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REVIEW PAPER

Challenges, progress, and future perspectives for cyanobacterial polyhydroxyalkanoate production

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Abstract Polyhydroxyalkanoates (PHA) are a promising bio-based alternative to traditional plastics derived from petroleum. Cyanobacteria are photosynthetic organisms that produce PHA from CO_2 and sunlight, which can potentially reduce production costs and environmental footprint in comparison to heterotrophic bacteria cultures because (1) they utilize inorganic carbon sources for growth and (2) they do not require intensive aeration for oxygenation. Moreover, supplementing precursors such as propionate, acetate, valerate, etc., can be used to obtain various copolymers with plastic customizable properties in comparison to the classical homopolymers, such as polyhydroxybutyrate, PHB. This critical review covers the latest advances in PHA production, including recent discoveries in the metabolism interplay between PHA and glycogen production, and new insights into cultivation strategies that enhance PHA accumulation, and purification processes. This review also addresses the challenges and suggests potential solutions for a viable industrial PHAs production process.

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Abbreviations

2-OG	2-Oxoglutarate
2-PGA	2-Phosphoglycerate
c-di-AMP	Second messenger nucleotide
CBC	Calvin–Benson cycle
DIC	Dissolved inorganic carbon
ED	Entner–Doudoroff
EMP	Emden–Meyerhof–Parnas
GlgA	Glycogen synthase
glob	Glycogen branching enzyme
GlgC	Glucose-1-phosphate adenylyltransferase
GlgP	Glycogen phosphorylase
GlgX	Glycogen debranching enzyme
GltA	Citrate synthase
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
mcl-PHA	Medium-chain length polyhydroxyalkanoates
OPP	Oxidative pentose phosphate
P(3HB-co-3HV)	Co-polymer of polyhydroxybutyrate and polyhydroxyvalerate
P(3HB)	Poly(3-hydroxybutyrate)
P(3HD)	Poly(3-hydroxydecanoate)
P(3HHx)	Poly(3-hydroxyhexanoate)
P(3HO-co-3HD)	Co-polymer of poly(3-hydroxyoctanoate) and poly(3-hydroxydecanoate)
P(3HO)	Poly(3-hydroxyoctanoate)
P(3HV)	Poly(3-hydroxyvalerate)
PBAT	Polybutylene adipate terephthalate
PBR	Photobioreactor
PBS	Polybutylene succinate
PCL	Polycarbonate
PE	Polyethylene
PEP	Phenolpyruvate
PET	Polyethylene terephthalate
PGAM	2,3-Phosphoglycerate-independent phosphoglycerate mutase
pgm	Phosphoglucomutase
PHA	Polyhydroxyalkanoates
PhaB	Acetyl-CoA reductase
PhaC/PhaE	Poly(3-hydroxyalkanoate) synthase
phaF	Phasin
PhaP	Poly(3-hydroxyalkanoate) inclusion protein
phoR	Phosphate transport
PHV	Polyhydroxyvalerate
PirC	PII interacting regulator of carbon storage
PLA	Polylactic acid
PP	Polypropylene
ppx	Polyphosphate phosphatase
pstA	Phosphate transport
pstC	Phosphate transport
SBR	Sequencing batch reactor
sbtA	HCO_3^- transporter
scl_PHA	Short-chain length polyhydroxyalkanoates
Slr0058	Phasin family protein
sphU	Phosphate regulator
TCA	Tricarboxylic acid cycle
Tg	Glass transition temperature
Thl/PhaA	Acetyl-CoA acetyltransferase
TIN	Total inorganic nitrogen
TIP	Total inorganic phosphate
Tm	Melting temperature
Trk AG/H	K^+ transporter

1 Introduction

Nowadays plastics are an essential material for many manufacturing sectors, such as packaging, construction, automotive, electrical, electronics, household, medical, and agriculture. Unfortunately, nearly ~ 55% of plastic waste is disposed of in landfills or discarded in natural ecosystems (Hannah Ritchie and Max Roser 2019). Plastic waste accumulation in the ecosystems leads to adverse effects on human health and negative impacts on ecosystems, as well as disruption of economic activities, among others (Cook and Halden 2020). The current methods used to tackle plastic pollution, such as recycling and incineration, are inadequate in addressing the global scale of plastic usage and fall short of being ideal solutions. For example, plastic incineration can produce toxic components (e.g., Dioxins, Furans, Mercury, and Polychlorinated Biphenyls) (Castilho et al. 2009), while recycling can change the properties of the plastic, limiting their downstream applications (Castilho et al.

2009). Therefore, conventional petroleum-based plastics must be replaced with sustainable alternatives.

Bio-based polymers are a promising alternative to reduce the dependency on fossil resources and to address the accumulation and management challenges of plastic waste disposal (Tharanathan 2003; Abe et al. 2021). Out of these, PHAs are the only biopolymers directly synthesized by bacteria.

Polylactic acid (PLA), Polybutylene adipate terephthalate (PBAT) and Polybutylene succinate (PBS) are formed by the chemical polymerization of their monomers (Shaikh et al. 2021).

The conventional PHAs production process is sourced from heterotrophic microorganisms (e.g. *Cupriavidus necator)* (Chen 2009). Heterotroph PHAs commercialization is hampered by high costs, 2.4–7.5 € kg^{-1} (Manikandan et al. 2021; Rajendran and Han 2022) compared to 1.9 € kg^{-1} for Polylactic acid (PLA). The need for organic carbon sources accounts for 30–50% of the total production costs (Lee and Choi 1998; Lynd et al. 1999; Mudliar et al. 2008; Halami 2008; Singh et al. 2017; Troschl et al. 2017). In this context, cyanobacteria present a promising alternative to overcome these drawbacks as they can produce PHAs autotrophically, using inorganic C sources (CO_2 or HCO_3^-) and sunlight without requiring intense aeration. However, compared to heterotrophic PHAs production, the process of PHAs production using cyanobacteria is not as well-developed. According to data from *Web of Science© (consulted in November 2023)*, less than 5% of the scientific publications dealing with PHAs production were related to cyanobacteria. This means, that from the 8192 publications on PHAs production, only 208 were related to PHAs production with cyanobacteria.

Recently there have been many studies focused on improving the PHA yields in cyanobacteria in connection with the understanding of the effect of different cultivation factors in the process (Kamravamanesh et al. 2018b; Arias et al. 2020; Carpine et al. 2020; Koch et al. 2020a; Price et al. 2020; Afreen et al. 2021). However, an industrial PHAs production process with cyanobacteria will only be possible by optimizing the entire process, which includes cyanobacteria cultivation, PHAs production stimulation, PHAs extraction, and purification. This review analyses all aspects of PHAs production via cyanobacteria, from optimization of cultivation conditions to the PHAs purification process. To this aim, first, it describes the main characteristics and advantages of PHAs in front of other plastics. Later, the recently discovered genetic regulators of PHA and glycogen metabolism in cyanobacteria are discussed. As well as the strategies to enhance the PHAs accumulation in cyanobacteria and the recommendations to maintain a cyanobacteria-dominated culture when wastewater is used as cultivation broth. Finally, the recent advances and challenges in PHAs purification and recovery are presented, including prospects, challenges, and recommendations for the future development of biopolymer production with cyanobacteria.

2 Polyhydroxyalkanoates (PHAs)

PHAs are a family of polyesters synthesized naturally by many different prokaryotic microorganisms. PHAs can be classified as medium-chain (mcl-PHAs) if their monomers contain six or more carbons (e.g. poly(3-hydroxyhexanoate)-P(3HHx), poly(3-hydroxyoctanoate)-P(3HO) or poly3-hydroxydecanoate-P(3HD)), or as short-chain (scl-PHAs) if they have 4 or 5 carbons (e.g. polyhydroxybutyrate-PHB and polyhydroxyvalerate-PHV) (Fig. 1) (Naser et al. 2021). Based on the types of microorganisms, carbon source, and the culture condition, PHAs of different chain lengths can be produced (Jiang et al. 2016) (Table 1).

PHAs are a promising source of plastic (Naser et al. 2021). PHB shares similar properties with polypropylene (PP), except for its elongation at break, which can be enhanced by enriching it with HV to create the copolymer P(HB-co-HV). mcl-PHAs do not become brittle even at low temperatures, a property similar to that of rubber and latex (Muhr et al. 2013). Moreover, PHAs can be processed into final plastic products with the same techniques used for conventional polymers (Muthuraj et al. 2021). Moreover, differently than other biopolymers such as PLA, PHAs can be readily degraded in a variety of environments, such as marine, freshwater, landfill, soil, or home composting (Muthuraj et al. 2021). Additionally, PHA-based polymers have a low permeability to water vapour, CO_2, and O_2, comparable to those of synthetic plastics, and higher than those of other biodegradable polymers. These characteristic features are highly attractive to the food packaging industry (Byrom 1993; Naser et al. 2021). The major

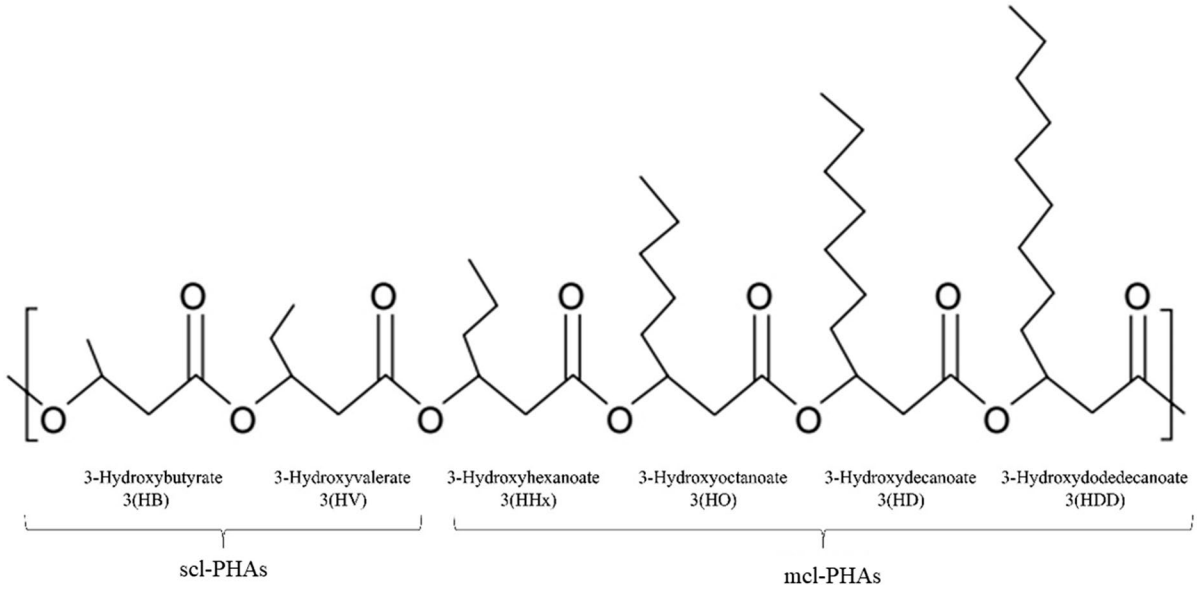


Fig. 1 Classification of the different types of PHA according to the number of carbons in their monomers (Wang and Chen 2017)

barrier to their further commercialization is production costs (Panuschka et al. 2019; Naser et al. 2021). Table 2 shows a comparison between PHAs and other plastics.

