Chapter

Cyanobacteria as a Source of Biodegradable Plastics

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Abstract

Polyhydroxyalkanoates (PHAs) are a group of biopolymers produced from various microorganisms that attracted many researchers for their use as a substitute for conventional petrochemical plastics. PHA possesses similar material properties to petrochemical plastics with the added benefits of biocompatibility, biodegradability, hydrophobicity, thermoplasticity, piezoelectricity, and stereospecificity. The first discovery of PHA production in cyanobacteria was in 1969, and the commercialization of PHA produced from cyanobacteria is not feasible to date. The difficulty with the commercial production of cyanobacterial PHA is due to the low biomass production and lower PHA accumulation than the heterotrophic bacteria. The biosynthesis of PHA, production of cyanobacterial PHA, and strategies to improve the production of PHA and commercialization are discussed in this chapter.

Keywords: cyanobacteria, Polyhydroxyalkanoates, biodegradable polymers, bioplastics, bioprocess, PHB, P(HB-HV), PHA properties

1. Introduction

Bioplastics are a type of plastic that can be produced from natural materials like plant starches and oils. By 2025, it is anticipated that the amount of petroleum used to produce plastic would have decreased by 15–20% due to the use of bioplastics, which are made from plants. Asia and Europe will hold the biggest market share for bioplastics by 2025. Asia will make up 32% of the market, followed by Europe at 31% and the United States at 28%. The market for bioplastics is now growing at a rate of 10% per year, accounting for 10–15% of the entire plastics business in 2016 and increased to 25–30% in 2020 [1]. Synechocystis, Spirulina, Anabaena, and Nostoc muscorum are cyanobacteria that can serve as bio-factories for the production of biofuel and bioplastic. They can produce biopolymers like polyhydroxybutyrate (PHB) and polyhydroxyalkanoates (PHAs), among other copolymers, that are both affordable and sustainable [2].

Recent bioplastics like Bio-PET are only called biobased since their monomers are made from corn, but the polymerization process is chemical, and the final polymer has the same qualities as traditional PET, making it nondegradable [3]. Scytonema geitleri and other cyanobacterial species can store internal poly-hydroxybutyrate granules for

energy and carbon reserve when under stress. The environmentally benign and biodegradable PHB can then be collected and utilized to create biocompatible thermoplastics [4]. Polyhydroxyalkanoates (PHAs) are a type of polymer produced by cyanobacteria. PHAs are lipid compounds that a variety of microbes accumulate when there are abundant carbon sources present. They can be used for a variety of purposes, including the creation of bioplastics [5]. Cyanobacteria need only a small amount of nutrients to develop, and they produce PHAs through oxygenated photosynthesis [6].

Biochemical processes can naturally recycle bioplastics manufactured from renewable resources, reducing the need for fossil fuels and preserving the environment. Bioplastics are therefore environmentally friendly, generally biodegradable, and biocompatible. In many industrial applications today, including horticulture, food packaging, hygiene, AND composting bags, bioplastics have become essential. Additionally, bioplastics are utilized in biological, structural, electrical, and other consumer goods. With the demand for plastic usage increasing globally, a lot of research is being done to investigate green materials and novel processing techniques.

Chlorosis is the term for the dormant state that occurs when nutrients are scarce, such as nitrogen. During chlorosis, cyanobacteria deteriorate their photosynthetic machinery. Beyond this breakdown, there is a significant buildup of glycogen for the storage of carbon and energy. The process ends with the cells starting to break down the glycogen and turn it into PHB [7]. The sole PHA synthesized under the described photoautotrophic state out of the several PHAs is PHB. It is possible to add organic carbon precursors like valerate to make the additional short-chain-length PHAs (scl-PHAs), such as P(3HB-co-3 HV). Long-chain-length PHA or mcl-PHA have not yet been found in cyanobacteria. The most effective known catalyst for PHB synthesis in cyanobacteria is nitrogen restriction [8]. It has been noted that elements including culture conditions, such as N, P, light exposure, and CO2 dynamics, have an impact on cyanobacteria's ability to produce PHA. Additionally, it has been noted that additional elements including two-stage (growth and PHA accumulation) processes, metabolic inhibitors for other pathways, and bioengineering have a favorable effect on PHA production [9–13].

1.1 Types of bioplastics

Following is a classification of bioplastics based on the wide definition:

i. Starch-Based Bioplastics—Starch-based polymers are defined as those that contain either natural or modified starch moieties. This group comprises polymers made from the fermentation of starch as well as mixtures of starch and natural or manufactured plastics. This makes up many of the thermoplastics already in use and represents around 50% of the worldwide bioplastics market such as thermoplastic starch (TPS) and Bio-PET.

ii. Bioplastics made of cellulose that is derived from cellulose esters or other cellulose derivatives. Because cellulose comprises glucose molecules linked together by a linkage [1, 14], certain symbiotic microorganisms are necessary for ruminants to digest it. For instance, cellulose acetate and methylcellulose.

iii. Aliphatic Polyesters—Materials that have more resistance to hydrolytic degradation, e.g., PHA and PLA.

iv. Protein-Based Bioplastics—Derivative of sources such as milk, wheat gluten, and other sources of protein. Very similar to the process of cheese-making, e.g., casein bioplastics.

v. Lignin-Based Bioplastics—Although lignin has long been a byproduct of cellulose manufacturing, it has only recently become important due to the development of biorefinery projects. For instance, PP- and PHA- and lignin polymer blends.

vi. Chitin-based bioplastic—The second most prevalent biopolymer after cellulose, chitin is comprised of N-acetyl-D-glucosamine units connected by linkages [1, 14]. Although chitin is found in the exoskeletons of arthropods and the cell walls of yeast and fungi, the shells of crustaceans like crabs, prawns, and shrimps are the main source of its extraction. For instance, bioplastics made of chitosan, chitin blended with PP, etc. [3].

1.2 Sources of bioplastics

Microbial biopolymers are natural polymers that are produced and broken down by a variety of species; they do not harm the host and have some benefits over petroleum-based plastics [14].

Because of their potential for usage and quick destruction by microorganisms, especially bacteria, biopolymers are innovative and promising. Under stressful circumstances, these biopolymers build up in microbial cells as store resources [15].

