

MINIREVIEW

Rational engineering of natural polyhydroxyalkanoates producing microorganisms for improved synthesis and recovery

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Abstract

Microbial production of biopolymers derived from renewable substrates and waste streams reduces our heavy reliance on petrochemical plastics. One of the most important biodegradable polymers is the family of polyhydroxyalkanoates (PHAs), naturally occurring intracellular polyoxoesters produced for decades by bacterial fermentation of sugars and fatty acids at the industrial scale. Despite the advances, PHA production still suffers from heavy costs associated with carbon substrates and downstream processing to recover the intracellular product, thus restricting market positioning. In recent years, model-aided metabolic engineering and novel synthetic biology approaches have spurred our understanding of carbon flux partitioning through competing pathways and cellular resource allocation during PHA synthesis, enabling the rational design of superior biopolymer producers and programmable cellular lytic systems. This review describes these attempts to rationally engineering the cellular operation of several microbes to elevate PHA production on specific substrates and waste products. We also delve into genome reduction, morphology, and redox cofactor engineering to boost PHA biosynthesis. Besides, we critically evaluate engineered bacterial strains in various fermentation modes in terms of PHA productivity and the period required for product recovery.

INTRODUCTION

Petrochemical plastics are increasingly accumulating in soil and aquatic environments, with recent studies demonstrating that nearly 80% of the ever-produced plastics are intact across the planet (Geyer et al., 2017). A sustainable alternative to deriving polymers from renewable carbon sources is the use of microbial cell factories (Choi et al., 2020). Among them,

polyhydroxyalkanoates (PHAs) are polyoxoesters that accumulate intracellularly as carbon and energy form in bacteria and archaea (Wilkinson, 1963) when they encounter famine and stress conditions (Mason-Jones et al., 2021). They also display similar mechanical and physical properties to various synthetic plastic like polypropylene and polystyrene (Van de Velde & Kiekens, 2002) and proven biodegradable in landfills, soil, and water ecosystems (Meereboer et al., 2020).

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Some microbes can amass more than 90% on a cell mass basis as PHA, and the hydrophobic granules are prone to hydrolysis to satisfy cell's metabolic demands (Madison & Huisman, 1999). Initially described as a nongrowth associated intracellular product where the limitation of an inorganic nutrient is necessary to trigger PHA accumulation (Sudesh et al., 2000), yet several studies showed that in some environmental microbes, PHAs are intrinsic parts of the cell components during replication (Godard et al., 2020; Poblete-Castro et al., 2012a; Shrivastav et al., 2010). PHA polymer molecular structures display desired technological features such as water, oxygen and flavour barriers (Follain et al., 2014) along with suitable melting points and molecular weights for manufacturing containers and packaging materials (Israni & Shivakumar, 2019). Despite these advantages, the global polymer market still relies on materials originated from petrochemical sources, accounting for 99% of the worldwide production volume (Chen et al., 2020). In fact, these polyoxoesters share nearly 1% of the biopolymer market.

In this review, we underline the principles and rationale behind metabolic and genetic engineering strategies to enhance PHA production in natural biopolymer producers belonging to different bacterial genus and archaea. The construction of novel pathways to generate not naturally occurring metabolites of these biocatalysts' metabolic networks is also highlighted. We also delve into designing strategies to program cell autolysis and discuss the lytic system's efficiency during PHA accumulation.

FUNDAMENTALS OF PHA METABOLISM AND KEY PRECURSORS

There is a remarkable diversity in the monomer composition of the biosynthesized PHA across bacteria. The final chemical structure depends greatly on the type of PHA synthase (Chek et al., 2019). These enzymes determine whether the precursors for PHA synthesis yield short-chain- (*scl*-PHA, C4-C5 carbon atoms) or medium-chain length (*mcl*-PHA, C6-C14 atoms) of 3-hydroxy acids (Rehm, 2003). Early research demonstrated that the substrate for class I PHA synthetase, present in bacteria like *Cupriavidus necator* (formerly *Ralstonia eutropha*) and *Azotobacter vinelandii* (Senior et al., 1972), is (*R*)-3-hydroxybutyryl-CoA, which finally result in poly(3-hydroxybutyrate) (3HB or PHB, C4) (Rehm & Steinbüchel, 1999; Slater et al., 1988) (Figure 1). *Pseudomonas putida* GPo1 (formerly *Pseudomonas oleovorans*) produced an alternative PHA to those characteristic of *C. necator* and *A. vinelandii*, which presented a different monomeric chemical structure as they harbour Class II PHA synthases that catalyse the transformation of 3-hydroxyenoyl-CoAs,

ranging from 3-hydroxyhexanoyl-CoA (C6-CoA) to 3-hydroxytetradecanoyl-CoA (C14-CoA) (Figure 1). PHA synthase classes III and IV are usually heterodimers, PhaC-PhaE and phaC-PhaR, respectively, tending to prefer short-chain length monomers and being present in bacteria like *P. megaterium* and *Bacillus cereus* (Lawrence et al., 2005; Tsuge et al., 2015). Another important class of PHA-related proteins is the amphipathic phasin. These proteins cover the PHA granule exerting control on the size, shape and the number of inclusion bodies in the cell (Velázquez-Sánchez et al., 2020), where their repression avoids PHA formation (Mitra et al., 2021). It also influences the molecular weight of the resulting polyester (Tian et al., 2005).

Several monomers that serve as substrates for PHA synthases (PhaC) are 3-hydroxypropionyl-CoA, (*R*)-3-hydroxyacyl-CoA, 3-hydroxybutyryl-CoA, 3-hydroxyvaleryl-CoA and 4-hydroxybutyryl-CoA. Conversion of fatty acids and n-alkanes through the β -oxidation pathway generates several precursors (*trans*-2-enoyl-CoA, [*S*]-3-hydroxyacyl-CoA and 3-ketoacyl-CoA) for the synthesis of (*R*)-3-hydroxyacyl-CoA, the PHA polymerase substrate (Figure 2). On the other hand, glucose, fructose, xylose, methanol, glycerol, CO₂ and organic acids result in the central intermediate acetyl-CoA to initiate the bioconversion towards PHA synthesis (Figure 2). A conserved pathway involving acetyl-CoA condensation into acetoacetyl-CoA is present in *C. necator*, *P. megaterium*, *Burkholderia*, *H. elongata* and *A. vinelandii* (Doi et al., 1988). *Pseudomonas* and related bacteria usually lack this PHB biosynthetic route (Prieto et al., 2016). Instead, a biotin-, ATP-dependent carboxylase (ACC) catalyses the conversion of acetyl-CoA to malonyl-CoA, being the rate-limiting step in the fatty acid de novo synthesis (Magnuson et al., 1993; Schweizer et al., 1978). The presence of PhaG transacylase allows the conversion of (*S*)-3-hydroxyacyl-ACP into (*S*)-3-hydroxy-fatty acids, which are transformed by the AlkK ligase (Wang et al., 2018) yielding (*R*)-3-hydroxyacyl-CoA moieties that supply the PHA synthase (Figure 2). Excellent reviews covering PHA metabolic pathways in detail include (Mezzina et al., 2021; Winnacker, 2019).

The archaea *H. mediterranei* is unique among biopolymer producer as it accumulates the copolymer PHBV when cultured on glucose as the sole carbon source (Nicolaus et al., 1999). Together with the archetypic PHB pathway, it operates four different routes to generate propionyl-CoA (Figure 2). Acetyl-CoA can turn into malonyl-CoA, (*R*)-methylmalate or acetoacetyl-CoA to initiate the PHA synthesis (Han et al., 2013). Two precursors belonging to the TCA cycle, oxaloacetate and succinyl-CoA also result in subsequent enzymatic steps into propionyl-CoA. The archaeal strain can produce 3-ketovaleryl-CoA directly from propionyl-CoA and acetyl-CoA by the enzyme β -ketothiolase (PhaA) (Han et al., 2013). Given the broad substrate spectrum of these particular PhaA and PhaB1/B2

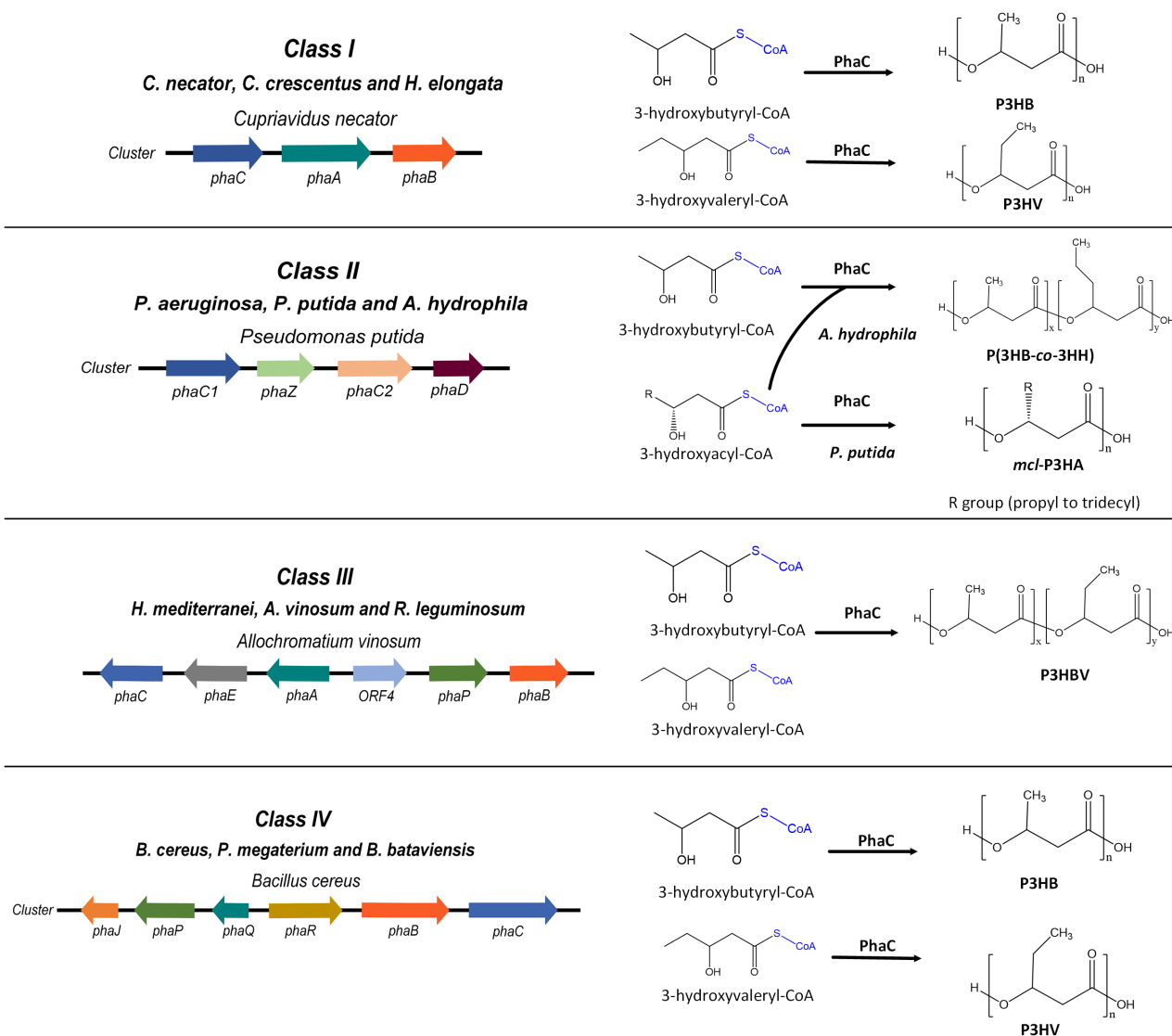


FIGURE 1 PHA cluster and PHA polymerase classes and substrates required in diverse native PHA-producing strains. Class I (*phaC*: PHA polymerase, *phaA*: β -ketothiolase, *phaB*: NADPH-dependent acetoacetyl-CoA reductase). Class II (*phaC1* and *phaC2*: PHA polymerases, *phaZ*: PHA depolymerase, *phaD*: transcriptional regulator). Class III (*phaC* and *phaE*: PHA synthase subunits, *phaA*: β -ketothiolase, *phaB*: NADPH-dependent acetoacetyl-CoA reductase, *phaP*: PHA phasin). Class IV: (*phaC* and *phaR*: PHA synthase subunits, *phaB*: NADPH-dependent acetoacetyl-CoA reductase, *phaP*: PHA phasin, *phaJ*: (*R*)-3-hydroxyacyl-CoA hydratase, *phaQ*: PHB-responsive transcriptional repressor).

enzymes, (*R*)-3-hydroxyvaleryl-CoA is formed from 3-ketovaleryl-CoA, enabling the incorporation of HV monomers by the class III PHA enzymes (PhaCE) from non-related substrates (Poli et al., 2011) (Figure 2).

