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Cyanobacteria for PHB Bioplastics Production: A Review

Erich Markl, Hannes Grünbichler and Maximilian Lackner

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 CO₂ 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 CO₂ 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 bio-medical, 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 × 10 ⁵	2 × 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**).

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 competitiveness	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 CO₂ as sole carbon source are described as strong potential here.

In general, organic carbon feedstocks can yield high PHB contents in micro-organisms. 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 CO₂ 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 CO₂ 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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