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Reviewing Biopolymers: Pioneering Sustainable Alternatives to Traditional Plastics Using Algae

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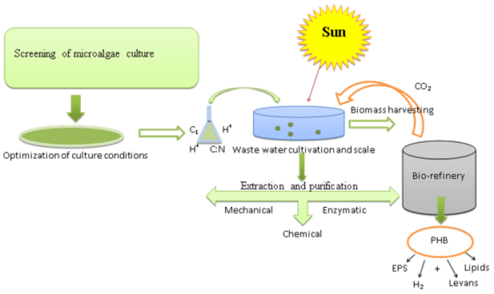
Graphical abstract



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

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

1. Introduction

The global demand for plastic has risen, driven by plastic-based goods, exerting strain on the existing waste treatment infrastructure. Plastics typically consist of synthetic and semi-synthetic materials, mainly composed of polymers. Plastics include lightweight, cost-effective, easily manufacturable, flexible, and long-lasting characteristics, making them widely utilized by several individuals [Abdo and Ali 2019]. Plastics consist of artificial polymers, including polyesters, polyurethanes, halogenated plastics, and acrylics. Plastics primarily impact the living environment of humans, wildlife, and numerous marine species. Plastic deterioration has a direct impact on human life via the drinking of tap water and an indirect effect through the consumption of animals [Abu 2020]. Plastic trash is categorized into main and secondary forms. Primary plastics were retrieved from their initial location, and the deterioration of these primary plastics resulted in the formation of secondary plastics. Plastics are often categorized as micro, macro, and mega trash. The plastic particles range from 2 mm to 5 mm and are classified as microtrash. Mega debris refers to plastic particles with a dimension of 20 mm and are primarily utilized in food stores [Abu 2020].

Annually, about 8 million metric tonnes of plastic trash are disposed of in the ocean, with the potential for repurposing through the creation of inventive packaging materials [Alaerts *et al.* 2018]. In general, plastics may be

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categorized into two types: derived from petroleum and biodegradable. Annually, around 1% of the world's bioplastic is manufactured. The starch blend that provides 21% of the total bioplastic is anticipated to grow in the market. Bioplastics are primarily used in packaging, textile, construction, and car industries [Alaerts *et al.* 2018]. Natural polymers, such as proteins and carbohydrates, were the primary source of bioplastic synthesis.

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

Furthermore, historically, the marketing of bio-based products encountered significant challenges on a widespread level [Barros *et al.* 2015]. Microalgae are regarded as a promising biomass resource for the development of bioplastics due to their lack of association with food sources. This organism exhibits the ability to thrive in aquatic environments and can generate substantial amounts of lipids. Moreover, bioplastic production using microalgae is considered more environmentally friendly and may be readily introduced and distributed within the economy and the bio-economy [Beckstrom 2016]. Bioplastics are applicable in the medicines, cosmetics, and food packaging sectors. Research efforts encompass the processing of microalgae bioplastic within a bio-refinery framework, the formulation of microalgae-polymer composites, and the genetic modification of microalgae strains to produce biopolymers [Biron 2016].

The wastewater from the industrial sector was treated under specific conditions (stress, pH, temperature, aeration duration, and agitation speed) to optimize the synthesis of PHB (polyhydroxybutyrate), presumably by microorganisms present in the wastewater. Hydroxy butyryl CoA, a precursor that undergoes polymerization to generate polyhydroxybutyrate (PHB), was utilized in this process. PHB exhibits insolubility in water and solubility in hydrolytic compounds [Brányiková *et al.* 2011]. It exhibits superior durability against UV light but displays reduced resilience to acidic and alkaline environments. It is non-toxic and readily dissolves in chloroform and other chlorinated hydrocarbons. The melting point of PHB is 175°C, and its glass transition temperature is 2°C. The tensile strength of PHB measures 40 MPa, which is comparable to that of polypropylene [Brányiková *et al.* 2011].

Additionally, PHB exhibits reduced stickiness when heated compared to other plastics. This study involved the synthesis of polyhydroxybutyrate from algae, which was then combined with natural polymers in varying ratios. The aim was to survey the resulting mix's physical, chemical, and mechanical characteristics and degradation time. This technology has the potential for several commercial applications, including agriculture, medicine, and the food business. However, it is crucial to implement efficient technology to improve industrialization, commercialization, and scaling-up processes. This study exhaustively scrutinizes several microalgae species used in bioplastic production to uncover any research deficiencies in this emerging field. The text explains opportunities associated with the expansion of microalgae. This review study comprehensively examines all facets of the technique and production.