Microbiologically synthesized PHAs have shown considerable potential for various applications in the fields of (i) pharmaceuticals: controlled release and drug delivery systems; (ii) agriculture: regulated discharge of pesticides, plant growth regulators and herbicides, and fertilizers; (iii) biofuel: methyl ester of 3-hydroxybutyrate and methyl esters of 3-hydroxyalkanoate (MCL) can be used as biofuels; (iv) medicine: PHAs can be used to create absorbable sutures, bone plates, surgical pins, films, and staples, bone marrow supports, tendon repair tools, ocular implant implants, skin substitutes, cardiac valves, tissues for cardiovascular use, vascular grafts, tissue engineering applications, nerve guides, adhesion barriers, etc.; **(v)** disposable: PHAs may be utilized in the production of razors, food trays, diapers, hygiene items, cutlery, cosmetic packaging, glasses, medical surgical clothes, furniture, carpets, packaging, bags, compostable lids, and other items; and **(vi)** chromatography—Additionally, PHAs may be used as a stationary phase for chromatographic columns [16].

2. Polyhydroxyalkanoates

Bio-polymers such as polyhydroxyalkanoates (PHAs) are produced by microorganisms as lipid inclusions for granular types of energy storage inside the cellular structure [17]. PHAs are natural polyesters made from thermoplastic 3-, 4-, 5-, and 6-hydroxy alkanoic acids. More than 90 genera of bacteria, both Gram-positive and Gram-negative, have been found to produce PHAs in both aerobic and anaerobic conditions thus far. Some native bacterial strains, recombinant bacterial strains, and recombinant eukaryotes can all manufacture polyhydroxyalkanoates (PHAs). These bio polyesters are created by metabolically converting different carbon sources.

Numerous PHA polymers also offer intriguing characteristics, such as the ability to biodegrade, and they can be used for a variety of purposes, from single-use bulk plastics to specialized medicinal applications [18].

2.1 Structure of PHA

A total of 150 distinct PHA congeners have been identified. The resulting polymer is known as polyhydroxybutyrate or polyhydroxybutyric acid if the group is R = CH_3, polyhydroxyoctanoate (PHO) if R = C_3H_7, and so on.

2.2 Classification of PHA

PHAs are classified into three classes short, medium, or long chain length (scl, mcl, and lcl), respectively. It is based on the number of carbon atoms as short-chain-length PHA (scl-PHA), medium-chain-length PHA (mcl-PHA), and long-chain length PHA (lcl-PHA). Scl-PHA refers to PHA comprised of monomers having 5 or fewer carbon atoms [19]. These include 3-hydroxybutyrate and 3-hydroxyvalerate. The mcl-PHA is comprised of monomers having 6 to 14 carbon atoms. These include 3-hydroxyhexanoate, 3-octanoate, and 3-hydroxydecanoate. The lcl-PHA, which is uncommon and least studied, consists of monomers with more than 14 carbon atoms [20] (**Figure 1**).

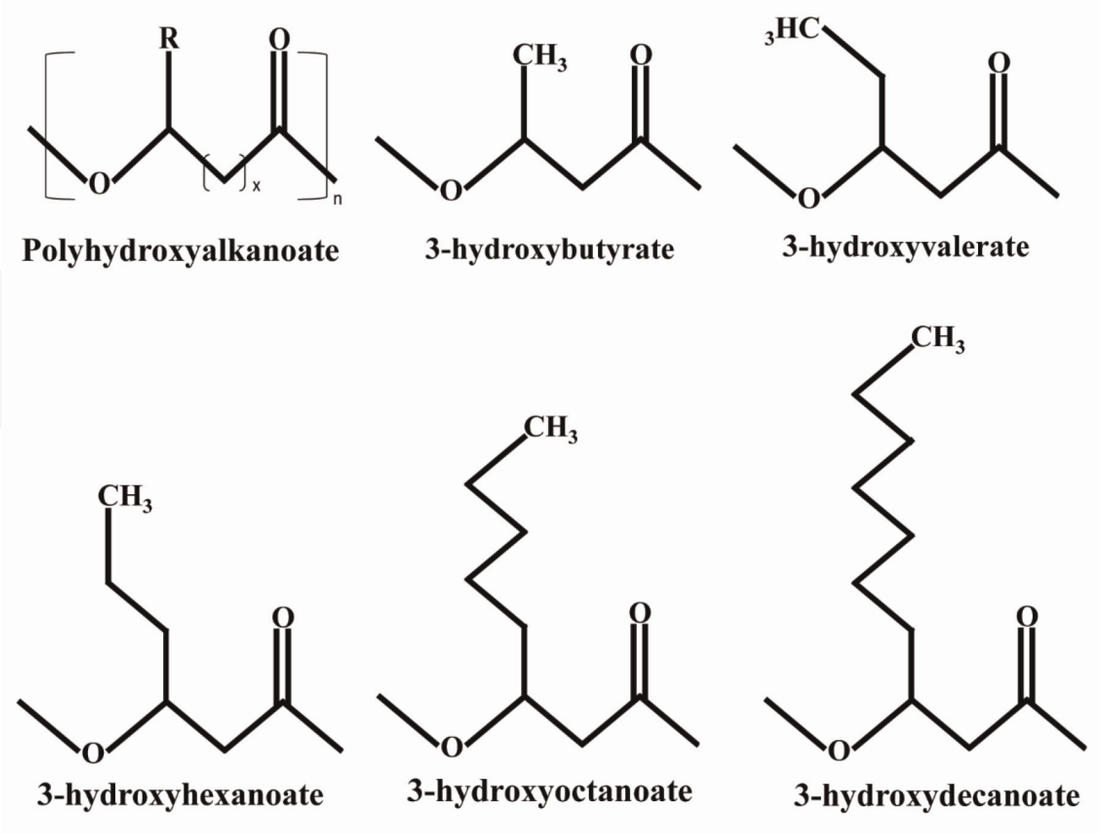

Figure 1.
General structures of polyhydroxyalkanoates.

2.3 Biosynthesis of PHA

PHAs are produced from two molecules of acetyl-CoA by three enzymatic reactions. The classical polyhydroxybutyrate (PHB) biosynthesis pathway consists of the following reactions:

1. β-ketothiolase (encoded by the phaA gene) catalyzes the formation of acetoacetyl-CoA by the condensation of 2 acetyl-CoA molecules.

2. Acetoacetyl-CoA dehydrogenase reduces acetoacetyl-CoA to R-3-hydroxybutyryl-CoA with the reduction of NADP(H) to $NADP^+$ (encoded by phaB gene).

3. PHA synthase polymerizes R-3-hydroxybutyryl-CoA to 3-hydroxyacid units (3HAs) or polyhydroxybutyrate (PHB) polymer (encoded by phaC gene).

