Advanced Industrial and Engineering Polymer Research 3 (2020) 1–7

Contents lists available at ScienceDirect

Advanced Industrial and Engineering Polymer Research

journal homepage: http://www.keaipublishing.com/aiepr

Polyhydroxyalkanoates (PHA) toward cost competitiveness and functionality

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ARTICLE INFO

Article history:
Received 5 September 2019
Received in revised form
21 October 2019
Accepted 10 November 2019

Keywords:
PHB
Polyhydroxyalkanoates
Extremophiles
Halophiles
Next generation industrial biotechnology
NGIB
Metabolic engineering
Pathway engineering
Morphology engineering
Functional polymers

ABSTRACT

Polyhydroxyalkanoates (PHA) have been produced by several bacteria as bioplastics in industrial scales. PHA commercialization has been challenging due to its complexity and the associated high cost together with instabilities on molecular weights (Mw) and structures, thus instability on thermo- and mechanical properties. PHA high production cost is related to complicated bioprocessing associated with sterilization, low conversion of carbon substrates to PHA products, and poor growth of microorganisms as well as complicated downstream separation. To reduce complexity of PHA production, robust microorganisms that are contamination resistant bacteria have been targeted especially extremophiles, developments of engineering approaches for extremophiles especially *Halomonas* spp. for better PHA production have been successfully conducted and termed as "next generation industrial biotechnology" (NGIB). Diverse PHA can also be produced by engineering *Halomonas* or *Pseudomonas* spp. This review introduces recent advances on engineering bacteria for enhanced PHA biosynthesis and diversity.

1. Introduction

Polyhydroxyalkanoates (PHA), a family of intracellular biopolyesters, have been produced as bioplastics with limited success in market [1–3] (Table 1), mostly due to the high cost of production and instability on thermo-mechanical properties resulted from changing molecular weights (Mw) and structures, that are related to varying PHA synthase activity [4–10]. Lots of studies have since been conducted to address these challenges [5,11–13].

High production cost of PHA have been related to high energy demand for high temperature sterilization using super-hot steam and intensive aeration under pressure, slow growth of microorganisms, discontinuous production processes and complicated downstream processing et al. (Table 1) [14,15]. Extremophile bacteria, especially *Halomonas* spp., have been successfully engineered to reduce the process complexity and extend the PHA diversity [16,17].

Plastic pollution has increasingly becoming a global concern. It calls for low cost biodegradable plastics to replace non-degradable petrochemical plastics. The developments of extremophiles based "Next Generation Industry Biotechnology" or "NGIB", can reduce PHA production complexity and thus PHA production cost combined with more diversity (Table 1), promising to make PHA competitive in cost. In this review, *E. coli, Pseudomonas* spp., and halophiles *Halomonas* spp. were used as examples to show how to engineer bacteria for better PHA biosynthesis, more PHA accumulation and downstream purification so as to increase PHA application competitiveness.

2. Engineering PHA synthesis pathways

Substrates contribute most to the high PHA production [11]. Usually, glucose and/or fatty acids are commonly used as substrates for PHA synthesis. For example, the formation of PHA consisting of

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https://doi.org/10.1016/j.aiepr.2019.11.001

Table 1
Possibilities to reduce PHA production complexity and cost.

Problems	Reasons	Solutions	Ref.
High energy consumption	Sterilization and aeration	Open and weak aeration	[17]
Substrates consumed not for PHA	Substrates consumed for growth	Weakening non-PHA pathways	[18,19]
Unstable PHA structures	Pathways consuming PHA precursors	Engineering PHA unrelated pathways	[19,20]
Unstable PHA molecular weights	Unstable PHA synthase activity	Stabilizing the synthase activity	[8,10]
Slow growth	Binary fission and slow division	Multiple fission	[21]
Discontinuous processes	The demand of avoiding possible contamination	Contamination resistant strains for continuous processes	[16,17,22]
Expensive downstream processes	Difficulty of extracting and purifying PHA	Morphology engineering	[23,24]
Difficulty of cell high density growth	Oxygen supply limitation	Bacterial hemoglobin	[25]
Hard to disrupt cells	Strong bacterial cell walls	Weakening cell walls	[26]
Waste water	Culture and downstream water	Recycling wastewater	[27]

non 3-hydroxubutyrate (3HB) monomers needs fatty acid(s) as precursors for formation of other short-chain-length (scl) or medium-chain-length (mcl) monomers [28–32]. Cells will conduct β-oxidization to turn most fatty acids into acetyl-CoA for cell growth, the costly fatty acids are wasted for generating acetyl-CoA but not for mcl PHA synthesis (Fig. 1) [20], while acetyl-CoA can also be generated from cheaper substrates as glucose [18,33]. Because of the β-oxidation, fatty acids conversion to PHA are very inefficient, leading to the high cost of mcl PHA production. Thus, engineering the β-oxidation could help enhance PHA efficiency.

Deletions of enzymes FadA and FadB in the β-oxidation pathway of *Pseudomonas putida* or *P. entomophila* can enhance the substrate to PHA conversion efficiency significantly (Fig. 1) [34], as fatty acid substrates were mostly converted into 3-hydroxyacyl-CoA for mcl PHA formation instead of being oxidized to generate acetyl-CoA [20,35,36]. *Pseudomonas* spp. fatty acid mutants were found to produce mcl-PHA containing 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and 3-hydroxydodecanoate (3HDD) in the forms of homopolymers, block- or random copolymers [37].

Recombinant *Escherichia coli* co-expressing genes related to succinate degradation in *Clostridium kluyveri* and poly-3-hydroxybutyrate (PHB) accumulation pathway of *Ralstonia eutropha* produces copolymers poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P3HB4HB) from glucose. When the native succinate semi-aldehyde dehydrogenase genes *gabD* and *sad* were both deleted, the carbon flux was channeled to 4HB biosynthesis [18]. Terpolymer P(3HB-*co*-3HV-*co*-4HB) consisting of 3-hydroxybutyrate, 3-hydroxyvalerate (3HV) and 4-hydroxybutyrate (4HB) can be synthesized directly based on lactose or waste raw materials as carbon sources [38]. A mannitol rich ensiled grass press juice (EGPJ) was successfully used as a renewable carbon substrate for PHA production [39]. Fed-batch culture of *Burkholderia sacchari* IPT101 grown on EGPJ as sole carbon source could produce 45 g/L dry cells with 33% PHA in 36 h. In *Ralstonia eutropha* NCIMB11599 and *R. eutropha* 437−540, a sucrose utilization pathway was constructed by introducing the *Mannheimia succiniciproducens* MBEL55E *sacC* gene encoding β-fructofuranosidase [40], which was excreted into the culture to hydrolyze sucrose into glucose and fructose for better cell growth. 73 wt% PHB was obtained when *Ralstonia eutropha* NCIMB11599 was grown in nitrogen-free chemically defined medium containing 20 g/L of sucrose. *E. coli* was successfully engineered to produce mcl PHA from glucose by reversing fatty acid β-oxidation cycle [41]. After deleting major thioesterases and expressing a PHA synthase with low substrate specificity from *Pseudomonas stutzeri* 1317, the recombinant *E. coli* synthesized 12 wt% scl-mcl PHA copolymers, of which 21 mol% was 3HB and 79 mol% mcl monomers.

