Chapter

# Cyanobacteria for PHB Bioplastics Production: A Review

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## Abstract

Cyanobacteria, or blue-green algae, can be used as host to produce polyhydroxyalkanoates (PHA), which are promising bioplastic raw materials. The most important material thereof is polyhydroxybutyrate (PHB), which can replace the commodity polymer polypropylene (PP) in many applications, yielding a bio-based, biodegradable alternative solution. The advantage from using cyanobacteria to make PHB over the standard fermentation processes, with sugar or other organic (waste) materials as feedstock, is that the sustainability is better (compare first-generation biofuels with the feed *vs.* fuel debate), with $CO_2$ being the only carbon source and sunlight being the sole energy source. In this review article, the state of the art of cyanobacterial PHB production and its outlook is discussed. Thirty-seven percent of dry cell weight of PHB could be obtained in 2018, which is getting close to up to 78% of PHB dry cell weight in heterotrophic microorganisms in fermentation reactors. A good potential for cyanobacterial PHB is seen throughout the literature.

**Keywords:** polyhydroxybutyrate (PHB), bioplastics, EN13432, biodegradability, organic carbon content, microplastics, cyanobacteria

## 1. Introduction

Bioplastics [1–3] are either biodegradable, e.g., according to the standard EN13432 [4], or at least partly made from renewable raw materials, e.g., according to ASTM D6866 [5]. Although their market share today is only approx. 2%, they see two-digit growth figures [6]. The sustainability of bioplastics is reviewed in [7]. Plastics in general and their composites are a large and important class of materials. The global production volume exceeds 300 million tons/year [8]. For a bioplastics material to have a major impact, it has to match the key properties of one of the commodity plastics such as PP, PE, PVC, PS or PET. This is the case with polyhydroxyalkanoates (PHA), which have the potential to replace mass polymer PP in many applications. Polyhydroxybutyrate (PHB) is the most important representative of PHA.

*Cyanobacteria* [9–11] are a phylum of bacteria that obtain their energy through photosynthesis, and they are the only photosynthetic prokaryotes that can produce oxygen. The name "cyanobacteria" is derived from the Greek word for "blue," which is the color of cyanobacteria. Cyanobacteria are prokaryotes, and they are also called "blue-green algae," though the term "algae" is not correct technically, as it only includes eukaryotes.

It was discovered that cyanobacteria can produce polyhydroxyalkanoates (PHA) photoautotrophically [12], with the potential for $CO_2$ recycling and bioplastics production. This chapter is an up-to-date review on PHB production from cyanobacteria, since the last review article on this topic [13] was written already 5 years ago.

## 2. PHB, a commodity bioplastics for mass market products?

Today, thermoplastic starch (TPS) and polylactic acid (PLA) are the two dominant biodegradable bioplastics materials. Partly, bio-based PET (see, for example, the PlantBottle™ project) and "Green PE," a polyethylene made from sugarcane-derived ethanol in Brazil, are the two most common nondegradable, but bio-based plastics. PHB has striking similarities to PP and has therefore been envisaged as potential replacement candidate for PP by Markl et al. [14], for instance, in biomedical, agricultural, and industrial applications [15]. The following **Table 1** shows a comparison of PHB and PP.

| Property | PHB | PP |
|---|---|---|
| Crystalline melting point (°C) | 175 | 176 |
| Crystallinity (%) | 80 | 70 |
| Molecular weight (Daltons) | $5 \times 10^5$ | $2 \times 10^5$ |
| Glass transition temperature (°C) | 4 | −10 |
| Density (g/cm$^3$) | 1.250 | 0.905 |
| Flexural modulus (GPa) | 4.0 | 1.7 |
| Tensile strength (MPa) | 40 | 38 |
| Extension to break (%) | 6 | 400 |
| Ultraviolet resistance | good | poor |
| Solvent resistance | poor | good |

**Table 1.**
*Properties of PHB compared to those of PP (source: [16]).*

| Properties | PHB | PHBV | PHB4B | PHBHx | PP |
|---|---|---|---|---|---|
| Melting temperature (°C) | 177 | 145 | 150 | 127 | 176 |
| Glass transition temperature (°C) | 2 | −1 | −7 | −1 | −10 |
| Crystallinity (%) | 60 | 56 | 45 | 34 | 50–70 |
| Tensile strength (MPa) | 43 | 20 | 26 | 21 | 38 |
| Extension to break (%) | 5 | 50 | 444 | 400 | 400 |

**Table 2.**
*Property modification by copolymerization (source: [13]).*

The low elongation and break and the brittleness of PHB are limitations. These, however, can be overcome by using other PHA, blends of copolymers, see **Table 2**.