However, apart from the classical pathway, there are other biosynthetic pathways involved in PHA production that differs based on the substrates, enzymes, and microorganisms used. The enzyme PHA synthase plays the most crucial role in PHA synthesis since it can polymerize 3-HA units obtained from different pathways such as fatty acid β-oxidation pathway, methylmalonyl-CoA pathway, and de novo fatty acid synthetic pathway [21, 22]. Numerous studies conducted on heterophilic bacteria revealed the classification of PHA synthase based on the specificity of 3-HA (C-Chain Length) substrate, amino acid sequence, and constituent subunits to have four classes [23]. Class I PHA synthases are encoded by phaC and polymerize scl-3HA units, monomers with approx 64 kDa MW. Class II PHA synthases polymerize mcl-3HA and are also encoded by phaC genes. These are monomers and have similar MW of ~63 kDa. Class III PHA synthases are heteromeric with ~40 kDa two subunits encoded by phaC and phaE genes each. They polymerize scl-3HA units. Class IV PHA synthases are similar to Class III and are encoded by either phaEC genes or phaRC genes. They polymerize scl-3HA to mcl-3HA and scl-3HA alone, respectively.

The acetyl-CoA utilized in the classical pathway of PHB synthesis is acquired as precursors derived from the tricarboxylic acid (TCA) cycle. This type of pathway is most commonly found in cyanobacteria, archaea, and heterophilic bacteria such as *Cupriavidus metallidurans*. Lipid metabolism is also used for the production of PHA which are mostly medium chain length (MCL) —PHAs. Different hydroxyalkanoates are generated from the β-oxidation pathway of fatty acids by the biotransformation of alkanes, alkenes, and alkanoates. The conversion of the β-oxidation intermediate trans-2-enoyl-CoA into (R)-hydroxyacyl-CoA is catalyzed by an R-specific enoyl-CoA hydratase (encoded by phaJ gene) and is the crucial step in this type of pathway. Studies conducted on *Aeromonas caviae* and *Pseudomonas putida* strains reported the (R)-specific manner of action of the phaJ enzyme [24, 25]. The PHA synthase (encoded by phaC genes) polymerizes (R)-hydroxyacyl-CoA into PHAs. MCL -3HA is produced in this type of pathway where both sugars and lipids are utilized. Glycolic precursor and fatty acid biosynthesis intermediates are converted to 3-hydroxyacyl-ACP by 3-hydroxyacyl-ACP-CoA transferase and malonyl-Coa-ACP transacylase. These key enzymes are encoded by phaG gene and are (R)-specific reactions by acyl-ACP-CoA transacylase. The 3-hydroxyacyl-ACP is converted into 3-hydroxyacyl-CoA and then polymerized to PHAs by PHA synthase.

Apart from the biosynthesis pathways and carbon source, other nutrients such as phosphate, nitrogen, oxygen, and sulfur also play a major role in PHA accumulation [26]. Limiting nitrogen and/or phosphorus with an excess carbon source is favorable for cell growth, along with C: N ratio changes showing better beneficial stress for PHA accumulation [15, 27, 28]. Under nitrogen deprivation, the conversion of α-ketoglutarate to glutamate is decreased causing accumulation of NAD(P)H by absorption of ammonium ions into cells. Similarly, the supplement of citrate reduces citrate synthase activity, thereby increasing the concentration of NAD(P)H. These high concentrations of NADPH result in increased PHB production since the reduction of acetoacetyl-CoA to R-3-hydroxybutyryl-CoA is increased [29]. Limiting phosphorus to a minimum level needed for cell maintenance restricts the Krebs cycle by promoting NADH accumulation, inhibiting citrate synthase and isocitrate dehydrogenase with increased acetyl-CoA. Nutritional stress induced by phosphorus limitation is sometimes more significant than nitrogen as a limiting factor in cyanobacteria and proved to be a good strategy for inducing PHA production [29] (**Figure 2**).

2.4 General properties of PHA

PHA properties are very indistinguishable from that of conventional plastics since it has great chemical diversity of radicals [17]. The ranges of these polymers vary from rigid and brittle thermoplastics to elastomers, rubbers, and adhesives which is totally based on their composition. Depending on the kinds of aromatic monomers used, aromatic PHAs exhibit a variety of properties. A lot of research has been done on the thermal characteristics of aromatic PHAs, which show behavior that is particular to the structure. Due to the increase in chain length and increase in the number of comonomers in a copolymer, its elasticity increases, and thus, PHAs have different properties according to their monomeric composition.

The physical properties of PHAs are as follows:

1. molecular mass

2. thermal properties

3. crystallinity index.

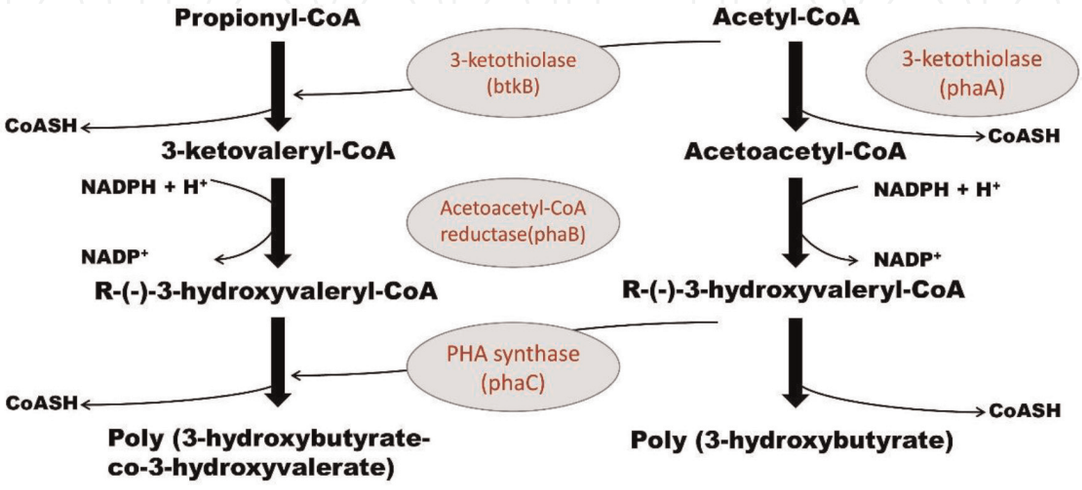

Figure 2.
Biosynthesis pathway of PHB and P(3HB-co-3 HV) copolymer. Adopted from [30].

Commercial suitability of molecular mass and molecular weight distribution of a polymer plays a vital role in characterization., and polymers with molecular mass less than 4×10^4 Da have their mechanical properties deteriorated.

The molecular weight of the compounds differs from 2×10^5 to 3×10^5 Da which depends on the type of microbial species used and growth conditions like pH, cultivation modes, and type and concentration of the carbon source. The properties of the PHA depend on the size of the polymer chains, whose structural rearrangements may depend on the degree of polymerization [31].