ENHANCED PHA PRODUCTION ON PURE SUBSTRATES

Engineering strategies to increase PHA synthesis from carbohydrates

Cupriavidus necator H16 is one of the most prominent PHB producers on simple sugars and oleaginous compounds. The prokaryote can cope with high fructose

and gluconate concentrations, showing biopolymer accumulations above 90% of its cell dry weight (CDW). In fed-batch processes, the most utilized fermentation mode in industry, the strain reaches more than 150 gCDW L⁻¹ (Table 1). However, the cells are not able to propagate on glucose nor xylose, the major components of cellulosic material. Sequencing genetic regions of a glucose-utilizing *C. necator* NCIMB 11599, subjected to UV and spontaneous mutagenesis (Kim et al., 1995), revealed mutations in the *nagE* and *nagR* genes belonging to the EIIC-EIIB component (Orita et al., 2012). The insertion of a functional *nagE* and deletion of the *nagR* gene in H16 allowed PHB synthesis from glucose to similar amounts to fructose. In another round of engineering, the expression of the *xyIAB*

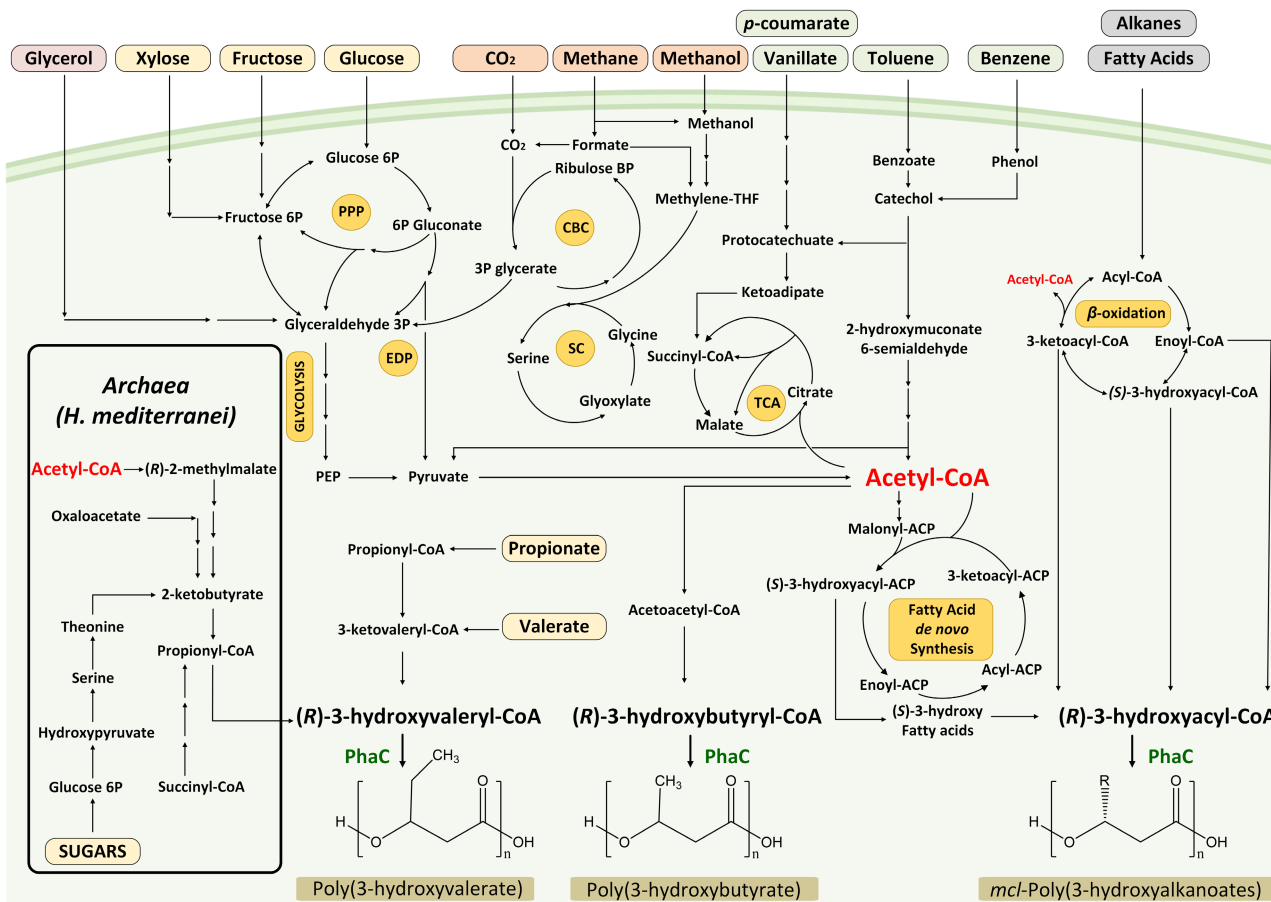


FIGURE 2 Metabolic pathways of different substrates towards poly(3-hydroxyalkanoates) biosynthesis in bacteria and archaea. CBC, Calvin Benson cycle; ED, Entner–Doudoroff pathway; PEP, Phosphoenolpyruvate; PhaC, PHA synthase; PPP, Pentose phosphate pathway; SC, Serine cycle; TCA, Tricarboxylic acid cycle.

genes of *E. coli* in *C. necator* NCIMB 11599 (Figure 3) converted the strain in a PHB producer using xylose as the only substrate (Kim et al., 2016). In a remarkably successful case, the researchers challenged the engineered bacterium to produced PHB from sunflower hydrolysates, which resulted in 7.68 gPHB L⁻¹ in batch cultures and attained similar biopolymer content (~73, wt%) as with pure sugars (Table 1).

The metabolically versatile and robust chemical-producing platform *Pseudomonas putida* strains (Nikel & de Lorenzo, 2018) are superb *mcl*-PHA accumulators (Poblete-Castro et al., 2012b; Prieto et al., 2016) where multiple rational engineering strategies keep advancing the development of superior biocatalysts (Poblete-Castro et al., 2017; Weimer et al., 2020). In these bacteria, the glyoxylate shunt is an active metabolic reaction consuming acetyl-CoA and isocitrate in a non-oxidative manner. Inactivation of the anaplerotic reaction via the insertion of a mini-transposon in the *aceA* gene encoding for an isocitrate lyase of KT2442 improved the *mcl*-PHA synthesis using gluconate under nitrogen limitation (Klinke et al., 2000). The mutant strain produced 70% more PHA (g L⁻¹) than the parental *P. putida* and increased the specific growth rate

by 31% and biomass yield from 0.57 to 0.78 (gCDW L⁻¹) (Table 1). In silico-aided metabolic engineering strategies have proven successful to predict genetic modifications to overproduce fuels, amino acids and health compounds (Ko et al., 2020). Optimization methods using large metabolic networks like those found in genome-scale models, or small networks comprising dozens of reactions, enable targeting gene deletions in host strains with effective cases applying minimization of metabolic adjustment (MOMA) (Segre et al., 2002), OptKnock (Burgard et al., 2003). Expanded algorithms involve the use of elementary modes analysis (Schuster & Hilgetag, 1994; Trinh et al., 2009) to prioritize genes for inactivation or overexpression (Melzer et al., 2009). Optimization of the metabolic network of *P. putida* KT2440 growing on glucose using flux design (Melzer et al., 2009) revealed that the removal of glucose dehydrogenase (*gcd* gene) responsible for oxidizing glucose into gluconate in the periplasm of the cell could increase the PHA flux as gluconate secretion was abolished (Poblete-Castro et al., 2013). The engineered strain finally doubled the product titre (1.8 g L⁻¹) compared to the wild-type in batch bioreactors (Table 1). In another study, this mutant strain performed

TABLE 1 Genetic modification of host cells for enhanced PHA synthesis under various fermentation modes

Organism	Modification	C-source	Fermentation mode	PHA type	CDW (g L ⁻¹)	PHA content PHA wt%	Productivity (g L ⁻¹ h ⁻¹)	References
<i>C. necator</i> H16	Δ nagR & nagE	Soya hydrolysates	Fed-batch	PHB	150.0	90.0	0.33	Orita et al. (2012)
<i>C. necator</i> 11,599	pKM212-Xyl/AB _{Ec}	Xylose	Fed-batch	PHB	88.7	72.5	0.15	Kim et al. (2016)
<i>P. putida</i> KT217 (KT2440)	Δ aceA	Glucuronate	Batch culture	mcI-PHA	0.8	41.0	0.25	Klinke et al. (2000)
<i>P. putida</i> KT2440	Δ gcd	Glucose	Batch culture	mcI-PHA	4.6	38.0	0.53	Poblete-Castro et al. (2013)
<i>P. putida</i> KT2440	Δ gcd	Glucose	Fed-batch	mcI-PHA	61.8	67.0	0.83	Poblete-Castro et al. (2014a, 2014b)
<i>P. putida</i> KT2440	Δ gcd	Glucose	Batch culture	mcI-PHA	4.7	42.1	0.04	Borrero-de Acuña et al. (2014)
<i>P. putida</i> KTU-U13	Δ gcd	Glucose	Batch culture	mcI-PHA	4.0	39.3	0.26	Liang et al., 2020
<i>P. mendocina</i> NKU421	7.7% genome reduction	Glucose	Batch culture	mcI-PHA	1.3	35	0.01	Fan et al. (2020)
<i>P. putida</i> EM42	bgIC ⁺	Cellobiose + Xylose	Batch culture	mcI-PHA	1.2	21.0	0.01	Dvořák et al. (2020)
<i>P. putida</i> KT2440- S. elongatus	cscAB	Sucrose-CO ₂	Fed-batch (Photobioreactor)	mcI-PHA	n.s	n.s	n.s	Löwe et al. (2017a, 2017b)
<i>P. putida</i> EM178 cscRABY & S. elongatus	Δ nasT	Sucrose-CO ₂	Batch culture	mcI-PHA	0.18	14.8	n.s	Hobmeier et al. (2020)
<i>A. vinelandii</i> UW	<i>A. vinelandii</i> 113 DNA	Glucose	Batch culture	PHB	3.5	65.0	0.08	Page and Knosp (1989)
<i>A. vinelandii</i> OPNA	PTSNtr and RsmA-RsmZ/Y extract	Sucrose + Yeast	Fed-batch	PHB	37.2	73.3	0.46	García et al. (2014)
<i>A. vinelandii</i>	AlgK	Burk + glucose	Batch culture	PHB	3.2	68	0.05	Segura et al. (2003)
<i>H. boliviensis</i>	Wild type	Glucose	Fed-batch	PHB	44	81	1.1	Quillaguamán et al. (2008)
<i>H. campaniensis</i> LS21	phbCAB	Glucose mineral	Batch culture	PHB	7.8	82.9	n.s	Yue et al. (2014)
<i>H. bluephagenesis</i> TD40	P _{lac} :orfZ	Glucose + corn steep liquor	Fed-batch	P(3HB-co-4HB)	99.6	60.4	1.66	Ye et al. (2018)
<i>H. bluephagenesis</i> TD08AB	scpA, argK and scpB	Glucose + glutarate	Batch culture	PHBV	10.6	84.3	0.19	Chen et al. (2019)
<i>C. necator</i> H16_glpFK	glpF & glpK	Glycerol	Batch culture	PHB	2.3	63.9	1.4	Fukui et al. (2014)
<i>P. putida</i> KT40GlpR	Δ glpR	Glycerol	Batch culture	mcI-PHA	1.2	39.3	0.01	Escapa et al., 2012
<i>P. putida</i> KT2440	Δ phaZ	Raw Glycerol	Batch culture	mcI-PHA	4.2	46.8	0.03	Poblete-Castro et al. (2014a, 2014b)
<i>P. putida</i> KT2440	Δ phaZ	Raw Glycerol	Batch culture	mcI-PHA	52.4	38.9	0.34	Borrero-de Acuña et al., 2021a, 2021b
<i>C. necator</i> H16	can	Glycerol	Batch culture	PHB	0.4	68	n.s	Thorbecke et al. (2021)