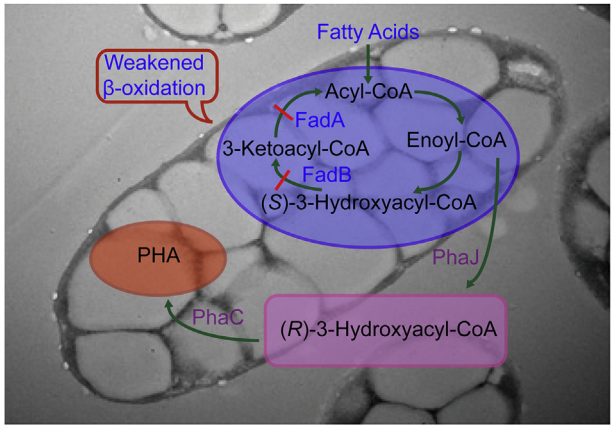

Fig. 1. Biosynthesis of PHA from fatty acids via β-oxidation pathway. Deleting enzymes FadA and FadB in β-oxidation pathways channels most fatty acid(s) to PHA synthesis, resulting in significant improvement in substrates conversion efficiency.

Varying monomer supplies and *in vivo* PHA synthase activity changes monomer ratios in PHA copolymers and Mw, leading to unstable PHA structures and Mw. Lots of studies have thus been conducted to stabilize the PHA monomer ratios and Mw. Recombinant *Pseudomonas putida* KT2442 with its β-oxidation deleted was constructed as a platform for PHA synthesis with controllable monomer contents and compositions [19]. Monomer ratios of PHA can be precisely adjusted by feeding fatty acids with a predefined ratio, random and block copolymers PHBHHx consisting of precisely adjustable 3HB and 3HHx were synthesized. The materials demonstrated steady performances if the monomer ratios were stable [19,42]. Homopolymers of C5 (3HV) to C14 (3-hydroxytetradecanoate) were synthesized by β-oxidation deficient *P. entomophila* LAC23 fed with different fatty acids as precursors, respectively [43]. These researches clearly demonstrated that β-oxidation deficient mutants allowed controllable PHA monomer structures, and these were also supported by several other studies [44]. Similarly, one can synthesize block copolymers of poly-3-hydroxypropinate-*block*-poly-4-hydroxybutyrate (P3HP-*b*-P4HB) using recombinant *E. coli* S17-1 by feeding precursors with a predefined ratio (Fig. 2) [45,46].

Activities of PHA synthase and depolymerase affect PHA Mw [47], which needs to be controlled to tailor PHA physical properties. PHA Mw can be reduced in the presence of chain transfer agents such as poly(ethylene glycol) (PEG), methanol, ethanol and isopropanol in the culture or via mutations in the N-terminus of PHA synthase [48,49]. CRISPRi (clustered regularly interspaced short palindromic repeats interference) was employed to control the *phaC* transcription and thus PhaC activity, as supported by evidences that PHB contents, Mw and polydispersity approximately in direct and reverse proportion to the PhaC activity, respectively [10]. A higher PhaC activity leads to more intracellular PHB accumulation yet with less PHB Mw and wider polydispersity. PHB was controlled in the ranges of 2.0−75% cell dry weights, Mw from 2 to

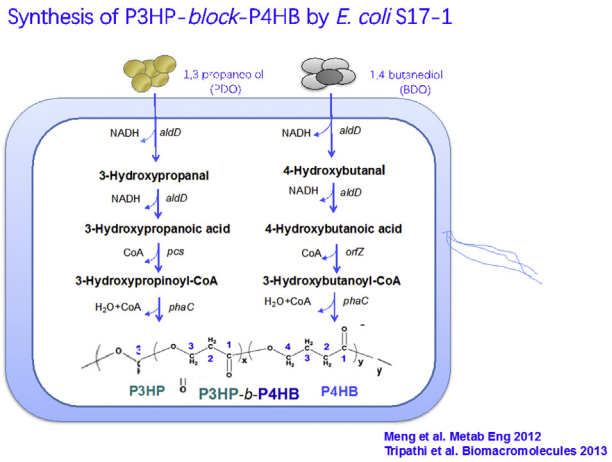

Fig. 2. Recombinant *E. coli* synthesizes block-copolymers of poly-3-hydroxypropionate (P3HP) and poly-4-hydroxybutyrate (P4HB) [45,46].

6 millions Dalton and a polydispersity of 1.2–1.43 in 48 h shake flask studies using PhaC activity regulation [10].

3. Engineering the cell growth pattern

Commonly, most bacteria grow in a binary fission way (Fig. 3). Wu et al. [21] deleted fission related genes *minC* and *minD* together to change the binary fission into multiple fission, resulting in the formation of multiple fission rings (Z-rings) in several positions of an elongated cell, achieving cell division with more than two daughter cells in one cell cycle (Fig. 3). Additionally, several cell division process related genes including *ftsQ*, *ftsL*, *ftsW*, *ftsN* and *ftsZ*, together with the cell shape control gene *mreB*, were all overexpressed in *E. coli* JM109 *ΔminCD* to further improve cell growth and PHA production, leading to more cell dry weights (CDW) and more than 80% PHB accumulation increases compared to its original binary fission pattern. This study has demonstrated that changing the cell division pattern and cell morphology could enhance cell growth and PHB production. In another related research, Wu et al. [50] further demonstrated that combining the multiple division pattern with elongated cell shape of *E. coli* could improve PHB accumulation.

In addition, Tyo group [51] developed a toggle switch which utilizes glucose sensing to decouple growth and production phase. This industrially relevant auto-inducible genetic switch responded to glucose to precisely control the expression of enzymes in burdensome pathways for enhancing bio-production, which improved growth by 2-folds with comparable PHB production yields to a constitutively expressing system. Those provided a new vision for enhancing PHA production.