2. Characteristics and attributes of poly-β-hydroxybutyrates in terms of their chemical and physical qualities

The synthesis of Poly-β-hydroxybutyrates begins with the precursor molecule acetyl coenzyme A (acetyl-CoA). This process involves three consecutive enzymatic reactions. The first step is the conversion of 2-acetyl CoA to 1-acetyl CoA, which is catalyzed by the enzyme 3-ketothiolase. The second step is the production of D-3-hydroxybuturylCoA, achieved through converting NADPH-dependent acetoacetyl–CoA reductase to acetoacetyl CoA [Brennan and Owende 2010]. The D-3-hydroxy butyryl moiety was connected to the existing PHB molecule by an ester bond facilitated by the last enzyme, PHB synthases. Therefore, substituents or unsaturation were detected in the fatty acids at positions 4, 5, and 6 of the pendant group, where a hydroxyl group is located. Simultaneously, PHB and poly three hydroxy valerates (PHV) were categorized as short-chain length PHAs and merged to constitute a PHA class [Bussa *et al.* 2010]. On the other hand, it has been reported that the typical chain length of PHAs ranges from carbon six to carbon 16, explicitly referring to 3-hydroxy fatty acids. Additionally, it has been proposed that the PHB homopolymer synthesized by bacteria consists of more than one molecule and contains 3% hydroxy valerate monomers [Carlozzi *et al.* 2003].

The combination of Valerate and glucose served as the substrate for the microorganisms. These microorganisms play a crucial role in synthesizing small-chain-linked PHAs [Chandra and Mohan 2014]. The formation of PHBHx - copolymers, consisting of 3-hydroxyl hexanoate units in combination with PHAs copolymers of PHB, was described [Charlie *et al.* 2019]. Random copolymers were formed by alternatingly introducing different substrates into the current culture. This process led to the synthesis of a PHA block by bacteria. The molecular mass of PHBs generated by bacteria is typically 4.6 × 106 Da, with a poly disparity (Mw/Mn) of around 2.0 [Chen *et al.* 2011]. The biomaterial polypropylene has comparable material properties [Chisti 2008, 2007]. The PHB homopolymer [Ciapponi *et al.* 2019] exhibited a high degree of crystallinity. This material is rigid and brittle, yet it possesses elastic capabilities. When twisted into fibers, it was seen to have an elastic behavior [Costa *et al.* 2019]. The helical crystalline structure is a characteristic of homopolymers. This copolymer, characterized by its structural composition, exhibits superior durability against ultraviolet light but reduces resilience to acidic and alkaline environments. It shares structural similarities with many other copolymers [Das *et al.* 2018]. A recent study analyzed PHB's melting behavior and crystallization [Di Caprio *et al.* 2016] and examined its physical properties.

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

Microalgae gained attention for research following the discovery of *Spirulina* in Spain before 1519 [Dianursanti *et al.* 2019]. Scientists have extensively studied the nutritional properties of microalgae and made significant discoveries, such as the PHB homopolymer, which holds great potential for various applications benefiting humankind [Dianursanti *et al.* 2018]. Microalgae have been employed for bioremediation in wastewater treatment for an extended period. Using wastewater to culture microorganisms has become a leading practice in resource recovery—the authors of this publication aimed to highlight twenty years of trends in PHB research. A comprehensive evaluation was conducted on indexed publications published between 1999 and 2020, focusing on the search for microbial plastics and biosynthesis. The keywords used for the search were PHB, microbial bioplastics, and biosynthesis [DiGregorio 2009]. This assessment provides a comprehensive analysis of the research and development activities from a chronological standpoint. The literature research indicated that "PHBs" was the most often utilized keyword during twenty years, accounting for 50% of the occurrences. The process of synthesizing polyhydroxybutyrate (PHB) is seen in Figure 1.

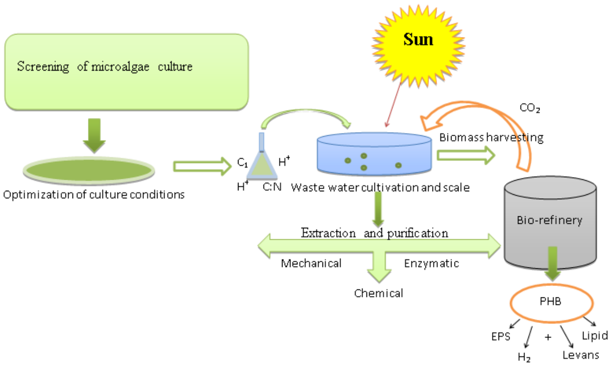

Figure 1. Biosynthesis of PHB

While algae are well-known for their ability to produce polyhydroxybutyrate (PHB), other microorganisms, including certain types of bacteria, can also synthesize PHB. Activated sludge, a mixture of bacteria, fungi, protozoa, and other microorganisms used in wastewater treatment, can contain bacteria capable of producing PHB. Several types of bacteria, such as species of Bacillus, Pseudomonas, and Alcaligenes, are known to produce PHB as intracellular storage granules when they are under conditions of nutrient limitation, mainly when there is an excess of carbon source available. This ability makes them useful in various biotechnological applications, including producing biodegradable plastics like PHB [Dianursanti 2018; DiGregorio 2009].