TABLE 1 (Continued)

Organism	Modification	C-source	Fermentation mode	PHA type	CDW (g L ⁻¹)	PHA content PHA wt%	Productivity (g L ⁻¹ h ⁻¹)	References
<i>C. necator</i> H16 Reh01	ΔIdh -p2m-pj-V	CO ₂	Batch culture	PHB	0.6	50.4	n.s	Tang et al., (2020)
<i>C. necator</i> H16	<i>phaCCn</i> , <i>pBAD_{chim4}</i> , <i>phaC1P</i>	CO ₂	Batch culture	PHB	0.8	41.2	n.s	Nangle et al. (2020)
<i>Synechocystis</i> sp. WDKOX	Δach -pta & <i>xfpk</i>	CO ₂	Batch culture	PHB	2.4	12.4	0.01	Carpine et al. (2017)
<i>Synechocystis</i> sp. PCC6714 (Mt_a24)	<i>pstA</i>	CO ₂	Batch culture	PHB	3.6	37	n.s	Kamravamaneh et al. (2018)
<i>Synechocystis</i> sp. PCC6803	Δgcd	CO ₂ +Glucose	Batch culture	PHB	1.6	5.5	n.s	Monshupanee et al. (2019)
<i>Synechocystis</i> sp. PCC6803	$\Delta pirC$ -RE <i>phaAB</i>	CO ₂	Batch culture	PHB	n.d	63.0	n.s	Koch et al. (2020)
<i>M. extorquens</i> AM1	$\Delta phaC_{Me}$ & <i>phaC_{Ac}</i>	Methanol	Batch culture	P(3HB-co-3 HV-co-3HHX)	0.9	34.7	n.s	Orita et al. (2014))
<i>I. sakaiensis</i>	Wild type	PET	Batch culture	<i>mcl</i> -PHA	1.2	25	n.s	Fujiwara et al. (2021)
<i>P. putida</i> GO16	Wild type	TA	Fed-batch	<i>mcl</i> -PHA	8.2	32	0.05	Kenny et al. (2012)
<i>P. frigusceleri</i> MPC6	Wild type	Ethylene glycol	Batch culture	P(scl-co-mcl)	1.4	15.5	n.s	Orellana-Saez et al. (2019)
<i>P. putida</i> MLF185 (KT2440)	<i>Pt_{ac}::gcl-hy1-glxR-ttuD-pykF-gclDEF</i>	Ethylene glycol	Batch culture	<i>mcl</i> -PHA	1.8	32.2	n.s	Franden et al. (2018)
<i>P. putida</i> A514	pTPxy AphaJ4C1	Vanillic acid	Batch culture	<i>mcl</i> -PHA	0.714	35	n.s	Wang et al. (2018)
<i>P. putida</i> AG2162 (KT2440)	$\Delta phaZ$, $\Delta fadBA1$, $\Delta fadBAE2$, $\Delta aldB::P_{rac}$, <i>phaG-alkK-phaC1-phaC2</i>	Lignin stream	Batch culture	<i>mcl</i> -PHA	0.7	17.7	n.s	Salvachúa et al. (2020)
<i>P. putida</i> H	$\Delta catA2$	Benzoate	Fed-Batch	<i>mcl</i> -PHA	21.1	28.9	0.08	Borrero-de Acuña et al. (2021a, 2021b)
<i>P. putida</i> H	$\Delta catA2$	Enriched lignin stream	Fed-Batch	<i>mcl</i> -PHA	5.4	25.9	0.03	Borrero-de Acuña et al. (2021a, 2021b)
<i>C. necator</i> PHB-4/pJRDEE32d1 (H16)	$\Delta phaC$ & <i>phaC_{Ac}</i>	Soybean oil	Batch culture	P(3HB-co-3HHX)	138.0	74.0	1.06	Kahar et al. (2004)
<i>P. putida</i> KT2440	$\Delta tctA$	Waste vegetable oil	Batch culture	<i>mcl</i> -PHA	5.1	38	0.03	Borrero-de Acuña et al. (2019)

Abbreviations: *mcl*-PHA, medium-chain-length poly(3-hydroxyalkanoates); N.S., Not shown; PHB, poly(3-hydroxybutyrate); P(3HB-co-3 HV-co-3HHX), poly(3-hydroxybutyrate-co-3-hydroxybutyrate-co-3-hydroxyhexanoate); P(3HB-co-3HHX), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate).

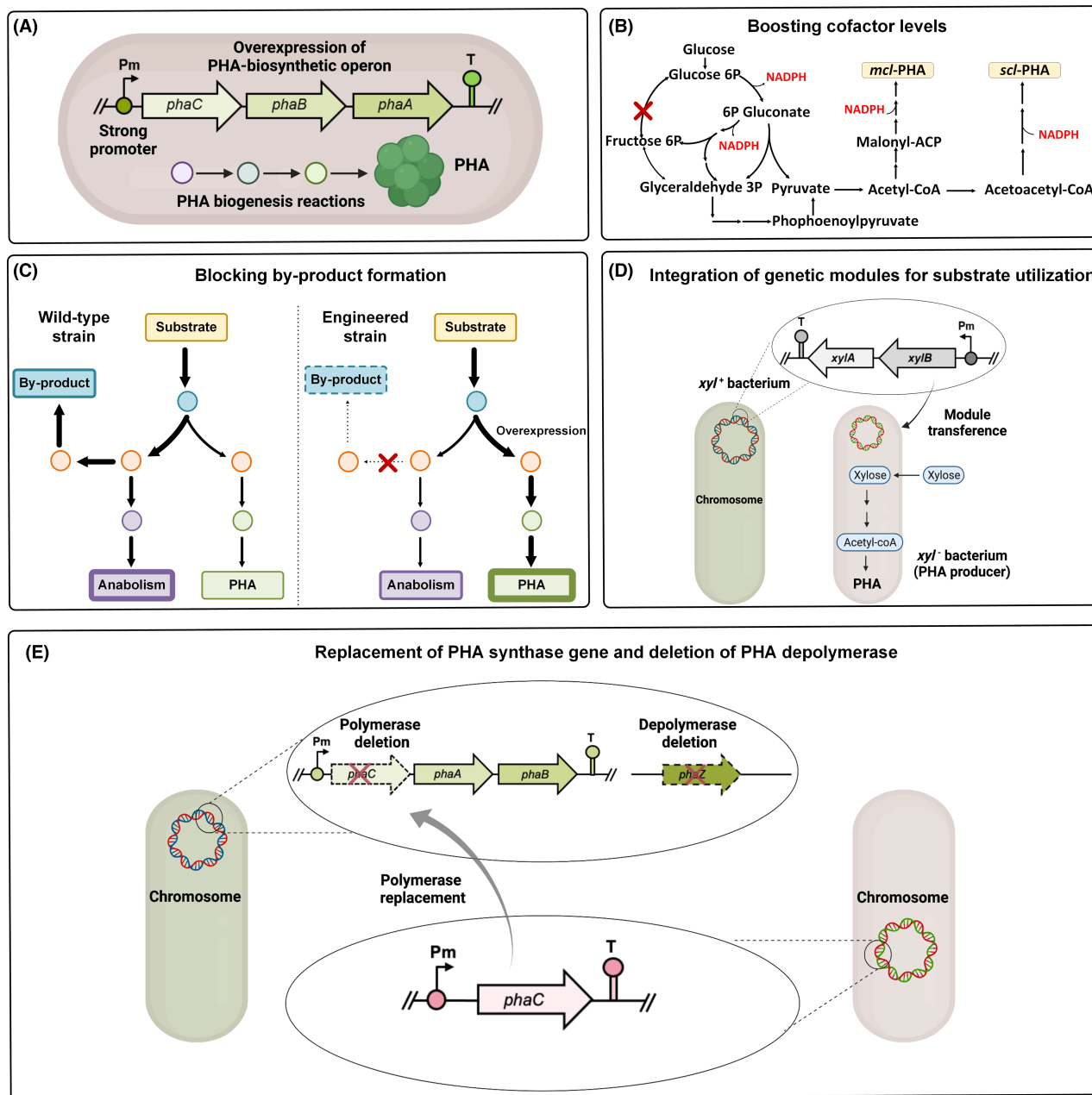


FIGURE 3 Successful metabolic engineering approaches to improve PHA synthesis in microorganisms. (A) Overexpression of the PHA operon, Pm: promoter, T: terminator. (B) Increased cofactor levels, *mcl*-PHA: medium-chain-length poly(3-hydroxyalkanoates), *scl*-PHA: short-chain-length poly(3-hydroxyalkanoates), X: gene deletion. (C) Blocking by-product synthesis, X: gene deletion. (D) Integration of genetic modules for metabolizing novel substrates in PHA-producing microorganisms. (E) Replacement of the native PHA synthase and deletion of the PHA depolymerase. Pm, promoter; T, terminator.

well in a glucose-fed-batch fermentation synthesizing $0.83 \text{ (gPHA L}^{-1} \text{ h}^{-1}\text{)}$ and intracellular content of 67 wt% (Poblete-Castro et al., 2014a). Furthermore, based on the flux design predictions, the pyruvate dehydrogenase overexpression enabled a 60% *mcl*-PHA increase in glucose-grown Δgcd *P. putida* cells (Borrero-de Acuña et al., 2014) (Table 1). Although successful, these model-aided engineering approaches do not consider pivotal regulatory elements during PHA synthesis (Velázquez-Sánchez et al., 2020). Incorporating them into the mathematical problem might precisely capture

the metabolic response of the cell (Kwon et al., 2020), enabling the identification of novel genetic targets to increase PHA accumulation.

Bacteria expend considerable amounts of energy for protein synthesis to satisfy multiple cellular functions (Maitra & Dill, 2015). Eliminating counterproductive genetic elements by precise deletion of large DNA segments of *P. putida* – in some cases removing ~8% of the total genome – generated promising biocatalysts for various applications (Lieder et al., 2015). Several groups have attempted to reduce the genome of *P. putida*

(Leprince et al., 2012; Martínez-García et al., 2014), demonstrating that such strains produce higher levels of *mcl*-PHAs. Targeting non-essential genes of genomic islands for exclusion, the mutant *P. putida* KTU-U13 reached a genome reduction of 4.12% and showed 40% and 26% improvement in *mcl*-PHA and biomass synthesis on glucose (Table 1), respectively (Liang et al., 2020). Nonetheless, it was not explained why this mutant strain had improved PHA accumulation. Likewise, the genome of *Pseudomonas mendocina* also underwent genome reduction (7.7%), where the most important traits of the mutant NKU421 strain comprise a boosted intracellular ratio of ATP/ADP, 2-fold enhancement in *mcl*-PHA synthesis (Table 1), and 28% increase in oligosaccharides (Fan et al., 2020). Somehow the genes removed from the genome of *P. mendocina* boosted transcription levels of PHA-related genes, especially the PHA polymerases. Strikingly, the PHA yields reached by the minimal genome *Pseudomonas* strains (Fan et al., 2020; Liang et al., 2020) were quite low ($<0.8 \text{ g L}^{-1}$) and far from the titers attained by other native *Pseudomonas* bacteria (Borrero-de Acuña et al., 2014). Therefore, unveiling the genetic regulation prohibiting higher PHA synthesis would be critical in understanding biopolymer synthesis using glucose as carbon substrate.