4. Morphology engineering for more PHA accumulations and better separation

Tiny cell sizes of bacteria bring difficulties to downstream separation. Morphology engineering can change cell sizes and shapes, allowing easier downstream separation [24]. Genes *ftsZ* and *mreB* which respectively encode proteins of bacterial fission ring and skeletons are essential for cell growth and for keeping the bacterial shapes [52–56]. Clustered regularly interspaced short palindromic repeats interference (CRISPRi) was applied to regulate expression intensities of *ftsZ* or/and *mreB* in *E. coli* leading to various decreased expression levels of *ftsZ* or/and *mreB*, respectively [57]. It was found that with the increasing repression on genes *ftsZ* or/and *mreB*, *E. coli* fibers got longer and cell got larger [57]. Combined repressions on expressions of *ftsZ* and *mreB* leaded to longer and larger *E. coli* with numerous morphologies including various sizes of gourds, bars, coccus, spindles, multi-angles and ellipsoids (Fig. 4). In all these cases, PHB accumulations were improved. Enlarged morphology increased PHB synthesis from 40% to 80%, and it also promotes gravity separation of cells from fermentation broth [23,24,58].

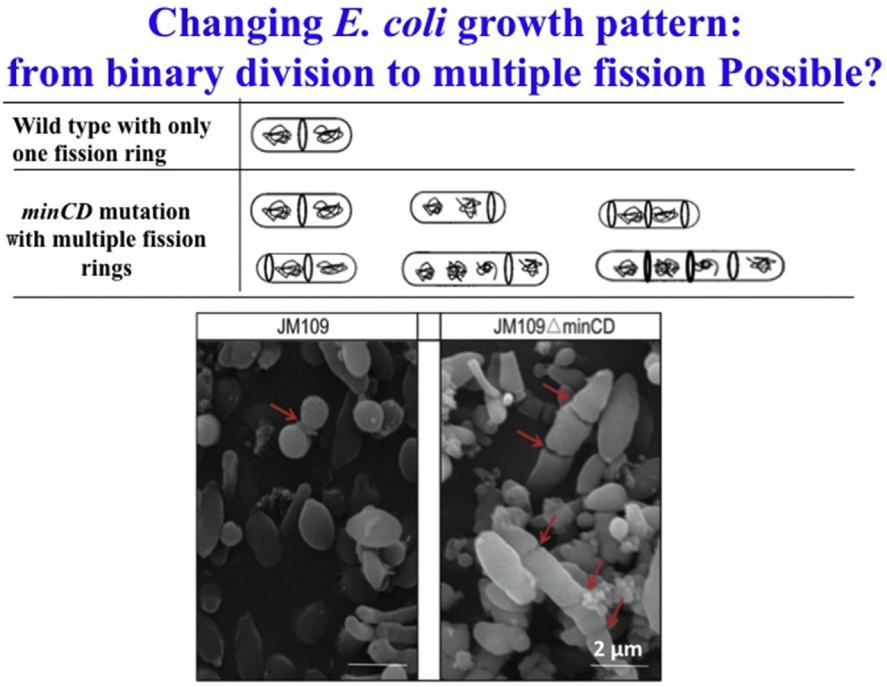

Fig. 3. Binary fission has been changed to multiple fission by deleting fission related genes *minC* and *minD* together, resulting in the formation of multiple fission rings (Z-rings) in several positions of an elongated cell for formation of more than two daughter cells [34].

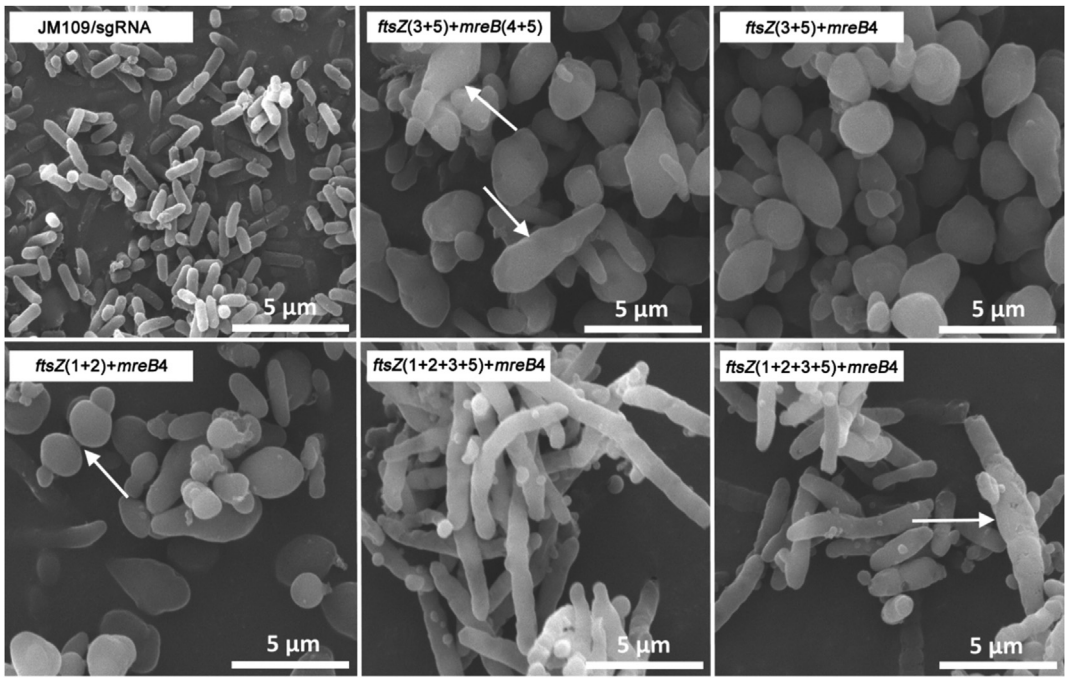

Fig. 4. CRISPRi represses expression intensities of *ftsZ* or/and *mreB* in *E. coli* [57]. Combined repressions on expressions of *ftsZ* and *mreB* leaded to longer and larger *E. coli* with numerous morphologies resulting in enhanced PHB accumulations. Larger cells are prone to separate from broth via gravity or filtration [34].

5. Next generation industry biotechnology (NGIB) based on halophiles

Extremophiles can be cultured under extreme conditions, so that they are more resistant to microbial contamination in fermentation. Among them, halophilic bacteria are able to grow rapidly in medium with high salt concentrations and high pH [16], resulting in their excellent contamination resistance that few other microorganisms can have.

Halophilic bacteria were found able to grow in open and continuous fermentation processes in unsterile seawater medium without contamination for at least two months [59]. Their potentials could be further developed by introducing new pathways or adding new genetic parts. Recombinant *Halomonas* spp. can now be designed for producing multiple products separately or simultaneously as described below [62–68].