3 Cyanobacteria as PHAs producers

Most of the tested cyanobacteria can produce PHA. Indeed, Kaewbaingam et al. (2016) found that 134 strains from the 137 studied produced PHA. *Synechocystis* sp. PCC 6803 is the most studied cyanobacteria to produce PHA, as it is a well-studied model organism, easy to grow and genetically modify (Koch et al. 2020b). Nevertheless, other strains, such as *Calothrix scytonemicola, Nostoc* sp. or *Oscillatoria subtilissima,* may have higher PHA yields than *Synechocystis* sp. PCC 6803, but they are much less studied (Ansari and Fatma 2016; Kaewbaingam et al. 2016).

Cyanobacteria photosynthetically synthesize PHA (in the form of PHB) and can also produce other PHA co-polymers by supplementing organic carbon sources. PHAs production via cyanobacteria requires generally the cells to be in a state of nutrient starvation. First, cyanobacteria are grown in a medium rich in nitrogen and phosphorus. Once an adequate concentration of microorganisms is achieved, they are collected and placed in nutrient-limited media (two-step cultivation), or allowed to deplete the nitrogen or/and phosphorus (one-step cultivation). In one-step cultivation, initial N and P concentrations should be optimized to reach this depletion by the due time. Implementing a one-step strategy could significantly reduce production costs since less amount of nutrients are needed and energy consumption due to biomass harvesting is reduced (Drosg et al. 2015; Kamravamanesh et al. 2019). However, slightly higher biomass productivity is expected in the two-stage cultivation, due to the higher nutrient availability which implies a higher growth rate. For instance, Kamravamanesh et al. (2017) reported a biomass-specific growth rate of 0.67 d^{-1} using two-step cultivation, while using a one-step strategy and similar light conditions, Rueda et al. (2020), observed a specific growth rate of 0.08 d^{-1}.

Once nutrients become limiting, biopolymers start to accumulate in cyanobacteria. Cells first accumulate glycogen and during prolonged chlorosis, a part of the accumulated glycogen is gradually catabolized and transformed into PHAs. Several strategies can be applied during the accumulation phase, also named the starvation phase to further enhance the PHAs accumulation such as the addition of organic carbon

Table 1 Examples of different carbon sources and microorganisms on the production of different PHAs

Carbon substrate	Strain	PHA polymer	PHA yield as $\%_{dcw}$	Productivity (mg L^{-1} h^{-1})	Cultivation conditions	Ref
Inorganic carbon						
CO_2	*Synechocystis* sp. PCC 6714*	P(3HB)	28	0.23	Photoautotrophic; N, P starvation	Kamravamanesh et al. (2019)
$CO_2 + HCO_3^-$	*Synechocystis* sp.*	P(3HB)	14	0.1	Photoautotrophic; N, P starvation	Rueda et al. (2022a, b, c)
Sugars						
Molasses (37% sucrose)	*Cupriavidus necator*	P(3HB)	27.3	13	Mineral salt medium (MSM) + Pre-treated molasses	Sen et al. (2019)
Fructose	*Pseudomonas* sp. B14-6	Mix mcl-PHAs	35	4.6	Aerobic	Choi et al. (2021)
Triacylglycerols						
Waste cooking oil	*Pseudomonas chlororaphis* 555	P(3HO-co-3HD)	20.8	36	Aerobic	Ruiz et al. (2019)
Glycerol	*Cupriavidus necator* DSM 545	P(3HB)	64.5	76	Aerobic	Rodríguez-Contreras et al. (2015)
Hydrocarbons						
Polycyclic aromatic hydrocarbons	Mixed culture (*Pseudomonas aeruginosa PAH-P02, Pseudomonas sp. PAH-P04* and *Ralstonia* sp. *PAH-P08*)	P(3HD)	50	40	Aerobic	Sangkharak et al. (2020)
Oily wastewater	Mixed culture of oil-degrading aerobic granules	P(3HB-co-3HV)	71	–	Aerobic	Ghosh and Chakraborty (2020)
Biogas (70% CH_4)	*Methylocystis hirsuta*	P(3HB)	45	99	Aerobic N starvation	López et al. (2018)
Organic acids						
Decanoic acid + Acetic acid	*Pseudomonas putida* KT2440 ΔfadBA	P(3HD)	35	190	Aerobic; C starvation	Oliveira et al. (2020)
Fatty acid methyl esters	*Pseudomonas citronellolis* DSM 50332	P(3HO-co-3HD)	26.6	50	Aerobic; N starvation	Muhr et al. (2013)
Acetate	PAOs	P(3HB-co-3HV)	42	–	Anaerobic/aerobic	Yuan et al. (2015)
Acetate	*Synechocystis* sp.	P(3HB)	31	2	Photomixotrophic; N starvation	Gracioso et al. (2021)
Acetate + Valerate	*Nostoc muscorum*	P(3HB-co-3HV)	78	4.6	Photomixotrophic; N starvation	Bhati and Mallick (2015)
Acetate	*Mixed photosynthetic culture*	P(3HB)	60	8.3	Photomixotrophic; High light intensity	Fradinho et al. (2016)
Acetate	*Rhodopseudomonas palustris SP5212*	P(3HB-co-3HV)	15	0.4	Photomixotrophic; N-limitation	Mukhopadhyay et al. (2005)

P(3HB): poly(3-hydroxybutyrate); P(3HHx): poly(3-hydroxyhexanoate); P(3HD): poly(3-hydroxydecanoate); P(3HV): poly(3-hydroxyvalerate); P(3HO): poly(3-hydroxyoctanoate)

Table 2 Processability, production process, properties, application, and price (according to Zhao et al. 2020) of some typically used polymers

Polymer	Processing possibilities	Production process	Properties						Applications	Average Price ($/kg)
	1-Injection moulding 2-Extrusion 3-Extrusion blowing 4-Extrusion casting 5-Blowing 6-Fibre Spinning 7-Thermo forming		Tg (°C)	Tm (°C)	Tensile Strength (MPa)	Tensile modulus (MPa)	Elongation at break (%)	Biodegradable	1-Flexible packaging 2-Rigid Packaging 3-Textiles 4-Consumer goods 5-Agriculture and transport 6-Automotive and transport 7-Coating and adhesives 8-Building 9-Electronics 10-Medical 11-Others	
Polypropylene (PP)	1, 2, 3, 4, 5, 6, 7	Petrochemical	−10	171	33	1800	150	No	1, 2, 4, 6, 9	0.97–1.2
Polyethylene Terephthalate (PET)	1, 2, 3, 4, 5, 6, 7	Petrochemical/ fermentation + polymerization	73–80	245–265	48–72	2800–4100	30–300	No	2, 3, 6	1.51–2.12
Low-density polyethylene (LDPE)	1, 2, 3, 4, 5, 6, 7	Petrochemical/ fermentation + polymerization	−120	98–115	8–20	300–500	100–1000	No	1, 4, 8	0.95–1.18
High-density polyethylene (HDPE)	1, 2, 3, 4, 5, 6, 7	petrochemical/ Fermentation + polymerization	−120	129	39	600–1500	650	No	4, 8, 11	0.82–1.01
Polybutylene adipate terephthalate (PBAT)	2, 3, 4	Petrochemical	−30	110–115	34–40	–	500–800	Yes	1, 2, 5, 7	2.5–5
Polylactic acid (PLA)	1, 2, 5, 6, 7	Fermentation + polymerization	40–70	130–180	48–53	3500	30–240	Yes	1, 2, 3, 4, 5, 7, 8, 9, 11	3.31–3.53

Table 2 (continued)

Polymer	Processing possibilities	Production process	Properties						Applications	Average Price ($/kg)
	1-Injection moulding 2-Extrusion 3-Extrusion blowing 4-Extrusion casting 5-Blowing 6-Fibre Spinning 7-Thermo forming		Tg (°C)	Tm (°C)	Tensile Strength (MPa)	Tensile modulus (MPa)	Elongation at break (%)	Biodegradable	1-Flexible packaging 2-Rigid Packaging 3-Textiles 4-Consumer goods 5-Agriculture and transport 6-Automotive and transport 7-Coating and adhesives 8-Building 9-Electronics 10-Medical 11-Others	
Polyhydroxybutyrate (PHB)	1, 2, 3, 4, 5, 7	Heterotrophic Microorganisms	0	140–180	25–40	3500	5–8	Yes	1, 2, 4, 5, 9, 10	–
Polyhydroxybutyrate (PHB)	1, 2, 3, 4, 5, 7	Cyanobacteria	0.9	175	30.2	3.8	4.9	Yes	1, 2, 4, 5, 9, 10	7.7 (estimated in Price et al. (2022)
Polyhydroxybutyrate-valerate (P(3HB-co-3HV)	1, 2, 3, 4, 5, 6, 7	Heterotrophic Microorganisms	0–30	100–190	25–30	600–1000	7–15	Yes	1, 2, 4, 5, 9, 10	6.86–20.26
mcl-Polyhydroxyalkanoates (mcl-PHA)	–	Heterotrophic Microorganisms	−46.9–43.5	48.6–86	10	1200	100–300	Yes	3, 5, 7, 8, 10	–
Starch blends	1, 2, 3, 4	Agro-polymers	–	110–115	35–80	600–850	580–820	Yes	1, 2, 3, 4, 5, 6, 7	2.43–4.85
Cellulose-based	1, 2, 5	Agro-polymers	–	–	55–120	3000–5000	18–55	Yes	1, 3, 4, 8, 10, 11	2.49–3.40
Polycaprolactone (PCL)	1, 2, 3, 5, 6, 7	Petrochemical	−60	59–64	4–28	390–470	700–1000	Yes	1, 2, 10, 11	–

Tg: glass transition temperature, Tm: melting temperature, B: biodegradability (Achilias et al. 2007; Agawin et al. 2007; Muhr et al. 2013; Jiang et al. 2016; Poltronieri et al. 2017; Santos et al. 2017; Zhang et al. 2018; Panuschka et al. 2019; Rueda et al. 2020; Muthuraj et al. 2021; Naser et al. 2021)

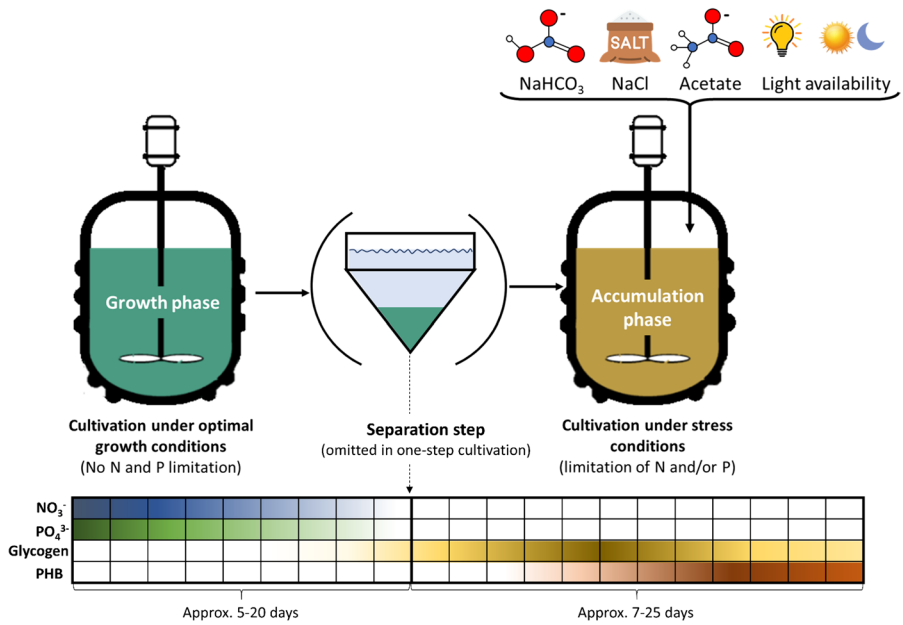


Fig. 2 Schematic representation of the PHA production process using cyanobacteria. First, cyanobacteria are grown with light and sufficient nutrients (growth phase). Nutrients are reduced with time. Next, the biomass is separated from the cultivation medium and placed in a new medium without N or/and P (two-step cultivation). It is possible to omit the separation step by optimizing the initial nutrient concentration so they are completely exhausted by the end of the growth phase. Without nutrients, the accumulation phase starts and other PHA-stimulating conditions can be applied (e.g., high salinity, acetate, light availability). During this phase, glycogen and PHAs accumulate. If the chlorosis persists for a long time, glycogen is degraded and converted into PHAs

sources, inorganic carbon, salinity, or light intensity (Fig. 2) (Eberly and Ely 2012; Ansari and Fatma 2016; Gracioso et al. 2021; Rueda et al. 2022a, c).

