

Reviewing Biopolymers: Pioneering Sustainable Alternatives to Traditional Plastics Using Algae

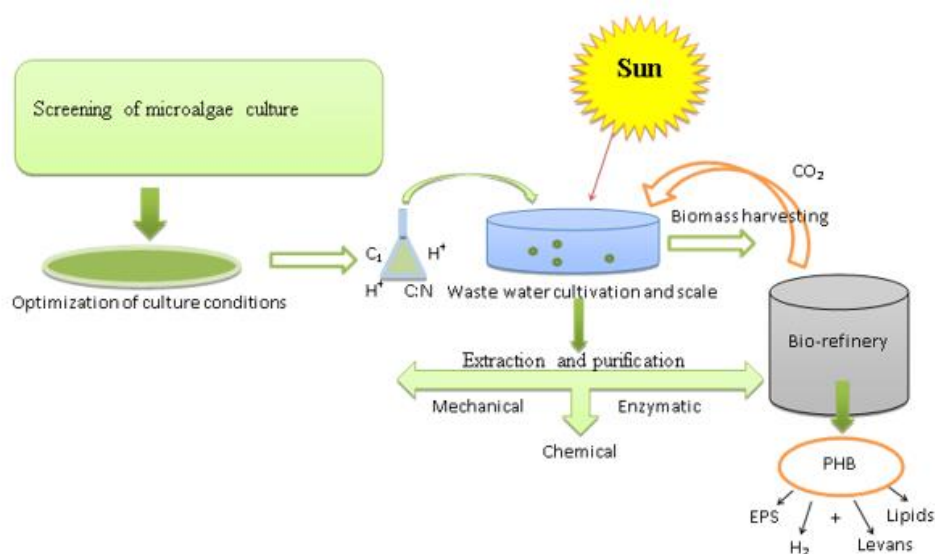
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Graphical Abstract



Abstract

In the wake of escalating environmental concerns associated with conventional plastics, exploring sustainable alternatives has garnered significant attention. Biopolymers, particularly those derived from algae, have emerged as promising substitutes due to their renewable nature and biodegradability. This review synthesizes current research endeavours aimed at harnessing algae-based biopolymers as sustainable alternatives to traditional plastics. The utilization of algae in biopolymer production capitalizes on their inherent capacity for rapid growth and efficient carbon fixation through photosynthesis. Algae offer diverse species, each possessing unique biochemical compositions conducive to biopolymer synthesis. This diversity enables the customization of biopolymer properties to suit various applications, ranging from packaging materials to biomedical implants. The synthesis pathways for algae-based biopolymers, notably polyhydroxyalkanoates, including polyhydroxybutyrate, are elucidated, highlighting the role of genetic engineering and process optimization in enhancing production yields. Furthermore, advancements in downstream processing techniques for extracting and

23 purifying biopolymers from algae biomass are discussed, addressing challenges related to
24 scalability and cost-effectiveness. Environmental sustainability considerations, such as life
25 cycle assessments and end-of-life disposal options, are integral to evaluating algae-based
26 biopolymers. Comparative analyses with conventional plastics underscore the environmental
27 benefits of biopolymer adoption, including reduced greenhouse gas emissions and decreased
28 dependence on finite fossil resources. Moreover, this review examines algae-based
29 biopolymers' economic viability and market potential, exploring factors influencing
30 commercialization, such as production costs, regulatory frameworks, and consumer
31 acceptance. Algae-based biopolymers represent a promising avenue for pioneering sustainable
32 alternatives to traditional plastics.

33 **Keywords:** *Polyhydroxybutyrate, polyhydroxyalkanoates, Biopolymers, Algae-based,*
34 *Sustainable alternatives, Environmental sustainability*

35 **Introduction**

36 The global demand for plastic has risen, driven by plastic-based goods, exerting strain on the
37 existing waste treatment infrastructure. Plastics typically consist of synthetic and semi-
38 synthetic materials, mainly composed of polymers. Plastics include lightweight, cost-effective,
39 easily manufacturable, flexible, and long-lasting characteristics, making them widely utilized
40 by several individuals [1]. Plastics consist of artificial polymers, including polyesters,
41 polyurethanes, halogenated plastics, and acrylics. Plastics primarily impact the living
42 environment of humans, wildlife, and numerous marine species. Plastic deterioration has a
43 direct impact on human life via the drinking of tap water and an indirect effect through the
44 consumption of animals [2]. Plastic trash is categorized into main and secondary forms.
45 Primary plastics were retrieved from their initial location, and the deterioration of these primary
46 plastics resulted in the formation of secondary plastics. Plastics are often categorized as micro,
47 macro, and mega trash. The plastic particles range from 2 mm to 5 mm and are classified as
48 microtrash. Mega debris refers to plastic particles with a dimension of 20 mm and are primarily
49 utilized in food stores [2].

50 Annually, about 8 million metric tonnes of plastic trash are disposed of in the ocean, with the
51 potential for repurposing through the creation of inventive packaging materials [3]. In general,
52 plastics may be categorized into two types: derived from petroleum and biodegradable.
53 Annually, around 1% of the world's bioplastic is manufactured. The starch blend that provides
54 21% of the total bioplastic is anticipated to grow in the market. Bioplastics are primarily used

55 in packaging, textile, construction, and car industries [3]. Natural polymers, such as proteins
56 and carbohydrates, were the primary source of bioplastic synthesis.

57 The manufacturing of bioplastics has had a parallel growth, reaching around 2 million tonnes
58 in 2014 and 6.5 million tonnes in 2018. These bioplastics are primarily composed of polylactic
59 acid-based polymers and starch. Recently, bioplastics have been derived from crops such as
60 maize and potatoes, creating competition with the food business. Bioplastic manufacturing
61 from crops requires extensive land area, fertilizers, and water resources [4].