In addition to defining some mechanical characteristics of a material at ambient temperature, a polymer's thermal properties, such as its melting and glass transition temperatures as well as crystallinity and crystallization time, also serve as useful factors for the thermal processing of materials [32]. PHAs have melting points between 50 and 180°C and crystallinities between 30 and 70%, depending on the polymer's composition. PHAs are categorized as stiff if their crystallinity is between 60 and 80%. Medium (30–40%) and short (30%) polymer lengths characterize flexible and more elastic PHAs, respectively [31]. PHA's industrial applications are expanded thanks to its reduced degree of crystallinity, which also enhances its processing properties [32].

Semicrystalline polymers, the most popular type of PHA, are more brittle and less solvent-resistant but have tensile qualities that are comparable to those of polypropylene and polyethylene. PHB and its copolymers, which are made by cyanobacteria, have physical characteristics that can be linked to those of synthetic polymers like polypropylene and high-density polyethylene [33]. The creation of polymers with the appropriate properties will be aided by a good understanding of the connections between the PHA crystallinity and the polymer composition.

PHA is a suitable substitute for synthetic polymers due to its natural origin, biodegradability, biocompatibility, piezoelectricity, optical purity, and thermoplasticity [34]. Additionally, they are thermoplastic and/or elastomeric, nontoxic, and have a very high purity inside the cell. They are also hydrophobic, insoluble in water, inert, and indefinitely stable in the air [35]. PHA is less solvent resistant than polypropylene but has a substantially higher resilience to ultraviolet (UV) radiation degradation [36].

Numerous microorganisms in distinct situations have the ability to break down PHAs. PHA breakdown generates carbon dioxide and water under aerobic settings, whereas it generates carbon dioxide and methane under anaerobic ones [37]. The degradation time depends on a number of variables, including surface area, microbial activity of the environment, pH, temperature, humidity, the presence of other nutrients, and the properties of the polymer, such as composition and crystallinity, and can range from months (anaerobic digestion) to years (marine environment), among others.

Due to their high density, PHAs do not float in aquatic settings; as a result, after being dumped there, they sink and are biogeochemically destroyed on the surface of the sediments [37]. The two main processes involved in the biodegradation of polymeric heterocomposites, such as cellulose, starch, and aliphatic polyesters, of which PHAs are typical, are biotic or abiotic hydrolysis followed by bio-assimilation (hydrobiodegradation), and the second is peroxidation followed by the bio assimilation of low molecular mass (oxybiodegradation) products, which is applied in particular. Despite their quick biodegradability, PHAs are exceedingly stable in the air and do not decay when stored normally.

2.4.1 Appearance

Depending on the types of integrated monomers, aromatic PHAs have a variety of physical appearances. PHAs made only of phenoxy or phenyl monomers (P(3H5PhV)) are sticky and supple. When the content of 3H5PhV was increased in the instance of P(3HA-3H5PhV), the polymer softened. P(3HA-3-hydroxyphenylalkanoate) [P(3HA-3HPhA)] changed from water-soluble to glue-like as the provided acyl chain length of phenylalkanoic acid was lengthened. PHAs with methylphenoxy groups are brittle, whitish substances [38]. PHAs that contain the 3H4BzB unit are similarly difficult. PHAs with thiophenoxygroups, however, are cream in color and elastomeric. The majority of PHAs that include the difluorophenoxy monomer is also cream-colored. Even with the addition of a small number of nitrophenyl units (1.2–6.9%), the physical properties of PHAs containing the nitrophenyl group diverged significantly from those of mcl-PHA [39].

2.4.2 Mechanical properties

The P(3-hydroxydodecanoate-3H5PhV) [P(3HDD-3H5PhV)] with varied 3H5PhV contents have different mechanical characteristics. The yield strength, maximum tension strength, and elongation at the break all decreased as a result of the addition of the 3H5PhV unit to P(3HDD). It is interesting to note that P(3HDD-18.70 mol% 3H5PhV) displayed a larger elongation at break than P(3HDD). On the other hand, except for P(3HDD-31.97 mol% 3H5PhV), Young's modulus increased above that of P (3HDD). These findings suggest a nonlinear relationship between the mechanical characteristics and the content of 3H5PhV [38].

2.4.3 Surface properties

Two fluorine atoms were added to P(3H5opFPxV), and its surface characteristics were assessed. This polymer has a surface contact angle of 104°, compared to 50° for PHAs having phenoxy or alkyl groups (C3 and C5) in the side chain [38]. A surface contact angle of more than 100 is typically insufficient to use the polymer as a non-wetting material. This difluorinated PHA thus possessed water-shedding qualities [39].

2.4.4 Degradability

The capacity of aromatic PHAs to degrade has also been investigated. One crucial quality of using PHAs as biodegradable materials is degradability. The stability of PHAs at physiological pH and the safety of the substance produced during hydrolysis should be assessed for medical applications such as medication delivery systems by analyzing the chemical degradation and microorganism-mediated degradation [38].

2.4.5 Chemical degradation

According to the literature, the P(3H6PhHx) homopolymer's chemical degradation was investigated. Around pH 7, this polymer is remarkably stable. It could therefore be utilized as a medication carrier to induce a delayed release of the active ingredient [39]. Additionally, the hydrolytic products of P(3H6PhHx) may have significant pharmacological effects that could enhance or expand the therapeutic effects of the drug that is encapsulated. These hydrolytic products can be oxidized in vivo to

phenylbutyric acid, phenylacetic acid, or trans-cinnamic acid. The antibacterial activity of (R)-3-hydroxy-phenylalkanoates (C5-C8), a hydrolytic product of PHAs bearing a phenyl group, is established. The relevant study showed that all (R)-3-hydroxy-phenylalkanoates inhibited the growth of Listeria species, attributed only (or mainly) to the phenyl group. Olivera et al. created polymeric microspheres of P(3H6PhHx) [38].

2.4.6 Solubility

Bacterial PHA copolymers often display a wide range of comonomer compositions, which may result from modifications in the bacterial metabolism during PHA production. The biosynthesized aromatic PHAs are not always formed as a copolymer, but rather occasionally as a combination of two distinct PHAs. These aromatic polymers were isolated by solvent fractionation in several investigations [38].

2.4.7 Thermal properties

PHAs are polymers that are only partly crystalline. Therefore, the Tg and Tm of the amorphous and crystalline phases are typically used to express the thermal characteristics of these materials. The results of several studies show that the properties of aromatic PHAs differ significantly from those of mcl-PHAs, which are elastomers with Tgs between 53 and 28 C and a Tm between 45 and 69 C, where the values change depending on the types of aromatic monomers used [38].

2.4.8 Extraction of PHA

Treatment of cellular disruption and/or instability, recovery, and purification of biopolymers are the steps involved in the PHA extraction process. These procedures allow for the use of chemical, physical, biological, or even a mix of these technologies to provide a product with high purity and preserved physical and thermal characteristics.