Apart from short-chain-length PHA, there are medium- and long-chain-length variants, too, [17], so that material properties can be tailored in a wide spectrum.

The majority of PP is used in short-lived plastic products such as rigid packaging, which partly end up in nature. A biodegradable alternative can be a sensible material solution. Since PHA can be selected and customized for various applications, and also blended, co-polymerized and compounded, it is estimated that up to 90% of all PP applications can be covered by PHA and to a large extent thereof by PHB. A disadvantage of PHB is its high production cost. In [15], ways to make PHA production more cost-competitive are listed (see **Table 3**).

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| Technology | Reasons and/or purpose | Methodology |
|---|---|---|
| High cell density fermentation | Achieve effective growth and cells recovery | Manipulation on quorum sensing and cell oxygen uptake mechanisms |
| Growth cells in low cost substrates or mixed substrates | Substrates contributed to over 60% of PHA cost | Screening targeted substrates utilizing bacteria able to produce high content PHA |
| Fast growing cells | Reduce fermentation duration and avoid microbial contamination | Minimizing bacterial genome, changing cell growth patterns |
| Fast growing $CO_2$ utilizing bacteria able to produce PHA | $CO_2$ is a free substrate | Manipulating the $CO_2$ uptake mechanism such as carboxysomes, etc. |
| Open (unsterile) and continuous fermentation process | To save sterilization energy, reduce fermentation complexity and improve process effectiveness | Screening for PHA producers able to grow fast in extreme environments such as high or low pH and temperature, high osmotic pressure, etc. |
| PHA synthesis induced by oxygen limitation | Oxygen is a limited factor in all high cell density growth | Place PHA synthesis operons behind microaerobic promotor |
| Ultrahigh PHA accumulation (over 95% PHA in cell dry weight) | To avoid expensive and complicated downstream PHA purification process | Manipulating the PHA synthesis mechanism and PHA synthases |
| Increase substrate (mostly carbon sources) to PHA conversion efficiency | Substrates contributed to over 60% of PHA cost | Removing pathways that consume substrates for non-PHA metabolisms, and/or reinforce PHA synthesis flux |
| Enlarging the PHA production cells | To allow more cellular space for PHA accumulation, this also allows easy cells recovery | Engineering the cell division patterns and/or cytoskeletons |
| Inducible cell flocculation | Allow easy biomass recovery after fermentation | Inducible expression of surface displaying adhesive proteins |
| Inducible cell lysis | Allow easy PHA granules recovery after biomass harvest | Inducible expression of cell lysis proteins |
| Cell disruption by PHA hyperproduction | Save the biomass harvest process | Manipulating the PHA synthesis mechanism and PHA synthases |
| Extracellular PHA production | Not limited by a small cellular space, also for easy PHA granule recovery | Need new PHA synthesis mechanisms |
| Large PHA granules | Allow easy recovery of PHA granules from lysis broth | Manipulating the formation of PHA granules associated proteins |
| A synthetic cell combining the above properties | Achieve up-stream and down-stream competitivenesses | An artificial cell with assembled functional DNA |

**Table 3.**
*Technology to be developed to lower PHA production cost (reproduced with permission from [15]).*

Avoiding feedstock costs and using $CO_2$ as sole carbon source are described as strong potential here.

In general, organic carbon feedstocks can yield high PHB contents in microorganisms. For instance, Bhati et al. produced 78% PHB of dry well weight with *Nostoc muscorum* Agardh [18].