62 Furthermore, historically, the marketing of bio-based products encountered significant
63 challenges on a widespread level [5]. Microalgae are regarded as a promising biomass resource
64 for the development of bioplastics due to their lack of association with food sources. This
65 organism exhibits the ability to thrive in aquatic environments and can generate substantial
66 amounts of lipids. Moreover, bioplastic production using microalgae is considered more
67 environmentally friendly and may be readily introduced and distributed within the economy
68 and the bio-economy [6]. Bioplastics are applicable in the medicines, cosmetics, and food
69 packaging sectors. Research efforts encompass the processing of microalgae bioplastic within
70 a bio-refinery framework, the formulation of microalgae-polymer composites, and the genetic
71 modification of microalgae strains to produce biopolymers [7].

72 The wastewater from the industrial sector was treated under specific conditions (stress, pH,
73 temperature, aeration duration, and agitation speed) to optimize the synthesis of PHB
74 (polyhydroxybutyrate), presumably by microorganisms present in the wastewater. Hydroxy
75 butyryl CoA, a precursor that undergoes polymerization to generate polyhydroxybutyrate
76 (PHB), was utilized in this process. PHB exhibits insolubility in water and solubility in
77 hydrolytic compounds [8]. It exhibits superior durability against UV light but displays reduced
78 resilience to acidic and alkaline environments. It is non-toxic and readily dissolves in
79 chloroform and other chlorinated hydrocarbons. The melting point of PHB is 175°C, and its
80 glass transition temperature is 2°C. The tensile strength of PHB measures 40 MPa, which is
81 comparable to that of polypropylene [8].

82 Additionally, PHB exhibits reduced stickiness when heated compared to other plastics. This
83 study involved the synthesis of polyhydroxybutyrate from algae, which was then combined
84 with natural polymers in varying ratios. The aim was to survey the resulting mix's physical,
85 chemical, and mechanical characteristics and degradation time. This technology has the
86 potential for several commercial applications, including agriculture, medicine, and the food
87 business. However, it is crucial to implement efficient technology to improve industrialization,

88 commercialization, and scaling-up processes. This study exhaustively scrutinizes several
89 microalgae species used in bioplastic production to uncover any research deficiencies in this
90 emerging field. The text explains opportunities associated with the expansion of microalgae.
91 This review study comprehensively examines all facets of the technique and production.

92 **Characteristics and attributes of poly- β -hydroxybutyrates in terms of their chemical and** 93 **physical qualities**

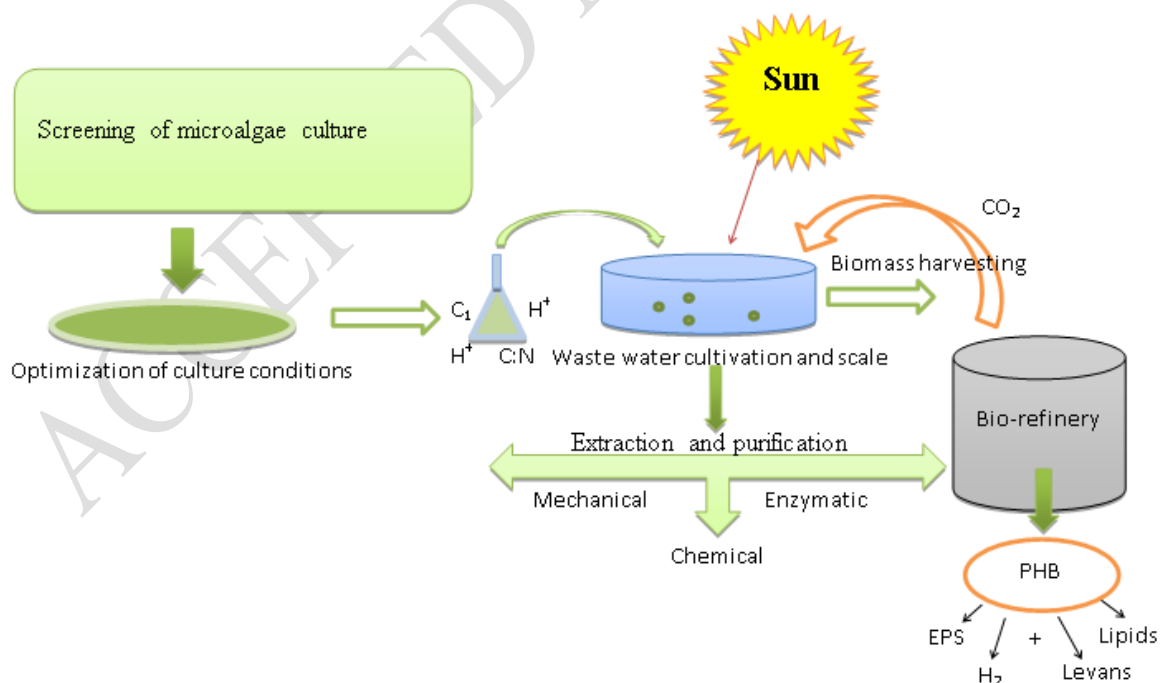
94 The synthesis of Poly- β -hydroxybutyrates begins with the precursor molecule acetyl coenzyme
95 A (acetyl-CoA). This process involves three consecutive enzymatic reactions. The first step is
96 the conversion of 2-acetyl CoA to 1-acetyl CoA, which is catalyzed by the enzyme 3-
97 ketothiolase. The second step is the production of D-3-hydroxybutyrylCoA, achieved through
98 converting NADPH-dependent acetoacetyl-CoA reductase to acetoacetyl CoA [9]. The D-3-
99 hydroxy butyryl moiety was connected to the existing PHB molecule by an ester bond
100 facilitated by the last enzyme, PHB synthases. Therefore, substituents or unsaturation were
101 detected in the fatty acids at positions 4, 5, and 6 of the pendant group, where a hydroxyl group
102 is located. Simultaneously, PHB and poly three hydroxy valerates (PHV) were categorized as
103 short-chain length PHAs and merged to constitute a PHA class [10]. On the other hand, it has
104 been reported that the typical chain length of PHAs ranges from carbon six to carbon 16,
105 explicitly referring to 3-hydroxy fatty acids. Additionally, it has been proposed that the PHB
106 homopolymer synthesized by bacteria consists of more than one molecule and contains 3%
107 hydroxy valerate monomers [11].