In the next section, research advances on the physiological function of cyanobacterial PHAs and strategies to improve PHA production and reduce costs in an engineered environment are presented.

3.1 PHA metabolism in cyanobacteria

The synthesis of PHA in cyanobacteria is a complex process that involves several metabolic pathways and has many regulators which are not completely known. The exact function of many of these regulators of the PHA metabolism is still unclear (Koch and Forchhammer 2021).

Cyanobacteria produce two main interrelated carbon storage compounds: glycogen and PHA. PHA biosynthesis is strongly related to glycogen synthesis, and both molecules compete for the metabolic intermediate 3-phosphoglycerate (3-PGA) as shown in Fig. 3. This intermediate is produced in the Calvin cycle during CO_2 fixation (Zhang and Bryant 2011; Singh and Mallick 2017). Recently, many attempts have been made to explain the interaction between the two polymers and how they are regulated. Dutt and Srivastava, (2018) observed that only 26% of the carbon used to synthesize PHA came from the fixation of inorganic carbon, while the rest came from internal carbon recycling. Koch et al., (2019), discovered that PHA was produced mainly by glycogen catabolism through the Emden–Meyerhof–Parnas (EMP) pathway (Fig. 3, the green pathway), rather than the Entner–Doudoroff (ED) or the oxidative pentose phosphate (OPP) routes. Rueda et al., (2022b) also observed a positive correlation between the expression of genes related to glycogen catabolism with the ones of the PHA metabolism. Considering altogether these evidences, it is hypothesized that the synthesis of PHA from Acetyl-CoA consumes one mol of NADPH per mol of HB, and is used as a sink of electrons to rebalance the ATP/NADPH ratio (Hauf et al. 2013). This fact means that PHA production, rather than other fermentative products is advantageous

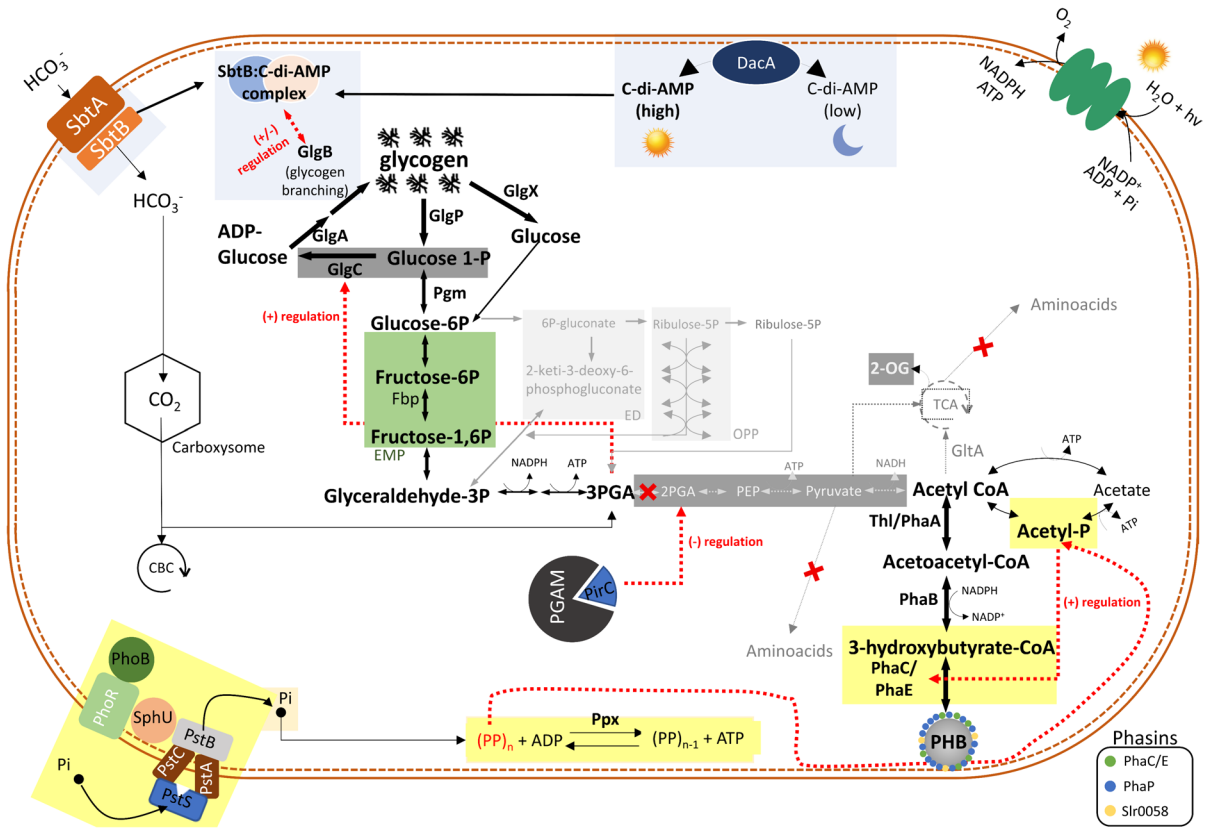


Fig. 3 Simplified representation of the glycogen/PHA metabolism pathways and regulators in the cyanobacteria taking research mostly conducted in *Synechocystis* sp. The metabolic pathways and enzymes highlighted in bold correspond to the routes upregulated during nutrient starvation. Red lines indicate molecules that cause the up or downregulation of different enzymes. The pathway highlighted in green corresponds to part of the EMP pathway, which is the main route used in cyanobacteria to transform glycogen into PHA (Koch et al. 2019). Pathways highlighted in blue light correspond to the day-night regulation of the GlgB enzyme, by the formation or disappearance of the SbtB:c-di-AMP complex as described by (Selim et al. 2021). The pathways highlighted in yellow correspond to the effect of phosphate inside the cell, that stimulates the synthesis of acetyl phosphate and this one activates the PHA synthase (Krasaesueb et al. 2021). Pathways in light grey correspond to the inhibition of the transformation of the 3-PGA to 2-PGA due to the bounding of the PirC to the PGAM. PirC is released from the P_{II} (and free to be bound to the PGAM) due to the accumulation of the 2-OG. The accumulation of 3-PGA, due to the inhibition of the 3-PGA to 2-PGA transformation, stimulates the activity of the GlgC (Orthwein et al. 2021). Pathways in dark grey are glycolytic pathways present in cyanobacteria but do not play an important role in forming PHA. Abbreviations: ADP-glucose: Adenosine diphosphoglucose; 3-PGA: glycerate-3P; 2-PGA: glycerate-2P; PEP: Phenolpyruvate; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; PP: Polyphosphate; PGAM: 2,3-phosphoglycerate-independent phosphoglycerate mutase; TCA: tricarboxylic acid cycle; 2-OG: 2-oxoglutarate; CBC: Calvin–Benson cycle; c-di-AMP: second messenger nucleotide; EMP: Emden–Meyerhof–Parnas; ED: Entner–Doudoroff; OPP: oxidative pentose phosphate; GlgA: glycogen synthase, GlgC: Glucose-1-phosphate adenylyltransferase, GlgP1 and GlgP2: glycogen phosphorylase, GlgX: glycogen debranching enzyme, GltA: Citrate synthase, Thl/PhaA: Acetyl-CoA acetyltransferase, PhaB*:* Acetyl-CoA reductase, PhaC/PhaE*:* poly(3-hydroxyalkanoate) synthase; PhaP*:* poly(3-hydroxyalkanoate) inclusion protein; Slr0058: Phasin family protein

for the cell, as the intracellular carbon is conserved while ATP is recovered, suggesting that PHA will be produced in those cultivation conditions that favor high reduction equivalents (high content of NAD(P)H or $FADH_2$) (Koch and Forchhammer 2021). PHA is, therefore, an efficient way to recover the reducing power needed in the cell metabolism while keeping the stored carbon.

PHA may also serve as a valuable structural component for the cell to survive stressful situations

and changing environments, such as nutrient depletion and osmotic shock (Damrow et al. 2016; Obruca et al. 2017). In *Synechocysts* sp. PCC 6803, during resuscitation from chlorosis, it was observed that PHAs are not degraded, but they are equally distributed among daughter cells (Koch et al. 2020c). Indicating that PHAs may not serve as a carbon source used during chlorosis recovery, but as a structural component related to the nucleotide and essential to restart cell proliferation.

Many PHA and glycogen metabolism regulators control the production and transformation of these two polymers (Fig. 3). For instance, P transport and content in the cell play an important role in PHA and glycogen metabolism (Kamravamanesh et al. 2018a; Krasaesueb et al. 2021). Kamravamanesh et al., (2018a) produced a random UV mutated *Synechocystis* sp. PCC 6714 strain with 2.5-fold higher PHA production, increasing from 14%$_{dcw}$ in the wild-type strain to 37%$_{dcw}$. This strain had a mutation in the *pstA* gene, which is a membrane protein related to the phosphate transport system. This mutation derived from a complex change of the cell metabolism which included the downregulation of the *sphU* gene, which is a regulator of the P transport that prevents the assimilation of additional phosphate by closing the transporter when inorganic phosphorus is abundant in the culture medium. The authors could not completely link the metabolic changes to the new genotype, but it was hypothesized that imbalanced P transport caused the increased PHA production (Kamravamanesh et al. 2018a). Subsequently, Krasaesueb et al., (2021) further studied the effect of this SphU regulator on PHA production. Under normal photosynthetic conditions (wild-type) the SphU regulon is activated in response to phosphate-limiting conditions leading to the upregulation of genes involved in the phosphate transportation and to the accumulation of polyphosphate and acetyl phosphate in the cell (Summers et al. 1999). Silencing the sp*hU* gene in *Synechocystis* sp. PCC 6803 allowed the incorporation of P inside the cell even when P was abundant in the cultivation media, which translated in a 15-fold increase in PHA production, which means a 14.5%$_{dcw}$ compared to a 1%$_{dcw}$ in the wild type under non-producing conditions. This increased phosphate inside of the cell activated the synthesis of acetyl phosphate, which is an intermediate for the synthesis of acetyl-CoA and intracellular acetate (Fig. 3, yellow pathway). This discovery may be of great interest to produce PHA under non-limited nutrient conditions, which means that there would be no need to disconnect the growth and accumulation phase. PHA would be produced directly during growth, which would increase productivity.