4. Biological production of polyhydroxybutyrate

Multiple studies were conducted in biosynthesis to generate polyhydroxybutyrate via the microbial fermentative method [Dixon *et al.* 2018]. The production of polyhydroxybutyrate involves three fundamental steps catalyzed by three different enzymes. Firstly, the enzyme B-keto thiolase catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. Secondly, the enzyme phaB, encoded with NADPH-dependent acetoacetyl CoA dehydrogenase, reduces acetoacetyl-CoA. Lastly, the enzyme PHA synthase, attached with phaC, catalyzes the polymerization of 3-hydroxy acid (3HA) units, specifically (R)-3-hydroxy butyryl-CoA. The result is the transformed acetyl-CoA used in the PHB biosynthetic pathway.

4.1. Microalgae and additives

The Algae species are classified as microalgae and macroalgae, with one million species identified [Draaisma]. Microalgae, microscopic organisms, utilize solar energy to make adenosine triphosphate (ATP), a compound that may be found in fresh waters and coastal locations [Eagan *et al.* 2017]. Microalgae are utilized as a substitute for several sources in biofuel manufacture. Pharmaceutical formulations extensively use it, while cosmetics might be a supplementary product in the food business [European Bioplastics]. The development of the bio-economy using microalgae has shown constant improvement [Berlin European Bioplastics 2018]. Microalgae consist primarily of proteins (6–52%), lipids (7–23%), and carbohydrates (5–23%) [Fabra *et al.* 2018]. In addition, microalgae have a composition that includes iron (1395–11,101 mg·kg^{-1}), phosphorous (0.7–1.5%), manganese (45–454 mg·kg^{-1}), copper (18–102 mg·kg^{-1}), magnesium (0.3–0.7%), selenium (0–0.5 mg·kg^{-1}), calcium (0.1–3.0%), sulphur (0.4–1.4%), potassium (0.7–2.4%), sodium (0.8–2.7%), and zinc (28–64mg·kg^{-1}) [Dianursanti *et al.* 2019]. The subsequent sections will provide a detailed analysis of bioplastic manufacture, focusing on using additives, microalgae species, and chemicals [Fabra *et al.* 2018].

4.2. Chlorella

Chlorella is a genus of photosynthetic microorganisms that belong to the green algae family. It predominantly inhabits aquatic environments and primarily comprises around 58% protein. Its fracture resistance is highly effective because of its clustered cell walls and exceptional thermal stability compared to *Spirulina* [Fu *et al.* 2012]. These species are primarily used in mixes of biomass and polymers. The comparison between blends consisting mainly of polymers/additives and microalgae biomass (100%) for bioplastic manufacture demonstrated the necessity of efficient combining for commercial use [Gifuni *et al.* 2017]. The test findings showed that using *chlorella vulgaris* yielded superior bioplastics to *Spirulina*. Nevertheless, *Spirulina* has superior mixing characteristics compared to *Chlorella* [Gilbert 2016]. The study suggested that a high-quality product can be produced using a compatibilizer (maleic anhydride) at a concentration of 6% in the proposed mixture composition [Gozan 2018].

Starch, a notable biopolymer, is primarily used in the chemical, bioplastic, and food production industries. The gelatinization temperature of this substance is estimated to be around 110◦C. This temperature is suitable for the production of bioplastics, mostly made from starch [Hai *et al.* 2000]. Combining *Chlorella* cells with hydrogen ions creates a homogeneous mixture without empty spaces [Harun *et al.* 2009]. Prior use of ultra-sonic homogenization as a pretreatment step before combining significantly enhanced *Chlorella*-PVA blends' uniformity and surface characteristics. This technique was suggested as a potential substitute for food packaging [Hempel *et al.* 2011]. The researchers examined the disparity between *Chlorella*–PE-based composites with and without modifications of PE using maleic anhydride. The tensile strength of composites is enhanced by introducing PE Modification [Huo *et al.* 2011].