108 The combination of Valerate and glucose served as the substrate for the microorganisms. These
109 microorganisms play a crucial role in synthesizing small-chain-linked PHAs [12]. The
110 formation of PHBHx -copolymers, consisting of 3-hydroxyl hexanoate units in combination
111 with PHAs copolymers of PHB, was described [13]. Random copolymers were formed by
112 alternatingly introducing different substrates into the current culture. This process led to the
113 synthesis of a PHA block by bacteria. The molecular mass of PHBs generated by bacteria is
114 typically 4.6×10^6 Da, with a poly disparity (M_w/M_n) of around 2.0 [14]. The biomaterial
115 polypropylene has comparable material properties [15, 16]. The PHB homopolymer [17]
116 exhibited a high degree of crystallinity. This material is rigid and brittle, yet it possesses elastic
117 capabilities. When twisted into fibers, it was seen to have an elastic behavior [18]. The helical
118 crystalline structure is a characteristic of homopolymers. This copolymer, characterized by its
119 structural composition, exhibits superior durability against ultraviolet light but reduces
120 resilience to acidic and alkaline environments. It shares structural similarities with many other

121 copolymers [19]. A recent study analyzed PHB's melting behavior and crystallization [20] and
122 examined its physical properties.

123 Investigate trends in the generation of Poly- β -hydroxybutyrates.

124 Microalgae gained attention for research following the discovery of *Spirulina* in Spain before
125 1519 [21]. Scientists have extensively studied the nutritional properties of microalgae and made
126 significant discoveries, such as the PHB homopolymer, which holds great potential for various
127 applications benefiting humankind [22]. Microalgae have been employed for bioremediation
128 in wastewater treatment for an extended period. Using wastewater to culture microorganisms
129 has become a leading practice in resource recovery—the authors of this publication aimed to
130 highlight twenty years of trends in PHB research. A comprehensive evaluation was conducted
131 on indexed publications published between 1999 and 2020, focusing on the search for
132 microbial plastics and biosynthesis. The keywords used for the search were PHB, microbial
133 bioplastics, and biosynthesis [23]. This assessment provides a comprehensive analysis of the
134 research and development activities from a chronological standpoint. The literature research
135 indicated that “PHBs” was the most often utilized keyword during twenty years, accounting
136 for 50% of the occurrences. The process of synthesizing polyhydroxybutyrate (PHB) is seen in
137 Figure 1.



138

139

Fig. 1 Biosynthesis of PHB

140 While algae are well-known for their ability to produce polyhydroxybutyrate (PHB), other
141 microorganisms, including certain types of bacteria, can also synthesize PHB. Activated
142 sludge, a mixture of bacteria, fungi, protozoa, and other microorganisms used in wastewater
143 treatment, can contain bacteria capable of producing PHB. Several types of bacteria, such as
144 species of *Bacillus*, *Pseudomonas*, and *Alcaligenes*, are known to produce PHB as intracellular
145 storage granules when they are under conditions of nutrient limitation, mainly when there is an
146 excess of carbon source available. This ability makes them useful in various biotechnological
147 applications, including producing biodegradable plastics like PHB [22,23].

148 **Biological production of polyhydroxybutyrate**

149 Multiple studies were conducted in biosynthesis to generate polyhydroxybutyrate via the
150 microbial fermentative method [24]. The production of polyhydroxybutyrate involves three
151 fundamental steps catalyzed by three different enzymes. Firstly, the enzyme B-keto thiolase
152 catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. Secondly,
153 the enzyme *phaB*, encoded with NADPH-dependent acetoacetyl CoA dehydrogenase, reduces
154 acetoacetyl-CoA. Lastly, the enzyme PHA synthase, attached with *phaC*, catalyzes the
155 polymerization of 3-hydroxy acid (3HA) units, specifically (R)-3-hydroxy butyryl-CoA. The
156 result is the transformed acetyl-CoA used in the PHB biosynthetic pathway.

157 **Microalgae and additives**

158 The Algae species are classified as microalgae and macroalgae, with one million species
159 identified [26]. Microalgae, microscopic organisms, utilize solar energy to make adenosine
160 triphosphate (ATP), a compound that may be found in fresh waters and coastal locations [27].
161 Microalgae are utilized as a substitute for several sources in biofuel manufacture.
162 Pharmaceutical formulations extensively use it, while cosmetics might be a supplementary
163 product in the food business [28]. The development of the bio-economy using microalgae has
164 shown constant improvement [29]. Microalgae consist primarily of proteins (6–52%), lipids
165 (7–23%), and carbohydrates (5–23%) [30]. In addition, microalgae have a composition that
166 includes iron (1395–11,101 mg·kg⁻¹), phosphorous (0.7–1.5%), manganese (45–454 mg·kg⁻¹),
167 copper (18–102 mg·kg⁻¹), magnesium (0.3–0.7%), selenium (0–0.5 mg·kg⁻¹), calcium (0.1–
168 3.0%), sulphur (0.4–1.4%), potassium (0.7–2.4%), sodium (0.8–2.7%), and zinc (28–
169 64mg·kg⁻¹) [21]. The subsequent sections will provide a detailed analysis of bioplastic
170 manufacture, focusing on using additives, microalgae species, and chemicals [30].