Metabolic regulations directly impacting glycogen synthesis and catabolism have been also studied. Cyanobacteria coordinate the glycogen metabolic switch to survive the day-night cycles by implication of the second messenger nucleotide (c-di-AMP) (Selim et al. 2021). During the day, SbtA (HCO_3^- transporter) interacts with the c-di-AMP to form SbtB:c-di-AMP complex. This SbtB:c-di-AMP complex further stimulates the glycogen-branching enzyme (GlgB). During the night, glycogen synthesis is stopped due to the reduction of the c-di-AMP and the subsequent absence of the SbtB:c-di-AMP complex. The energy required to survive the night is produced through the catabolism of the glycogen stored during the day (Fig. 3, blue pathway) (Selim et al. 2021). When there is a nitrogen shortage, cyanobacteria switch their metabolism to produce glycogen and PHA (Orthwein et al. 2021). Under these circumstances, a small inhibitory protein known as PirC, a regulator of carbon storage, inhibits phosphoglycerate mutase (PGAM), slowing the flow of carbon toward lower glycolysis through PGAM. Upon N-starvation, the levels of the Tricarboxylic Acid Cycle (TCA) intermediate, 2-oxoglutarate (2-OG), increase, which is sensed by the signalling protein PII (Forchhammer et al. 2022). The PII-PirC protein complex, which prevails under normal growth conditions, dissociates at increased 2-OG levels, releasing the PGAM inhibitor PirC. The binding of PirC to PGAM inhibits the conversion of 3-phosphoglycerate (3-PGA) into 2-phosphoglycerate (2-PGA). Consequently, the increase in the concentration of 3-PGA stimulates glycogen synthesis. The metabolic disruption of *PirC*, allows more carbon to flow towards 2-PGA which finally leads to PHA levels that are increased up to 49%$_{dcw}$ from a 29%$_{dcw}$ in the wild-type, during prolonged nitrogen starvation (Orthwein et al. 2021) (Fig. 3, grey pathway).

The formation of PHA granules is also complex and puzzling. The surface of PHA is coated by different proteins, called phasins, which are involved in the PHA metabolism. Phasins regulate transcriptional response, depolymerization of the PHA, and regulation of the formation of granules (Hauf et al. 2015;

Koch et al. 2020b). The open reading frame (ORF) *ssl2501* encodes for phasin PhaP in *Synechocystis sp.* PCC 6803 (Hauf et al. 2015). PhaP modifies the size and number of granules in the cell by regulating the PHA synthetase activity (Hauf et al. 2015). Slr0060 is a PHA depolymerase, however, its function is unclear, as its deletion did not cause an increase in the amount or size of PHA granules (Koch et al. 2020b). Slr0058 expresses the phasin, PhaF, which may be involved in the initiation of PHA granule formation as PHA is not properly aggregated in its absence (Koch et al. 2020b).

The precise regulation of cyanobacteria PHA metabolism and its physiological functions are still puzzling, however, many research advances have been made in recent years, benefitting the further development of industrial cyanobacteria PHA production and the development of new modified strains. There is still work to be done to thoroughly understand the regulation of the PHA metabolism under different cultivation conditions.

3.2 Effect of the cultivation conditions on cyanobacterial PHAs production

Several strategies have been applied during the accumulation phase (Fig. 2) to enhance the PHA production in cyanobacteria, among them, nutrient deficiency and supplementation of organic carbon sources like glucose, acetate, fructose, or valerate are the most prevalent (Carpine et al. 2020). Recently, other factors, such as the content of inorganic carbon, salinity, or light intensity, have also been seen to have a role in accumulation. This section critically reviews the most recent findings on the effect of different factors on cyanobacteria PHA.

3.2.1 Effect of inorganic carbon

Inorganic carbon (CO_2 or HCO_3^-) is essential for the generation of new organic molecules during photosynthesis (Markou et al. 2014). Nevertheless, only a few studies have evaluated the effect of varying amounts of inorganic carbon on PHA production, and the results are somehow contradictory (Eberly and Ely 2012; Kamravamanesh et al. 2017; Rueda et al. 2022b). Kamravamanesh et al., (2017) and Eberly and Ely, (2012), found that the increase in DIC concentration harmed PHA production (Table 3). On the contrary, Rueda et al. 2020, 2022b, reported a positive correlation between carbon and PHA production. The cause of the differing effects of DIC found in these studies remains unclear. One possible explanation is that adding high concentrations of CO_2 can drastically lower the pH in the culture, which may lead to metabolic activity decrease or culture crash if pH is not well controlled. Another explanation is the use of various inorganic carbon sources (CO_2 or $NaHCO_3$). Nevertheless, CO_2 exists as HCO_3^- at pH values between 6.5 and 9, which is the typical pH at which cyanobacteria are cultivated. Therefore, the only difference between the two DIC sources should be the increase in the ionic strength when $NaHCO_3$ was added. The different capacities of the different strains to resist the osmotic shock may explain these contradictory results.

3.2.2 Effect of salinity

Several studies on heterotrophic bacteria indicate that the presence of PHAs in the cell enhances their survival under high osmotic pressure by improving the cell's ability to maintain water content (Obruca et al. 2017).

Cyanobacteria also present several strategies for their protection against osmotic stress: (1) they produce compatible solutes (low molecular weight compounds such as sucrose, trehalose, glucosylglycerol, glucosylglycerate or glycine betaine, that increase osmolarity (Klähn and Hagemann 2011), (2) they activate or inactivate the ion transporters to regulate the water content inside the cell (Pade and Hagemann 2015), (3) they reorganize the thylakoid structure (Pandhal et al. 2008); (4) they increase the amount of nutrient binding proteins in the membrane (Pandhal et al. 2008); and (5) they modify the extracellular layers of the periplasmic space (Pandhal et al. 2008). By analogy to the observations made for heterotrophic bacteria, it can be hypothesized that PHAs accumulation also serves as a mechanism for cyanobacteria to adapt to osmotic stress. Meixner et al. (2022), observed that the optimal amount of salt to stimulate PHA production depends on the strain. For instance, *Synechocystis* IFA3 required 20 g NaCl L^{-1} to reach its maximum PHA content, a $3.2\%_{dcw}$. *Synechococcus* sp., required an optimal NaCl content of 9 g L^{-1} (Rueda et al. 2022a). When 1 g L^{-1} of NaCl was added to *Nostoc muscorum* NCCU- 442,

Table 3 Summary of the PHA accumulation as a percentage of dry cell weight and productivity using different cultivation strategies to enhance the production

Strain	Culture properties					PHA production			Reference
	Light (μmol m^{-2} s^{-1})	Hydraulic regime	System	Diameter (cm)	Volume (L)	Conditions	PHA ($\%_{dcw}$) ($\%_{dcw}$ of co-polymers)	Productivity (mg L^{-1} d^{-1})	
Synechocystis sp. PCC 6714	40 (24:0h)	Two-steps Batch	Water-jacket column	–	1	2% CO_2	16.4	59	Kamravamanesh et al. (2017)
Thermosynechococcus elongatus	180 (24:0h)	One-step Batch	Water-jacket column	–	2.7	–	14.5	–	Eberly and Ely (2012)
Synechocystis sp. R2020	36 (15:9h)	One-step Batch	Column	11	2.5	N and P limitation C feeding feast-famine	5	–	Rueda et al. (2020)
Synechocystis sp. R2020	73 (15:9h)	One-step Batch	Column	11	3	N, P limitation 2 gC L^{-1}	14	2.45	Rueda et al. (2022b)
Nostoc muscorum NCCU-442	25 (24:0h)	One-step Batch	–	–	–	N limitation 1 g NaCl L^{-1}	8.15	–	Ansari and Fatma (2016)
Spirulina subsalsa	n.m. (14:10h)	Two-steps Batch	Erlenmeyer	8.4	0.25	N limitation 50 g NaCl L^{-1}	7.45	9.8	Shrivastav et al. (2010)
Synechocystis sp. R2020	73 (15:9h)	One-step Batch	Column	11	3	N and P limitation 2 gC L^{-1}/12 gNaCl L^{-1}	7.5	4.9	Rueda et al. (2022c)
Synechocystis sp. R2020	73 (15:9h)	One-step Batch	Column	11	3	N and P limitation 0.1 gC L^{-1}/ 15 gNaCl L^{-1}	7.2	3.6	Rueda et al. (2022c)
Synechocystis sp. R2020	500 (15:9h)	One-step Batch	Column	11	3	N, P limitation 2 gC L^{-1} High light intensity	6.4	15.7	Rueda et al. (2022c)
Nostoc muscorum	75 (14:10h)	Two-steps Batch	Erlenmeyer	7.5	0.05	P limitation 4 g L^{-1} acetate/ 7 days darkness	35.1	–	Sharma and Mallick (2005)
Nostoc muscorum	75 (14:10h)	Two-steps Batch	Erlenmeyer	7.5	0.05	1.7 g L^{-1} acetate 1.6 g L^{-1} glucose 4 days darkness	45.6	–	Sharma et al. (2007)
Synechocystis sp. PCC 6803	200 (24:0h)	Two-steps Batch	Erlenmeyer	8.4	0.25	N and P limitation High light intensity	1.8	–	Monshupanee and Incharoensakdi (2014)

Table 3 (continued)

Strain	Culture properties					PHA production			Reference
	Light (µmol m^{-2} s^{-1})	Hydraulic regime	System	Diameter (cm)	Volume (L)	Conditions	PHA ($\%_{dcw}$) ($\%_{dcw}$ of co-polymers)	Productivity (mg L^{-1} d^{-1})	
Synechocystis sp. B12	300 (24:0h)	Two-steps Batch	Erlenmeyer	8.4	0.25	N limitation 4 g L^{-1} acetate High light intensity	31.5	–	Gracioso et al. (2021)
Aulosira fertilisima CCC 444	75 (14:10h)	Two-steps Batch	Erlenmeyer	7.5	0.05	N limitation 2.6 g L^{-1} citrate 2.8 g L^{-1} acetate 5.58 mg L^{-1} K_2HPO_4	85	–	Samantaray and Mallick (2012)
Aulosira fertilisima CCC 444	75 (14:10h)	Two-steps Batch	Erlenmeyer	7.5	0.05	N limitation 4 g L^{-1} valerate 5 g L^{-1} fructose	61.8 (73% 3HB 27% HV)	–	Samantaray and Mallick (2014)
Synechococcuss sp. R2020	500 (15:9h)	One-step Batch	Column	11	3	N and P limitation 1.2 g L^{-1} acetate 0.05 gC L^{-1} 9 gNaCl L^{-1}	26.1	42.3	Rueda et al. (2022a)
Synechocystis sp. R2020	500 (15:9h)	One-step Batch	Column	11	3	N and P limitation 1.2 g L^{-1} acetate 4 gC L^{-1} 18 gNaCl L^{-1}	5.6	4	Rueda et al. (2022a)
Nostoc muscorum	75 (10:14h)	Two-steps Batch	Erlenmeyer	7.5	0.05	N limitation 1.1 g L^{-1} acetate 0.8 g L^{-1} propionate	31.4 P(3HB-co-3HV)	–	Mallick et al. (2007)
Nostoc muscorum	75 (10:14h)	Two-steps Batch	Erlenmeyer	7.5	0.05	N limitation 2.8 g L^{-1} acetate 3.8 g L^{-1} glucose 3 g L^{-1} valerate	78 (73% 3HB 27% HV)	110	Bhati and Mallick (2015)
Synechocystis sp. PCC 6803	75 (14:10h)	Two-steps Batch	Erlenmeyer	7.5	0.05	N, P limitation 4 g L^{-1} acetate 0.1 g L^{-1} γ-butyrolactone	37.64 (95% 3HB 5% 4HB)	–	Tanweer and Panda (2020)
Synechococcus sp. MA19	~14.7 (24:0h)	Two-steps Batch	Oblong flask	1	0.45	N limitation 82 mg L^{-1} acetate	18	–	Miyake et al. (1997)