However, genetic parts are often influenced by host strains, and altering activity of biological parts frequently causes failures in process control [60,61]. Therefore, in order to fully realize the potential of *Halomonas* spp., genetic parts with tight regulation and high efficiency need to be developed. Recently, Technology has also been developed for genetic manipulation of halophilic bacteria [22,62]. Molecular engineering tools have been developed to construct recombinant *Halomonas* spp. for production of foreign proteins [63], small molecular compound 5-aminolevulinic acid [64], and PHBV copolymers with high substrate to PHA conversion efficiency [22,65,66]. More studies on genetic engineering will lead to the generation of new products produced by recombinant halophiles. The PHA granules synthesized by *Halomonas* spp. can also be produced in large size for convenient separation and drying processes [69].

The biotechnology based on extremophiles that grow under open unsterile conditions will surely promotes the emerging "Next Generation Industrial Biotechnology" or NGIB for bio-production with reduced cost and thus improved competitiveness.

6. Engineering *Halomonas* spp. for PHA diversity

PHBV is a promising biopolyester which has good mechanical properties and biodegradability. CRISPR/Cas9 method was applied to edit the TCA cycle in *Halomonas bluephagenesis* to produce PHBV from glucose as a sole carbon source. Two TCA cycle related genes *sdhE* and *icl* encoding succinate dehydrogenase assembly factor 2 and isocitrate lysase were deleted, respectively, in *H. bluephagenesis* TD08AB containing PHBV synthesis genes on the chromosome, to channel more flux to increase the 3HV ratio of PHBV. Supported by the synergetic function of phosphoenolpyruvate carboxylase and *Vitreoscilla* hemoglobin encoded by genes *ppc* and *vgb* inserted into the chromosome of *H. bluephagenesis* TY194 (Δ*sdhE*) serving to enhance TCA cycle activity, a series of strains were generated that could produce PHBV containing 3–18 mol% 3HV using glucose as a sole carbon source. Next-generation industrial biotechnology based on recombinant *H. bluephagenesis* grown under unsterile and continuous conditions, reduces complexity and cost of PHBV production [67].

P3HB4HB is another promising biomaterial with wide applications. The high cost is still the barrier blocking its large-scale production. Pathway debugging using structurally related carbon source allows more 4HB accumulation. Whole genome sequencing and comparative genomic analysis found multiple orthologs of succinate semialdehyde dehydrogenase (*gabD*) that compete with 4HB synthesis flux in *H. bluephagenesis*. Therefore, combinatory gene-knockout strains were constructed to increase the molar fraction of 4HB by 24-folds in shake flask studies. The best-performing strain was grown on glucose as the single carbon source for 60 h under non-sterile conditions in a 7 L bioreactor, reaching 26 g/L of dry cell mass containing 61% P(3HB-*co*-17 mol% 4HB). 4HB molar fraction in P3HB4HB can be tuned from 13 mol% to 25 mol% by controlling the glucose concentration in the cultures [68].

7. Functional PHA and chemical modifications

Functional PHA homo- and copolymers can be synthesized by genome engineered *Pseudomonas entomophila* [44,69]. A thermo-

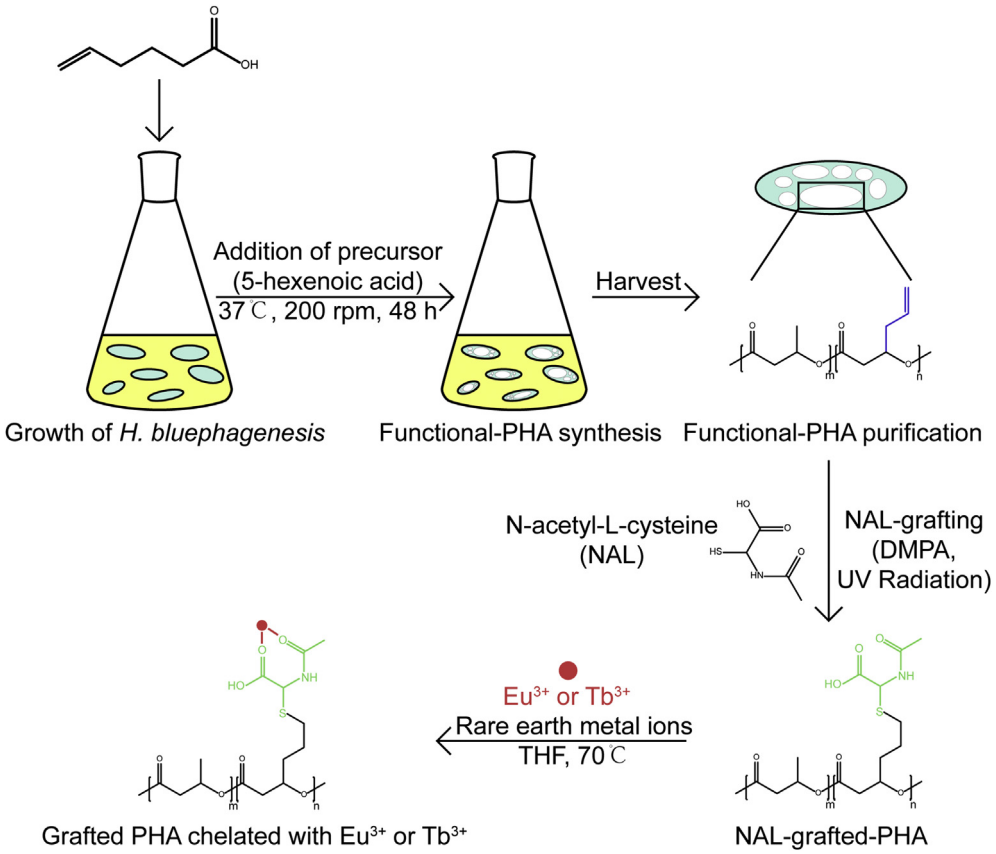

Fig. 5. Process flowsheet for preparation of functional NAL-grafted PHA chelated with rare earth metal ions (Eu^{3+} and Tb^{3+}) [72].