171 **Chlorella**

172 *Chlorella* is a genus of photosynthetic microorganisms that belong to the green algae family.
173 It predominantly inhabits aquatic environments and primarily comprises around 58% protein.
174 Its fracture resistance is highly effective because of its clustered cell walls and exceptional
175 thermal stability compared to *Spirulina* [31]. These species are primarily used in mixes of
176 biomass and polymers. The comparison between blends consisting mainly of
177 polymers/additives and microalgae biomass (100%) for bioplastic manufacture demonstrated
178 the necessity of efficient combining for commercial use [32]. The test findings showed that
179 using *chlorella vulgaris* yielded superior bioplastics to *Spirulina*. Nevertheless, *Spirulina* has
180 superior mixing characteristics compared to *Chlorella* [33]. The study suggested that a high-
181 quality product can be produced using a compatibilizer (maleic anhydride) at a concentration
182 of 6% in the proposed mixture composition [34].

183 Starch, a notable biopolymer, is primarily used in the chemical, bioplastic, and food production
184 industries. The gelatinization temperature of this substance is estimated to be around 110°C.
185 This temperature is suitable for the production of bioplastics, mostly made from starch [35].
186 Combining *Chlorella* cells with hydrogen ions creates a homogeneous mixture without empty
187 spaces [36]. Prior use of ultra-sonic homogenization as a pretreatment step before combining
188 significantly enhanced *Chlorella*-PVA blends' uniformity and surface characteristics. This
189 technique was suggested as a potential substitute for food packaging [37]. The researchers
190 examined the disparity between *Chlorella*-PE-based composites with and without
191 modifications of PE using maleic anhydride. The tensile strength of composites is enhanced by
192 introducing PE Modification [38].

193 **Spirulina**

194 *Spirulina* can adapt to severe settings and has been used as a critical source of protein in the
195 food industry for many years [39]. *Spirulina platensis* has a higher protein concentration [40].
196 Several experiments were conducted to assess the potential of *Spirulina* for manufacturing bio-
197 plastics. The comparable cell size of *Spirulina* and *Chlorella* makes them more desirable for
198 creating bio-plastic blends [41]. Although *Spirulina* and *Chlorella* share commonalities, they
199 exhibit different behaviours and bio-plastic capabilities, mainly when mixed with PE, which
200 can be attributed to their differing amino acid compositions. The use of compatibilizers [42]
201 can improve the characteristics of *Chlorella*-based bioplastic. The assimilation rate of the
202 compatibilizer into *Spirulina* was approximately 6% by weight—a mixture of *platensis* with
203 PVA.

204 Consequently, a bioplastic sheet with superior tensile strength was produced compared to the
 205 commercially available plastic bag. Using a compatibilizer enhances the plastic's potential,
 206 particularly in terms of elongation, and helps the formation of delicate layers [43].
 207 Nevertheless, the capacity for reinforcement was diminished when the particle size of
 208 microalgae biomass exceeded five μm [44]. The smaller particles were efficiently mixed with
 209 various materials [45]. Glycerol, with percentages ranging from 15% to 30%, is primarily used
 210 to enhance the flexibility of the plastic. It is predominantly derived from *S. Platensis*, a term.
 211 In bioplastic production, including glycerol at a concentration of 30% resulted in reduced
 212 elongation and increased tensile strength compared to conventional plastic bags [46]. This
 213 finding provides evidence that bioplastic may be extensively employed in the food sector for
 214 packaging, cosmetic, and medicinal purposes, particularly in cases where a significant amount
 215 of elongation is unnecessary [47]. The plasticization of *Spirulina* yields two primary outcomes:
 216 enhanced inter-surface adhesion and uniform phase distribution. They are utilizing mixing
 217 processes that improve both the uniformity and tensile strength.
 218 However, adding a compatibilizer [48] does not enhance the mechanical characteristics of the
 219 bioplastic. Adding maleic anhydride-grafted PBS to the biomass as a primary compatibilizer
 220 improves the product's strength, particularly in tensile strength, and decreases the temperature,
 221 leading to deterioration [49]. The table provided, labeled Table 1, illustrates the process of
 222 characterizing and extracting PHB using several species of algae.

223

Table 1 Characterization and Extraction Method of PHB

SL .N O	Algae name	% Of PHB & PHA production	Extraction method	Studies carried	References
1	<i>Chlorella pyrenoidosa</i>	27%	Fogg's media	Analysis of PHB content	[50]
2	<i>Chlorella fusca</i>	17.4%	D-xylose	Characterization and quantification of polyhydroxybutyrate	[51]
3	<i>Spirulina platensis</i>	6.20%	Zarrouk medium	PHB Production and comparison	[52]
4	<i>Sargassum</i> sp.	5.36%	Culture Mediasea weed	Characterization of PHB (Fourier transform infrared (FTIR)), Differential scanning calorimetry, Nuclear magnetic resonance.	[53]