An alternative production pathway for PHB is a catalytic one [19, 20]. Both the fermentation and the catalytic process yield an expensive PHB product, which is hard to sell as it competes with low-price commodities such as PE and PP for packaging applications, which are very cost-sensitive.

## 3. PHB production by cyanobacteria: current state of knowledge

It is known that cyanobacteria can produce PHB as an intracellular energy and carbon storage compound [21] (see **Figure 1**).

Reference [23] discusses the use of cyanobacteria to produce chemicals. Cyanobacteria show several industrially relevant benefits compared to their plant counterparts, including a faster growth rate, higher $CO_2$ utilization and greater amenability to genetic engineering [24, 25].

**Table 4** shows compounds that can be produced by cyanobacteria photoautotrophically [26].

In 2013, a review on the production of poly-β-hydroxybutyrates from cyanobacteria for the production of bioplastics was published [13]. Meanwhile, significant improvements have been implemented.

In 2018, Troschl et al. could report 12.5% PHB cry well weight [21]. In the same year, Kamravamanesh et al. have shown that the cyanobacterium *Synechocystis* sp. PCC 6714 can produce up to 37% dry cell weight of PHB with $CO_2$ as the only carbon source [27, 28], which is significantly above the other reported values from literature. The strain had been subjected to UV light mutations to increase the PHB productivity. Prior to that work, the thermophilic cyanobacterium,