Table 1. Characterization and Extraction Method of PHB

SL.NO	Algae name	% Of PHB & PHA production	Extraction method	Studies carried	References
1	*Chlorella pyrenoidosa*	27%	Fogg's media	Analysis of PHB content	[Murilo Moraes Mourão n.d.]
2	*Chlorella fusca*	17.4%	D-xylose	Characterization and quantification of polyhydroxybutyrate	[Bhati and Mallick 2014]
3	*Spirulina platensis*	6.20%	Zarrouk medium	PHB Production and comparision	[Clemens Trosch 2018]
4	*Sargassum* sp.	5.36%	Culture Mediaseaweed	Characterization of PHB (Fourier transform infrared (FTIR)), Differential scanning calorimetry, Nuclear magnetic resonance.	[Monshupanee 2016]
5	High-rate algal pond	17%	Different media	Characterization (SEM analysis, Mechanical properties)	[Monshupanee 2016]
6	*Chroococcus*	12%	----	Characterization (SEM analysis, Mechanical properties)	[Monshupanee 2016]
7	*Haematococcus*	35%	----	Characterization (SEM analysis, Mechanical properties)	[Monshupanee 2016]
8	*Microcystis*	45%	----	Characterization (SEM analysis, Mechanical properties)	[Monshupanee 2016]
9	*Chlorella vulgaris*	----	Sudan black B and Nile Blue Stain	Optimization	[Ranjana Bhati 2010]
10	*Stigeoclonium sp.*	----	Nile Red	Optimization	[Martins *et al.* 2014]
11	*Nostoc muscorum*	71%	----	Optimization	[Gopi *et al.* 2014]
12	*cyanobacterium Synechocystis sp.*	12.5%	Culture medium	Analysis and characterization of PHB	[Quines *et al.* 20015]
13	*Chlorogloea fritschii*	51%	heterotrophy	Material properties of PHB	[Riedel n.d.]
14	*Phaeodactylum tricornutum*	10.6%	Cell culture	Fluorescence and electron microscopy	[Riedel n.d.]
15	*Anabaena cylindrica*	2.8%	Yellore and Desia	----	[Knuckey *et al.* 2006]
16	*Anabaena doliolum*	3.5%	Yellore and Desia	----	[Knuckey *et al.* 2006]
17	*Anabaena variabilis*	3.1%	Yellore and Desia	----	[Knuckey *et al.* 2006]
18	*Anacystis nidulans*	4.4%	Yellore and Desia	----	[Knuckey *et al.* 2006]
19	*Aphanocapsa sp.,*	3.3%	Yellore and Desia	----	[Knuckey *et al.* 2006]
20	*Aulosira fertilissima*	6.5%	Yellore and Desia	----	[Knuckey *et al.* 2006]
21	*Calothrix sp.*	6.8%	Yellore and Desia	----	[Knuckey *et al.* 2006]

22	*Fischerella muscicola*	2.4%	Yellore and Desia	----	[Knuckey *et al.* 2006]
23	*Microcystis aeruginosa*	4.0%	Yellore and Desia	----	[Knuckey *et al.* 2006]
24	*Microcystis sp.*	3.6%	Yellore and Desia	----	[Knuckey *et al.* 2006]
25	*Nostoc linckia*	3.6%	Yellore and Desia	----	[Knuckey *et al.* 2006]
26	*Nostoc muscorumc*	8.5%	Yellore and Desia	Quantification PHB	[Knuckey *et al.* 2006]
27	*Oscillatoria limosa*	2.9%	Yellore and Desia	----	[Knuckey *et al.* 2006]
28	*Pleurocapsa sp*	2.9%	Yellore and Desia	----	[Knuckey *et al.* 2006]
29	*Rivularia sp*	2.7%	Yellore and Desia	----	[Knuckey *et al.* 2006]
30	*Scytonema sp.*	7.4%	Yellore and Desia	----	[Knuckey *et al.* 2006]
31	*Spirulina platensis*	2.5%	Yellore and Desia	----	[Knuckey *et al.* 2006]
32	*Synechocystis sp. PCC 6803*	4.3%	Yellore and Desia	----	[Knuckey *et al.* 2006]
33	*Tolypothrix sp*	2.2%	Yellore and Desia	----	[Knuckey *et al.* 2006]
34	*Westiellopsis prolifica*	2.9%	Yellore and Desia	----	[Knuckey *et al.* 2006]
35	*Synechocystis PCC6803*	----	----	Analytical methods, Theoretical framework	[Knuckey *et al.* 2006]
36	*Spirulina sp. LEB 18*	44.2%	----	Sodium hypochlorite	[Kutz 2011]
37	*Cyanobium sp*	2.9%	----	10–12% active chlorine, Sodium hypochlorite	[Kutz 2011]
38	*Nostoc ellipsosporum*	19.2%	----	10–12% active chlorine, Sodium hypochlorite	[Kutz 2011]
39	*Synechococcus nidulans*	10.2%	----	10–12% active chlorine, Sodium hypochlorite	[Kutz 2011]
40	*Phormidium sp*	7.6%	----	Chloroform -Soxhlet method (hot extraction)	[Lee2001]
41	*Synechococcus sp.*	4.5%	----	Chloroform -Soxhlet method (hot extraction)	[Lee2001]
42	*Synechocystis sp*	3.7%	----	Chloroform -Soxhlet method (hot extraction)	[Lee2001]
43	*Anabaena sp*	2.3%	----	Chloroform -Soxhlet method (hot extraction)	[Lee2001]
44	*Ralstonia eutropha*	25 – 99.0%	----	Propylene carbonate mainly with thermally treated biomass	[Lupatini *et al.* 2017]
45	*Ralstonia eutropha*	84%	----	Methyl isobutyl ketone mainly for cell disruption & ethyl acetate for recovery	[MacArthur 2017]