Light is expressed as the intensity in µmol m^{-2} s^{-1} (hours of light: hours of darkness). A two-step batch means that during the process of PHA production, there is a separation step between the growth and the accumulation phases. One-step batch means that both growth and accumulation phases are done in the same reactor (without separation step). Data are sorted by PHA stimulation strategies (remarked as "effects"), which are the different stress strategies applied to enhance PHAs accumulation (see the main text). Feast-famine means that carbon is added in an intermittent regime, thus there are periods when C is depleted (famine) and periods when C is abundant (feast). Abbreviations: HB: Hydroxybutyrate; HV: Hydroxyvalerate. nm is "not measured"

PHB content increased from 7.6%$_{dcw}$ to 8.15%$_{dcw}$ (Ansari and Fatma 2016). The addition of 50 g NaCl L^{-1} increased the PHB concentration from 5.9%$_{dcw}$ to 7.45%$_{dcw}$ in *Spirulina subsalsa* (Shrivastav et al. 2010). The halotolerant cyanobacterium *Synechocystis* cf. salina CCALA 192 required 40 g NaCl L^{-1} to

reach a 6.9%$_{dcw}$ of PHA (Meixner et al. 2022). These results suggest that higher salinities are needed to boost the PHAs production in cultures adapted to salinity, indicating that this polymer may play a role in protecting the cell against osmotic stress. This agrees with the fact that *Synechocystis* sp., a moderately halotolerant cyanobacteria, requires a higher NaCl concentration to stimulate its PHAs production than *Synechococcus* sp. or *N. muscorum* NCCU- 442, non-halotolerant species, but less than the amount required by *Spirulina subsalsa or Synechocystis* cf. salina CCALA 192, both halotolerant strains.

However, the observed effect of salinity on cyanobacterial PHAs production is very limited (Table 3) (Shrivastav et al. 2010; Ansari and Fatma 2016; Rueda et al. 2022a, c). Although a salinity increase stimulates PHA production, it may also affect the properties of the polymer (Rueda et al. 2022c). Hence, the NaCl concentration should be wisely selected and controlled for each strain, since above certain values it harms polymer accumulation.

3.2.3 *Effect of light*

The presence of day and night cycles, instead of constant illumination, increased PHA production (Arias et al. 2018b; Koch et al. 2020a). The need for dark cycles seems to be related to the fact that the resulting low oxygen, or even anoxic conditions, boosts glycogen catabolism (glycolysis) to produce ATP. The reducing power needed in glycolysis can be regenerated by converting pyruvate into PHA. In this process, the carbon is conserved in the form of PHA, rather than being consumed by other fermentative processes (Koch et al. 2020a). A series of studies done with *Nostoc muscorum* and *Synechocytis* sp. CCALA192, indicate that a darkness period (3–5 days) at the end of the accumulation phase stimulates PHA production (Sharma and Mallick 2005; Sharma et al. 2007; Troschl et al. 2018). Another study observed a negative effect of a dark period for *Synechocystis* sp. and *Synechococcus* sp. cultures on PHAs accumulation (Rueda et al. 2022a). These differences may be explained by the presence of oxygen in the cultures of *Synechocystis* sp. and *Synechococcus* sp. due to the bubbling of compressed air (Rueda et al. 2022a). Dark cultivation must be performed under anoxic conditions since, otherwise, glycogen will be used during respiration, hindering its transformation into PHAs if oxygen is present.

Light intensity had differing effects on PHAs accumulation. Similar PHAs concentrations were obtained when light intensity was increased from 100 to 500 μmols m^{-2} s^{-1} in cyanobacteria cultivated under autotrophic conditions (Monshupanee and Incharoensakdi 2014; Rueda et al. 2022c). While, a higher PHA content was obtained by increasing light intensity in cultures grown mixotrophically (Faradinho et al. 2016; Gracioso et al. 2021). These differences may be caused by the fact that increased light intensity increases cyanobacteria growth rate and nutrient consumption, which will cause stronger nutrient starvation forcing the PHAs accumulation, rather than by the effect of light by itself. Further research is, therefore, necessary to clarify why light becomes beneficial under mixotrophic growth.

3.2.4 *Effect of organic carbon sources*

Organic carbon sources such as acetate, valerate, glucose, and fructose have been extensively used in many investigations to enhance PHAs production (Carpine et al. 2020) (Table 3). The supplementation of these compounds increases the pool of acetyl-CoA and the [acetyl CoA]/[CoA] ratio. PHA synthesis is subsequently activated to rebalance this ratio (Ren et al. 2009; Yashavanth et al. 2021). The positive effect of these organic carbon supplementations is extendible to most cyanobacteria studied (Miyake et al. 1997; Panda and Mallick 2007; Samantaray and Mallick 2014; Taepucharoen et al. 2017; Rueda et al. 2022a), however, the PHA yield increase depends on the cyanobacteria strain, culture conditions and probably other unknown factors. The amount of supplemented organic carbon plays an important role in PHA accumulation. In general, the higher the amount of organic carbon added, the higher the amount of PHA accumulated (Table 3).

Although the supplementation of organic carbon sources is a promising alternative to achieve high PHAs contents, their usage could increase production costs, which would hinder the competitivity of cyanobacteria PHAs production process compared to heterotrophic bacteria (Price et al. 2022). Moreover, the addition of organic carbon would increase the potential for bacterial growth when the process is scaled up to non-sterile outdoor conditions (Troschl

et al. 2017). Therefore, the addition of organic carbon in cyanobacterial cultures should be minimized and very well controlled to the point necessary to achieve good productivity.

3.2.5 *Effect of cultivation conditions on polymer properties*

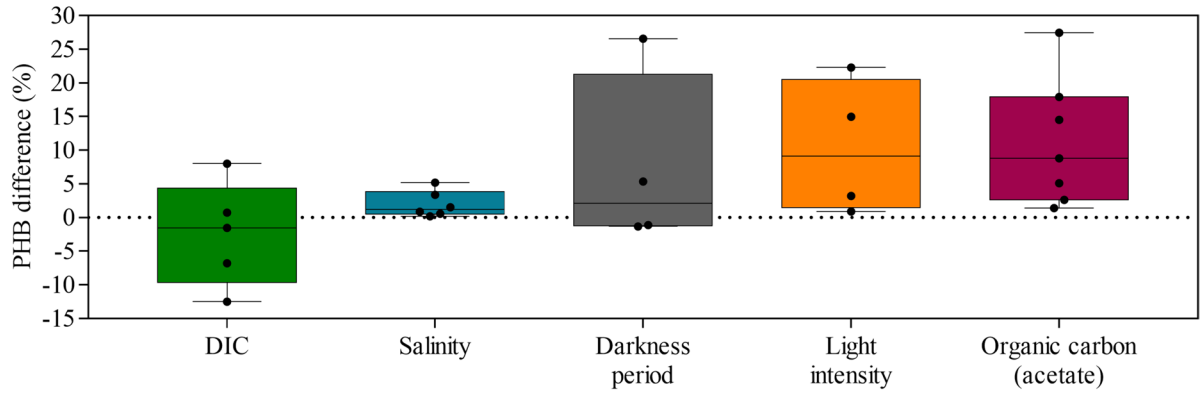
The quality and mechanical properties of the produced biopolymer are determinants for their final application. Polymer properties are affected by their monomer composition and chain length. Polyhydroxybutyrate (PHB) is the primary PHA polymer synthesized by cyanobacteria, but it is known to be highly brittle and crystalline. Adding other monomers different than the 3-hydroxybutyrate (3HB) to the chains can improve PHB properties. For instance, the introduction of 3-hydroxyvalerate (3HV) improves PHB crystallinity and thermal properties (Chan et al. 2019). On the other hand, the incorporation of 3-hydroxyvalerate resulted in a decrease in Young's Modulus and tensile strength, indicative of a heightened elasticity in the material (Bhati and Mallick 2015). Elongation to break can also be increased by adding a higher proportion of co-monomers (such as 3-hydroxyhexanoate) (Asrar et al. 2002). Variations in biopolymer molecular weight can also significantly influence mechanical properties. PHAs exhibiting high molecular weights (600 kDa) can serve as thermoplastics, whereas those with low molecular weight (< 400 kDa) are characterized as brittle (Luzier 1992; COX 1994; Penloglou et al. 2012).

The type of cyanobacteria strains and culture conditions affect the polymer composition and properties (Miu et al. 2022). For instance, Rueda et al., (2022c), observed that for *Synechocystis* sp. cultivated under a salinity of 12 mS cm^{-1} (approximately 40 g salt L^{-1}) and a salinity of 60 mS cm^{-1} (approximately 40 g salt L^{-1}) the molecular weight of the produced polymer was reduced from around 100 to 38 kDa. The type of carbon source especially affects the type of co-polymer. For example, *Nostoc muscorum* produced the homopolymer PHB under photosynthetic conditions (CO_2) and also under acetate supplementation, while the co-polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV) was produced when propionate was added (Mallick et al. 2007). On the other hand, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) was obtained by supplementing *Synechocystis* sp. PCC 6803 cultures with γ-butyrolactone (Tanweer and Panda 2020). The amount of each co-polymer could be tailored by changing the concentration of each of the organic carbon sources. For instance, Tanweer and Panda, (2020), found that by increasing the concentration of γ-butyrolactone from 0.1 to 0.4%, the monomer 4HB increased from 16 to 43%mol. When both acetate and γ-butyrolactone were added at a concentration of 0.4% and 0.1% respectively, the content of 4HB decreased from 16 to 11%mol and the 3HB monomer content increased from 84 to 89%mol (Tanweer and Panda 2020). A strong relationship of the carbon source with polymer properties has also been observed for heterotrophic bacteria (Penloglou et al. 2012; McAdam et al. 2020). Penloglou et al. (2012), observed that by increasing the C/N and the C/P ratio the molecular weight of the synthetized polymer can be increased. Sanhueza et al.(2020) reported that for P. *xenovorans* LB400, the use of mannitol, xylose or glucose as a carbon source changed the molecular weight of the polymer from, 2122 kDa, 1708 kDa and 1589 kDa respectively. Garcia-Gonzalez et al. (2015) tested the possibility of producing PHB autotrophically using *Cupriavidus necator* DSM 545 previously grown using different carbon sources. This study demonstrates that the polymers produced autotrophically have a lower molecular weight compared to polymers produced from organic substrates. They also reported that cells previously grown on glucose accumulated polymers with a higher molecular weight and melting temperature than the cells previously grown on glycerol (Garcia-Gonzalez et al. 2015).

Culture conditions seem to have a pivotal role in customizing bioplastic production to meet specific requirements for each of the desired applications. However, there is a notable absence of comprehensive information regarding the influence of cultivation conditions on the thermo-mechanical properties of PHA, especially for PHB produced for cyanobacteria, but also for heterotrophic bacteria (McAdam et al. 2020).