Most *Pseudomonas* bacteria are not able to use the pentose sugars xylose and arabinose as substrates. As a first attempt, KT2440 expressing the *xyIAB* genes of *E. coli* grew on xylose but showed no accumulation of PHAs in minimal salt medium (Le Meur et al., 2012). Insertion of foreign genetic modules, carrying genes for cellobiose and xylose conversion, in the genome-reduced *P. putida* EM42 conferred the ability to propagate in both substrates (Dvořák & de Lorenzo, 2018). By knocking out the *gcd* gene, coding for the glucose dehydrogenase, the cells no longer synthesized xylonate. The research team then sought to produce *mcl*-PHA on the pentose as feedstock using resting cells but failed to mitigate the synthesis of the coproduct xylonate achieving only traces (0.2 g L^{-1}) of the polyester (Dvořák et al., 2020) (Table 1). Similarly, *P. putida* KT2440 also exhibits impaired sucrose metabolism. To circumvent this restriction, equipping the environmental strain with an invertase enzyme (*CscA*) to cleave first the disaccharide and a permease (*CscB*) enabled an active sucrose metabolism (Löwe et al., 2017b). To produce PHA in a novel fashion, the cyanobacterial strain *Synechococcus elongatus* – a sucrose producer from CO_2 – and the natural polyester synthesizer *cscAB P. putida* were grown together in a synthetic bacterial consortium. While the maximal PHA titre was low (0.16 g L^{-1}) (Table 1), the biopolymer was entirely derived from CO_2 in the recombinant *P. putida* (Löwe et al., 2017a). The same group next deleted the *nasT* gene coding for a nitrate regulator, thus the sucrose-consumer $\Delta\text{nasT}:\text{cscRABY}$ *P. putida* lost its ability to utilize nitrate. Compared with the nitrate metabolizing *cscRABY*-positive *P. putida*,

the nitrate-blind strain improved the polyester formation by 8.8-fold (Table 1) and amassed 14.8% of its CDW as PHA (Hobmeier et al., 2020).

Azotobacter vinelandii strains are non-pathogen nitrogen-fixing bacteria known for their capacity to simultaneously polymerize the commercial macromolecules alginate and PHB. For optimal PHB production, initial studies restricted the O_2 supply as a standard procedure given the bacterium's natural capacity to incorporate atmospheric nitrogen. In a seminal work, the use of chemical-based mutagenesis granted a PHB overproducing *A. vinelandii* that accumulated the biopolymer without nutrient limitation (Page & Knosp, 1989). It appears that the mutation affected the respiratory NADH oxidation leading to an increase in NADH levels, thus inhibiting citrate synthase and forming 2.26 gPHB L^{-1} compared to 0.17 gPHB L^{-1} at 24h in the parental strain from glucose (Table 1). Indeed, it seemed also consistent to pursue the impairment of alginate production in *A. vinelandii*, aiming to drain more intracellular carbon into PHB biosynthesis. The *algK* gene's inactivation boosted PHB production by 50%, with still considerable amounts of alginate being secreted by the cell while consuming sucrose (Martínez et al., 1997) (Table 1). Mutations of other genes belonging to the *alg* cluster had different effects on PHB synthesis, where *algA* mutant improved PHB productivity by 5-fold in Burk's medium (Table 1) (Segura et al., 2003). Another strategy involved engineering the anaplerotic reactions of *A. vinelandii* guaranteeing less carbon entering the TCA cycle. Knocking the *pycA* genes, coding for the pyruvate carboxylase an enzyme converting pyruvate into oxaloacetate, entailed diverting more acetyl-CoA into the PHB biosynthetic pathway, enhancing the PHB yield from 1 to $3.5 \text{ gPHB gProtein}^{-1}$ in the engineered strain (Segura & Espín, 2004).

Engineering regulatory elements orchestrating several metabolic traits have also led to the creation of better PHB producers (Velázquez-Sánchez et al., 2020). Particularly, enzymes of the nitrogen phosphotransferase system play a critical role in carbon storage in *Azotobacter*. The OPN strain lacking the *ptsN* gene activated the *phbBAC* operon's expression and, in turn, elevated PHB synthesis (Noguez et al., 2008). Engineering other repressing genetic elements for biopolymer formation (*RmsA-RmsY/Z*) in the OPN mutant, a fed-batch process attained a polyester volumetric productivity of $0.46 \text{ (gPHB L}^{-1} \text{ h}^{-1})$ and $27.3 \text{ (gPHB L}^{-1})$ (García et al., 2014) (Table 1).

Bacteria of the halophilic genus *Halomonas* can efficiently propagate in a wide range of NaCl concentrations (0–25%) as they synthesize the compatible-solute ectoine, which confers osmoprotection and resistance. *Halomonas boliviensis* produced high PHB levels in a glucose-based fed-batch fermentation attaining $1.1 \text{ g L}^{-1} \text{ h}^{-1}$ (Quillaguamán et al., 2008). *Halomonas bluephagenesis* TD01 achieved similar productivities ($0.96 \text{ gPHB L}^{-1} \text{ h}^{-1}$) in a continuous open system by

coupling two bioreactors (Tan et al., 2011). The same research group then evaluated the PHB production performance of a recombinant *H. campaniensis* LS21 overexpressing the PhaCAB-Porin enzymes using synthetic seawater on glucose and mixed substrates. The engineered LS21 continuously formed 35.5 gPHB L⁻¹ (Yue et al., 2014) (Table 1). There are few examples of using an engineered PHA-producing bacterium at a semi-industrial scale. In a 5-m³ bioreactor set in continuous and fed-batch modes the recombinant *H. bluephagenesis* amassed 60% of the CDW as P(HB-co-4HB, 4-hydroxybutyrate) and reached an exceptional biopolymer productivity of 1.68 gL⁻¹ h⁻¹ under non-sterile conditions (Ye et al., 2018). Recently, the application of the versatile editing technology CRISPR/Cas9 permitted the rapid and precise creation of mutant strains and insertion of genomic elements to enhance and tailor PHA synthesis in *Halomonas* (Qin et al., 2018). The capitalization of *Halomonas* as a manageable chassis for bioproduction befell when researchers engineered the TCA genes *sdhE* and *icl*, coding for succinate dehydrogenase and isocitrate lyase enzymes, respectively, to modulate carbon partitioning. Blocking the methyl citric acid cycle (MCC) along with the overexpression of the native phosphoenolpyruvate carboxylase (*ppc*) and the operation of the haemoglobin (*vgb*) of *Vitreoscilla* in *H. bluephagenesis* enabled carbon flux funnelling towards 3-hydroxyvalerate to improved PHBV production from glucose (8.7 gPHB L⁻¹), displaying up to 18% mol content of HV (Chen et al., 2019). Expression of the PhaC synthase and a PhaJ hydratase of *A. hydrophila* in a PhaC minus *H. bluephagenesis* strain brought about the formation of 0.1 gL⁻¹ h⁻¹ copolymer of P(3HB-co-3HHx) from glucose in open bioreactors (Yu et al., 2020). Together, these studies position *Halomonas* species as one of the best industrial hosts to manufacture high PHA levels with varying monomer contents more cost-effectively.

Engineering strategies to increase PHA synthesis from Glycerol

Worldwide glycerol production is 42 billion litres annually (Kaur et al., 2020), with projections forecasting 4% growth by 2025, with the biodiesel industry being the main driver of this oversupply. Each batch producing 9 tons of biodiesel after *trans*-esterification of fatty acids results in 1 ton of the polyol as a side-product. *C. necator* H16 grows poorly on glycerol yet produces 60% of the cell mass as PHB in 10 days of cultivation. The main reason H16 strain exhibits insufficient phosphorylation activity (glycerol kinase, GlpK). Thus, the expression of the *glpK* from *E. coli* turned *C. necator* into a fast-growing bacterium (Fukui et al., 2014). Another unusual trait is the low molecular weights (Mw) of the biosynthesized PHB derived from the polyol. H16 carrying the

foreign *glpK* and *glpF* (coding for a glycerol uptake facilitator of *E. coli*) synthesized 1.44 gPHB L⁻¹ in 72 h – 33% more biopolymer than the parental strain – with a Mw of 2.2 × 10³ KDa, which is more suitable for thermoforming (Table 1).

High tolerance to toxic compounds and harsh environmental conditions are prerequisites of the hosts to bioconvert crude glycerol into valuable industrial chemicals (Poblete-Castro et al., 2017). *Pseudomonas* bacteria own these qualities and efficiently catabolize raw glycerol derived from the biodiesel industry (Fu et al., 2015; Kenny et al., 2012; Liu et al., 2018; Poblete-Castro et al., 2014b). So far, *P. putida* KT2440 represents the best *mcl*-PHA producer using raw glycerol. In this strain, the *glp* gene cluster harbours the *glpR* regulator that plays a crucial role in glycerol catabolism. Glyceraldehyde 3-phosphate, the product of glycerol's conversion by glycerol kinase, seems to control the mRNA level of *glpR*, which represses the expression of *glpF* and *glpD*. Once glyceraldehyde 3-phosphate reaches a certain threshold, it blocks the transcription of *glpR* eliciting expression and further synthesis of the necessary enzymes for glycerol metabolism. Escapa and co-workers reduced the lag phase in glycerol-grown *P. putida* cells by knocking out the *glpR* gene. Comparison between the wild-type and the Δ *glpR* KT2440 showed an improvement in the *mcl*-PHA volumetric productivity from 0.13 to 0.24 (g L⁻¹ day⁻¹) on glycerol (Escapa et al., 2012), but with a very small quantity over time. In another study, the deletion of the *phaZ* depolymerase in KT2440 improved polyester synthesis by 36%, resulting in 2.2 gPHA L⁻¹ in batch cultures (Poblete-Castro et al., 2014b) (Table 1). Likewise, assessing different feeding modes in fed-batch bioreactors, the Δ *phaZ* *P. putida* produced 0.34 (gPHA L⁻¹ h⁻¹) on crude glycerol, displaying a Mw of 506 KDa and a PDI of 2.6, desirable values for industrial thermoforming (Borrero-de Acuña et al., 2021a, 2021b). Nevertheless, the high secretion rate of the side-product citrate during crude glycerol bioconversion represents a challenge since it had a product yield of 0.6 (g gGlycerol⁻¹). During PHA production nitrogen limitation inhibits the isocitrate dehydrogenase enzyme in *P. putida* leading to citrate accumulation (Beckers et al., 2016; Poblete-Castro et al., 2012a). Possible solutions involve the overexpression of the acetyl-CoA carboxylase to channel more acetyl-CoA into the de novo synthesis of fatty acids or by inactivating the transcription of citrate synthase enzyme thorough antisense or interference RNA (Ko et al., 2020).