4.3. Spirulina

Spirulina can adapt to severe settings and has been used as a critical source of protein in the food industry for many years [Johnsson and Steuer 2018]. *Spirulina platensis* has a higher protein concentration [Kalia and Avérous 2016]. Several experiments were conducted to assess the potential of *Spirulina* for manufacturing bio-plastics. The comparable cell size of *Spirulina* and *Chlorella* makes them more desirable for creating bio-plastic blends [Kaparapu 2018]. Although *Spirulina* and *Chlorella* share commonalities, they exhibit different behaviours and bio-plastic capabilities, mainly when mixed with PE, which can be attributed to their differing amino acid compositions. The use of compatibilizers [Kato 2019] can improve the characteristics of *Chlorella*-based bioplastic. The assimilation rate of the compatibilizer into *Spirulina* was approximately 6% by weight—a mixture of *platensis* with PVA.

Consequently, a bioplastic sheet with superior tensile strength was produced compared to the commercially available plastic bag. Using a compatibilizer enhances the plastic's potential, particularly in terms of elongation, and helps the formation of delicate layers [Khanra *et al.* 2018]. Nevertheless, the capacity for reinforcement was diminished when the particle size of microalgae biomass exceeded five µm [Sruthy 2016]. The smaller particles were efficiently mixed with various materials [Cassuriaga 2018]. Glycerol, with percentages ranging from 15% to 30%, is primarily used to enhance the flexibility of the plastic. It is predominantly derived from *S. Platensis*, a term. In bioplastic production, including glycerol at a concentration of 30% resulted in reduced elongation and increased tensile strength compared to conventional plastic bags [Uma Maheswari 2011]. This finding provides evidence that bioplastic may be extensively employed in the food sector for packaging, cosmetic, and medicinal purposes, particularly in cases where a significant amount of elongation is unnecessary [Nahid Azizi n.d.]. The plasticization of *Spirulina* yields two primary outcomes: enhanced inter-surface adhesion and uniform phase distribution. They are utilizing mixing processes that improve both the uniformity and tensile strength.

However, adding a compatibilizer [Sayeda M. Abdo 2019] does not enhance the mechanical characteristics of the bioplastic. Adding maleic anhydride-grafted PBS to the biomass as a primary compatibilizer improves the product's strength, particularly in tensile strength, and decreases the temperature, leading to deterioration [Rebecca Robert 2018]. The table provided, labeled Table 1, illustrates the process of characterizing and extracting PHB using several species of algae.

5. Alternative Microalgae Species Employed for Bioplastic Manufacturing

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

5.1 Hybrid Biological System

The heterotrophic culture of PHA has successfully met its growth objective, but the expense associated with acquiring feed remains a considerable concern [Mata *et al.* 2010]. Open pond culture often yields economic advantages, while ensuring a contamination-free monoculture requires constant monitoring and maintenance, which can be challenging. Likewise, the unpredictable variations in candlepower, pH, temperature, and carbonic acid gas levels caused by daily and cyclical swings lead to limited economic structures [McBride 2014]. Therefore, creating a fusion organic structure presents difficulties in utilizing event capability in both heterotrophic and photoautotrophic culture requirements [Medeiros *et al.* 2015]. Researchers have discovered that the claims on the potential of PHA production by photoautotrophic scientific agriculture are limited and can only be attained up to 55% in *Synechococcus sp* the number 68. Therefore, the scientific agricultural structures and procedures that were suggested to improve PHA production are as follows [Mohan 2019].