responsive graft copolymer PHA-*g*-poly(N-isopropylacrylamide) (short as PHA-gPNIPAm), was successfully synthesized by a three-step reaction based on unsaturated P(3HDD-*co*-3H10U), a PHA random copolymer of 3-hydroxydodecanoate (3HDD) and 3-hydroxy-10-undecylenate (3H10U). Started with PNIPAm oligomer with a trithiocarbonate-based chain transfer agent (CTA), short as PNIPAm-CTA, a reversible addition fragmentation chain transfer (RAFT) polymerization was completed. Subsequently, the PNIPAm-CTA was treated with n-butylamine for aminolysis in order to obtain a pendant thiol group at the end of the chain (PNIPAm-SH). At the end, the PNIPAm-SH was grafted onto P(3HDD-*co*-3H10U) via a thiol ene click reaction. Enhanced hydrophilicity and thermo-responsive property of the resulted PHA-g-PNIPAm were obtained [70]. Similarly, Yao et al. [71] employed a PHA copolymer P(3HDD-*co*-3H9D) consisting of 3-hydroxydodecanoate (3HDD) and 3-hydroxy-9-decenoate (3H9D) synthesized by engineered bacterium *Pseudomonas entomophila* to generate a comb-like temperature-responsive graft polymer P(3HDD-*co*-3H9D)-*g*-poly(2-dimethylamino-ethylmethacrylate), abbreviated as PHA-*g*-PDMAEMA, via a three-step reaction. The grafted material shows a tendency of increasing protein adsorptions over the lower critical solution temperature (LCST 47.5 °C) on PHA-g-PDMAEMA with an increasing percentage of DMAEMA in the copolymers. At a temperature below LCST, PHA-*g*-PDMAEMA demonstrated poor protein absorption in contrast to P(3HDD-*co*-3H9D). The comb-like graft polymer PHA-*g*-PDMAEMA can be manipulated toward controllable protein adsorption for biomedical usages [71].

Unsaturated poly(3-hydroxydodecanoate-*co*-3-hydroxy-9-decenoate), short as P(3HDD-*co*-3H9D), were explored for synthesis of PHA-graft-graphene nanocomposites with graphene content ranging from 0.2 to 1.5 wt%. PHA-graft-graphene nanocomposites exhibited higher thermal degradation temperature and enhanced electricity conductivity compared with that of neat PHA [71].

A highly efficient rare-earth-modified fluorescent material was successfully designed and fabricated based on functional-PHA containing terminal double bonds to generate the rare-earth-modified PHA [72]. N-Acetyl-L-cysteine-grafted PHA (NAL-grafted-PHA) was first produced via a UV-initiated thiol-ene click reaction and the rare earth ions (Eu^{3+} and Tb^{3+}) were subsequently chelated onto the NAL-grafted-PHA. The composite material exhibited intense photoluminescence properties under UV laser

Table 2
Functional PHA and chemical modifications with purposes.

PHA Modification	New properties	References
RPHA-*g*-PNIPAm	Enhanced hydrophilicity and thermoresponsibility	[70]
PHA-*g*-PDMAEMA	Temp controllable protein adsorption	[71]
PHA-*g*-graphene	Higher thermal degradation temperature and electricity conductivity	[74]
Rare-earth-modified PHA	Intense photoluminescence under UV laser excitation	[72]
PHA modified with PDT	PHA organo-hydrogel	[73]
PHA physical surface modification	Superhydrophobicity	[75]

excitation, indicating its excellent features as a fluorescent material with enhanced hydrophilicity and superior biocompatibility for potential applications in biomedical fields [72]. Fig. 5.

A new family of PHA-based organogels was synthesized via UV photo-crosslinking, using unsaturated PHA copolymer Poly[(*R*)-3-hydroxyundecanoate-*co*-(*R*)-3-hydroxy-10-undecenoate] (PHU10U) and polyethylene glycol dithiol (PDT) as precursors [73]. Increasing the densities of the organogel networks resulted in a higher compressive modulus. Both the PHU10U precursor and PHU10U/PDT hydrogels showed relatively good biocompatibility. Table 2.

8. Conclusion and perspective

With the increasing diversity of PHA, the application of PHA will be more and more extensive. However, the large-scale industrialization and commercialization of PHA has been restricted by the production cost, especially the production cost of the new PHA, which is much higher than the traditional PHA. By means of synthetic biology, systems biology, morphology engineering and next generation industry biotechnology, the synthesis of all kinds of PHA can be integrated to realize a low-cost production platform, which bases on one chassis bacteria harboring multiple metabolic pathways and can synthesize certain kind of PHA on demand. Eventually, it will be possible to reduce the production cost of all kinds of PHA, thus promoting the application of different types of PHA in different fields.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

This research was financially supported by grants from National Key Project of Synthetic Biology in China (2018YFA0900200), and National Natural Science Foundation of China (Grant No. 21761132013 and NSFC-EU, 31961133019).