3.2.6 *Optimal cultivation conditions*

Figure 4 shows the relative effect of each of the reviewed parameters on PHAs accumulation. The addition of Na^+ rather than the increase in carbon availability itself was primarily responsible for how



Range studied	CO_2 → 0.04-20% HCO_3 → 0-2 gC L^{-1}	1-50 gC L^{-1} NaCl	0-12 days in darkness	200-500 µmol m^{-2} s^{-1}	1.2-4 gC L^{-1} Acetate
Strain	*Synechocystis* sp. R2020 *Synechococcus* sp. R2020 *Thermosynechococcus elongatus* *Synechocystis* sp. PCC 6714	*Synechocystis* sp. R2020 *Synechococcus* sp. R2020 *Nostoc muscorum* NCCU- 442 *Spirulina subsalsa*	*Synechocystis* sp. R2020 *Synechococcus* sp. R2020 *Synechocystis* sp. PCC 6803	*Synechocystis* sp. R2020 *Synechocystis* sp. B12 *Synechocystis* sp. PCC 6803	*Aulosira fertilissima* CCC 444 *Synechococcus* sp. MA19 *Synechocystis* sp. R2020 *Synechococcus* sp. R2020 *Synechocystis* sp. PCC 6803

Fig. 4 Box-plot of the difference in the PHA content when modifying the studied parameters. The dotted line indicates the limit between positive and negative effects. Lower and upper box boundaries represent the 25th and 75th percentiles, respectively; the line inside the box represents the average; lower and upper error lines show the maximum and minimum of the studied data; and black circles represent the actual value of each study. The PHA difference was obtained by comparing the optimal results with the control condition obtained in each of the studies reviewed in Table 3 (Miyake et al. 1997; Sharma and Mallick 2005; Sharma et al. 2007; Eberly and Ely 2012; Samantaray and Mallick 2012, 2014; Monshupanee and Incharoensakdi 2014; Kamravamanesh et al. 2017; Gracioso et al. 2021; Rueda et al. 2022c, a, b)

$NaHCO_3$ increased PHAs formation (Fig. 4). Optimal salinity is strain-dependent and strongly related to the capacity of each strain to survive osmotic stress (Rueda et al. 2022c; Meixner et al. 2022). A period without illumination enhanced PHAs production in some studies (Sharma and Mallick 2005; Sharma et al. 2007; Troschl et al. 2018). It is possible that in the dark and anoxic conditions are appropriate for PHA accumulation. The supplementation of organic carbon sources (citrate, acetate, fructose, glucose, valerate) stimulates PHA production in all cyanobacteria, however, the extent of this effect depends on the strain and the amount of organic carbon. Moreover, the modification of the cultivation conditions, especially the addition of different organic carbon sources at different concentrations can also be used to tailor the polymer properties.

Although general trends can be observed, there is high variability between studies using similar cultivation conditions. Indeed, using the same cultivation conditions and strain, Rueda et al., (2022b) obtained 14%$_{dcw}$ of PHA, while Rueda et al., (2022c) obtained only 6.4%$_{dcw}$ PHA. These differences may be related to uncontrolled variables, reactor design, previous cultivation modes, temperature, other microorganisms growing in the culture, nutrient consumption, etc. On the other hand, in some cases, the maximum PHA accumulated was maintained for some period (Miyake et al. 1997; Carpine et al. 2018; Kamravamanesh et al. 2018a; Rueda et al. 2020, 2022a), while in other studies, a PHAs production peak was obtained followed by a rapid decline (Samantaray and Mallick 2012; Monshupanee and Incharoensakdi 2014; Rueda et al. 2022b). The explanation for these mixed results is unclear, but it does not appear to be related to the amount of PHA accumulated or the carbon source used. A feasible hypothesis is that PHA is degraded and cells begin to lyse after a certain time under nutrient limitation. The extent to which this happens may depend on the age and the state of the

culture. Future research should target the effects of varying parameters to create a standardized process that will allow better comparison between studies.

3.3 Use of wastewater to produce PHA with cyanobacteria

The use of wastewater has been reported for microalgae biomass production to be an excellent strategy to reduce production costs from 3.2 to 1.8 € kg^{-1} of biomass (Acién et al. 2012). In alignment with this trend, cyanobacteria can potentially grow and treat wastewater at a reduced cost with higher efficiency than traditional wastewater treatment processes (Oswald 1988; Selvaratnam et al. 2015; Li et al. 2019). Cyanobacteria are useful bioaccumulators for the bioremediation of emerging contaminants such as dyes, hydrocarbons, phenolic compounds, pesticides, and herbicides (Touliabah et al. 2022). However, using wastewater to grow cyanobacteria for PHA production presents several challenges. One of the most crucial is the need to keep cultures cyanobacteria-dominated during extensive, non-sterile cultivation.

There are several strategies/recommendations to maintain cyanobacteria as the dominant microorganism in cultures: (1) use of wastewaters with low organic carbon content to reduce the growth of heterotrophic bacteria (Arias et al. 2020); (2) keep a high N:P ratio (> 32:1 molar) and low phosphorus concentrations (Arias et al. 2017); (3) optimize the hydraulic (HRT) and solids retention times (SRT) to retain filamentous cyanobacteria and avoid the growth of unicellular microalgae and (4) as usually done with mixed heterotrophic bacterial cultures, a feast-famine strategy could be applied to specifically select those microorganisms that accumulate more PHAs (Fardinho et al. 2016; Arias et al. 2018a). This strategy consists of adding the substrate in an intermittent regime. During the feast phase, microorganisms accumulate PHA. Then, in the famine phase, only the microorganisms that can grow using stored PHAs will survive (Fradinho et al. 2016).

Although several studies have evaluated the potential of cyanobacteria to treat wastewater, only a few of them assess the use of nutrients from wastewater to grow and produce PHA. The results of these few studies are found in Table 4. *Synechocystis* sp. PCC 6803 *ΔSphU*, which is a mutant with phosphate transport regulation genes deleted, growing in filtered shrimp wastewater produced 32.5%$_{dcw}$ of PHA after 14 days of cultivation (Krasaesueb et al. 2019). *Aulosira fertilissima* cultivated outdoors for 27 days in aquaculture wastewater reached an 80%$_{dcw}$ PHA (Samantaray et al. 2011). *Synechocystis salina* accumulated 5.5%$_{dcw}$ of PHA in 36 days growing in sterilized digestate from the anaerobic digestion of thin stillage (Meixner et al. 2018). *Nostoc muscorum* obtained 65%$_{dcw}$ of PHA accumulated in 20 days (Bhati and Mallick 2016). Wastewater generated during PHA extraction can also be used for cyanobacteria cultivation. During PHAs extraction, many chemicals and non-polymeric biomass are generated. This waste can potentially be recirculated as a source of nutrients for new cultures (Da Silva et al. 2018). All the reviewed studies evidenced that PHA production using cyanobacteria cultivated in wastewater is a promising strategy to enhance the environmental benefits and reduce production costs. There are still numerous issues that need to be addressed further, particularly those related to the robustness and scaling up of the process (Arias et al. 2020). Additionally, the use of wastewater also presents sociological challenges, such as consumers' acceptance of products produced from wastewater, the lack of a robust regulatory framework, and the interest of investors in these processes (Josa and Garfí 2023).

4 PHA recovery

PHA recovery consists of 4 main steps: (1) biomass harvesting, (2) pre-treatment, (3) PHA extraction, and (4) PHA purification (Fig. 5). PHA recovery from cyanobacteria is a relatively immature process, and only a few studies have evaluated its efficiency. Recovery is a crucial phase of the PHA production process because it affects product yield, quality, environmental impact, and process costs (Koller et al. 2016). In this section, the strategies used in each step of PHA recovery will be reviewed.

The first step of the recovery process is harvesting the cyanobacteria biomass. This step is challenging as cyanobacteria cells are small and may have relatively low cell densities (Drosg et al. 2015). 20–30% of the costs associated with the production of microalgae and cyanobacteria are due to the harvesting of the cell (Gudin and Thepenier 1986). Microalgal and cyanobacteria species characteristics, such as cell size,

Table 4 Summary of the PHA percentage and productivity obtained in cyanobacteria cultures cultivated by reusing the nutrients from different waste streams or using wastewater-borne cyanobacteria

Specie	Cultivation conditions			Photobioreactor				PHA production			Reference
	Wastewater	WW characteristics (mg L^{-1})	Removal (%)	System	Hydraulic regime	Light (µmol m^{-2} s^{-1})	Volume (L)	Stimulation strategy	PHA ($\%_{dcw}$)	Productivity (mg L^{-1} d^{-1})	
Synechocystis sp. PCC 6803 ΔSphU	Shrimp aquaculture	N-NO_3^-: 55 P-PO_4^{3-}: 8.2	NO_3^-: 80 PO_4^{3-}: 97	Flat-plate	One step batch	40	15	N, P limitation/12.3[2] mS cm^{-1}	32.48	12.73	Krasaesueb et al. (2019)
Aulosira fertilissima	Aquaculture wastewater	COD: 228 N-NO_3^-: 11.8 P-PO_4^{3-}:3	COD: 82–77 NO_3^-: 80–88 PO_4^{3-}: 100	Tank	Two steps batch	Sunlight (India)	112.5	N limitation/2.6 g L^{-1} citrate/2.8 g L^{-1} acetate	80.4	23	Samantaray et al. (2011)
Synechocystis salina CCALA 192	Sterile digestate supernatant	COD: 18 TN: 35 P: 1.3	-	Tubular	One-step batch	41–51 (16:8 h)[1] (9.3 W m^{-2})	200	–	5.5	17	Meixner et al. (2018)
Mixed culture	Agricultural run-off + NO_3^-	COD: 73 TIN: 7.5 TIP: 0.4	TIN: 95 TIP: 99	Tubular horizontal semi-closed	Semi-continuous	Sunlight (Spain)	3 × 11,700	N, P limitation/feast/famine (HCO_3^- addition)	4.5	0.55	Rueda et al., (2020)
Wastewater-borne mixed culture	Non-sterile growth media supplemented with Na_2CO_3	N-NH_4^+: 13 P-PO_4^{3-}:42–1	–	Column	SBR (HRT = 2 days, SRT = 10 days)	~ 190–720[1] (31–343 W m^{-2})	2 + 0.4	Feast-aerobic-light/famine-anoxic-dark	3.9	–	Arias et al. (2018a)
Synechoystis sp. CCALA192	Non-sterile BG-11	N-NO_3^-: 66 P-PO_4^{3-}: 5.3	NO_3^-: 100	Tubular	SBR	Sunlight + artificial light	200	N, P limitation	13.7	8.5	Troschl et al. (2018)
Nostoc muscorum	Poultry litter (10 g L^{-1})	TOC: 105 N-NH_4^+: 85 N-NO_3^-:2 P-PO_4^{3-}: 31	TOC: 72 NO_3^-: 100 NH_4^+: 97 PO_4^{3-}: 98	Cylinder	One-step Batch	75 (14:10 h)	4	10% of CO_2/2.8 g L^{-1} acetate/3.8 g L^{-1} glucose/0.3 g L^{-1} valerate	65		Bhati and Mallick (2016)

Table 4 (continued)

Specie	Cultivation conditions			Photobioreactor				PHA production			Reference
	Wastewater	WW characteristics	Removal	System	Hydraulic regime	Light	Volume	Stimulation strategy	PHA	Productivity	
		($mg\ L^{-1}$)	(%)			($\mu mol\ m^{-2}\ s^{-1}$)	(L)		($\%_{dcw}$)	($mg\ L^{-1}\ d^{-1}$)	
Spirulina sp. LEB 18	25% Biopolymer extraction waste	TOC: 1000 N-NO_3^-: 3.5 P-PO_4^{3-}: 32	TOC: 20 NO_3^-: 65 PO_4^{3-}: 0	Column	One-step Batch	2,600[1] (12 kW m^{-2}) (12:12 h)	1.5	–	10.6		Da Silva et al. (2018)

Light is expressed as intensity in $\mu mol\ m^{-2}\ s^{-1}$ (hours of light: hours of darkness). A two-step batch means that during the process of PHA production, there is a separation step between the growth and the accumulation phases. One-step batch means that both growth and accumulation phases are done in the same reactor (without separation step). PHAs stimulation strategies are the different stress strategies applied to enhance PHAs accumulation. Feast-famine means that carbon is added in an intermittent regime, thus, there are periods when C is depleted (famine) and periods when C is abundant (feast). Abbreviations: WW: Wastewater, SBR: Sequencing batch reactor; COD: chemical oxygen demand; HRT: hydraulic retention time; TIN: total inorganic nitrogen; TIP: total inorganic phosphorus

[1]The units were converted from $W{\cdot}m^{-2}$ assuming that the lamps used in these studies had a spectrum similar to sunlight. Original values were specified in brackets

[2]Salinity of the culture media used

affect the harvesting efficiency. Larger microalgae or cyanobacteria (such as *Spirulina platensis*) are usually easier to recover than smaller diameter species (such as *Synechocystis* and *Synechococcus*) (Drexler and Yeh 2014).