5.2 Mixotrophy

Mixotrophic or heterotrophic agronomy has consistently led to significant improvements in PHA earnings [Monshupanee *et al.* 2016]. However, there is still potential for a 2 to 9-fold increase in PHA output. This concept aimed to incorporate heterotrophic (tilling) and photoautotrophic elements inside the same structure for practice. The proposed design includes a live bacterium that functions as a PHA producer. This bacterium undergoes photoautotrophic cultivation during the day, using light, and then switches to heterotrophic cultivation at night. [Moreno-Garcia 2017] Bacteria may utilize carbon dioxide in sunlight as a carbon source. On the other hand, in the dark portion, an external supply of carbon (such as glucose, acetate, polymer precursors, etc.) is also introduced. An alternative improvement method involves enhancing biomass growth and reducing PHA production in the existing bacteria by cultivating photoautotrophic organisms. In this configuration, the bacteria are exposed to photoautotrophic conditions during the daytime without any restrictions on nitrogen/phosphorus (N/P) levels, resulting in significant biomass accumulation until the late-night exponential or dormant phase. During the dark phase, the bacteria can be in a heterotrophic state, but their growth is limited by N/P availability. It suggests the presence of a distinct segment within the structure, characterized by a notable difference in intensity, with one part appearing more prominent or darker and the other appearing more limited or brighter. This structural contrast is believed to promote the production of polyhydroxyalkanoates (PHA) in bacteria [Musa *et al.* 2019].

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

The main goal of establishing an association was to create an additional economic framework that would provide greater access to resources, higher productivity, cost-effective food production, social stability, non-competitive segregation, and the distribution of carbon or energy resources among members of a community-based metabolic system [Oh *et al.* 2018]. The reciprocal relationship refers to a mutual interaction or influence between different elements or factors. In biotechnology advancements, this reciprocal relationship has been observed as the primary driving force behind numerous developments in areas such as bioprocessing, biofuel production, and the creation of various other biotechnological products. Essentially, these advancements in one area often contribute to and benefit from progress in other related fields, leading to a symbiotic relationship that fuels innovation in biotechnology [Oh *et al.* 2018]. The photosynthetic association consists of an alga and a bacterium that consumes it. This association produces PHA, which makes up 20-30% of the cell dry weight without needing other elements. The method of metabolic network modelling is illustrated in Figure 2.

7. Impact of extraction techniques on the quantity and quality of Poly-β-hydroxybutyrate

Microorganisms that produce PHB can store as much as 90% of their mass in biopolymers [Osanai *et al.* 2013]. However, it is challenging to remove the polymer from inside the cell. According to reports, harvesting and extraction activities make up around 60-80% of the total production expenses [Otsuki *et al.* 2004]. Enhancements in extraction techniques are required to achieve a significant recovery yield while ensuring the desired polymer properties [Pal *et al.* 2019]. Chemical, surfactant-chelate, enzyme, and solvent extraction are commonly used techniques for extracting PHB. Over the years, several advanced methods for cell disruption and solvent procedures have been developed and employed to effectively release the desired product by lysing cells [Pandey *et al.* 2017]. The solvent extraction process usually entails the slow dissolution of microbial biomass in a combination of several solvents, which is then followed by the precipitation of polymers [Park and Seo 2011]. The robustness of the cell wall is crucial for disrupting microbial cells throughout the process of biopolymer recovery. Alterations in the structure of the microbial cell wall can be induced by modifying the content of the growth medium [Pragya *et al.* 2013]. Different circumstances, such as action duration, solvent type, concentration, and temperature range, impact a biopolymer's extraction rate, molecular weight, and purity [Proshad *et al.* 2018]. These parameters influence biopolymers' cost, characteristics, and monomeric composition, which affect their industrial applications [Pulz 2001].

