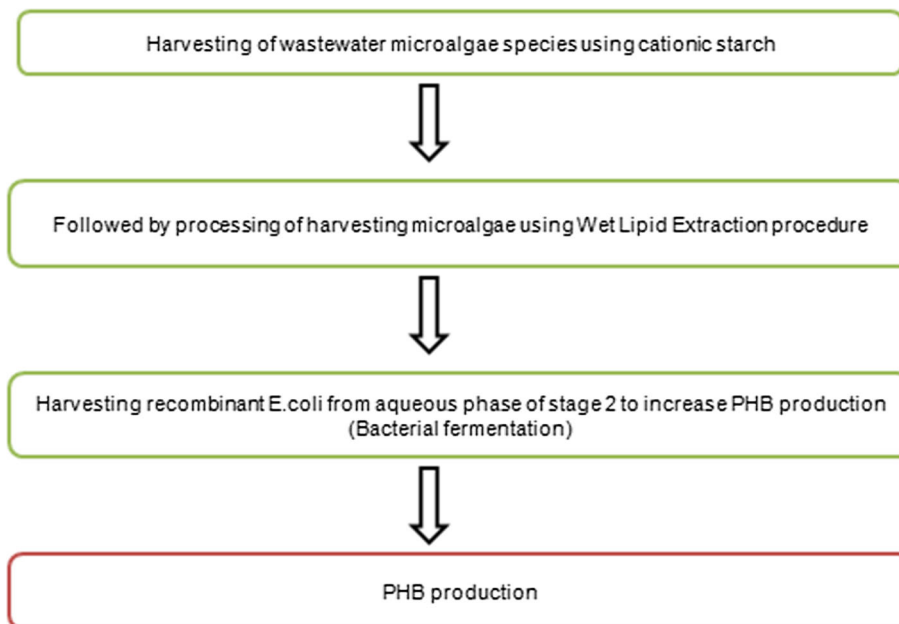


Fig. 4 Different strategies of bioplastic production using algal biomass

Fig. 5 Steps for bioplastic (PHB) production through wastewater biorefinery approach



approach is in huge demand because of its potential of generating high-value products at a time and it is quite sustainable as saves energy, time, and labor costs for setting individual setups for different products [98].

7.5 Hydrolysis of Microalgae Biomass for PHA Production

Hydrolysis of wastewater microalgal biomass as machinery for PHA production is also being used for bioplastic production. Studies showed that PHB biopolymer production can be achieved when recombinant *E. coli* is grown on hydrolyzed wastewater microalgal biomass (microalgae-based media as microalgae provide nourishment to growing *E. coli*). This

approach gives a PHB yield of 31% [99] given the fact that it is economical and can yield a wide range of bioproducts.

8 Issues Faced in Up-Scaling of Algal Biomass

The most economical method of up-scaling algae biomass based on non-sterile conditions is using an open-pond system using wastewater, which is an environmentally and economically feasible solution to eliminate the risk of industrial hazards. Also, this type of cultivation often demands lower energy inputs than other cultivation strategies which include the use of bioreactors [100, 101]. However, there are several technical challenges to the harvesting strategy that limits algae scale-up under non-sterile conditions. Challenges such as:

- Separating harvesting algae from water and energy used for separating algae biomass is quite high, making algae harvesting processing somewhat costly [102].
- Risk of genetic contamination and other microbial contamination.
- Not all microalgae species can be grown in these conditions [103].
- Challenges in the selection of strains as there is no guarantee that strains with specific traits will prevail under these conditions [1, 103, 104].
- Other limitations include; difficulty in temperature control, lessening algal photosynthetic efficiency, and hydrodynamic stress [103].

For these reasons, the need for bioengineering gene-editing tools to develop strains with specific traits arises, ensuring high polymer productivity in desired algae strains. Moreover, as non-sterile harvesting conditions enable only some specific algae species to grow, more limited by-products will be given by those algal strains.

9 Modern Bioengineering Techniques to Enhance Production of Bioplastic

Reports have indicated that even if PHA/PHB is present naturally in several species of microalgae, they are accumulated inside a cell at a very low percentage by weight. A screening test is done on 137 microalgae strains for analyzing their PHA content, of which 133 strains have PHA content lower than 3.5% w/w [105]. Another study was done on chlorella species for PHB content. Results indicated it has 2.15 g/500 mL PHB content [39]; thus, there rises a need for gene-editing tools like CRISPR for modifying enzyme-producing PHB/PHA

content in microalgae species for up-scaling bioplastic production.