The first step in the PHA extraction method is to centrifugate the solid material, which is made up of cells containing intracellular biopolymer, from the culture broth. Additionally, the microbial cell wall may be punctured or disturbed through biological, physical, or chemical means [40]. A suspension of bio-polymer, cells containing biopolymer (cells that destabilize but do not break cell walls), and cell debris form upon rupture or instability of the cell wall (mixture of proteins, nucleic acids, lipids, and cell-wall fragments). The next stage is to recover the biopolymer, which can be done in a variety of ways, including chemically, biologically, physically, or utilizing a combination of approaches like physical and chemical, biologically and chemical, among others [16].

2.4.9 Chemical methods

Isolated or coupled solvents are used in the chemical processes of removing PHAs from the cells of the microorganisms [40]. Chloroform, acetone, methyl isobutyl ketone, methylene chloride, propylene carbonate, ethyl acetate, and isoamyl alcohol are the most often used solvents. It is vital to assess the contact time and heating temperature of the polymer with the solvent to gauge the efficacy of the extraction process and the quality of the resulting product [16].

2.4.10 Physical methods

The use of homogenizer mills and ultrasound is among the most popular physical techniques used in PHA extraction. These methods are typically used at the beginning of the extraction procedure to disrupt and weaken the microorganisms' cell membranes. When compared to chemical extraction techniques, mechanical extraction yields polymers with higher thermal characteristics while also being more cost-effective and less hazardous. If an appropriate chemical method is used in conjunction with the mechanical method to extract biopolymers, which allows for high PHA recovery without significantly altering its features, the possibility for recovery will be increased [16].

2.4.11 Biological methods

The biological method of microbial PHA extraction is a complicated procedure that relies on the use of enzymes including lysozymes, nucleases, and proteases to recover the biopolymer. The culture broth is supplemented with enzymes to hydrolyze PHA-containing cells [16]. The gentle operating conditions, great selectivity of the enzymes in hydrolyzing the microorganisms' cell wall proteins without affecting the breakdown of the polymer, and high quality of the recovered polymer make this technology appealing [40].

3. Cyanobacteria as a source of bioplastics

The PHB accumulation in cyanobacteria was first reported by Carr G.N. in 1966 with up to 10% (dcw) in *Chlorogloea fritschii* [41]. In photoautotrophic culture *Artrospira platensis (Spirulina)* accumulated a maximum PHB of 6%, and it is very less for exploiting cyanobacteria for PHA thermoplastics production. However, PHA biosynthesis in, *Synechocystis sp*. PCC 6803*, Nostoc muscorum*, and *Synechococcus sp.* MA19 produced up to 38, 46, and 55% (dcw), respectively, under different limiting culture conditions reported in studies [9, 42, 43]. The production of PHA reported in several other strains in photoautotrophic and also with supplementation of acetate or other organic carbon sources are profoundly lower compared to heterotrophic bacteria. Chen et al. reported a maximum accumulation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) [P(3HBco-3HHx)] co-polymer up to 50% (dcw) in *Aeromonas hydrophila* 4AK4 grown in 5% glucose medium with 5% lauric acid under phosphorous limitation with a productivity of 540 $mg.L^{-1}.h^{-1}$ [44]. Despite the high PHA accumulation, bacterial PHA thermoplastic has commercial limitations since the organic carbon substrate itself accounts for ~30–50% of the total cost of production on a large-scale [15]. For example, PHB production of up to 77% (dcw) was reported in recombinant *Escherichia Coli* using glucose as substrate with a productivity of 3200 $mg.L^{-1}.h^{-1}$; however, the carbon source used accounts for 38% of the overall cost of production [45]. Compared to heterotrophic bacteria (4–5% carbon substrate), cyanobacteria required significantly lower carbon substrate at about 0.4% [44, 46]. Thus, cyanobacteria are a more promising candidate for the large-scale production of bioplastics.

3.1 General Cultivation of cyanobacteria

Cyanobacteria are photosynthetic prokaryotes found in both fresh and marine water, soil, etc., and they have a unique physiology that makes them survive even in

harsh ecological habitats such as deserts, hot springs, volcanic substrates, and even in alkaline basins. The cyanobacteria can be cultivated in three different culture systems—an open-raceway pond (mostly preferred), a closed system (photobioreactor), and a hybrid system (combination of both open and closed systems) [47]. The widely used media for the cultivation of cyanobacteria is BG11 having the following composition (1500 mg.L^{-1} $NaNO_3$, 31.4 mg.L^{-1} K_2HPO_4, 36 mg.L^{-1} $MgSO_4$, 36.7 mg.L^{-1} $CaCl_2.2H_2O$, 20 mg.L^{-1} Na_2CO_3, 1 mg.L^{-1} NaMgEDTA, 5.6 mg.L^{-1} citric acid, 6 mg.L^{-1} ferric ammonium citrate, and 120 mg·L-1 $NaHCO_3$) (himedialabs). The components of the modified BG-11 used in the reactors are (K_2HPO_4, $NaNO_3$, $NaHCO_3$, $CaCl_2.2H_2O$, NaOH, Na_2EDTA, and NaHCO3). The optimum pH and temperature for the growth of cyanobacteria are 7.5–9 and 30 ± 2°C, respectively. The culture takes up to 7 days to reach the log phase, and the complete growth cycle ends in 20 days (after reaching the death phase).

3.1.1 Open systems

Open ponds are the natural ecosystem in which the algae tend to grow and develop. Open systems are classified into two types—natural (lakes and ponds) and artificial (containers and artificial ponds). There are several advantages of growing cyanobacterium in open systems which include low investment, construction of the pond being easier, and easy maintenance. Some of the drawbacks include a requirement for large land, poor light penetration, and low biomass productivity [48].

3.1.2 Closed systems

Photobioreactors are considered to be the closed system for the cultivation of cyanobacterium. By using this culture system, the drawbacks of the open system can be neglected. There are several advantages of using a closed system for algal cultivation which include control over culture parameters (pH, temperature, etc.,), low level of contamination, and good mixing that induces high gas exchange within the culture. There are various types of closed system available for the culture of algae which includes vertical column, tubular bioreactor, flat-plate bioreactor, etc [48].

3.1.3 Hybrid system

A combination of both open and closed systems is known as a hybrid system. There are two stages of cultivation in which the first stage involves a closed system and the second stage occurs in the open-raceway system. By utilizing this system, the advantages of both open and closed systems are possible. Many ongoing studies are designing a commercial-scale hybrid reactor that can be economical and can be easy to handle [49].