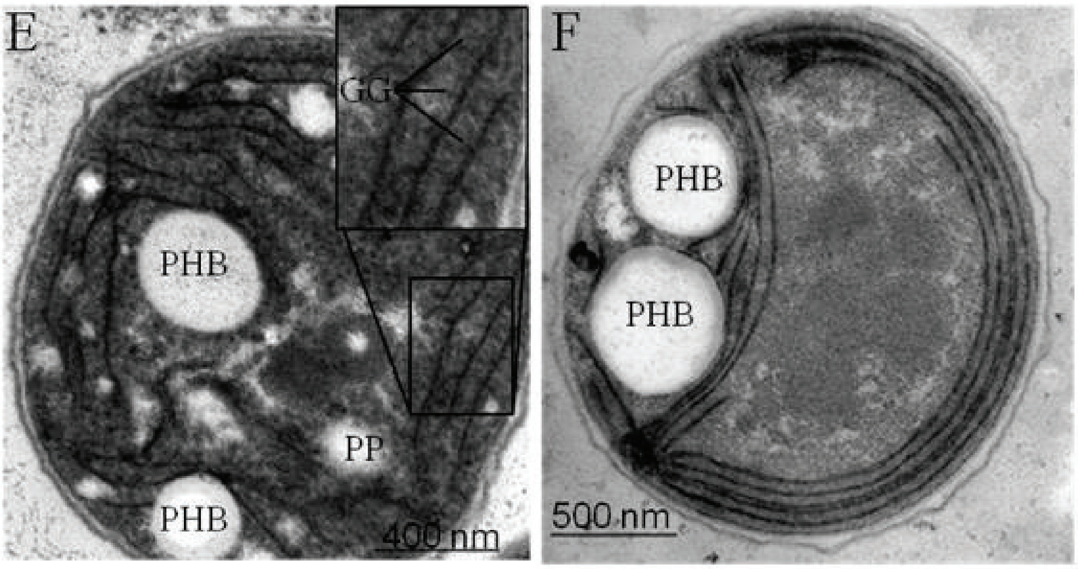


**Figure 1.**
*PHB granules in cyanobacteria. Left: Wild type. Right: Mutant (reproduced with permission from [22]).*

| Strain | Compound | Titer (g/L) |
|---|---|---|
| 2973 | sucrose | 3.3 |
| 6803 | 3-hydroxypropionic acid | 0.8 |
| | ethanol | 5.5 |
| | isobutanol | 0.6 |
| | lactic acid | 0.8 |
| | limonene | 0.007 |
| 7002 | 2,3-butanediol | 1.6 |
| | alpha bisabolene | 0.0006 |
| | fatty acids | 0.1 |
| | glycogen | 1.8 |
| | | 3.0 |
| | | 3.5 |
| | limonene | 0.004 |
| | lysine | 0.4 |
| | mannitol | 1.1 |
| | poly-3-hydroxybutyrate | 0.05 |
| 7942 | 2,3-butanediol | 3.0[a] |
| | | 5.7[b] |
| | | 12.6[a] |
| | alpha-farnesene | 0.005 |
| | ethanol | 0.07 |
| | fatty acid ethyl esters | 0.01 |
| | isobutyraldehyde | 1.1 |
| | isoprene | 1.3 |
| | limonene | 0.005 |
| | squalene | 0.05 |
| | succinate | 0.4 |
| | | 7.3 |
| | sucrose | 2.6 |
| | | 0.8 |

**Table 4.**
*Compounds that could be produced by cyanobacteria (reproduced with permission from [26]).*

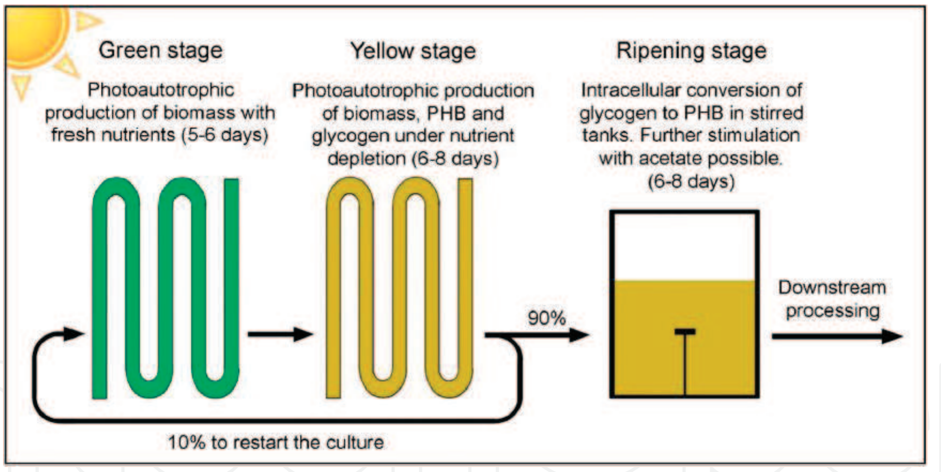


**Figure 2.**
*Operation mode for PHB production from cyanobacteria. The ripening tank is used for PHB production at a later stage, where no $CO_2$ is consumed, but glycogen gets converted into PHB (reproduced with permission from [18]).*

*Synechococcus* sp. MA19, was reported to have achieved 27% of dry cell weight PHB [29]. It was reported that, originally, the MA 19 was isolated from a hot spring in Japan (Miyakejima). However, neither the authors of this paper nor other researchers [30] were able to obtain a sample from that strain in 2016–2018, despite high efforts, so currently, Kamravamanesh's strain *Synechocystis* sp. PCC 6714 can be considered the cyanobacterium with the highest PHB content. A high PHB content is advantageous for downstream processing in terms of energy efficiency, for instance, or product quality.

Genetic engineering is commonly deployed to increase the yield of PHB compared to wild types [26, 31, 32]. Also, bioprocess optimization is carried out [27, 28]. Growth is typically followed by nitrogen and/or phosphorous limitation. Also, "feast and famine" strategies concerning the carbon source are applied [33].

Reference [34] discusses the use of consortia of cyanobacteria and heterotrophic bacteria for stable PHB production.

The modeling of cyanobacterial PHB production is discussed in [35].

A possible growth system for PHB from cyanobacteria is presented in [18], see **Figure 2** below.

The study in [18] uses long-term, non-sterile cultivation of *Synechocystis* sp. CCALA192 in a tubular photobioreactor for PHB production. Another concept would be open pond photobioreactors like open pond raceways. Different photobioreactor setups are reviewed in [18, 36–39]. A promising alternative is an integrated algae-based biorefinery, e.g., for the production of biodiesel, astaxanthin and PHB as presented by [40] or [41].

## 4. PHB production by cyanobacteria: an outlook

A major unsolved issue is the downstream processing of the cyanobacteria, i.e., how to get the bioplastics material out of the cyanobacteria (see **Figure 3**).