References

[1] G.Q. Chen, M.K. Patel, Plastics derived from biological sources: present and future: a technical and environmental review, Chem. Rev. 112 (2012) 2082–2099. https://pubs.acs.org/doi/10.1021/cr200162d.
[2] X. Gao, J.C. Chen, Q. Wu, G.Q. Chen, Polyhydroxyalkanoates as a source of chemicals, polymers, and biofuels, Curr. Opin. Biotechnol. 22 (2011) 768–774. https://www.sciencedirect.com/science/article/pii/S0958166911006173?via%3Dihub.
[3] B. Laycock, P. Halley, S. Pratt, A. Werker, P. Lant, The chemomechanical properties of microbial polyhydroxyalkanoates, Prog. Polym. Sci. 38 (2013) 536–583. https://www.sciencedirect.com/science/article/pii/S007967001200072X.
[4] S.J. Park, T.W. Kim, M.K. Kim, S.Y. Lee, S.C. Lim, Advanced bacterial polyhydroxyalkanoates: towards a versatile and sustainable platform for unnatural tailor-made polyesters, Biotechnol. Adv. 30 (2012) 1196–1206. https://www.sciencedirect.com/science/article/pii/S0734975011001996?via%3Dihub.
[5] Y. Wang, J. Yin, G.Q. Chen, Microbial polyhydroxyalkanoates, Challenges and opportunities, Curr. Opin. Biotechnol. 30C (2014) 59–65. https://www.sciencedirect.com/science/article/pii/S0958166914001037.
[6] H. Bernd, Polyester synthases: natural catalysts for plastics, Biochem 376 (2003) 15–33. http://www.biochemj.org/content/376/1/15.long.
[7] K.E. Tyo, C.R. Fischer, F. Simeon, G. Stephanopoulos, Analysis of polyhydroxybutyrate flux limitations by systematic genetic and metabolic perturbations, Metab. Eng. 12 (2010) 187–195. https://www.sciencedirect.com/science/article/pii/S1096717609000913.
[8] J.-i. Choi, S.Y. Lee, High level production of supra molecular weight poly (3-hydroxybutyrate) by metabolically engineered *Escherichia coli*, Biotechnol. Bioproc. Eng. 9 (2004) 196–200. https://link.springer.com/article/10.1007/BF02942292.
[9] S. Lee, A. Middelberg, Y. Lee, Poly (3-hydroxybutyrate) production from whey using recombinant *Escherichia coli*, Biotechnol. Lett. 19 (1997) 1033–1035. https://link.springer.com/article/10.1023/A%3A1018411820580.
[10] D. Li, L. Lv, J.C. Chen, G.Q. Chen, Controlling microbial PHB synthesis via CRISPRi, Appl. Microbiol. Biotechnol. 101 (2017) 5861–5867. https://link.springer.com/article/10.1007%2Fs00253-017-8374-6.
[11] J. Choi, S.Y. Lee, Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation, Appl. Microbiol. Biotechnol. 51 (1999) 13–21. https://link.springer.com/article/10.1007/s002530051357.
[12] A.A. Koutinas, A. Vlysidis, D. Pleissner, N. Kopsahelis, I. Lopez Garcia, I.K. Kookos, S. Papanikolaou, T.H. Kwan, C.S. Lin, Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers, Chem. Soc. Rev. 43 (2014) 2587–2627. https://pubs.rsc.org/en/content/articlelanding/2014/CS/c3cs60293a#!divAbstract.
[13] A. Steinbüchel, T. Lütke-Eversloh, Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms, Biochem. Eng. J. 16 (2003) 81–96. https://www.sciencedirect.com/science/article/pii/S1369703X03000366.
[14] K. Sudesh, H. Abe, Y. Doi, Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters, Prog. Polym. Sci. 25 (2000) 1503–1555. https://www.sciencedirect.com/science/article/pii/S0079670000000356?via=ihub.
[15] L.S. Serafim, P.C. Lemos, M.G. Albuquerque, M.A. Reis, Strategies for PHA production by mixed cultures and renewable waste materials, Appl. Microbiol. Biotechnol. 81 (2008) 615–628. https://link.springer.com/article/10.1007/s00253-008-1757-y.
[16] J. Yin, J.C. Chen, Q. Wu, G.Q. Chen, Halophiles, coming stars for industrial biotechnology, Biotechnol. Adv. 33 (2014) 1433–1442. https://www.sciencedirect.com/science/article/pii/S0734975014001578.
[17] T. Li, X.b. Chen, J.c. Chen, Q. Wu, G.Q. Chen, Open and continuous fermentation: products, conditions and bioprocess economy, Biotechnol. J. 9 (2014) 1503–1511. https://onlinelibrary.wiley.com/doi/full/10.1002/biot.201400084.
[18] Z.J. Li, Z.Y. Shi, J. Jian, Y.Y. Guo, Q. Wu, G.Q. Chen, Production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) from unrelated carbon sources by metabolically engineered *Escherichia col*, Metab. Eng. 12 (2010) 352–359. https://www.sciencedirect.com/science/article/pii/S1096717610000194.
[19] L. Tripathi, L.P. Wu, M. Dechuan, J. Chen, Q. Wu, G.Q. Chen, *Pseudomonas putida* KT2442 as a platform for the biosynthesis of polyhydroxyalkanoates with adjustable monomer contents and compositions, Bioresour. Technol. 142 (2013) 225–231. https://www.sciencedirect.com/science/article/pii/S0960852413007876.
[20] A.L. Chung, H.L. Jin, L.J. Huang, H.M. Ye, J.C. Chen, Q. Wu, G.Q. Chen, Biosynthesis and characterization of poly (3-hydroxydodecanoate) by β-oxidation inhibited mutant of *Pseudomonas entomophila* L48, Biomacromolecules 12 (2011) 3559–3566. https://pubs.acs.org/doi/10.1021/bm200770m.
[21] H. Wu, Z. Fan, X. Jiang, J. Chen, G.Q. Chen, Enhanced production of polyhydroxybutyrate by multiple dividing *E. coli*, Microb. Cell Factories 15 (2016) 128. https://link.springer.com/article/10.1186/s12934-016-0531-6.
[22] X.Z. Fu, D. Tan, G. Aibaidula, Q. Wu, J.C. Chen, G.Q. Chen, Development of *Halomonas* TD01 as a host for open production of chemicals, Metab. Eng. 23 (2014) 78–91. https://www.sciencedirect.com/science/article/pii/S1096717614000123.
[23] X.R. Jiang, G.Q. Chen, Morphology engineering of bacteria for bio-production, Biotechnol. Adv. 34 (2016) 435–440. https://www.sciencedirect.com/science/article/pii/S073497501530063X.
[24] X.R. Jiang, H. Wang, R. Shen, G.Q. Chen, Engineering the bacterial shapes for enhanced inclusion bodies accumulation, Metab. Eng. 29 (2015) 227–237. https://www.sciencedirect.com/science/article/pii/S1096717615000439.
[25] P.F. Ouyang, H. Wang, I. Hajnal, Q. Wu, Y.Y. Guo, G.Q. Chen, Increasing oxygen availability for improving PHB production by *Halomonas*, Metab. Eng. 45 (2018) 20–31. https://www.sciencedirect.com/science/article/pii/S1096717617303543.
[26] X.C. Zhang, Y.Y. Guo, X. Liu, X.G. Chen, Q. Wu, G.Q. Chen, Engineering cell wall synthesis mechanism for enhanced PHB accumulation in *E. coli*, Metab. Eng. 45 (2018) 32–42. https://www.sciencedirect.com/science/article/pii/S1096717617303385.
[27] C. Ling, G.Q. Qiao, B.W. Shuai, K.N. Song, X.R. Jiang, G.Q. Chen, Engineering self-flocculating Halomonas campaniensis for wastewaterless open and continuous fermentation, Biotechnol. Bioeng. 116 (2019) 805–815. https://onlinelibrary.wiley.com/doi/full/10.1002/bit.26897.
[28] B. Andreeßen, A.B. Lange, H. Robenek, A. Steinbüchel, Conversion of glycerol to poly (3-hydroxypropionate) in recombinant *Escherichia coli*, Appl. Environ. Microbiol. 76 (2010) 622–626. https://aem.asm.org/content/76/2/622.long.
[29] R.J. Sánchez, J. Schripsema, L.F. da Silva, M.K. Taciro, J.G. Pradella, J.G.C. Gomez, Medium-chain-length polyhydroxyalkanoic acids (PHA mcl) produced by *Pseudomonas putida* IPT 046 from renewable sources, Eur. Polym. J. 39 (2003) 1385–1394. https://www.sciencedirect.com/science/article/pii/S0014305703000193.
[30] A.B. Kroumova, G.J. Wagner, H.M. Davies, Biochemical observations on medium-chain-length polyhydroxyalkanoate biosynthesis and accumulation in *Pseudomonas mendocina*, Arch. Biochem. Biophys. 405 (2002) 95–103. https://www.sciencedirect.com/science/article/pii/S0003986102003508.
[31] J.M. Cavalheiro, R.S. Raposo, M.C.M. de Almeida, M.T. Cesário, C. Sevrin, C. Grandfils, M. Da Fonseca, Effect of cultivation parameters on the production of poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) and poly (3-hydroxybutyrate-4-hydroxybutyrate-3-hydroxyvalerate) by *Cupriavidus necator* using waste glycerol, Bioresour. Technol. 111 (2012) 391–397. https://www.sciencedirect.com/science/article/pii/S0960852412002271.