4. PHA from cyanobacteria

4.1 Biosynthesis of PHA in cyanobacteria

For decades it was believed that cyanobacteria possess an incomplete Kerbs cycle like some prokaryotes due to the absence of the 2-oxoglutarate dehydrogenase

complex which performs the conversion of 2-oxoglutarate to succinyl-CoA in the TCA cycle [50]. Since the TCA cycle is incomplete, it is assumed that the breakdown of PHB polymers generating acetyl-CoA could be utilized neither for the production of cell components nor for energy generation [51]. It was hypothesized that this cycle was closed by the glyoxylate stunt of aspartate transaminase reactions [52]. However, recent studies reported that the Kerbs cycle was completed with help of γ-aminobutyric acid shunt and 2 enzymes 2- oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase found in *Synechocystis sp.* PCC 6803 [53] and *Synechococcus sp.* PCC 7002 [54], respectively. The later reported protein-encoding genes are present in most cyanobacteria with variation in their organization.

The PHA polymer biosynthesis is linked with mobilization or depolymerization [5]. The PHA polymers usually undergo a cyclic process of biosynthesis and depolymerization, where the PHA is formed from acyl-CoA precursors via different metabolic routes under nutrient depletion/limitation conditions as the carbon source is stored as polymer granules in the cells. The mobilization of PHB polymers is carried out by intracellular PHB depolymerase generating acetyl-CoA which is used to generate oxidation via the Krebs cycle. Many studies reported the regulatory effect of acetyl phosphate produced by the phosphotransacetylase catalytic activity on the post-translation of PHB synthase enzyme [55–59]. The exploitation of exogenous carbon sources such as glucose, fructose, and acetate showed decreased mobilization and increased biosynthesis of PHA [60–63].

4.2 PHA production

The PHA-producing cyanobacterium is classified into two groups—one group requires a limitation of an essential media component for PHA production, and another group does not require any limitation in nutrients for the production of PHA. The cyanobacterium that can be cultivated without nutrient limitation is preferred on an industrial scale. A few studies have been conducted to optimize the nutrients for the production of PHA and PHB on large scale in batch mode. In a study, *Synechocystis* sp. PCC 6803 was cultivated in BG11 media with reduced nitrogen concentration and showed a maximum PHB accumulative of 180 $mg.ml^{-1}$ [47], *Synechocystis* sp. CCALA192 was cultivated in a 200 L tubular photobioreactor in batch mode and accumulated a maximum of 125 $mg.ml^{-1}$ of PHB, and a wild-type cyanobacterial strain *Synechocystis* sp. PCC 6714 produced a maximum of 640 $mg.L^{-1}$ of PHA when cultivated in optimized growth media [64].

Several studies reported that higher PHA accumulation in cyanobacteria occurs under nutritional stress activating the PHA biosynthesis pathway. According to Mendhulkar and Shetye [65], the metabolic pathways are diverted to produce carbon-rich compounds for energy storage, such as PHAs, and glycogen, when the cyanobacteria experience nutrient deficiency (nitrogen and/or phosphorus). The study on cyanobacteria *Synechococcus subsalsus* and *Spirulina sp.* LEB18 in nitrogen-deficient environment revealed that the carbon source is diverted to other metabolic pathways for biopolymer production which is used as energy storage and reused in favorable conditions [66]. PHA accumulation in *Botryococcus braunii* and *Synechocystis salina* grown in BG-11 medium without any nutritional limitation was reported [67, 68]. Different nutritional conditions are employed to increase the production of PHA such as excess or limited levels of nitrogen and/or phosphorus, acetate, and propionate, and various other conditions like salinity, gas exchange, wastewater as a source, etc., were summarized in **(Table 1).** Apart from culture condition variations,

Cyanobacteria	PHB content (% DCW)	Substrate	Production condition	Polymer composition	Reference
Synechocystis sp. PCC 6803	38	Acetate	P limitation and gas exchange limitation	PHB	[69]
Synechocystis sp.	11		Nitrogen and phosphorous deficiency	PHB	[69]
Synechococcus sp.MA19	55		Phosphorous deficiency	PHB	[43]
Synechocystis sp. PCC 6714	16	CO_2	N^2 and P^3 limitation	PHB	[12]
Spirulina platensis	6.0	CO_2	Not given	PHB	[70]
Spirulina platensis UMACC 161	10	Acetate and CO_2	N starvation	PHB	[71]
Botryococuus braunli	16.4	Sewage wastewater	BG 11 medium	PHB	[67]
Spirulina sp. LEB-18	12		Nitrogen deficiency	—	[66]
Spirulina platensis	10		Addition of acetate and CO_2	PHB	[71]
Synechocystis salina	5.5–6.6	CO_2	BG 11 medium	PHB	[68]
Synechococcus subsalsus	16		Nitrogen deficiency	—	[66]
Spirulina maxima	7–9	CO_2	N and P limitation	PHB	[72]
Synechocystis sp. PCC6803	5		BG 11 medium	—	[73]
Synechococcus elongates	17.15	Sucrose	Nitrogen deficiency	PHA	[65]
Synechococcus elongates	7.02	Sucrose	Phosphorous deficiency	—	[65]
Gloeothece sp. PCC 6909	9.0	Acetate	—	—	[15]
Microalgae consortium	43	Agro-based industrial wastewater and activated sludge	Wastewater	PHB	[74]
Microalgae consortium	31	Agro-based industrial wastewater and activated sludge	Wastewater	PHB	[75]
Nostoc muscorum	69		Phosphorous deficiency	P(3HB-co-3 HV)	[10]
N. muscorum	31	Acetate and propionate	Addition of acetate and propionate	P(3HB-co-3 HV)	[9]

Cyanobacteria	PHB content (% DCW)	Substrate	Production condition	Polymer composition	Reference
N. muscorum Agardh	60	Acetate and valerate	N deficiency	PHB-co-PHV	[11]
N. muscorum	22	CO_2	P starvation	PHB	[61]
Spirulina subsalsa	7.45	Acetate and CO_2	Increased salinity	PHB	[76]
Spirulina sp. LEB18	30.7	Nitrogen deficiency	PHB	[77].
Aulosira fertilissima	49	Acetate	Gas exchange limitation	PHB	[78]
Alusira fertilisim CCC444	77	Fructose and valerate	N deficiency	PHB-co-PHV	[79]
Alusira fertilisima CCC444	85	Citrate and acetate	P deficiency	PHB	[80]
Synechocystis PCC 7942	3	CO_2	N limitation	PHB	[81]
Synechocystis PCC 7942	25.6	Acetate	N limitation	PHB	[81]
Synechocystis sp. CCALA192	12.5	CO_2	N limitation	PHB	[13]
Anabaena cylindrica	<0.005	CO_2	Balanced Growth	PHB	[82]
A. cylindrica	2.0	Propionate	N limitation	PHB + PHV	[82]
Synechococcus elongatus	17.2	CO_2 and sucrose	N deficiency	—	[65]
Caltorix scytonemicola TISTR 8095	25	CO_2	N deficiency	PHB	[83]

Table 1.
PHA production in cyanobacteria under different culture conditions.

highly productive strain selection can also increase the PHA accumulation yields ranging from 5.0% to about 70% (dcw).