Engineering strategies to increase PHA accumulation in CO₂ utilizing microbes

The current carbon dioxide rate due to anthropogenic activities has no precedent where the abundance of

atmospheric CO₂ is more than 400 ppm – 250 ppm is the estimated average concentration of CO₂ in the last 800,000 years – contributing enormously to global warming as a greenhouse gas. The natural metabolic routes for CO₂ catabolism are slow and less efficient, including the widely distributed ribulose biphosphate cycle (RuBP or Calvin-Benson cycle), to that displayed by heterotrophic microorganisms. This statement arises since Rubisco, the most abundant enzyme on Earth, has carboxylation k_{cat} values ranging from 1 to 10 s⁻¹, while enzymes belonging to the central carbon metabolism reach values superior to 80 s⁻¹ (Flamholz et al., 2019). The well-suited PHA producer, *C. necator*, can fix CO₂ in a hydrogen-oxidizing fashion, but high O₂ levels are detrimental to CO₂ catabolism as it represses the Rubisco enzyme. Hence to propagate *C. necator* at its maximal growth rate (0.42 h⁻¹), supplying a gas mixture of H₂, O₂, CO₂ (7:1:1 ratio) appears to be optimal (Ishizaki & Tanaka, 1990). The success of *C. necator* to propagate on atmospheric CO₂ highly rely on the use of β -carbonic anhydrases (CA, encoded by *can* genes) enzymes that catalyse the conversion of bicarbonate into CO₂ in the intracellular milieu (Kusian et al., 2002). Tailoring the *can* gene dosage in H16 strain yielded a 1.5-fold increase in PHA production (66 wt%) (Thorbecke et al., 2021) (Table 1). In another work, RNA sequencing analysis enabled to unveil transcriptome signatures of *C. necator* during PHA overproduction under oxygen limitation, showing that the genes *ldh* and *ackA2*, encoding L-lactate dehydrogenase and acetate kinase, respectively, were upregulated and suitable candidates for deletion to prevent byproduct formation (Tang et al., 2020). Additionally, the gene cluster encoding for membrane-bound hydrogenase (MBH) and *b*-, *c*-type cytochrome oxidase enzymes were repressed, explaining the low energy and biomass synthesis in the autotrophic growth condition. Heterologous expression of the *vhb* gene (vitreoscilla haemoglobin, VHb) using a promoter-optimized system in the Δ *ldh* *C. necator* strain ensured proper energy generation by binding to the cytochrome subunit *o* promoting enhanced ATP levels (Tang et al., 2020). This transcriptome-aided engineering strategy provided a deep understanding of the genes encoding biosynthetic machinery operations during biopolymer production, allowing to accumulate PHB at 50% on a cell dry basis and 0.3 (gPHB L⁻¹) using CO₂ as the only substrate (Table 1). Expanding the assortment of PHA, copolymers of *mcl*-PHAs were also biosynthesized directly from CO₂ and H₂ by co-expressing thioesterase acyl carrier proteins to generate specific fatty acids along with different PHA polymerizing enzymes in *C. necator*. The tailored strain accumulated 60% of the biomass as *mcl*-PHA displaying 49% of 3-hydroxyoctanoate in the polymeric chain (Nangle et al., 2020). Although these studies present promising results, the PHA productivities are insufficient to meet industrial PHA productivities given the technical setting required to derive the biopolymers from CO₂ and

the low enzyme capacity to uptake this abundant greenhouse gas (GHG).

Another promising group of microbes for PHA production is the photoautotrophic cyanobacterium *Synechocystis*. During nitrogen limitation, the cells synthesize various carbon storage compounds, including glycogen, lipids, and PHB, accounting for 34% of the mole fraction of the CO₂ fixed (Hendry et al., 2017). This so-called chlorosis metabolic state initiates accumulating large quantities of glycogen (Allen & Smith, 1969), which is then transformed into PHB in a later phase (Koch et al., 2019). Although the PHB titre is still low in the photoautotrophic PHB-producing cyanobacteria 27% of the cell mass as PHB (Miyake et al., 1996). Most of the engineering strategies in this cyanobacterium have involved the disruption or inactivation of pathways generating alternative metabolites. For example, overexpression of a foreign XfpK from *Bifidobacterium* in *Synechocystis* led to an increased acetyl-P and, subsequently, acetate production (Carpine et al., 2017). Deletion of the acetyl-CoA hydrolase (Ach) and the phosphotransacetylase (PtA) in the recombinant cyanobacterium boosted PHA accumulation by sixfold compared to the wild type (Carpine et al., 2017) (Table 1). Another study achieved increased PHA synthesis from CO₂ by random mutagenesis in *Synechocystis* PCCC6714 using UV light. Genome sequencing of the mutant *Synechocystis* producing photoautotrophically 2.5-fold higher PHB revealed a point mutation in the ABC-specific transport system gene (*pstA*) (Table 1), impairing polyphosphate accumulation (Kamravamanesh et al., 2018). More related to the central carbon metabolism, disrupting the γ -aminobutyric acid (GABA) shunt of *Synechocystis* sp. PCC6803, the level of metabolites belonging to the TCA cycle decreases due to the silencing of the glutamate decarboxylase enzyme provoking a 2.5-fold increase in PHB synthesis (Table 1) without nutrient limitation (Monshupanee et al., 2019). Perturbation of *Synechocystis* regulatory network also has an impact on PHA accumulation. For instance, PirC, a P_{II}-interacting metabolic regulator, is a molecular switch that inhibits phosphoglycerate mutase when the cells generate high levels of 2-oxoglutarate – caused under nitrogen limitation – triggering glycogen storage (Orthwein et al., 2021). Taking advantage of this trait, a *pirC*-minus *Synechocystis* strain, overexpressing the *phaAB* genes of *C. necator* was created and cultured under dual N-P limitation, producing a 63% PHB content from CO₂ in 20 days of cultivation in dark/light regimes (Koch et al., 2020).

Engineering strategies to improve PHA synthesis from methane and methanol

Methane (CH₄) emissions result from natural and anthropogenic origins. Anaerobic biomass decomposition

in wetlands is the major source of methane production (Kirschke et al., 2013). The contributions of industrial outputs have given rise to unprecedented methane levels in the atmosphere where this GHG exhibits a Global Warming Potential (GWP) 28–36 higher than CO₂ as methane absorbs more heat than CO₂ (Anthony & Silver, 2021). There are two recognized classes of methanotrophs regarding methane bioconversion, which is oxidized to methanol by methanol dehydrogenases and then to formaldehyde; (i) Type I correspond to cells converting formaldehyde via the ribulose monophosphate pathway (RumP) and (ii) Type II genera, whose mode of catalysing formaldehyde is through the serine cycle (Strong et al., 2016). Interestingly, the Type II methanotrophs exhibit a better ability to synthesize PHB (Geşicka et al., 2021). Biopolymer content can reach nearly 60% and 70% of the CDW in the *Methylocystis* and *Methylosinus* genus, respectively, at mesophilic conditions using methane as the only C source (Asenjo & Suk, 1986) (Sundstrom & Criddle, 2015). However, engineering methanotrophs for increased PHAs synthesis are still in its infancy. One of the few studies manipulating the genome and overexpressing native and foreign enzymes in *Methylosinus trichosporium* focused on the production poly(3-hydroxybutyrate-co-4-hydroxybutyrate) exclusively from methane (Nguyen & Lee, 2021) but producing the biopolymer at the mg L⁻¹ range.

Another important C-1 carbon substrate for the bioproduction of chemicals is methanol, given its low reduction degree and price and well-established industrial production capacity from natural gas and biomass (Olah, 2005). As for methanotrophs, methylotrophs metabolize methanol via the (RumP) and serine pathways (Figure 2). Notably, *Methylobacterium extorquens* AM1 forms PHB under nitrogen deprivation (Natalia, 2001) and PHBV when calcium is the limiting nutrient of cells growing on methanol, evidencing the existence of an alternative pathway for HV precursor generation via the ethylmalonyl-CoA (EMC) route (Orita et al., 2014). Remarkably, without the need of supplying pure oxygen to the fed-batch bioreactor, *M. extorquens* and *Pseudomonas* sp. K can produce 53 gPHB L⁻¹ and 136 gPHB L⁻¹ (Table 1), respectively, in less than 4–5 days (Bourque et al., 1995; Suzuki et al., 1986). Exchanging the native PHA synthase of *M. extorquens* for a PHA polymerase of *Aeromonas caviae* yielded a superior PHBV producer using methanol, improving the biopolymer titre from 0.05 to 0.19 gPHBV L⁻¹ (Orita et al., 2014) (Table 1). The next step seems to establish fed-batch processes where engineered methylotrophs can produce the more desirable thermoforming PHVB. Positioning these cell factories as efficient biopolymer producers also requires increasing the product yield which is currently lower than 0.3 gg⁻¹.

ENGINEERING MICROBES TO INCREASE PHA SYNTHESIS FROM WASTE STREAMS

PET waste plastic into PHA: ethylene glycol and terephthalic acid

Poly(ethylene terephthalate) (PET) has spread worldwide as it is the main component of soft drinks and water bottles. Nearly 30% of PET bottles are recycled annually, with 21 million metric tons of PET ending up in the environment (Hamade et al., 2020). Scientists and industrial researchers have proposed that the recovery of these waste materials and further microbial hydrolysis of PET with low-crystallinity degrees at mesophilic and thermophilic conditions can yield ethylene glycol (EG) and terephthalic acid (TA), and thus obtaining inexpensive feedstocks for the synthesis of a wide array of valuable biochemicals (Ellis et al., 2021). In this sense, *I. sakaiensis* can assimilate both compounds to sustain growth (Yoshida et al., 2016) and recently demonstrated to accumulate PHB at 25 (wt%) (Fujiwara et al., 2021). Other native PHA synthesizer bacteria can also employ ethylene glycol and terephthalic acid as the only C source. For instance, *Pseudomonas umsongensis* GO16 produces 2.6 g *mcl*-PHA L⁻¹ from TA (Kenny et al., 2012) and the Antarctic psychrotolerant *Pseudomonas frigusceleri* MPC6 (Pacheco et al., 2019) biosynthesized 0.21 g L⁻¹ of *mcl*-PHA from EG, but not from TA (Orellana-Saez et al., 2019). The model organism *P. putida* KT2440 was engineered to metabolize EG by overexpressing the glyoxylate carboligase and bypassing glycolate formation by expressing the glycolate oxidase operon (*glcDEF*) (Franden et al., 2018). Remarkably, the EG consuming *P. putida* strain synthesized 1.8 gPHA L⁻¹ from 500 mM EG after 72 h in batch cultures. Adaptive laboratory evolution of *P. umsongensis* GO16 enabled the microorganism to grow more efficiently from a μ_{max} of 0.14 to 0.4 h⁻¹ on EG (Tiso et al., 2021). Hence, it is important to realize that improving the specific growth rate usually results in lower biomass given the yield-rate threshold phenomenon (Lipson, 2015). The evolved *Pseudomonas* strain was capable of metabolizing both monomers yielding a *mcl*-PHA production of nearly 7 (wt%) from hydrolyzed PET in batch culture (Tiso et al., 2021). This low PHA titre obtained in the evolved strain emphasizes the complexity of using waste hydrolysates and the little knowledge concerning the simultaneous degradation of EG and TA in *Pseudomonas* bacteria that restricts full exploitation of these biocatalysts where carbon catabolic repression or generation of inhibiting compounds can occur.