5	High-rate algal pond	17%	Different media	Characterization (SEM analysis, Mechanical properties)	[53]
6	<i>Chroococcus</i>	12%	----	Characterization (SEM analysis, Mechanical properties)	[53]
7	<i>Haematococcus</i>	35%	----	Characterization (SEM analysis, Mechanical properties)	[53]
8	<i>Microcystis</i>	45%	----	Characterization (SEM analysis, Mechanical properties)	[53]
9	<i>Chlorella vulgaris</i>	----	Sudan black B and Nile Blue Stain	Optimization	[54]
10	<i>Stigeoclonium sp.</i>	----	Nile Red	Optimization	[55]
11	<i>Nostoc muscorum</i>	71%	----	Optimization	[56]
12	<i>cyanobacterium Synechocystis sp.</i>	12.5%	Culture medium	Analysis and characterization of PHB	[57]
13	<i>Chlorogloea fritschii</i>	51%	heterotrophy	Material properties of PHB	[58]
14	<i>Phaeodactylum tricornutum</i>	10.6%	Cell culture	Fluorescence and electron microscopy	[58]
15	<i>Anabaena cylindrica</i>	2.8%	Yellore and Desia	----	[59]
16	<i>Anabaena doliolum</i>	3.5%	Yellore and Desia	----	[59]
17	<i>Anabaena variabilis</i>	3.1%	Yellore and Desia	----	[59]
18	<i>Anacystis nidulans</i>	4.4%	Yellore and Desia	----	[59]
19	<i>Aphanocapsa sp.</i>	3.3%	Yellore and Desia	----	[59]
20	<i>Aulosira fertilissima</i>	6.5%	Yellore and Desia	----	[59]
21	<i>Calothrix sp.</i>	6.8%	Yellore and Desia	----	[59]
22	<i>Fischerella muscicola</i>	2.4%	Yellore and Desia	----	[59]
23	<i>Microcystis aeruginosa</i>	4.0%	Yellore and Desia	----	[59]
24	<i>Microcystis sp.</i>	3.6%	Yellore and Desia	----	[59]

25	<i>Nostoc linckia</i>	3.6%	Yellore and Desia	----	[59]
26	<i>Nostoc muscorum</i>	8.5%	Yellore and Desia	Quantification PHB	[59]
27	<i>Oscillatoria limosa</i>	2.9%	Yellore and Desia	----	[59]
28	<i>Pleurocapsa sp</i>	2.9%	Yellore and Desia	----	[59]
29	<i>Rivularia sp</i>	2.7%	Yellore and Desia	----	[59]
30	<i>Scytonema sp.</i>	7.4%	Yellore and Desia	----	[59]
31	<i>Spirulina platensis</i>	2.5%	Yellore and Desia	----	[59]
32	<i>Synechocystis sp. PCC 6803</i>	4.3%	Yellore and Desia	----	[59]
33	<i>Tolypothrix sp</i>	2.2%	Yellore and Desia	----	[59]
34	<i>Westiellopsis prolifica</i>	2.9%	Yellore and Desia	----	[59]
35	<i>Synechocystis PCC6803</i>	----	----	Analytical methods, Theoretical framework	[59]
36	<i>Spirulina sp. LEB 18</i>	44.2%	----	Sodium hypochlorite	[60]
37	<i>Cyanobium sp</i>	2.9%	----	10–12% active chlorine, Sodium hypochlorite	[60]
38	<i>Nostoc ellipsosporum</i>	19.2%	----	10–12% active chlorine, Sodium hypochlorite	[60]
39	<i>Synechococcus nidulans</i>	10.2%	----	10–12% active chlorine, Sodium hypochlorite	[60]
40	<i>Phormidium sp</i>	7.6%	----	Chloroform -Soxhlet method (hot extraction)	[61]
41	<i>Synechococcus sp.</i>	4.5%	----	Chloroform -Soxhlet method (hot extraction)	[61]
42	<i>Synechocystis sp</i>	3.7%	----	Chloroform -Soxhlet method (hot extraction)	[61]
43	<i>Anabaena sp</i>	2.3%	----	Chloroform -Soxhlet method (hot extraction)	[61]
44	<i>Ralstonia eutropha</i>	25 – 99.0%	----	Propylene carbonate mainly with thermally treated biomass	[62]
45	<i>Ralstonia eutropha</i>	84%	----	Methyl isobutyl ketone mainly for cell disruption & ethyl acetate for recovery	[63]

224

225 **Alternative Microalgae Species Employed for Bioplastic Manufacturing**

226 As mentioned earlier, most of the research in this emerging subject focused on studying bio-
227 plastic production using *Spirulina* and *Chlorella*. Regarding these efforts, several research have
228 examined the efficacy of several microalgae species [64].

229 **Hybrid Biological System**

230 The heterotrophic culture of PHA has successfully met its growth objective, but the expense
231 associated with acquiring feed remains a considerable concern [65]. Open pond culture often
232 yields economic advantages, while ensuring a contamination-free monoculture requires
233 constant monitoring and maintenance, which can be challenging. Likewise, the unpredictable
234 variations in candlepower, pH, temperature, and carbonic acid gas levels caused by daily and
235 cyclical swings lead to limited economic structures [66]. Therefore, creating a fusion organic
236 structure presents difficulties in utilizing event capability in both heterotrophic and
237 photoautotrophic culture requirements [67]. Researchers have discovered that the claims on the
238 potential of PHA production by photoautotrophic scientific agriculture are limited and can only
239 be attained up to 55% in *Synechococcus sp* the number 68. Therefore, the scientific agricultural
240 structures and procedures that were suggested to improve PHA production are as follows [69].