[32] Y. Doi, A. Segawa, M. Kunioka, Biodegradable poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) produced from γ-butyrolactone and butyric acid by *Alcaligenes eutrophus*, Polym. Commun. 30 (1989) 169–171.

[33] H.E. Valentin, D. Dennis, Production of poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) in recombinant *Escherichia coli* grown on glucose, J. Biotechnol. 58 (1997) 33–38. https://www.sciencedirect.com/science/article/pii/S0168165697001272?via%3Dihub.

[34] G.Q. Chen, X.R. Jiang, Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis, Synth. Syst. Biotechnol 2 (2017) 192–197. https://www.sciencedirect.com/science/article/pii/S2405805X17300819.

[35] Q. Liu, G. Luo, X.R. Zhou, G.Q. Chen, Biosynthesis of poly (3-hydroxydecanoate) and 3-hydroxydodecanoate dominating polyhydroxyalkanoates by β-oxidation pathway inhibited *Pseudomonas putida*, Metab. Eng. 13 (2011) 11–17. https://www.sciencedirect.com/science/article/pii/S1096717610000893.

[36] H.H. Wang, X.T. Li, G.Q. Chen, Production and characterization of homopolymer polyhydroxyheptanoate (P3HHp) by a *fadBA* knockout mutant *Pseudomonas putida* KTOY06 derived from *P. putida* KT2442, Process Biochem. 44 (2009) 106–111. https://www.sciencedirect.com/science/article/pii/S135951130800295X.

[37] G.Q. Chen, I. Hajnal, H. Wu, L. Lv, J. Ye, Engineering biosynthesis mechanisms for diversifying polyhydroxyalkanoates, Trends Biotechnol. 33 (2015) 565–574. https://www.sciencedirect.com/science/article/pii/S0167779915001596?via%3Dihub.

[38] S. Povolo, M.G. Romanelli, M. Basaglia, V.I. Ilieva, A. Corti, A. Morelli, E. Chiellini, S. Casella, Polyhydroxyalkanoate biosynthesis by *Hydrogenophaga pseudoflava* DSM1034 from structurally unrelated carbon sources, N. Biotech. 30 (2013) 629–634. https://www.sciencedirect.com/science/article/pii/S187167841200876X.

[39] F. Cerrone, R. Davis, S.T. Kenny, T. Woods, A. O'Donovan, V.K. Gupta, M. Tuohy, R.P. Babu, P. O'Kiely, K. O'Connor, Use of a mannitol rich ensiled grass press juice (EGPJ) as a sole carbon source for polyhydroxyalkanoates (PHAs) production through high cell density cultivation, Bioresour. Technol. 191 (2015) 45–52. https://www.sciencedirect.com/science/article/pii/S0960852415006628.

[40] S.J. Park, Y.A. Jang, W. Noh, Y.H. Oh, H. Lee, Y. David, M.G. Baylon, J. Shin, J.E. Yang, S.Y. Choi, Metabolic engineering of *Ralstonia eutropha* for the production of polyhydroxyalkanoates from sucrose, Biotechnol. Bioeng. 112 (2015) 638–643. https://onlinelibrary.wiley.com/doi/full/10.1002/bit.25469.

[41] Q. Zhuang, Q. Wang, Q. Liang, Q. Qi, Synthesis of polyhydroxyalkanoates from glucose that contain medium-chain-length monomers via the reversed fatty acid β-oxidation cycle in *Escherichia coli*, J. Metab. Eng. 24 (2014) 78–86. https://www.sciencedirect.com/science/article/pii/S1096717614000639.

[42] L. Tripathi, L.P. Wu, J. Chen, G.Q. Chen, Synthesis of Diblock copolymer poly-3-hydroxybutyrate-*block*-poly-3-hydroxyhexanoate [PHB-*b*-PHHx] by a β-oxidation weakened *Pseudomonas putida* KT2442, Microb. Cell Factories 11 (2012) 44. https://microbialcellfactories.biomedcentral.com/articles/10.1186/1475-2859-11-44.

[43] Y. Wang, A. Chung, G.Q. Chen, Synthesis of medium-chain-length polyhydroxyalkanoate homopolymers, random copolymers, and bBlock copolymers by an engineered strain of *Pseudomonas entomophila*, Adv. Healthc. Mater. 6 (2017), https://doi.org/10.1002/adhm.201601017. https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.201601017.

[44] S. Li, L. Cai, L. Wu, G. Zeng, J. Chen, Q. Wu, G.Q. Chen, Microbial synthesis of functional homo-, random, and block polyhydroxyalkanoates by β-oxidation deleted *Pseudomonas entomophila*, Biomacromolecules 15 (2014) 2310–2319. https://pubs.acs.org/doi/10.1021/bm500669s.

[45] D.C. Meng, Z.Y. Shi, L.P. Wu, Q. Zhou, Q. Wu, J.C. Chen, G.Q. Chen, Production and characterization of poly(3-hydroxypropionate-*co*-4-hydroxybutyrate) with fully controllable structures by recombinant *Escherichia coli* containing an engineered pathway, Metab. Eng. 14 (2012) 317–324. https://www.sciencedirect.com/science/article/pii/S1096717612000468.

[46] L. Tripathi, L.P. Wu, D.C. Meng, J.C. Chen, G.Q. Chen, Biosynthesis and characterization of diblock copolymer of p(3-Hydroxypropionate)-*block*-P(4-hydroxybutyrate) from recombinant *Escherichia coli*, Biomacromolecules 14 (2013) 862–870. https://pubs.acs.org/doi/10.1021/bm3019517.

[47] T. Tsuge, Fundamental factors determining the molecular weight of polyhydroxyalkanoate during biosynthesis, Polym 48 (2016) 1051–1057. https://www.nature.com/articles/pj201678.