Engineering strategies for increasing PHA synthesis from waste aromatic streams

The peripheral pathways for aromatic catabolism in bacteria present several interconnections given the high diversity of aromatic compounds. The intrinsic harmful nature of these chemicals makes it challenging to derive PHAs from aromatics. Bacteria of the genus *Rhodococcus*, *Cupriavidus*, *Pseudomonas*, and *Burkholderia*, *Achromobacter* are specialized aromatic degraders along with the ability to form PHA under nutrient imbalance from styrene (Ward et al., 2005), benzene-toluene-ethylbenzene-*p*-xylene (BTEX) (Nikodinovic et al., 2008), toluene (Hori et al., 2009), furfural (Pan et al., 2012), 4-hydroxybenzoic acid, vanillic acid (Tomizawa et al., 2014), and phenol (Zhang et al., 2018), but to limited amounts – less than 0.5 g L⁻¹ of the polyester. Nevertheless, in a fed-batch process, one of the highest *mcl*-PHA titre (3.4 g L⁻¹) was obtained from styrene in *P. putida* CA-3 (Nikodinovic-Runic et al., 2011). In the last decade, the focus of research has turned into the use of aromatics derived from lignin for obtaining next-generation biochemicals (Becker & Wittmann, 2019; van Duuren et al., 2020). A seminal work involving the bioconversion of lignin into *mcl*-PHA used *P. putida* KT2440, which synthesized 0.25 g L⁻¹ from lignin derivatives composed of *p*-coumarate, ferulic acid, acetate and vanillic acid (Linger et al., 2014). Exploiting an alternative method to generate lignin from hydrolyzed corn stover, a lignin-based fed-batch process produced 1 gPHA L⁻¹ in 36 h employing KT2440 as a cell factory (Liu et al., 2017). One of the first attempts to increase *mcl*-PHA in *P. putida* A514 (Lin et al., 2016) involved the overexpression of the *phaC1* and *phaJ4* genes and the use of an optimized xylose-based expression system (Table 1), which led to a moderate PHA production of 0.25 g L⁻¹ from vanilate (Wang et al., 2018). Similar PHA concentrations were obtained by engineering the PHA-related genes *phaZ*, *phaG*, and the PhaC polymerases in conjunction with inactivating the operation of the β -oxidation pathway in *P. putida* KT2440 (Table 1), the hyper-producer biosynthesized 0.25 g L⁻¹ of the elastomer polymer on lignin streams (Salvachúa et al., 2020).

Pseudomonas putida H, a natural phenol degrader, displaying the *meta*- and *ortho*-cleavage pathways for catechol catabolism (Vizoso et al., 2015). There are two copies of the *catA* gene in *P. putida* H coding for catechol 1,2-dioxygenase (C12DO) in the *ortho*-catechol catabolism, where the deletion of *catA2* gene activated the parallel catechol 2,3-dioxygenase enzyme (C23DO) fully, showing similar enzyme activity values when both genes were inactive accompanied by a slightly reduced enzyme activity (25%) of the C12DO in benzoate-grown cells. Most importantly, the mutant Δ *catA2*-H synthesized double amounts of *mcl*-PHA than the parental H on benzoate (Table 1), and when

cultured in fed-batch mode, synthesized 6.1 gPHA L⁻¹ (Borrero-de Acuña et al., 2021a). The bioconversion of lignin aromatics is problematic because their toxicity provokes extra ATP consumption, thus compromising biomass yield and PHA formation. Applying a DO-stat feeding approach, the Δ *catA2*-H *P. putida* produced 1.4 g L⁻¹ of *mcl*-PHA in fed-batch mode, the highest titre so far achieved, using the kraft lignin hydrolysate with catechol being the main component of the aromatic mixture (Borrero-de Acuña et al., 2021a). However, secretion of intermediate aromatics still occurs when metabolizing aromatic varieties, thus overexpression or replacement of more efficient enzymes appears necessary to funnel the carbon flux to the central metabolism. In line, the operation of the anaplerotic reactions and TCA enzymes needs more investigation under nitrogen limitation when the cells feed on complex aromatic mixtures to identify possible carbon loss through competing pathways.

Engineering strategies for increasing PHA synthesis from waste oils

The largest industrial production of PHAs, established by Danimer Scientific (GA, USA), relies on the use of fatty acids. Plant seeds are the primary source of fatty acids and require large arable land areas for their production. Another alternative is the use of waste oils to derive PHAs in submerged fermentation. Several decades of research have resulted in engineered bacteria with disrupted or diminished oxidation rates via the β -oxidation pathway converting pure fatty acids into biopolymers to increasing or obtaining defined monomers in the polymeric chain of PHAs, mainly in *Pseudomonas putida* being broadly covered in various reviews (Mezzina et al., 2021; Zheng et al., 2020). Improving PHA synthesis on waste oils implies seeking for novel genetic targets independent of the β -oxidation pathway. Early studies demonstrated that *P. aeruginosa* (Fernandez et al., 2005) and *C. necator* H16 (Kahar et al., 2004) bioconvert waste soybean oils into PHAs attaining 1.6 gPHA L⁻¹ and 96 gPHB L⁻¹, respectively. Replacing the PHA synthase of the H16 strain with the *A. caviae* PHA synthase, the transformant strain produced one of the highest biopolymer titers from waste oils (74 wt%) of the more elastic poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and 138 g L⁻¹ of biomass (Table 1), improving 6% the PHA yield (Kahar et al., 2004). Given the hydrophobic nature of the waste oils, some bacteria have difficulties accessing the carbon substrate that negatively impacts PHA productivity. Moreover, secretion of lipases by bacteria to the extracellular milieu enables triglyceride breakdown into fatty acids (Rosenau & Jaeger, 2000). By taking advantage of the lipase secretion capacity of *P. putida* KT2440 and deleting the constitutively expressed *tctA* gene

TABLE 2 Cofactor and morphology engineering targets of bacteria for increased PHA synthesis in different fermentations modes

Organism	Modification	C-source	Target	Fermentation mode
<i>A. Beijerinckii</i> 9067	<i>Lakcs</i> capsule of the wild type	Glucose	NADH (-)	Batch culture
<i>H. bluephagenesis</i> TD-Δβ	Δ <i>etf-x-β</i>	Glucose	NADH (+)	Batch culture
<i>P. putida</i> KT2440	Δ <i>gcd</i> , <i>acoA</i>	Glucose	NADH (+)	Batch culture
<i>C. necator</i> H16	<i>phbC</i> and <i>zwf</i>	Fructose + Valerate	NADPH (+)	Batch culture
<i>C. necator</i> H16	<i>tktA</i> _{ec}	Gluconic acid	NADPH (+)	Fed-batch
<i>H.</i> TD08	<i>pSEVA341-LaIq-Ptrc-MinCDt</i>	Glucose	Cell Elongation	Batch culture
<i>P. mendocina</i> NK-01	Δ <i>minCD</i> pBBR- <i>ftsZ</i>	Glucose	Cell Elongation	Batch culture
<i>H. campaniensis</i> LS21	Δ <i>pyrF</i> Δ <i>mreB</i> (<i>pTKmf/p341pphb</i>)	Glucose	Increased cell lengths	Batch culture

Abbreviations: *mcl*-PHA, medium-chain-length poly(3-hydroxyalkanoates); NADH (-), reduced level of NADH; NADH (+), increased level of NADH; NADPH (+), increased level of NADPH; PHB, poly(3-hydroxybutyrate); P(3HB-co-3 HV), poly(3-hydroxybutyrate-co-3-hydroxyvalerate).

(encoding for the carboxylate transport system), which is not necessary during fatty acid metabolism, it was possible to almost double the *mcl*-PHA productivity (Table 1), achieving 0.65 g L⁻¹ day⁻¹ on waste vegetable oil (Borrero-de Acuña et al., 2019). Despite the increased polyester synthesis, the cells only amassed 38 (wt%) and the *mcl*-PHA titre were relatively low, pointing to the need of making the vegetable oils more prone to bioconversion into elastomer *mcl*-PHAs in high cell density cultures.

COFACTOR AND MORPHOLOGY ENGINEERING STRATEGIES

Redox cofactor modulation

Biological systems exhibit complex and highly connected biosynthetic pathways serving to produce energy mainly in the form of ATP, essential metabolites, and cofactors to sustain cellular self-replication. In this sense, most of the characterized PHA biosynthetic routes consume NADPH due to its need as a cofactor during the conversion of acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA mediated by the PhaB enzyme and the transformation of 3-ketoacyl-ACP into (*R*)-3-hydroxyacyl-ACP (catalysed by FabG enzyme) for *scl*- and *mcl*-PHAs synthesis, respectively (Figure 2). Other natural PHA-producing bacteria like *Azotobacter beijerinckii*, *A. vinelandii*, *H. bluephagenesis*, and *Allochromasia vinosum* harbour an NADH-dependent PhaB reductase instead. The need for either redox cofactor for PHA formation severely impacts the metabolic operation of the bioproducer as the pathway topology plays a crucial role in supplying different levels of reducing equivalents (NADH and NADPH) during growth and product formation. Inorganic nutrient limitations like P, N, O₂ all cause higher ratios of NADH/NAD⁺ resulting in the repression of the TCA cycle enzymes in varying manners. Early attempts to take

advantage of this metabolic regulation in *A. beijerinckii* demonstrated that oxygen-limited conditions triggered elevated biopolymer content (50 wt%) in chemostat cultures (Table 2) due to the reoxidation of NADH via PHB synthesis (Senior et al., 1972). Disruption of the respiratory chain in *A. beijerinckii* led to the regeneration of NADH into NAD⁺ through biopolymer accumulation in the cytosolic space, finally improving PHB yields without nutrient limitation (Steinbüchel, 1991). The halotolerant *H. bluephagenesis* shows better biopolymer production performance under oxygen limitation in unsterile bioreactors presenting a specific volumetric productivity of 1.2 (gPHB L⁻¹ h⁻¹) (Ling et al., 2018). By blocking an electron transfer flavoprotein pathway containing the *efg* genes, the NADH supply boosted, resulting in 90% of the biomass in PHA (Ling et al., 2018) (Table 2). In another study, the overexpression of the pyruvate dehydrogenase complex in *P. putida* KT2440 increased the NADH pool inhibiting the TCA cycle operation and therefore improving the PHA content of the cell by nearly 40% compared with the parental strain (Borrero-de Acuña et al., 2014) (Table 2). In light of these strategies, it appears adequate to dosage the expression of NADH-generating enzymes to induce repression of TCA cycle belonging enzymes that could allow rerouting the acetyl-CoA pool to PHA biosynthetic pathways even under nitrogen or oxygen sufficient conditions. A variety of engineering strategies for enhanced NADPH levels consisted of redirecting the carbon flux towards the NADPH-producing PP pathway. Overexpression of the *Zwf* and the ED belonging enzyme, 6-phosphogluconate dehydrogenase encoded by the *gnd* gene, is a successful approach for biodegradable polyester overproduction in *C. necator* (Choi et al., 2003; Lim et al., 2002). As the *Gnd* enzyme feeds the PP route, the concomitant amplification of *gnd* and the transketolase (*tktA* gene) of *E. coli* in *C. necator* H16 ensured carbon flux from glyceraldehyde 3-phosphate into acetyl-CoA of gluconate-grown cells; thus boosting the NADPH supply in the growth

PHA type	CDW (g L ⁻¹)	PHA (wt%)	PHA (g L ⁻¹)	Productivity (g L ⁻¹ h ⁻¹)	References
PHB	n.s	50	n.s	n.s	Senior et al. (1972)
PHB	11.9	92.0	10.9	0.22	Ling et al. (2018)
<i>mcl</i> -PHA	4.7	42.1	2.0	0.04	Borrero-de Acuña et al. (2014)
P(3HB-3 HV)	8.7	63.8	5.6	0.11	Choi et al. (2003)
PHB	15	52.0	7.8	0.11	Lee et al. (2003)
PHB	9.1	82	7.5	0.15	Tan et al. (2014)
<i>mcl</i> -PHA	1.6	23.7	0.4	0.01	Zhao et al. (2019)
PHB	16.6	75.9	12.6	0.26	Jiang and Chen (2016)

phase and PHA synthesis in the nitrogen-limiting stage (Lee et al., 2003). In a pH-stat fed-batch cultivation using the recombinant H16 strain, PHB reached a yield of 8 (g L⁻¹) in 48h, amassing 50% on a cell dry basis (Lee et al., 2003) (Table 2). This modest improvement and PHA synthesis demonstrate that the balance of NADPH/NADP⁺ and NADH/NAD⁺ ratios in the cell requires a fine-tuning strategy where metabolic models have proven powerful tools for predicting these cofactor availabilities which are crucial for *scl*- and *mcl*-PHA production.