As the demand for more and more good quality and quantity of bioplastic is arising, more techniques are being used for producing bioplastic with excellent properties. Of these, advanced cell or gene-editing techniques are widely being used because of their ability to produce hybrid materials with desired properties. Such emerging genetic engineering tools include clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 (RNA-guided cleaving enzyme) cloning systems, which can be used to genetically engineer microalgae strains to produce PHAs [106]. With the help of CRISPR–Cas9 (RNA-guided endonucleases), most of the challenges of bioplastic can be solved by editing the genes which code for enzymes responsible for producing a compound of interest in biopolymers like polyhydroxyalkanoates. This genetic tool can improve the quality and quantity of PHA by editing the genes for PHA Synthase, an enzyme accountable for PHA production in microorganisms resulting in the establishment of good quality bioplastic having huge market value [107]. Such targeted knock-in, knock-out, and knock-down of desired genes are done in microalgal strains like *Chlamydomonas*, *Phaeodactylum*, *Thalassiosira*, and *Nannochloropsis* [108]. But such application of CRISPR–Cas9 for genome engineering of algae is still at the preliminary stages of research and little success has been achieved since. Thus, the use of targeted CRISPR—Cas9 technology still requires significant research to improve current editing efficiencies with a good vector delivery mechanism [109].

Research on advanced techniques like metabolic engineering in which cellular machinery is genetically manipulated to enhance the production of valuable products should be employed in the bioplastic sector. Although many studies of metabolic engineering have been conducted on cyanobacteria for enhanced PHB production [110], but only limited literature is available on metabolic engineering of algal strains for bioplastics production. With metabolic engineering, the cellular processes could be modified in a specific organism through changes in DNA sequences by inducing specific mutations, changes in biosynthetic pathways, and changes at the gene level to elevate the production of effective products by inserting a gene of interest in the target organism. In a study, *Chlamydomonas reinhardtii* 849 algal strain was transformed with the plasmid pYearg4 for enhancing PHB accumulation. The cells after culturing were observed using TEM (transmission electron microscopy) and results showed transformed PHA synthase enzyme to elevate PHB levels inside cells. Thus, this emerging technology has made enormous advances in different fields of biotechnology and in the production of pharmaceutical products like drugs, vaccines, and other chemical compounds [111, 112].



Another emerging bioengineering technique includes Synthetic biology, which aids in improving the biological functions of cells to overcome their challenges and limitations of cell properties [113]. New artificial or existing cellular or biochemical pathways from other organisms can be introduced into algae via synthetic biology approaches to get the desired bioplastic from improvised algae. But if we look through economic perspective, these techniques are quite costly, especially if used on large-scale production of biopolymers. Thus, an extensive study is required for these current emerging techniques for effortless harvesting, smooth extraction of products, and highly efficient production of biopolymers.

10 LCA (Life Cycle Assessment) Analysis of Bioplastics

LCA is a quantitative method by which one can evaluate the potential impacts (positive and negative) of products on the environment throughout a product's life cycle. Two types of studies applied for bioplastic LCA study: Cradle-to-gate (upstream product process), which includes raw material of products, extraction, and manufacturing, and cradle-to-grave (downstream product process), which comprises end-of-life assessment assessing the impact of recycling or biodegradation process/ultimate removal of product at the end, followed by an analysis of bioplastic impact to the environment. Although LCA studies do not represent the actual process and accuracy of an assessment of the product, still several parameters can be considered to provide enough information (generally, assumptions are made) [114].

Like GHG emissions, toxicity, global warming, eutrophication, bioplastic, recycling, and biodegradation scenarios. In obedience to certain LCA studies, bioplastics reduce GHG emissions, considering raw materials extracted from renewable resources, and eliminating toxic production processes, which ultimately reduces the negative impacts of bioplastics on the environment. Such a scenario was observed when PET bottles were replaced with PLA bottles, which significantly decreased GHG emissions by 20%. Also, it saves two-thirds of the energy spent on petroleum-based plastics [59].

The end-of-life, which adversely impacts the environment, can also be studied to assess the environmental impact. LCA studies revealed that incineration and landfilling options are not suitable for bioplastic disposal. The treatment of bioplastic waste management differs from that of petrochemical plastics (unavailability of anaerobic digestion of plastics in most scenarios). While the recycling of bioplastics is costly and poorly established, biodegradation is considered to be a better option now. For example, PLA-based biopolymers biodegrade within 60 days [65] unlike petroleum-based plastics which take years to centuries for degradation. Future

LCA studies need to establish a systematic framework for each type of bioplastic available in the market and society and economic sustainability should also be considered for the evaluation of bioplastics as to whether they truly belong to a sustainable circular economy [59, 115].