Coelho et al., [77] reported higher percentages of PHA accumulation in *Spirulina sp.* using Zarrouk medium with nitrogen and phosphorus limitations of 30.7% and 14.1% (dcw), respectively. Phosphorus and gas exchange limitations along with additional acetate and nitrogen and phosphorus limitations in *Synechocystis sp.* PCC 6803 lead to PHA accumulation of about 38% and 11% (dcw), respectively [69]. Studies conducted by Bhati and Mallick on PHB-PHV co-polymer production in *N. muscorum* under nitrogen and phosphorus deficiency resulted in co-polymer accumulation of about 60% and 69% (dcw), respectively [10, 11]. Samantaray and Mallick reported a maximum of 85% (dcw) PHB and 77% (dcw) PHB-co-PHV in *Alusira fertilisima* CCC444 under nitrogen deficiency with fructose and valerate supplementation and phosphorus deficiency along with additional citrate and acetate, respectively [79, 80].

4.3 Strategies to improve cyanobacterial PHA production

4.3.1 Genetic manipulation

Many studies have been conducted on gene manipulation of cyanobacteria on metabolic engineering and PHB synthesis, *Synechhocystis sp.* PCC 6803 is the most studied strain. Insertion of *C. nectar* PHA operon into *Synechococcus* PCC 7942 increased the PHA production from 3 to 25% (dcw) [84]. The *Synechhocytis sp.* PCC 6803 was transfected with the PHA synthase gene from *C. nectar* and showed increased activity but net PHB content did not increase [73]. Overexpression of phaAB with 4 mM acetate supplementation showed an increase in PHB of up to 35% (dcw) in *Synechocytis sp. PCC6803* [85]. Wang et at. reported volumetric productivity of 263 $mg.L^{-1}.d^{-1}$ and a yield of 1.84 $g.L^{-1}$ by overexpression of the acetoacetyl-CoA reductase gene in *Synechocystis* [86]. **Table 2** summarizes further studies conducted on genetic manipulation for increasing PHB production.

4.3.2 Suppressing glycogen synthesis pathway

The 3PG intermediate is utilized for both glycogen and PHB polymer production. The productivity of glycogen is high and quicker than that of PHB in nitrogen deprivation conditions (30% PHB and 60% glycogen (dcw) is produced) [92]. Assimilation of CO_2 through ribulose-1,5-biphosphate carboxylation by the Rubisco produces 3PG which is directed to glycogen biosynthesis more than PHB accumulation. Grundel

Cyanobacteria	Genetic manipulation	Culture conditions	PHB content (% DCW)	Reference
Synechocystis sp. PCC 6803	Overexpression of PHA synthase	Direct photosynthesis	14	[87]
Synechocystis sp. PCC 6803	Transconjugant cells harboring expression vectors carrying PHA genes	CO_2	7.0	[88]
Synechocystis sp. PCC 6803	Introducing PHA biosynthetic genes from C. nectar	Acetate and nitrogen limitation	11	[73]
Synechocystis sp. PCC 6803	Increasing acetyl-CoA levels	CO_2	12	[89]
Synechocystis sp. PCC 6803	Overexpression of native PHA genes	CO_2 and nitrogen deprivation	26	[85]
Synechocystis sp.	Optimization of acetoacetyl-CoA reductase binding site	CO_2	35	[86]
Synechococcus sp. PCC 7942	Defective in glycogen synthesis	CO_2	1.0	[90]
Synechococcus sp. PCC 7942	Introducing PHA biosynthetic genes from C. nectar	Acetate and nitrogen limitation	26	[81]
Synechococcus sp. PCC 7002	Introduction of GABA Shunt	CO_2	4.5	[91]

Table 2.
Genetic manipulations to increase PHB biosynthesis.

et al. reported that there is no influence on growth under continuous light conditions while the biosynthesis pathway of glycogen was impaired in *Synechocystis sp.* PCC 6803 [93]. In the study conducted by Wu et. al, [94], an increase in PHB accumulation from 8–13% was observed in knockout mutants unable to produce glycogen and did not turn into dormant mode and was unable to recover from nitrogen scarcity. However, PHB-deficient mutants produced the same level of glycogen as the wild-type and recovered from scarcity once replenished with nutrients. A deficiency of growth was observed in the mutants with the knockout of genes involved in both polymer syntheses. Thus, it is important to improve the synthesis of PHB yield with robust PHB production and suppressed glycogen pathway.

4.3.3 Exploitation of metabolic inhibitors to increase cyanobacterial PHA

As the imbalance of C: N and NADPH: ATP ratios are contributing factors in stimulating PHB production many studies were carried out on the effect of the metabolic inhibitor on PHB production. Upon supplementing *N. muscorum* with carbonylcyanide m-chlorophenylhydrazone (CCCP) and dicyclohexylcarbodiimide (DCCD), the PHB pool was increased to 21% and 17% from 8.5%, respectively, were reported [55]. The addition of monofluoroacetate increased the PHB pool up to 19% (dcw), while Lmethionine-DL-sulfoximine (MSX) and azaserine addition also enhanced PHB production. Treatment with metabolic inhibitors such as DCCD, CCCP, and [3-(3,4-di chlorophenyl)-1,1- dimethylurea influenced the NADPH: NADP ratio along with PHB accumulation in *Synechocystis* PCC 6803 were reported [95]. This strategy of using metabolic inhibitors could help to enhance PHA accumulation in both wild-type and recombinant cyanobacteria.

4.3.4 Mixed consortium

The mixed consortium of cyanobacteria, bacteria, and algae is a feast-famine strategy where a sequencing batch reactor (SBE) without aeration is used for the cultivation [96, 97]. The concept of the consortium is developed to increase the system efficiency by enhancing productivity and accessibility of resources, community stability, efficient nutrient cycling, and partitioning, and distribution of carbon or energy source in a non-competitive manner. The oxygen produced by the algae cells during the famine phase is used to consume the NADPH reserves of the cells leading to around a 20% (dcw) increase in PHA accumulation [97]. A permanent feast regime under high light intensity conditions promoted PHA production to a maximum of 60% (dcw) in photosynthesis mixed culture. The famine phase can be eliminated using axenic dark feast conditions increasing productivity by up to 60% (dcw) by facilitating the acetate uptake [96, 97].