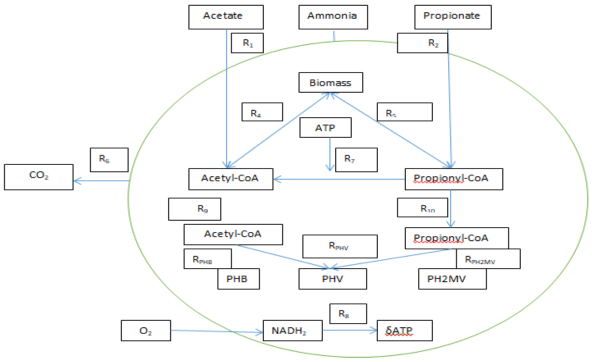

Figure 2. Metabolic network modelling mechanism

Unlike the usage of plastic bags, which are intended for single use, the medical industry requires a polymer that is devoid of toxins and has a high level of purity [Quinn and Davis 2015]. The monomeric composition and molecular mass of biopolymers may be significantly affected by extraction methods, as demonstrated by research [Rahman *et al.* 2015]. This suggests that the choice of extraction technique is crucial for achieving the required properties and functionality of the polymers in industrial applications. PHB derived from the combination of heated chloroform, diethyl ether, and sodium hypochlorite was utilized for cancer detection [Ranade 2009]. Compared to normal epithelial cells, breast cancer cells (T47D) demonstrated much stronger adherence to the PHB sheets [Richmond *et al.* 1990]. PHA polymers, namely PHB, have been employed in treating wounds, bone regeneration, surgical instruments, and drug delivery owing to their compatibility with mammalian cells [Rudnik 2019].

Additionally, it has widespread popularity within the agriculture sector [Sabathini *et al.* 2018]. While the result may be acceptable, it is crucial to consider the adverse ecological and financial repercussions of using highly concentrated chemicals in the extraction process [Salim *et al.* 2011]. Hydrothermal conversion and biological extraction, which are eco-friendly technologies, are being used because of the limitations associated with the mentioned approaches, such as high cost, time consumption, and toxicity. Predation systems, mealworm digesting systems, and Bacteriophage-mediated lysis systems are all instances of bio-extraction technologies [Sato *et al.* 2006]. These strategies provide economic benefits and minimize negative impacts on environmental and human well-being [Scott *et al.* 2010]. The PHB was obtained from Enterobacter *aerogenes* cultivated in sewage water using hypochlorite, yielding 96.25%. The biomass derived from PHB, employed in wastewater treatment (WWT), was efficiently transformed into propylene using hydrothermal conversion. This conversion process examined solvents, including phenetole, anisole, and cyclohexanone, often used in industrial settings for extended periods [Sereni 2016]. Additionally, the extraction of *Spirulina* LEB 18 was successfully carried out in wastewater treatment. To decrease the expenses associated with collecting microalgae, it is imperative to consider using environmentally benign and reusable harvesting techniques, such as crystalline nanocellulose [Lopez-Exposito *et al.* 2019; Shen *et al.* 2009].

8. Utilization of Microalgae Biomass in Material Blending

Conventional polymers are commonly employed to create composite bioplastics derived from microalgae sources to improve plastic properties. The most often employed polymers in the combining process are polyethylene (PE) and polypropylene (PP). They account for more than two-thirds of the worldwide demand for plastic. PE is utilized in several industries, such as medical items, food packaging, cosmetics, and prostheses. UHMW-PE is a high molecular weight polyethylene with a molecular weight ranging from 2 to 6 million. Bioplastic manufacturers favour this material due to its absence of toxins, odour, and low moisture absorption capacity. Wang conducted a trial where he mixed UHMW-PE with *Spirulina* at different ratios ranging from 20% to 80% with intervals of 15%. Using a PE-*Spirulina*-EG (Ethylene Glycol) ratio of 80:13:7 led to an increase in tensile strength, as reported in reference [Singh *et al.* 2017].

Polypropylene (PP) is particularly suitable for packaging yogurt, medicinal products, and beverages because of its somewhat translucent appearance and its resistance to the impact of heat and mechanical responses. A study was conducted to produce films formed of bioplastic containing *Chlorella* at various temperatures, using PVA as the primary material [Singh and Sharma 2012]. The adhesion between composite materials was less robust at elevated temperatures. Ultrasonication improved the quality of the blended material for blends by reacting to the uniformity of the whole mixture. Extensive studies have been conducted on the use of wheat gluten for the production of durable bioplastics. Although the material is inherently fragile, its structure can be strengthened by using additives and fillers. Wheat gluten has a substantial protein concentration, rendering it appropriate for various uses. PBS is a newly developed biopolymer that has the potential to fulfill the market's need for bioplastics. PBS is often favoured over low-density polyethylene and polypropylene (PP). Due to its efficient processing capabilities, PBS is especially favoured in the textile industry. Examples of materials derived from it are melt-blown and split yarn.

Additionally, it is employed to produce molded items in the plastics sector [Singh and Sharma 2012]. When PBS is combined with other polymers, its mechanical properties are enhanced, enabling its use in a broader range of applications. The amalgamation of PBS and *Spirulina* can improve the cost-effectiveness of *Spirulina*-based bioplastics. Multiple chemicals were employed in tests to enhance the combining process's efficiency and improve the result's quality [Subramanian 2017]. PVA is renowned for enhancing a product's strength, durability, and flexibility.