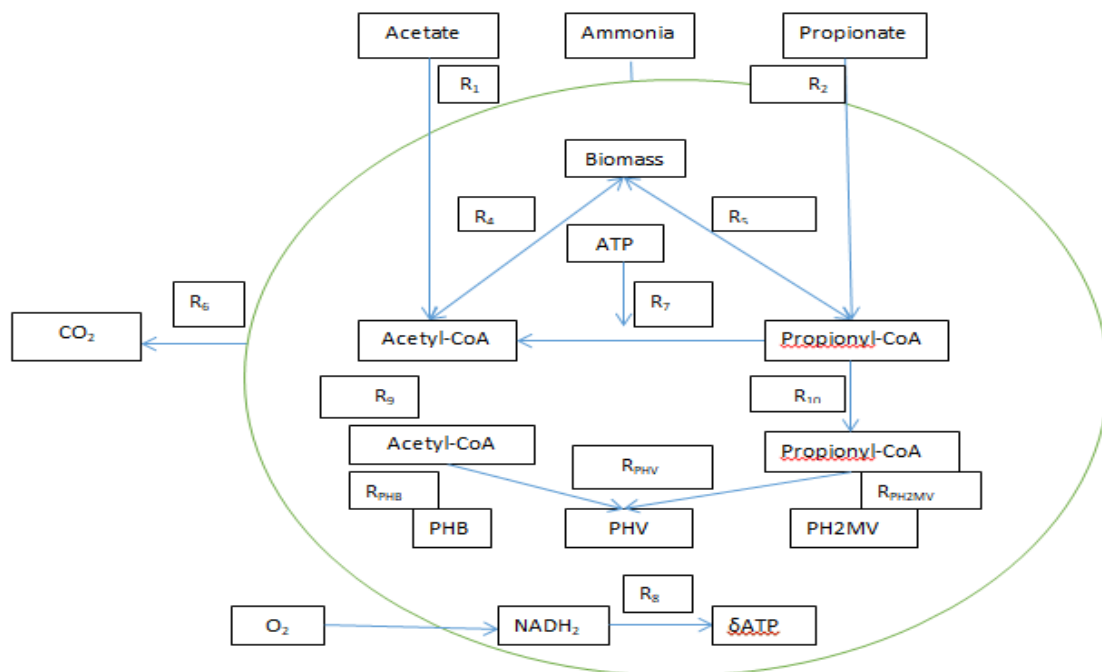
241 **Mixotrophy**

242 Mixotrophic or heterotrophic agronomy has consistently led to significant improvements in
243 PHA earnings [70]. However, there is still potential for a 2 to 9-fold increase in PHA output.
244 This concept aimed to incorporate heterotrophic (tilling) and photoautotrophic elements inside
245 the same structure for practice. The proposed design includes a live bacterium that functions
246 as a PHA producer. This bacterium undergoes photoautotrophic cultivation during the day,
247 using light, and then switches to heterotrophic cultivation at night. [71] Bacteria may utilize
248 carbon dioxide in sunlight as a carbon source. On the other hand, in the dark portion, an external
249 supply of carbon (such as glucose, acetate, polymer precursors, etc.) is also introduced. An
250 alternative improvement method involves enhancing biomass growth and reducing PHA
251 production in the existing bacteria by cultivating photoautotrophic organisms. In this
252 configuration, the bacteria are exposed to photoautotrophic conditions during the daytime
253 without any restrictions on nitrogen/phosphorus (N/P) levels, resulting in significant biomass
254 accumulation until the late-night exponential or dormant phase. During the dark phase, the
255 bacteria can be in a heterotrophic state, but their growth is limited by N/P availability. It
256 suggests the presence of a distinct segment within the structure, characterized by a notable
257 difference in intensity, with one part appearing more prominent or darker and the other

258 appearing more limited or brighter. This structural contrast is believed to promote the
259 production of polyhydroxyalkanoates (PHA) in bacteria [73].

260 **Association between organisms that are capable of photo autotrophy and heterotrophy.**

261 The main goal of establishing an association was to create an additional economic framework
262 that would provide greater access to resources, higher productivity, cost-effective food
263 production, social stability, non-competitive segregation, and the distribution of carbon or
264 energy resources among members of a community-based metabolic system [74]. The reciprocal
265 relationship refers to a mutual interaction or influence between different elements or factors.
266 In biotechnology advancements, this reciprocal relationship has been observed as the primary
267 driving force behind numerous developments in areas such as bioprocessing, biofuel
268 production, and the creation of various other biotechnological products. Essentially, these
269 advancements in one area often contribute to and benefit from progress in other related fields,
270 leading to a symbiotic relationship that fuels innovation in biotechnology [74]. The
271 photosynthetic association consists of an alga and a bacterium that consumes it. This
272 association produces PHA, which makes up 20-30% of the cell dry weight without needing
273 other elements. The method of metabolic network modelling is illustrated in Figure 2.



274

275

Fig. 2 Metabolic network modelling mechanism

276

277 **Impact of extraction techniques on the quantity and quality of Poly- β -hydroxybutyrate**

278 Microorganisms that produce PHB can store as much as 90% of their mass in biopolymers
279 [76]. However, it is challenging to remove the polymer from inside the cell. According to
280 reports, harvesting and extraction activities make up around 60-80% of the total production
281 expenses [77]. Enhancements in extraction techniques are required to achieve a significant
282 recovery yield while ensuring the desired polymer properties [78]. Chemical, surfactant-
283 chelate, enzyme, and solvent extraction are commonly used techniques for extracting PHB.
284 Over the years, several advanced methods for cell disruption and solvent procedures have been
285 developed and employed to effectively release the desired product by lysing cells [79]. The
286 solvent extraction process usually entails the slow dissolution of microbial biomass in a
287 combination of several solvents, which is then followed by the precipitation of polymers [80].
288 The robustness of the cell wall is crucial for disrupting microbial cells throughout the process
289 of biopolymer recovery. Alterations in the structure of the microbial cell wall can be induced
290 by modifying the content of the growth medium [81]. Different circumstances, such as action
291 duration, solvent type, concentration, and temperature range, impact a biopolymer's extraction
292 rate, molecular weight, and purity [82]. These parameters influence biopolymers' cost,
293 characteristics, and monomeric composition, which affect their industrial applications [83].
294 Unlike the usage of plastic bags, which are intended for single use, the medical industry
295 requires a polymer that is devoid of toxins and has a high level of purity [84]. The monomeric
296 composition and molecular mass of biopolymers may be significantly affected by extraction
297 methods, as demonstrated by research [85]. This suggests that the choice of extraction
298 technique is crucial for achieving the required properties and functionality of the polymers in
299 industrial applications. PHB derived from the combination of heated chloroform, diethyl ether,
300 and sodium hypochlorite was utilized for cancer detection [86]. Compared to normal epithelial
301 cells, breast cancer cells (T47D) demonstrated much stronger adherence to the PHB sheets
302 [88]. PHA polymers, namely PHB, have been employed in treating wounds, bone regeneration,
303 surgical instruments, and drug delivery owing to their compatibility with mammalian cells [89].