4.3.5 Two-Stage cultivation

The two-stage cultivation strategy is exploited for high biomass production and increased concentration of PHA thermoplastics. The cells are grown in optimal nutritional conditions in the first or growth stage to achieve high biomass concentration. The cells are recultivated in fresh media with the limitation of a specific nutrient (nitrogen and/or phosphorous) in the second or accumulation stage to induce stress and produce PHA. A study conducted on *Chlorogloea fritschii* TISTR 8527 in two-stage cultivation shows a maximum PHB accumulation of 25% (dcw) using acetate as

substrate with 51 ± 7% (w/w) of conversion efficiency [83]. As the first stage produces maximum biomass this strategy appears to be potentially viable for large-scale production, but the shear forces experienced by the cells during recultivated give rise to a new lag phase. Two-stage cultivation of *Synechocystis cf. salina* PCC 6909 operated in a single stage without recultivation of biomass produced about 90 $mg.L^{-1}$ of PHAs in 14 days. The cyanobacterium was grown in an optimized media such that the phosphorous and nitrogen were almost utilized by 7–8 days with a maximum biomass production of up to 2 $g.L^{-1}$ (dcw) and thereby entered the accumulation stage due to nutrient starvation without harvesting and transfer of biomass [98]. The overall production cost of PHA production can be reduced using such type of two-stage cultivation strategy.

5. Problems

Currently, the major bottleneck is the non-existence of an economical mass cultivation strategy for the commercial production of cyanobacterial PHAs. The two commercial-scale mass cultivation approaches as **(i)** closed photobioreactors and **(ii)** conventional open pond culture systems. The close photobioreactors are effective for monoculture cultivation as they are of more controlled types. An ideal photobioreactor should be flexible to all system requirements for different strains and specific growth environments for the production of the product of interest [99]. Open pond culture system is cheaper compared to photobioreactor which requires high construction, operation, and maintenance cost.

Biomass harvesting from the water on a commercial scale is still a major issue partly due to the low concentration (0.2–2 $g.L^{-1}$), small size, and colloidal stability [100]. Filtration, flocculation, gravity settling, and centrifugation are some of the techniques exploited for harvesting cyanobacterial biomass. Flocculation is cost-effective and energy efficient compared to centrifugation and it can also handle a huge volume of culture. Addition of inorganic salts such as $AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$, and so on, cationic starch and chitosan are used for the flocculation of biomass [101, 102]. Several research efforts are being carried out for developing cost-effective and efficient cyanobacterial biomass harvesting technologies. For example, the settling velocity distribution of flocculated microalgal/cyanobacterial biomass is a critical parameter for developing cost-effective gravity settlers for biomass recovery.

The drying of biomass is essential for further downstream processing and storage. Around 20% of the overall cost of PHA production from *Spirulina* is contributed to the drying process. The high-energy input process of drying is only required for PHA extraction. Air drying is quite feasible, but it requires a large area and a longer time. Solar or wind energy utilization for the drying process could overcome these limitations [6].

6. Applications

PHA has a lot of advantages over conventional plastics because of its sustainability, now fossil plastics are to be replaced the major obstacle to be faced is the reduction of the cost associated with microbiological plastic production. The cost of producing traditional petroleum-based plastic in 2002 was €1.00/kg, which was considerably less than the €9.00/kg cost of PHA. Even when compared to other sustainable polymers, like PLA, which costs €1.72/kg, microbiological manufacture of PHA costs €2.49/kg,

which is still pricey [103, 104]. Carbon source plays an important role in facing the obstacles such as yield of the input, fermentation, productivity, and downstream processing [105, 106].

7. Strategies to choose to face the obstacle related to a circular economy and industrial ecosystem

Keen interest in cyanobacteria is because of the production of different metabolites which works with more than one type of compound as a salable product this type of application use is called a "cradle to cradle" system (turning waste into a new product) that is bioplastic [107]. Another instance of turning waste into a new product is using microalgae, reusing the effluents from the refining of olive oil in the cultivation of microalgae for biodiesel and biopolymers [29].

Another beneficial environmental effect that makes the adoption of a circular bioeconomy more real is the uptake of ambient carbon dioxide for conversion into biotechnological products. Using by-products and leftovers from microbiological production, it is possible to integrate the creation of bioplastic with the manufacture of other desirable goods to reduce the cost of microbial PHB. An effective alternative is the cyanobacterial genus *Nannochloropsis sp.,* which produces eicosapentaenoic acid, and the cyanobacterial genus *Spirulina platensis*, which produces linoleic acid. This species is important for its expressive biomass output, which has a high protein content and can be used to make animal feed or nutraceuticals.

The construction of a biorefinery, merging *Synechocystis salina's* PHB synthesis with commercially valuable pigments, notably the commonly abundant phycocyanin and chlorophyll, and carotenoids, showed encouraging results. Since the quality of the resulting polymer is directly influenced by purification, which includes the removal of pigments that can be employed in manufacturing chains of higher value, the extraction of pigments without their degradation is not only feasible but also necessary. In addition to pigments, *S. salina* biomass contains carbohydrates, lipids, and proteins that can be used as animal feed as long as the necessary nutritional standards and laws regarding the presence of contaminants like heavy metals or mycotoxins are observed. In this case, cyanotoxins are given priority over cyanobacteria that do not produce toxins [108].

Cyanobacterial dietary supplements are also advantageous for animal health, with *Spirulina sp.* biomass enhancing hens' humoral and immunological responses. For cyanobacteria and microalgae in general, the dual benefit of production connected with bioremediation has already been discussed, with a focus on the creation of biodiesel. The same idea can be used to explain how naturally transformable organisms like cyanobacteria can produce biopolymers, opening new opportunities for genetic engineering.

8. Conclusion

PHA has turned out to be a substitute for conventional plastics. Cyanobacteria is becoming the alternative source of PHA production. The major cause of the production of PHA using microalgae is to reduce the cost. Now, cyanobacteria aids in the production of PHA as it collects a huge amount of PHS through photosynthesis which ultimately requires less nutritional content for growth. Cyanobacteria have a very low

yield of autotrophic PHA; in near future, a biological system can be constructed to make use of the resources and attributes which can increase in production of PHA through autotrophic and heterotrophic. Production of PHA using microalgae has many other advantages like industrial compounds, which include pigments, antioxidants, cosmetics, pharmaceuticals, polysaccharides, and so on. Additionally, it has been noted that these organisms create a variety of secondary metabolites, poisons, and other bioactive substances that are significant from a pharmacological perspective. The economics of cyanobacterial PHAs would unquestionably be improved by integrating all of these substances under a refining method.

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