However, it is necessary to modify PVA with MA to enhance the dimensional stability and surface properties of packaging materials and their mechanical characteristics. The biomass was rinsed with acetone to obtain tiny spherical particles suitable for mixing. Sodium sulphide was employed in bioplastic synthesis to cleanse biomass before amalgamating it with other constituents. To enhance the combiningproperties of the benzoyl peroxide (BPO) and *Chlorella*-PP composite, it was mixed with MA and acetone and then put onto powdered PE [Thielen 2014].

9. Plasticizers and compatibilizers

Plasticizers are expansive chemical molecules used in substances to enhance their flexibility and ease of processing. The efficacy of the plasticizer is judged by its ability to render the target material more pliable [Tibbetts 2015]. Glycerol, with the chemical formula C3H8O3, is a widely used plasticizer in the production of bioplastics derived from microalgae. Glycerol enhanced the accessibility of macromolecules for the process of breakdown [Torres *et al.* 2015], resulting in higher flexibility and primary extensibility. This led to the production of high-quality products and increased elongation in the study. The plasticization capabilities of octanoic acid and glycerol were also observed. A series of tests showed that 1, 4-butanediol, and glycerol are suitable for plasticization due to their high water permeability [Tredici 2004]. In addition, carboxymethylcellulose (CMC) was employed to augment the mechanical characteristics of the plastic. CMC is a compound formed by alkali, cellulose, and chloroacetic acid reactions. It dissolves rapidly in cold water, has low primary viscosity, and is resistant to changes in temperature [Klunklin *et al.* 2021]. Compatibilizers are employed to facilitate the bonding of two polymers. The process of compatibilization improved the mechanical strength of the heterogeneous biopolymers. 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 [Troschl 2018]. 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) [Uduman *et al.* 2010].

10. 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 [Bussa *et al.* 2019]. 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 [Ugwu *et al.* 2008].

Nevertheless, extensive studies on microalgae farming using Life Cycle Assessment (LCA) might provide insights into specific patterns and trends [Valerie 2007]. According to the analysis of the LCA trials, fossil fuels outperformed microalgae-derived biofuels [Ali *et al.* 2023]. According to their study, microalgal manufacturing systems show significantly more efficiency increases in greenhouse gas emissions than fossil fuels [Vonshak 1997]. Data uncertainty significantly affects life cycle assessments (LCAs) on microalgae biofuel production, leading to divergent outcomes [Wang 2014]. 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 [Wang *et al.* 2016]. The natural performance of microalgae-based products is primarily unclear. However, studies often highlight the possibility of improvement in microalgal production systems [Wijffels *et al.* 2010]. 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 [Wretfors 2009]. 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

11. Tissue engineering

Typically, the PHAs that were accessible were not used for medical implants. Consequently, the PHA quality evaluation has been postponed to obtain permission from Drug Administrators. It is necessary to generate PHAs with a high purity level and thoroughly examine their biodegradation in laboratory settings and their potential for use in scaffold creation and surface modification [Xu *et al.* 2010]. PHAs that undergo crucial modifications can substantially contribute to tissue engineering and the development of medicinal and therapeutic goods, particularly for applications such as vascular grafts, heart valves, and neural tissue engineering [Yan 2016]. PHAs with enhanced mechanical strength can be utilized to fabricate scaffolds for medicinal purposes. Scaffolds fabricated using PHAs facilitate cellular development by providing nourishment [Zeller *et al.* 2013]. The primary medical goods include screws, pins, sutures, films, and other similar items [Zhang *et al.* 2019]. Poly(3-hydroxybutyrate-4-hydroxybutyrate-3-hydroxyvalerate) promotes the proliferation and adherence of stem cells [Zhang, Endo *et al.* 2000]. The polymer (3HB-3HV-3HHx) can serve as a scaffold to build liver tissue [Zhang, Kabeya *et al.* 2000].

Additionally, the use of PHA nanofibers has led to the development of three-dimensional scaffolds [Zhou *et al.* 2011]. The study discovered that P(3HB-3HO) effectively repaired the cartilage using a scaffold made from PHAs [Zhu *et al.* 2017]. The recombinant organism produces the novel P(3-HB-3HV-2,3-diHB). To improve the capacity, inorganic bio ceramics have been integrated with PHAs to boost the mechanical strength and flexibility of PHAs, resulting in the development of innovative composites for engineering tissues. Blends consisting of PHA and ceramic composites are utilized to create various mixtures. Hydroxyapatite and PHA are employed in tissue engineering as well.

12. Conclusions

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

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