Cell morphology engineering

Bacteria have successfully thrived and evolved in the early phase of life owing to the ability to develop a polymeric structure that prevents direct contact with the surrounding environment. This shell enables to safeguard genetic information and defining the cell shape, preventing cell overexpansion, coping with turgor pressure, and crucial for cell viability and proliferation (Errington, 2021). The multiprotein network comprising the dynamic cytoskeleton governs two important cell morphogenesis events: elongation (spatial and temporal configuration of cell width and length) and division (cell fission to generate sister cells bearing identical genetic material) (Egan et al., 2020) (Figure 4). The first event is controlled by the divisome machinery, while the second is governed by the elongasome. The MreB protein forms helical filaments upon GTP or ATP binding oscillating along the cell circumference and pushes the bacterial edges that ultimately define the width and length of rod-shaped bacteria (Szwedziak & Löwe, 2013) (Figure 4A). Divisome molecular mechanics are orchestrated mainly by the tubulin-like GTPase FtsZ protein (Silber et al., 2020) (Figure 4) Meddling with certain elements of these systems derives in i) elongated microbes (FtsZ, its inhibitor MinCD,

MreB, SOS-responsive SulA, PBPs, MreB stabilizer RodZ), ii) spherically shaped bacteria (MreB, PBPs) or iii) reduced cell size owing to increased duplication rates (FtsZ, MinCD) (Huo et al., 2020) (Figure 4C). For instance, in *Pseudomonas mendocina* NK-01, the *ftsZ* gene was overexpressed, fostering cell division and improving *mcl*-PHA yield (Zhao et al., 2019). Likewise, atypical cell elongation of *Halomonas* spp. has also been shown when overexpressing *minCD* (Figure 4B, C), leading to PHB titre increase from 5.9 to 7.5 g L⁻¹ and boosting inclusion body accumulation from 69 to 82 wt% (Tan et al., 2014) (Table 2). To improve *mcl*-PHA accumulation in *Pseudomonas mendocina* NK-01 the *minCD* system was disrupted, shifting cell morphology from rod-shape to filamentous and increasing the CDW and PHA yield titers from 0.28 to 0.41 g L⁻¹ (Zhao et al., 2019) (Table 2).

However, such traditional molecular techniques are inefficient when dealing with essential cytokinesis genes as engineered bacteria exhibit poor growth and altered metabolism (Sperber & Herman, 2017; Westfall & Levin, 2018). To circumvent these phenotypes, the genetic systems can harbour control switches that enable activation at a given time during fermentation, usually when cells reach a considerable cell density (Batianis et al., 2020; Huo et al., 2020). In this sense, temperature-sensitive vectors are valuable tools to compensate for detrimental deletions. A controlled expression of *mreB* (Figure 4A) in the isogenic *H. campaniensis* LS21 *mreB* mutant led to a PHB yield of 12.6 g L⁻¹, a 74% increase contrasted to the wild-type strain (Table 2), which equals a PHB content from 56.8 wt% to 75.9 wt% (Jiang & Chen, 2016).

Due to their small sizes, separating the PHA granules from the broth has been always challenging. A recent study attempted to increase the elastomer size by manipulating the elements controlling this mechanism, namely the phasins (Shen et al., 2019).

Interestingly, the authors observed that removing each of the phasins (PhaP1, PhaP2 and PhaP3) from *Halomonas bluephagenesis* resulted in fewer numbers of biopolymer granules with larger size, the intrinsic cell size was still restricting further expansion of the granule. Thus, the authors resorted once again to the manipulation of components controlling the bacterial morphogenesis. By overexpressing the genes *minC* and *minD* in various *phaP* deprived strains of *H. bluephagenesis* TD01, the cell size of this bacterium was drastically enlarged allowing it to accommodate PHA granules of up to 10 μm . Besides, PHAs of higher molecular weight are easy to recover by centrifugation, filtration or sedimentation (Macagnan et al., 2019). Intense efforts have been invested in PHA granule enlargement to improve recovery (Cai et al., 2012; De Almeida et al., 2007; Melanie et al., 2018). Thus, further developments in controlling PHA molecular weight along with the cell size will lead to important reduction on the downstream costs of the bioprocess.

RATIONAL ENGINEERING FOR PROGRAMMABLE CELL LYSIS AND PHA RECOVERY

Conventional chemical or physical procedures to extract PHA encompass the addition of external hydrolytic enzymes, mechanical pressure, non- or halogenated solvents, and surfactants. These methods employed for biopolymer recovery are widely covered elsewhere (Koller, 2020). The use of such agents in industrial bioprocessing increases overall production expenses. Many studies endeavoured to devise genetic systems that trigger cell lysis on demand of the employed microbial strains to reduce the recovery-associated costs of biopolymer production.

Holin-endolysin systems

With the discovery of the molecular mechanism deployed by the bacteriophage T4 to lyse cellular membranes, scientists harnessed its potential to recover valuable chemicals. The system relies on the binary and stepwise action of the cell-wall perforating holins and the peptidoglycan-degrading endolysins (Figure 5). Remarkably, during the last stages of the lytic cycle, holins are produced in significant amounts inflicting internal membrane damage. After generating holes that allow penetration of the murein-hydrolases/endolysins produced in a second phase of the process, the hydrolytic enzymes act upon the peptidoglycan bonds (Figure 5A). One of the first examples for PHB extraction utilizing the bacteriophage-derived cell lytic system was in *P. megaterium* (Hori et al., 2002). Using

a xylose-responsive circuit harbouring the *Bacillus amyloliquefaciens* phage – a holin cassette that is part of the *amyE* engineered locus of the *Bacillus megaterium* chromosome. Glucose acted as an anti-inducer and supplemented to the minimal medium as a carbon source in the presence of xylose. Thereby, the expression of the system was timely modulated by glucose levels. Upon complete consumption of glucose, the system activated eliciting cell lysis.

Conducting similar studies in *P. putida* KT2440 the pneumococcal bacteriophage EJ-1 enzymatic battery was exploited – holin Ejh and endolysin Ejl (Martínez et al., 2011). The dual-circuit, inducible in the presence of 3-methylbenzoate, was inserted in the genome of *P. putida* KT2440. In the presence of the inducer cell lysis took place, but PHA recovery from the lysate of the genetically modified strain was not efficient. Transmission electron microscopy analysis revealed membrane disruption with the PHA granules tethered to the cells. As outlined previously, poor protein production occurred under nitrogen-limiting conditions, contributing to the low recovery PHA level (Martínez et al., 2011).

Lysozyme-based systems

One of the major constituents that provide integrity to the cell wall of bacteria is the peptidoglycan (murein) layer. Recently, a novel development on programmable cell lysis appeared to attain higher PHA recovery rates by conducting recombinant expression of the N-acetylmuramide glycanhydrolase (lysozyme) in *Pseudomonas putida* KT2440 (Borrero-de Acuña et al., 2017) (Figure 5B). This enzyme catalyses the cleavage of the β -1,4-linkages present between N-acetylmuramic acid and N-acetyl-D-glucosamine residues, which are the building blocks of the peptidoglycan sacculus. In the study, the authors recombinantly produced the lysozyme derived from *Gallus gallus*, which is N-terminally fused to the 21 a.a. signal peptide of the glucan 1,4- α -maltotetrahydrolase of *P. stutzeri* (Figure 5B). Translocation into the periplasm of the peptidoglycan-disrupting enzyme was mediated by the ultimately cleaved off leader sequence enabling catalytic action upon murein (Figure 5B). The efficiency of the cell-disruptive system was assessed in *P. putida* under nitrogen-limited PHA-producing conditions on glycerol. After 72-h fermentation the induction of the self-lytic circuit led to a 97% of cell mortality. Moreover, the PHA monomeric composition was uniformly maintained in comparison to the wild type. The recovery of the synthesized biopolymer was 75%. Hence, the most significant contributions of the study were unparalleled cell mortality rates over time under PHA-producing conditions and total PHA recoveries dispensing the use of external pressures (Borrero-de Acuña et al., 2017).

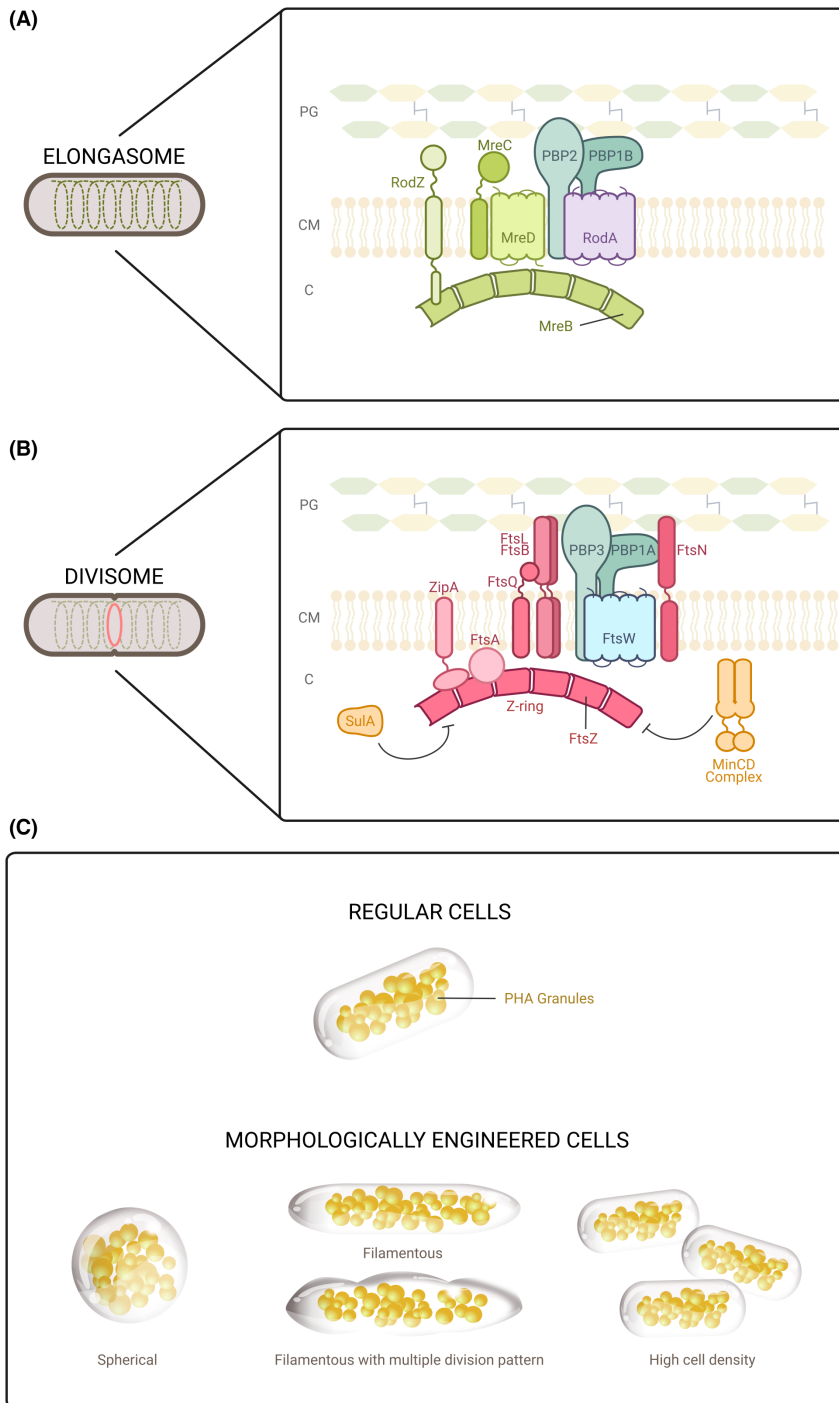


FIGURE 4 Schematic of the basic molecular components sustaining cell morphogenesis (elongasome; A) and cytokinesis (divisome; B) in rod-shaped bacteria, which have been successfully manipulated to date to achieve desired cell morphology phenotypes for increased PHA yields (C). The elongasome majorly comprises the polymeric MreB filaments, which determine cell elongation, its stabilizer RodZ, its ancillary proteins (MreCD) and enzymes involved in peptidoglycan biosynthesis (penicillin binding proteins “PBPs” and lipid flippase RodA). The divisome is primarily constituted of the master regulator FtsZ protein, which forms the Z-ring scaffold and its inhibitory systems (i) the constitutive MinCD complex and the (ii) SOS-responsive SulA merely induced upon DNA damage. A myriad accessory (FtsA, FtsABLQW), anchoring/stabilizing (ZipA) and penicillin binding proteins (PBP1A, PBP3) assist Z-ring formation and coordinated peptidoglycan biosynthesis to accomplish cell division. Meddling with these elements results in (i) filamentous microbes (single or multiple division patterns), (ii) spherical bacteria or (iii) boosted duplication rates leading to reduced cell size. CM, cytoplasmic membrane; C, cytoplasm; PG, peptidoglycan.