11 Challenges and Future Prospects

Bioplastics are gaining enormous attention nowadays because of their eco-friendly nature, positive influence on the environment by minimizing greenhouse gas emissions, and most importantly, plummeting the threat to marine and human health. Regardless of having numerous advantages of bioplastics, several challenges lie ahead:

- High production cost (twice the amount of traditional synthetic plastics) [116].
- Lack of ability to complete degradation in every probable state.
- Need to completely investigate and find all possible finest solutions for negative ecological impacts in which greenhouse gas emission (methane) is a crucial concern nowadays.
- Discovery of innovative inexpensive raw materials for economical production as an alternative to plant-based bioplastics, to save food sources, thus riding a few steps closer toward sustainable development.
- Improvement in algae-based biopolymer pathways (costly and low-efficient production process) for a sustainable future.
- Unawareness among people about the need for bioplastic and the public's negative response to its use. Certain strategies like an increase in marketing of these biopolymers, an economical production process with no greenhouse gas emission, and being completely biodegradable, may lead to a positive impact on society. And in fact, this positive impact was seen among the German population (< 60%) after improving the above-mentioned marketing strategies [117].
- Insufficient information on bioplastic labels by producers [73].
- Need to develop inexpensive genetically engineered microalgal strains for CRISPR and Cas9 systems to improve the quality and quantity of bioplastics.
- Poor water-resistant properties may interfere with bioplastic applications [107].

12 Conclusion

Based on the current scenario, our natural resources are on the verge of depletion because of their wide variety of applications, where the production of petroleum-based plastics holds a particular position. So the need for an alternative solution is frequently rising where, algae-based bioplastics are now a topic of attention because of their sustainable nature, easy availability throughout the year, and feedstock production. Although there are many methods by which bioplastic production from algal feedstock can be done, several technical challenges are there that need to be solved, keeping in mind the current environmental conditions. Moreover, Algae-based biopolymers are still in the experimental stage which makes them inadequate for market commercialization on a large scale. With the constant rise in technical issues caused by plastic recycling, there rises the need for fully biodegradable polymers which is a faster and more economical way of getting rid of plastic litter.

As mentioned above in the paper, despite the increasing plastic recycling rate, only a small proportion of plastic waste can be recycled and this process can be done in 4 ways. The first category belongs to primary recycling, where most intact plastics are subjected to recycling to form products with comparable features to the original product. The second category (most common) belongs to secondary recycling, where products are not fully intact and products produced are of inferior qualities when compared to the original product type. Also, the mechanical recycling method performed from mixed waste streams falls under this category only. Following are the third and fourth categories, which belong to tertiary (polymer not intact, but feedstock and monomers recovered) and quaternary recycling (least intact polymer, thus gets incinerated or destroyed). However, plastic recycling is not always an efficient process and the resulting products' quality can be of inferior grade. The main issue is that there are different categories of plastics with specific composition and characteristics. Also, they include various colorants and additives that cannot be recycled altogether and thus making it difficult for processing. Another major problem is that plastic is flammable in nature and the associated risks of fires at recycling facilities generally affect neighboring societies. Other serious concern is that the plastic recycling process is costly and recycled plastic costs are even very high because of different processes such as collection, sorting, and transportation, and thus reprocessing plastic is significantly expensive. Also, the reprocessed plastic is also itself a waste [118, 119]. Thus, due to all these serious issues, it is a pressing need to opt for more suitable alternative in the form of bioplastics. The bioplastics are much more eco-friendly and economical in nature and thus will eradicate the problems associated with conservative plastic.

So far, no algal bioplastic with a 100% biodegradability rate has been produced, which directs more research to develop fully biodegradable polymers by the scientific community. As mentioned earlier, the biodegradability of biopolymers depends on the type of material used; therefore, bioplastics with superior qualities are needed. The properties include high tensile strength, water- and heat-resistant biopolymers, high durability, high melting temperatures, high resistance toward UV radiations, resistance toward chemicals, longer shelf life with excellent rigidity, completely harmless while retaining its integrity until fully biodegradable, thermal stability, flexibility, and density. Future biopolymers should be 100% biodegradable, so to enable their breaking in a much faster way in every possible environmental condition and cause no harm to nature by eliminating any greenhouse gas emissions (if any). Although the plastic recycling rate is increasing widely, at a slow pace, and uses more energy it is not a sustainable approach. Plastic subjected to recycling is not able to get recycled at all. According to the European Bioplastics [120], existing recycling technologies mostly rely on mechanical recycling that demands separate recycling streams. Most bioplastic like PLA doesn't even have a distinct stream which makes them inefficient for recycling. Thus, only option left is to produce more and more biodegradable biopolymers which will have the potential to biodegrade when subjected to any kind of environmental conditions.

With the improvement in PHA/PHB algae-based biopolymers, a 100% biodegradability rate and high product yield with excellent properties can be achieved. It is quite possible with the invention of more algal strains with more innovative cheap efficient techniques for bioplastic production. Modern techniques like CRISPR–Cas9, synthetic biology, and metabolic engineering also hold exceptional significance. Moreover, there is a need for the exploration of new microbial pathways as well as enzymes to improve current conditions of biopolymer biodegradation on an urgent basis. With more research on algal bioplastics, more sustainable biopolymers can be produced.

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