304 Additionally, it has widespread popularity within the agriculture sector [90]. While the result
305 may be acceptable, it is crucial to consider the adverse ecological and financial repercussions
306 of using highly concentrated chemicals in the extraction process [91]. Hydrothermal
307 conversion and biological extraction, which are eco-friendly technologies, are being used
308 because of the limitations associated with the mentioned approaches, such as high cost, time
309 consumption, and toxicity. Predation systems, mealworm digesting systems, and

310 Bacteriophage-mediated lysis systems are all instances of bio-extraction technologies [92].
311 These strategies provide economic benefits and minimize negative impacts on environmental
312 and human well-being [93]. The PHB was obtained from *Enterobacter aerogenes* cultivated in
313 sewage water using hypochlorite, yielding 96.25%. The biomass derived from PHB, employed
314 in wastewater treatment (WWT), was efficiently transformed into propylene using
315 hydrothermal conversion. This conversion process examined solvents, including phenetole,
316 anisole, and cyclohexanone, often used in industrial settings for extended periods [94].
317 Additionally, the extraction of *Spirulina* LEB 18 was successfully carried out in wastewater
318 treatment. To decrease the expenses associated with collecting microalgae, it is imperative to
319 consider using environmentally benign and reusable harvesting techniques, such as crystalline
320 nanocellulose [95, 96].

321 **Utilization of Microalgae Biomass in Material Blending**

322 Conventional polymers are commonly employed to create composite bioplastics derived from
323 microalgae sources to improve plastic properties. The most often employed polymers in the
324 combining process are polyethylene (PE) and polypropylene (PP). They account for more than
325 two-thirds of the worldwide demand for plastic. PE is utilized in several industries, such as
326 medical items, food packaging, cosmetics, and prostheses. UHMW-PE is a high molecular
327 weight polyethylene with a molecular weight ranging from 2 to 6 million. Bioplastic
328 manufacturers favour this material due to its absence of toxins, odour, and low moisture
329 absorption capacity. Wang conducted a trial where he mixed UHMW-PE with *Spirulina* at
330 different ratios ranging from 20% to 80% with intervals of 15%. Using a PE-*Spirulina*-EG
331 (Ethylene Glycol) ratio of 80:13:7 led to an increase in tensile strength, as reported in reference
332 [97].

333 Polypropylene (PP) is particularly suitable for packaging yogurt, medicinal products, and
334 beverages because of its somewhat translucent appearance and its resistance to the impact of
335 heat and mechanical responses. A study was conducted to produce films formed of bioplastic
336 containing *Chlorella* at various temperatures, using PVA as the primary material [98]. The
337 adhesion between composite materials was less robust at elevated temperatures.
338 Ultrasonication improved the quality of the blended material for blends by reacting to the
339 uniformity of the whole mixture. Extensive studies have been conducted on the use of wheat
340 gluten for the production of durable bioplastics. Although the material is inherently fragile, its
341 structure can be strengthened by using additives and fillers. Wheat gluten has a substantial
342 protein concentration, rendering it appropriate for various uses. PBS is a newly developed

343 biopolymer that has the potential to fulfill the market's need for bioplastics. PBS is often
344 favoured over low-density polyethylene and polypropylene (PP). Due to its efficient processing
345 capabilities, PBS is especially favoured in the textile industry. Examples of materials derived
346 from it are melt-blown and split yarn.

347 Additionally, it is employed to produce molded items in the plastics sector [98]. When PBS is
348 combined with other polymers, its mechanical properties are enhanced, enabling its use in a
349 broader range of applications. The amalgamation of PBS and *Spirulina* can improve the cost-
350 effectiveness of *Spirulina*-based bioplastics. Multiple chemicals were employed in tests to
351 enhance the combining process's efficiency and improve the result's quality [99]. PVA is
352 renowned for enhancing a product's strength, durability, and flexibility.

353 However, it is necessary to modify PVA with MA to enhance the dimensional stability and
354 surface properties of packaging materials and their mechanical characteristics. The biomass
355 was rinsed with acetone to obtain tiny spherical particles suitable for mixing. Sodium sulphide
356 was employed in bioplastic synthesis to cleanse biomass before amalgamating it with other
357 constituents. To enhance the combining properties of the benzoyl peroxide (BPO) and
358 *Chlorella*-PP composite, it was mixed with MA and acetone and then put onto powdered PE
359 [100].

360 **Plasticizers and compatibilizers**

361 Plasticizers are expansive chemical molecules used in substances to enhance their flexibility
362 and ease of processing. The efficacy of the plasticizer is judged by its ability to render the target
363 material more pliable [101]. Glycerol, with the chemical formula $C_3H_8O_3$, is a widely used
364 plasticizer in the production of bioplastics derived from microalgae. Glycerol enhanced the
365 accessibility of macromolecules for the process of breakdown [102], resulting in higher
366 flexibility and primary extensibility. This led to the production of high-quality products and
367 increased elongation in the study. The plasticization capabilities of octanoic acid and glycerol
368 were also observed. A series of tests showed that 1, 4-butanediol, and glycerol are suitable for
369 plasticization due to their high water permeability [103]. In addition, carboxymethylcellulose
370 (CMC) was employed to augment the mechanical characteristics of the plastic. CMC is a
371 compound formed by alkali, cellulose, and chloroacetic acid reactions. It dissolves rapidly in
372 cold water, has low primary viscosity, and is resistant to changes in temperature [104].
373 Compatibilizers are employed to facilitate the bonding of two polymers. The process of
374 compatibilization improved the mechanical strength of the heterogeneous biopolymers.
375 Different types of blends can utilize various compatibilizers, including poly(ethylene-co-

glycidyl) meth acryloyl carbamate, grafted ethylene/propylene rubber, maleic anhydride, and diethyl succinate [105]. The use of maleic anhydride enhanced the flexibility and uniformity of the products. PE-g-MA was incorporated into UHMW-PE and *Spirulina* composites at eight different concentrations, with each concentration rising by 3%. The addition did not have a specific impact on the mechanical characteristics of the composite. In addition to the plasticizers and compatibilizers mentioned above, compatibilizer initiators such as potassium peroxide sulphate (KPS) were used. The process involved liquefying DMSO (15 mL), MA, and PVA, followed by the addition of KPS (1% PVA) [106].