Conventional methods for harvesting microalgae or cyanobacteria are membrane filtration, centrifugation or sedimentation usually enhanced with coagulation and flocculation (Pérez-Rivero et al. 2019). At present, novel cost-effective microalgal/cyanobacterial biomass harvesting technologies are being investigated. For instance, a new cyanobacteria concentrator based on microfluidics was developed in which a diluted suspension is introduced into a microchannel and the inertial forces generated from the structure geometry move the cyanobacteria laterally to a known position from where the concentrated stream is recovered (Wang and Dandy 2017). Magnetic nanoparticles were also used to improve harvesting, reaching a harvesting efficiency > 93% (Wang et al. 2020). Another possible alternative to reduce harvesting costs is to grow cyanobacteria in biofilm reactors (Strieth et al. 2018), however, not all cyanobacteria can easily form biofilms.

After harvesting, biomass needs to be pre-treated to enhance accessibility to the polymer and increase polymer properties. Cell disruption is needed to completely dissolve PHA (Meixner et al. 2018). Different mechanical methods such as freeze-thawing, sonication, homogenization, bead milling etc. have been seen to effectively disrupt the cell with low operational cost. However, they present certain drawbacks associated with their practical application in large-scale product recovery. Bead milling and homogenization, for instance, have a high energy demand. Ultrasonication is easily scalable but may be not effective in disrupting some strains and cause heat that may damage other sub-products (Corrêa et al. 2020). In this context, the pulsed electric field technique (PEF) has gained recognition for extracting a wide range of metabolites, including lipids, proteins, and carotenoids, from cyanobacteria. This approach utilizes an electric field to enhance the irreversible pore formation and the subsequent release of compounds from the cell. PEF has emerged as a prominent method due to its cost-effectiveness, high efficiency, minimal time requirements, and environmentally friendly characteristics (Corrêa et al. 2020). Another interesting alternative method is the

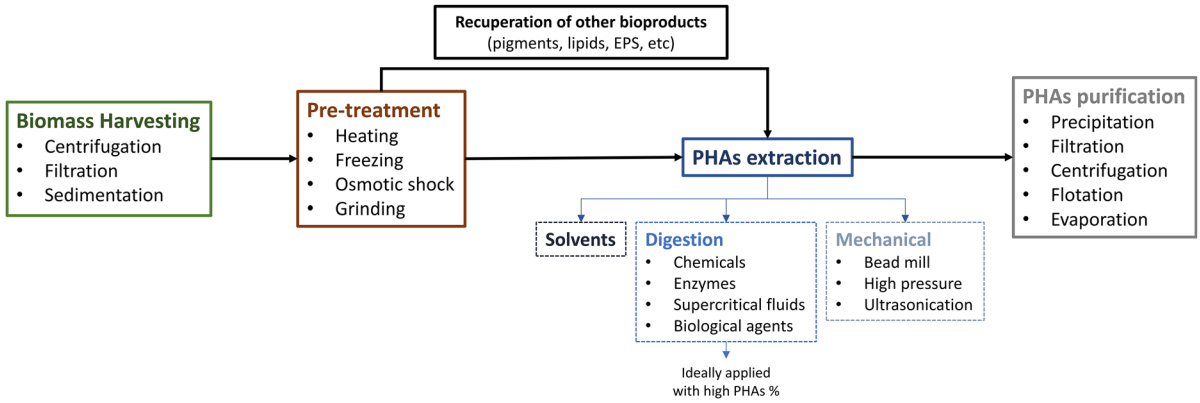


Fig. 5 Conventional steps for PHA downstream and most common methods for each stage (adapted from (Pérez-Rivero et al. 2019))

use of viruses that lyse cyanobacteria cells. This method significantly reduces the use of chemicals and energy consumption and they are not likely to cause health problems to humans or ecosystems. Moreover, viruses are easy to acquire and maintain, so they are cheaper than enzymes (Sun and Zhou 2019). The main drawback of this method is that it is a slow method; it requires several days, compared to ultrasonication which requires only some minutes.

Meixner et al., (2018) also observed that removal of carotenoids and chlorophyll was necessary before a complete PHA dissolution into chloroform. This pre-treatment step could be leveraged to recover other interesting bioproducts from cyanobacteria, such as pigments or exopolysaccharides (EPS), etc. After recovering all these co-products, PHAs could be purified from the remaining biomass (Strieth et al. 2018).

PHA can be extracted by solubilizing the polymer using organic solvents or by directly digesting the cellular components surrounding the polymer (Table 5). Solvent extraction is the most widely used method for PHA recovery, as it is simple, quick, and extracts polymers with a high purity and molecular weight (Pérez-Rivero et al. 2019; Carpine et al. 2020). The majority of the employed solvents are halogenated organic compounds, with chloroform being a particularly common choice. Halogenated solvents pose several challenges due to their high cost, adverse effects on aquatic environments, contribution to ozone layer depletion and they are potentially harmful to human health, as they are suspected carcinogens. Therefore, it is crucial to find safer alternatives (Pérez-Rivero et al. 2019). Methylene chloride or 1,2-dichloromethane were tested for PHA extraction from cyanobacteria and obtained similar yields to chloroform (7.11% and 6.98% of PHB extracted respectively, in front of 7.5% with chloroform) (Ansari and Fatma 2016). However, these are halogenated solvents that have the same aforementioned adverse effects. Alternative non-halogenated solvents for PHA extraction from cyanobacteria have never been evaluated. 1,3-dioxolane, which is a non-chlorinated green solvent, obtained recovery yields of 89.7% in front of 53.9% for chloroform and similar purity, 94% and 93.8% respectively for 1,3-dioxolane and chloroform when extracting PHA from *Cupriavidus necator*. Similar trends were obtained for PHA extracted from cyanobacteria using 1,3-dioxolane in a preliminary study (Bantysh 2021). Butyl acetate at 103 °C, for 30 min, was also found to be a good alternative to halogenated solvents in *C. necator*, leading to a recovery level of 96%, a purity of 99% and a higher molecular weight than chloroform. 1400 kDa using butyl acetate in front of 1000 kDa for chloroform (Aramvash et al. 2015). However, this solvent has not been yet tested to extract PHA from cyanobacteria.

Another strategy to recover PHA is to digest the cellular components surrounding the polymer. Kobayashi et al., (2015) evaluated ionic liquids as alternative solvents to dissolve cyanobacteria and recover PHA by filtration (Kobayashi et al. 2015). More than 98% of the PHA was retained after membrane filtration with the solvent 1-ethyl-3-methylimidazolium methylphosphonate. (Kobayashi et al. 2015). Other innovative biological methods have also been applied

Table 5 Comparison of different PHA extraction and purification methods

Strain	Methodology	PHA recovered ($\%_{\text{of initial cellular PHA}}$)	Purity (%)	Molecular weight (kDa)	PDI	Reference
Synechocystis salina	Cell disruption by milling, pigment removal by acetone/ethanol and PHA dissolution in hot chloroform and precipitation ice-cold ethanol	~61	–	987.5	1.6	Meixner et al. (2018)
Nostoc muscorum NCCU- 442	PHA purification using Soxhlet and different solvents	-Chloroform: 7.5 -1,2- Dichloroethane: 7.11 -Methylene Chloride: 6.98 -Dimethylforamide: 5.48 -Dimethylsulphoxide: 4.46 -Ethyl acetate: 3.19 -n-Hexane: 0	–	–		Ansari and Fatma (2016)
Synechocystis sp. PCC 6803	Dissolution in ionic liquid [C2mim][MeO(H)PO2] and filtration through PTFE membrane (3.0-µm pore size)	98	–	–		Kobayashi et al. (2015)
Cupriavidus necator	1 g of biomass was digested with 100 mL of Butyl Acetate. The mixture was centrifuged and the supernatant containing the PHA precipitated with acetone	97	99	1400		Aramvash et al. (2015)
Cupriavidus necator A-04	Cells digested with 1,3-dioxolane at 80 °C for 6 h and precipitation by water addition	92.7	97.9	486	1.3	Yabueng and Napathorn (2018)
Cupriavidus necator	Mealworms were fed with *Cupriavidus necator* enriched in PHA. The pellets excreted by mealworms were sieved using a 0.5 mm mesh and cleaned with SDS 1%	–	100	210	2.3	Murugan et al. (2016)
Pseudomonas putida	Modified bacterium *Bdellovibrio bacteriovorus* Bd3709 was used to predate bacteria enriched with PHA	80	–	124.8	1.62	Martínez et al. (2016)

Table 5 (continued)

Strain	Methodology	PHA recovered ($\%_{\text{of initial cellular PHA}}$)	Purity (%)	Molecular weight (kDa)	PDI	Reference
Ralstonia eutropha DSM545	Cells were first treated at 95 °C to denaturalize the outer membrane and enzymatically treated with Bromelian. After treatment, PHA pellets were recovered by centrifugation	88.8	66.6	618–622		Kapritchkoff et al. (2006)

PDI; Polydispersity Index; [C_2mim] [MeO(H)PO_2], 1-ethyl-3-methylimidazoliummethylphosphonate, SDS; Sodium dodecyl sulfate, PTFE; Polytetrafluoroethylene. PHA recovered refers to the amount of PHA extracted concerning the total amount of PHA inside of the cell before applying the extraction methodology

to recover PHAs. Murugan et al., (2016), demonstrated the feasibility of recovering PHA using mealworms, which eat and digest the non-PHA cell and excrete the PHA granules that can be cleaned using detergents. This process decreases the use of solvents and chemicals and reuses the non-PHA residual cellular materials to feed the worms, which can be an alternative source of proteins for animal feed. Using this methodology PHA was extracted from *C.necator* with a purity of 100% and a molecular weight of 240 kDa, equal to that of chloroform (Murugan et al. 2016). Genetically modified *Bdellovibrio bacteriovorus* Bd3709 was also used to recover PHA with an 80% efficiency from heterotrophic bacteria. These bacteria predate the gram-negative bacteria, releasing the PHA granules to the culture media (Martínez et al. 2016). Enzymes (i.e. proteases) are also promising to recover PHA, as they are very specific and use mild conditions, which give rise to high reaction rates with little product damage (Kapritchkoff et al. 2006). For instance, using 2.0% of bromelain per mass of biomass, 88% of PHA is recovered with a purity of 66% from *Ralstonia eutropha* DSM545 (Kapritchkoff et al. 2006).