In Ref. [23], photomixotrophic conditions to increase cyanobacterial production rate and yield are reviewed. Supplementation with fixed carbon sources gives additional carbon building blocks and energy to speed up production. Photomixotrophic production was found to increase titers up to fivefold over traditional autotrophic conditions [23], so there is a strong future potential in this mode for cyanobacteria.

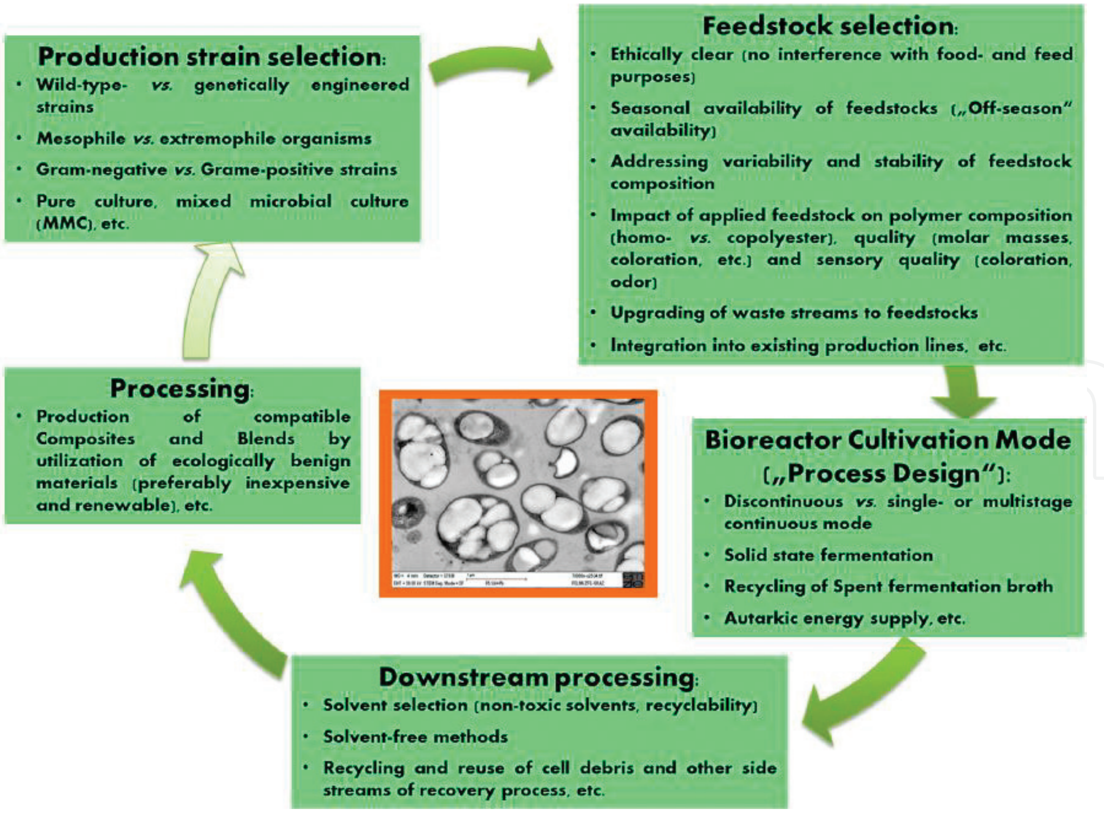


**Figure 3.**
*Schematic illustration of factors impacting sustainability of PHA production (reproduced with permission from [42]).*

## 5. Conclusions

This chapter has presented an update on PHB production by cyanobacteria, a process route which can be more sustainable than catalytic production from CO or fermentation from sugar compounds. It is expected that PHB and its compounds will gradually replace PP in many large volume applications. Genetic engineering can increase the yield of PHB in cyanobacteria; however, the downside is that approval for large-scale cultivation in (cost- and energy-efficient) open growth systems will be difficult to obtain in most countries, so technologies avoiding genetic engineering seem to be most promising for commercial development.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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