384 **Life Cycle Assessment Studies on Bioplastic Production from Microalgae**

Limited studies on Life Cycle Assessment (LCA) exist, particularly for bioplastics generated from microalgae. The researchers surveyed the production of PLA using microalgae and plant sources. They found that using microalgae significantly impacts land utilization and terrestrial ecotoxicity [107]. The investigation of the effects of greenhouse gases on several microalgae cultivation methods for bioplastic production revealed that various cyclic flow photobioreactors exhibited little variations compared to mixed systems and open raceway ponds. However, the study's findings do not reveal the comparative performance of microalgae-based bioplastics concerning conventional alternatives [108].

Nevertheless, extensive studies on microalgae farming using Life Cycle Assessment (LCA) might provide insights into specific patterns and trends [109]. According to the analysis of the LCA trials, fossil fuels outperformed microalgae-derived biofuels [110]. According to their study, microalgal manufacturing systems show significantly more efficiency increases in greenhouse gas emissions than fossil fuels [111]. Data uncertainty significantly affects life cycle assessments (LCAs) on microalgae biofuel production, leading to divergent outcomes [112]. Microalgae-based food production has high efficiency in land utilization, but it falls short in other critical areas, such as meeting the demand for freshwater [113]. The natural performance of microalgae-based products is primarily unclear. However, studies often highlight the possibility of improvement in microalgal production systems [114]. One way to establish synergies is by implementing bio-refineries that generate many byproducts and enhance farming techniques. Utilizing microalgal waste to produce bioplastics might enhance overall life cycle assessment (LCA) ratings. Microalgae production systems generally focus on minimizing land usage [115]. Table 2 demonstrates the diverse applications of PHB in different sectors.

Table 2 Way of using PHB

SL. NO	Area	Application
1	medical field	- tissue cultures for implants, - part of bones - Surgical implants And engineering of heart valves, pins, replanted veins, Sutures and dressings
2	Package	food package
3	Pharmacological	Encapsulation of different medicines for susceptible release
4	Environmental	Bottles, items of personal hygiene, involvement, remediation of areas affected by oil spills, bags, and disposal items.
5	Agricultural	Encapsulation of fertilizers
6	Industrial	Recovery of monomers and oligomers for new application in the synthesis of polymers
7	Agricultural	Encapsulation of fertilizers

409

410 Tissue engineering

411 Typically, the PHAs that were accessible were not used for medical implants. Consequently,
 412 the PHA quality evaluation has been postponed to obtain permission from Drug
 413 Administrators. It is necessary to generate PHAs with a high purity level and thoroughly
 414 examine their biodegradation in laboratory settings and their potential for use in scaffold
 415 creation and surface modification [116]. PHAs that undergo crucial modifications can
 416 substantially contribute to tissue engineering and the development of medicinal and therapeutic
 417 goods, particularly for applications such as vascular grafts, heart valves, and neural tissue
 418 engineering [117]. PHAs with enhanced mechanical strength can be utilized to fabricate
 419 scaffolds for medicinal purposes. Scaffolds fabricated using PHAs facilitate cellular
 420 development by providing nourishment [118]. The primary medical goods include screws,
 421 pins, sutures, films, and other similar items [119]. Poly(3-hydroxybutyrate-4-hydroxybutyrate-
 422 3-hydroxyvalerate) promotes the proliferation and adherence of stem cells [120]. The polymer
 423 (3HB-3HV-3HHx) can serve as a scaffold to build liver tissue [121].

424 Additionally, the use of PHA nanofibers has led to the development of three-dimensional
 425 scaffolds [122]. The study discovered that P(3HB-3HO) effectively repaired the cartilage using
 426 a scaffold made from PHAs [123]. The recombinant organism produces the novel P(3-HB-
 427 3HV-2,3-diHB). To improve the capacity, inorganic bio ceramics have been integrated with
 428 PHAs to boost the mechanical strength and flexibility of PHAs, resulting in the development
 429 of innovative composites for engineering tissues. Blends consisting of PHA and ceramic

430 composites are utilized to create various mixtures. Hydroxyapatite and PHA are employed in
431 tissue engineering as well.

432 **Conclusions**

433 This study examined the current state of bio-plastic production using resources derived from
434 microalgae. *Chlorella* and *Spirulina* were the dominant algae species that produced plastic
435 blends and biopolymers. To improve the overall quality of the final product, several additives
436 such as compatibilizers, plasticizers, and other chemicals were employed to combine the
437 ingredients. Based on the literature assessment of this study, further advancement of techniques
438 for producing bioplastics from microalgae is necessary to address economic feasibility
439 concerns in large-scale industrial applications, which hinder the general adoption of
440 microalgae-based bioplastic products in the market. Bioplastics were derived from the
441 byproduct of efficient chemicals produced from microalgae as part of a biorefinery concept.
442 Therefore, it may be seen as a very efficient product. In addition, different chemicals might
443 impose restrictions on the potential applications of microalgae products, particularly in areas
444 like healthcare and food packaging. Further research is required to enhance the efficiency of
445 industrial and manufacturing operations while minimizing the need for additives through more
446 inventive design.

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