Osmotic imbalance

Another work recently published delved into the potential of turning against the cell its inherent turgor preservation system, namely the porin and mechanosensitive channel (MSC) arrays, to elicit membrane disruption (Poblete-Castro et al., 2020) (Figure 5C). Porins and MSCs represent the frontline barriers responsible for counteracting osmotic up- or downshifts. In Gram-negative, porins comprise diverse outer membrane-spanning translocases that modulate uptake or efflux

of a wide spectrum of osmo-solutes upon intra- or extracellular osmotic pressures (Figure 5C). Additionally, gated-MSCs act as inner membrane safety valves that uncouple their channel under hypoosmotic endurance to relieve mechanical tension. The study revolved around the effects of concurrent overproduction of the major pseudomonads porin OprF and the narrowly channelled OprE (cation biased ionophore properties) and the genomic removal of the large-conductance mechanosensitive channel MscL-encoding ORF upon osmotic shifts (Figure 5C). The deletion of the *mscL* gene had

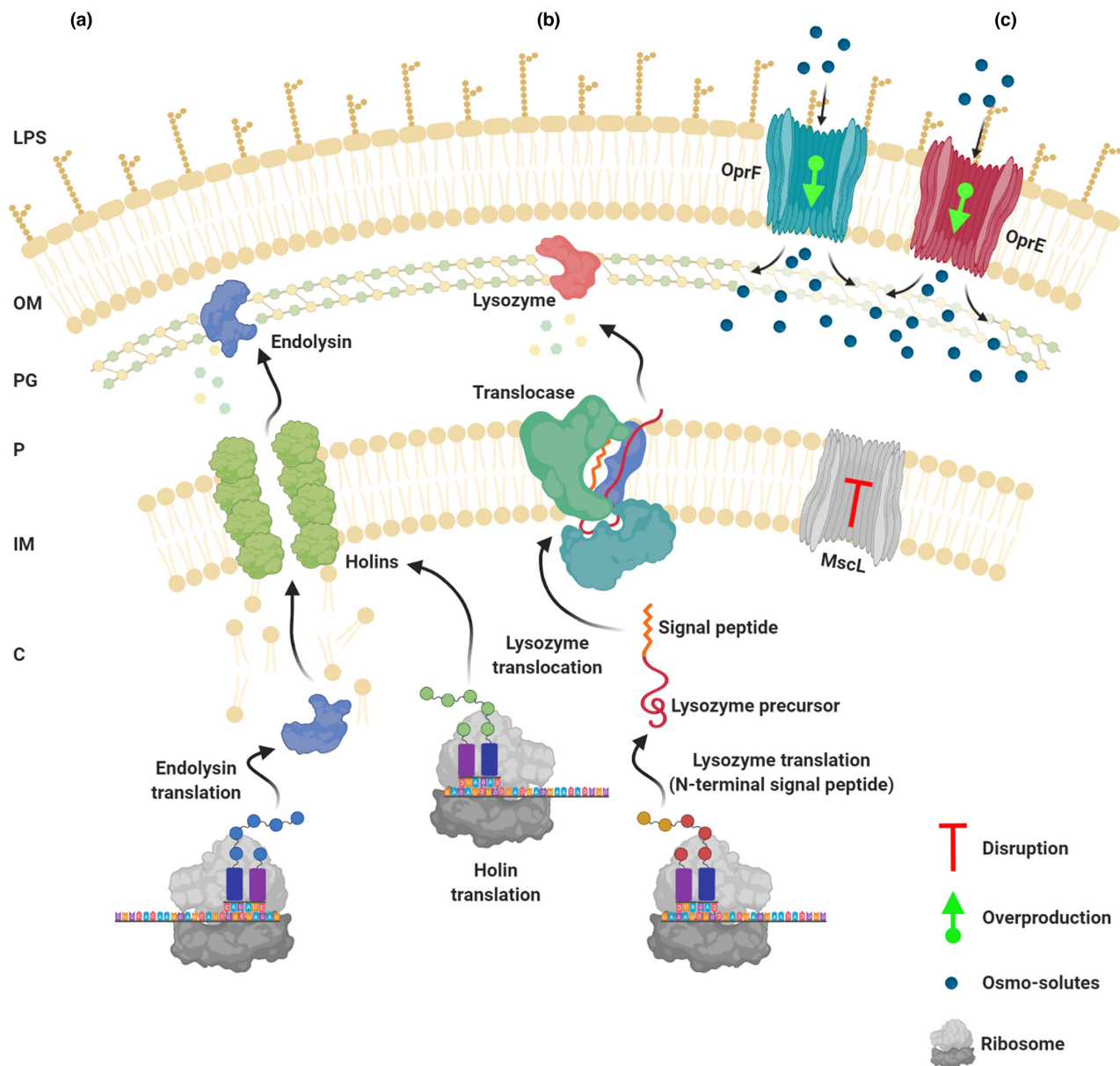


FIGURE 5 Programmable cell autolysis systems developed to date for PHA recovery. The use of such systems reduces considerably PHA production-associated costs. The dual bacteriophage-based system revolves around the action of the membrane perforating holin and the peptidoglycan degrading endolysin (A). The lysozyme-based system relies on the signal peptide-guided export of the peptidoglycan hydrolase into the periplasmic space (B). (A) and (B) approaches lead to the degradation of the peptidoglycan layer thereby reducing cell turgor, which results in cell wall weakening with its consequent lysis. The osmotic imbalance strategy relies on the deletion of the inner membrane mechanosensitive channel (MscL) and the overproduction of the outer membrane porins (OprE and OprF). In (C) excessive flux of osmolytes towards the periplasmic space is promoted, while the major barrier to compensate the detrimental effects of such osmotic shock has been disrupted resulting in cell membrane breakage. C, cytoplasm; IM, inner membrane; LPS, lipopolysaccharide; OM, outer membrane; PG, peptidoglycan; P, periplasm.

no impact on biomass production. Upon expression of the porin genes in the $\Delta mscL$ *P. putida*, the membrane hydrophobicity along with the cell permeability were heavily compromised during the shift from a hyperosmotic imbalance by NaCl supplementation followed by hypotonic stress (H_2O addition). The study reported a cell death of 96% of the overall population and a PHA

recovery of 93.3% (Poblete-Castro et al., 2020). This system is not exclusively restricted to *P. putida* since multiples MSCs and porins are present in all known bacteria. Additionally, the possible genetic strategies are countless due to many membrane-spanning osmotic transporters that might enable one-staged osmolarity shift (hyper- or hypotonic).

CONCLUSIONS AND FUTURE DIRECTIONS

Over the past decades, genetic interventions in natural PHA producers have resulted in superior cell factories with varying degrees of success (Tables 1 & 2). Most of the studies in the literature present the increased PHA synthesis as the weight percentage of the cell dry weight (wt%). However, enhancing the product titre, yield, and PHA-specific volumetric productivity (TYP) is imperative and a cornerstone in industrial biotechnology. In this sense, high PHA productivities have been achieved by engineered *C. necator*, *P. putida*, and *Halomonas* spp. employing glucose, sugar hydrolysates, crude glycerol, and waste vegetable oils (Orita et al., 2012; Poblete-Castro et al., 2014a; Ye et al., 2018). Most of these strategies relied on the overexpression of the native PHA biosynthetic genes, replacement of more efficient PHA polymerases, and the blockage of carbon fluxes towards competing pathways (Figure 3). Less successful has been the bioconversion of CO₂, lignin hydrolysates, PET derivatives into PHA and co-production in microbial consortia, which reached biopolymer titers lower than 2 (g L⁻¹) in several days given the toxic effect of some feedstocks or the slow uptake capacity of the host strains to metabolize inexpensive substrates. Overall, engineered microbes are still behind PHA productivities attained by natural PHA producers such as *C. necator* (3.1 g L⁻¹ h⁻¹) (Shang et al., 2003), *P. putida* (2.3 g L⁻¹ h⁻¹) (Maclean et al., 2008), and *P. megaterium* (1.7 g L⁻¹ h⁻¹) (Kanjanchumpol et al., 2013). Among strategies, adaptive laboratory evolution appears as a powerful tool to generate evolved lineages of cells that overcome growth arrest and low biomass yields (Portnoy et al., 2011). Resequencing more resistant bacterial strains might enable unveiling the genetic changes that confer such features and contribute the most to the desired phenotype. This reverse engineering approach combined with genome-scale metabolic modelling (GSMM) might deliver novel targets to optimize PHA synthesis (Sandberg et al., 2019). The elucidation of carbon partitioning through multiple metabolic pathways and intracellular modulators that preclude PHA accumulation in the cell can also benefit from using GSMM and regulatory models. For example, during genome reduction of PHA-producing bacteria many regulatory elements of unknown role in the PHA synthesis process were eliminated (Fan et al., 2020; Liang et al., 2020). Also, cofactor requirement is a crucial element when producing PHAs from waste streams as NADPH is involved in both fighting oxidative stress during replication and as a cofactor in many PHA biosynthetic pathways.

Concerning programmable cell lysis systems, lysozyme-based and phage holin-endolysin enzymes have been implemented in bacterial strains for cell

disruption and biopolymer recovery. Unfortunately, some of these genetic circuits arrest cell growth affecting the overall PHA formation. So far, the created genetic circuits exhibit a certain level of leakage. And activating the programmable circuits in nitrogen-limiting conditions compromises protein synthesis resulting in delayed cell disruption. Thus, further developing tightly regulated genetic circuits (on/off mode of action) that can be integrated into the chromosome of the biocatalyst might avoid antibiotic supplementation eluding unwanted cell growth impairment. Moreover, activating the cell lysis systems in high cell density cultures remain a challenge and it is an open question whether these genetic constructs function under such conditions.

Finally, another important aspect poorly covered in studies aiming to engineer PHA producers is the characterization and comparison of the biosynthesized PHA in terms of thermal and mechanical features. The polymerization process, catalysed by the PHA polymerases accompanied by the PHA phasin protein, defines in part the molecular weight of the formed biopolymer (Shen et al., 2019; Tian et al., 2005; Tsuge, 2016). Besides, fermentation conditions also influence the physical properties of the intracellular polyoxoester (McAdam et al., 2020). There is an interconnection between genetic manipulations and bioreactor settings that modulate the PHA polymerization process and possible applications. How the alteration of the genetic repertoire, metabolic operation, or regulatory elements affect the physical properties of the resulting PHA is still elusive. More than ever, there is a need to establish more efficient bioconversion processes of renewable materials into biopolymers for tackling the global plastic problem where metabolic engineering, synthetic biology, and mathematical modelling of cellular processes play a pivotal role in this endeavour.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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