Although these PHA extraction strategies seem promising to reduce PHA extraction costs, they have been tested only on heterotrophic bacteria. There are very few studies applied to cyanobacteria and most of them do not evaluate polymer properties after extraction. Compared to heterotrophic bacteria, cyanobacteria have a thicker and more complex cell wall, composed of peptidoglycan and cellulose, which is difficult to dissolve (Mehta et al. 2015). Therefore, methods used on heterotrophic bacteria may not be as efficient in cyanobacteria. It is, therefore, necessary to direct future research efforts in the evaluation of the new green purification protocols with cyanobacteria biomass. Moreover, the effect of the PHA extraction method applied, on the polymer properties and quality should be evaluated. Additionally, independently of the extraction method, PHA content in the cell has also a strong effect on the purity and extraction efficiency of the purification process. Cells that have a lower PHA content leave more cellular debris after extraction, thus, more energy and digesting agents are needed to break the cells, which leads to lower efficiency of the process and lower purity (Mohammadi et al. 2012). Therefore, to reach high purities and recovery yields, it is essential to still work on improving the PHA content in the cell.

5 Economic and environmental process sustainability

Cyanobacterial PHA offers a promising pathway towards the production of sustainable bioplastic, as they utilize inorganic carbon sources for growth (CO_2 and HCO_3^-) and they do not require intensive aeration for oxygenation. However, there are still significant technical barriers to be solved before production can be economically and environmentally viable (Price et al. 2022). In comparison with heterotrophic microorganisms, cyanobacteria growth rate is very slow, lower cell concentrations can be achieved in the photobioreactors due to light limitation (Acién

Fernández et al. 1999; Bähr et al. 2016) and PHA accumulated in the cell is in general lower than in heterotrophic microorganisms. These technical disadvantages can hinder the economic viability of the process and reduce its sustainability.

Recently, some authors have evaluated the economic viability of a hypothetical industrial plant for this process. Panuschka et al. (2019), determined that under optimal cultivation conditions (60%$_{dcw}$ of PHA, cell cultivated in a thin layer photobioreactor in south Europe) the minimum selling price for the produced PHB would be 24 € · kg^{-1} PHB. Similarly, Price et al. (2022), evaluated different plausible scenarios to reduce PHA production costs, such as the use of solar energy to reduce electricity costs, the use of wastewater as a cultivation media, deep 'PHB ripening ponds' to reduce capital and operating costs, biogas production from the remaining biomass and the pigment production using a biorefinery approach and carbon credits for the use of CO_2. The combination of all these improvements reduced the PHA production costs up to 7 €· kg^{-1} PHB, which is still approximately twice the current market price of PHA. Rueda et al. (2023), determined that to obtain competitive costs, PHA productivity should be increased up to 810 mg · L^{-1} · d^{-1}, while the actual maximum PHA productivity achieved is 59 mg L^{-1} d^{-1} (Kamravamanesh et al. 2017). The three studies agree that the highest costs were related to the major equipment costs.

Regarding process sustainability, (Rueda et al. 2023), observed that increasing PHB productivity drastically reduce all environmental impacts of the process. These impacts were mainly caused by construction materials and the use of chemicals, especially the use of chloroform for PHB purification. Compared with other PHA production processes and fossil-based polymers, the production of PHB with cyanobacteria has similar environmental impacts (Rueda et al. 2023). Energy Consumption is slightly lower than that of other PHA production processes and fossil-based plastics. Impacts on Fine Particulate Matter and Freshwater Eutrophication are similar to other PHA production processes. Indeed, the impact on these categories would be lower than the one of heterotrophic bacteria if the technology is further developed to become cost-competitive. Impacts in Stratospheric Ozone Depletion were higher for cyanobacteria. This is due to the chloroform extraction methodology used. Nevertheless, comparability between PHA production processes is limited. Differences in the technology maturity should also be considered. Those processes with a high maturity (i.e. fossil-based plastics production) are more likely to be highly optimized and to have improved efficiency concerning the immature processes (i.e. PHA production from cyanobacteria) (Walker and Rothman 2020).

Both environmental and economic evaluations of the process, underscore the necessity of increasing both the content of PHA and biomass productivity. This can be achieved by using genetically modified strains able to attain high PHA content, such as the strain recently developed by Koch et al. (2020b), which achieved an 81%$_{dcw}$ of PHA under photoheterotrophic growth conditions. Additionally, using reactors with a lower volume-to-surface ratio is also necessary to achieve these higher productivities. Alternative extraction methods, not using chloroform, as the ones described in the previous section, are also essential to reduce the environmental impact of the process.

6 Conclusions and future perspectives

The productive sectors of the 21st Century rely heavily on plastics; however, conventional petroleum-based plastics pose a growing environmental challenge due to their limited biodegradability and escalating pollution issues. PHA is a promising substitute for these plastics since they can be readily degraded in a variety of environments, such as marine waters, freshwaters, landfills, soils, or home composting devices. Moreover, PHA has similar properties to conventional polymers and can be processed with the same techniques.

PHA can be produced from cyanobacteria cultures from CO_2 and sunlight with the potential to reduce production costs and environmental footprint in comparison to heterotrophic bacteria because (1) utilize inorganic carbon sources for growth (CO_2 and HCO_3^-), and (2) they do not require intensive aeration for oxygenation. Achieving an industrial-scale production process for PHA with cyanobacteria necessitates a comprehensive optimization of the entire workflow, encompassing cyanobacteria cultivation, PHA production stimulation, as well as the extraction and purification of PHA. Concerns arise

from the fact that much of the existing literature predominantly addresses isolated aspects of the process, leaving uncertainties about the practical feasibility and applicability of the overall concept of the process. Also, the fundamental knowledge of the function and regulation of metabolisms is still incomplete and limits the development of industrial PHA production.

In the next paragraphs, we list and describe the crucial challenges that have to be overcome:

- *Increased biomass productivity:* cyanobacteria biomass productivity must be increased to make PHA from cyanobacteria commercially viable (Rueda et al. 2023). New photobioreactor designs should be, therefore, developed to obtain cultures with an increased cell density. This is especially challenging when using photosynthetic microorganisms, because of light limitation due to self-shading (Acién Fernández et al. 1999; Bähr et al. 2016). Therefore, new designs should consider an efficient light transmission meanwhile, they enable a good CO_2 supply and O_2 exchange (Hoschek et al. 2019). New promising reactor configurations reaching high cell densities have been recently developed. For instance, capillary microreactors made of borosilicate (2 mm inner diameter) (David et al. 2015) were able to reach up to 48 $g \cdot L^{-1}$ when cultivating S*ynechocystis* sp. PCC 6803 together with *P. taiwanensis* (Hoschek et al. 2019). Thin layer cascade reactors reached a cell density of 17 $g\ L^{-1}$ and biomass productivities of 4 $g\ L^{-1}\ d^{-1}$ with *Nannochloropsis salina* (Apel et al. 2017). Two-tier reactors consisting of two chambers divided by a membrane attained a cell density of 30 $g\ L^{-1}$ with *Synechococcus* sp. PCC 7002 (Bähr et al. 2016). However, none of them have been yet applied at an industrial scale, most of them had a volume < 1 L. Moreover, an economic analysis of the new alternative photobioreactors has not yet been done. It is essential to evaluate if the higher biomass production economically and environmentally compensates for the use of materials and energy consumption.
- *Improvement of PHA accumulation*: increasing PHA yield is one of the main challenges of cyanobacteria cultures for minimizing production costs and making the process economically feasible. Although literature describes general trends on the factors that affect PHA production, there is high variability in results between studies using similar cultivation conditions. Future research should target the effects of varying parameters to create a standardized process that will allow better comparison between studies.

PHA production has been seen to be stimulated by an increase in salinity, but the optimal salinity depends on the capability of each strain to resist osmotic stress. A period of darkness under anoxic conditions may also stimulate accumulation. Light intensity seems to enhance PHA production, especially under mixotrophic conditions. Organic carbon supplementations also stimulated PHB and other co-polymers synthesis. Culture conditions seem to have a pivotal role in customizing bioplastic production to meet specific requirements for each of the desired applications. However, there is a notable absence of comprehensive information regarding the influence of cyanobacteria cultivation conditions on quality and mechanical properties.

We envisage two different approaches to further increase the PHA content in cyanobacterial cells: (1) screening of new cyanobacteria strains and (2) genetically modifying the cells. There may be unexplored strains exhibiting higher productivity. Genetic modification, either by random mutagenesis or by target mutation, is also a good strategy to increase the PHA content of cyanobacteria (Kamravamanesh et al. 2018a; Koch et al. 2020b). As discussed in Sect. 3.1, PirC is a central carbon flow regulator that controls the carbon flux towards PHA production. When concentrations of 2-OG are high the PII-PirC complex, encountered under normal growth conditions, is broken and PirC inhibits PGAM and slows down the flow of carbon toward PHA formation. Therefore, the disruption of this gene increases the flux of newly fixed carbon towards the PHA building blocks. Indeed, Koch et al. (2020b) obtained a 63 $\%_{dcw}$ PHA content in *Synechocysts* sp. PCC 6803 by applying this modification. On the other hand, SphU regulon is activated in response to phosphate-limiting conditions leading to the upregulation of genes involved in the phosphate transportation and to the accumulation of polyphosphate and acetyl phosphate in the cell (Summers et al. 1999). Silencing this gene may allow PHA accumulation even when P is abundant, which can increase

productivity (Krasaesueb et al. 2019). Genes from other highly productive microorganisms, such as *Cupriavidus necator,* can also be introduced to increase the capacity of the modified strain to accumulate PHAs (Koch et al. 2020b). These genetic modifications will lead to strains with a high capability to produce PHA under laboratory conditions. Nonetheless, it is essential to test the stability of these new strains during long-term cultivation under real-scale photobioreactors, which are complex to control and the sterility is difficult to maintain (Troschl et al. 2018; Khan et al. 2019).

- *Wastewater as culture media*: Growth in wastewater to produce PHA has been a promising strategy to reduce production costs and environmental impact. The major challenge of using wastewater as a culture media is maintaining cyanobacteria as the dominant species during long cultivation periods. Most studies used lab-scale batch cultivation, where cultures were maintained for less than 30 days, so the stability of the culture in wastewater during long cultivation times needs further evaluation. Lastly, the social acceptance of the products coming from wastewater should be further improved by introducing more robust regulations.
- *Improvement of biomass and bioproducts recovery:* PHA extraction is one of the most important challenges to make cyanobacteria PHA production economically viable. The extraction of PHA from cyanobacteria has hardly been evaluated. Methods for heterotrophic bacteria cannot be directly transferred to cyanobacteria since they present a thicker and more complex cell wall. Therefore, in future research, the most promising and sustainable methods developed for heterotrophic bacteria, such as new green solvents, mealworms or enzymatic processes, should be further tested on cyanobacteria. In addition, the recuperation of other by-products (e.g. pigments) together with PHA should be addressed to increase process profitability.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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