[48] F. Shi, R. Ashby, R.A. Gross, Use of poly (ethylene glycol)s to regulate poly (3-hydroxybutyrate) molecular weight during *Alcaligenes eutrophus* cultivations, Macromolecules 29 (1996) 7753–7758. https://pubs.acs.org/doi/abs/10.1021/ma960805k.

[49] N.M. Thomson, A. Hiroe, T. Tsuge, D.K. Summers, E. Sivaniah, Efficient molecular weight control of bacterially synthesized polyesters by alcohol supplementation, Chem. Technol. Biotechnol. 89 (2014) 1110–1114. https://onlinelibrary.wiley.com/doi/full/10.1002/jctb.4198.

[50] H. Wu, J. Chen, G.Q. Chen, Engineering the growth pattern and cell morphology for enhanced PHB production by *Escherichia coli*, Appl. Microbiol. Biotechnol. 100 (2016) 9907–9916. https://link.springer.com/article/10.1007/s00253-016-7715-1.

[51] W. Bothfeld, G. Kapov, K.E. Tyo, A glucose-sensing toggle switch for autonomous, high productivity genetic control, ACS Synth. Biol. (2017), https://doi.org/10.1021/acssynbio.6b00257. https://pubs.acs.org/doi/abs/10.1021/acssynbio.6b00257.

[52] T.A. Henderson, K.D. Young, S.A. Denome, P.K. Elf, C. Amp, H. Amp, Proteins related to the class C β-lactamases, bind penicillin and contribute to normal morphology of *E. coli*, J. Bacteriol. 179 (1997) 6112–6121. https://jb.asm.org/content/179/19/6112.

[53] J. Dominguez-Escobar, A. Chastanet, A.H. Crevenna, V. Fromion, R. Wedlich-Soldner, R. Carballido-Lopez, Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria, Science 333 (2011) 225–228. https://science.sciencemag.org/content/333/6039/225.long.

[54] E.C. Garner, R. Bernard, W. Wang, X. Zhuang, D.Z. Rudner, T. Mitchison, Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*, Science 333 (2011) 222–225. https://science.sciencemag.org/content/333/6039/222.

[55] O. Kocaoglu, E.E. Carlson, Penicillin-binding protein imaging probes, Curr. Protoc. Chem. Biol. 5 (2013) 239–250. https://currentprotocols.onlinelibrary.wiley.com/doi/full/10.1002/9780470559277.ch130102.

[56] E. Bi, J. Lutkenhaus, Cell division inhibitors SulA and MinCD prevent formation of the FtsZ ring, J. Bacteriol. 175 (1993) 1118–1125. https://jb.asm.org/content/175/4/1118.long.

[57] D. Elhadi, L. Lv, X.R. Jiang, H. Wu, G.Q. Chen, CRISPRi engineering *E. coli* for morphology diversification, Metab. Eng. 38 (2016) 358–369. https://www.sciencedirect.com/science/article/pii/S1096717616301240.

[58] Y. Wang, H. Wu, X. Jiang, G.Q. Chen, Engineering *Escherichia coli* for enhanced production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) in larger cellular space, Metab. Eng. 25 (2014) 183–193. https://www.sciencedirect.com/science/article/pii/S1096717614001013.

[59] H.T. Yue, C. Ling, T. Yang, X.B. Chen, Y.L. Chen, H.T. Deng, Q. Wu, J.C. Chen, G.Q. Chen, A seawater-based open and continuous process for polyhydroxyalkanoates production by recombinant *Halomonas campaniensis* LS21 grown in mixed substrates, Biotechnol. Biofuels 7 (2014) 108. https://link.springer.com/article/10.1186/1754-6834-7-108.

[60] J.A. Brophy, C.A. Voigt, Principles of genetic circuit design, Nat. Methods 11 (2014) 508–520. https://www.nature.com/articles/nmeth.2926.

[61] J.T. Kittleson, G.C. Wu, J.C. Anderson, Successes and failures in modular genetic engineering, Curr. Opin. Chem. Biol. 16 (2012) 329–336. https://www.sciencedirect.com/science/article/pii/S1367593112000786.

[62] H. Zhao, H.M. Zhang, X. Chen, T. Li, Q. Wu, Q. Ouyang, G.Q. Chen, Novel T7-like expression systems used for *Halomonas*, Metab. Eng. 39 (2017) 128–140. https://www.sciencedirect.com/science/article/pii/S1096717616301367.

[63] L.H. Lan, H. Zhao, J.C. Chen, G.Q. Chen, Engineering *Halomonas* spp. as a low-cost production host for production of bio-surfactant protein PhaP, J. Biotechnol. 11 (2016) 1595–1604. https://onlinelibrary.wiley.com/doi/full/10.1002/biot.201600459.

[64] T. Li, Y.Y. Guo, G.Q. Qiao, G.Q. Chen, Microbial synthesis of 5-aminolevulinic acid and its coproduction with polyhydroxybutyrate, ACS Synth. Biol. 5 (2016) 1264–1274. https://pubs.acs.org/doi/abs/10.1021/acssynbio.6b00105.

[65] D. Tan, Q. Wu, J.C. Chen, G.Q. Chen, Engineering *Halomonas* TD01 for the low-cost production of polyhydroxyalkanoates, Metab. Eng. 26C (2014) 34–47. https://www.sciencedirect.com/science/article/pii/S1096717614001153.

[66] Y.X. Zhao, Z.M. Rao, Y.F. Xue, P. Gong, Y.Z. Ji, Y.H. Ma, Poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) production by *Haloarchaeon Halogranum* amylolyticum, Appl. Microbiol. Biotechnol. 99 (2015) 7639–7649. https://link.springer.com/article/10.1007%2Fs00253-015-6609-y.

[67] Y. Chen, X.Y. Chen, H.T. Du, X. Zhang, Y.M. Ma, J.C. Chen, J.W. Ye, X.R. Jiang, G.Q. Chen, Chromosome engineering the TCA cycle in *Halomonas bluephagenesis* for production of copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), Metab. Eng. 54 (2019) 69–82. https://www.sciencedirect.com/science/article/pii/S1096717619300631.

[68] J.W. Ye, D.K. Hu, X.M. Che, X.R. Jiang, T. Li, J.C. Chen, H.Q. Zhang, G.Q. Chen, Engineering of *Halomonas bluephagenesis* for low cost production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) from glucose, Metab. Eng. 47 (2018) 143–152. https://www.sciencedirect.com/science/article/pii/S1096717618300181.

[69] R. Shen, L.W. Cai, D.C. Meng, L.P. Wu, K. Guo, X.G. Dong, L. Liu, J.C. Chen, Q. Wu, G.Q. Chen, Benzene containing polyhydroxyalkanoates homo- and copolymers synthesized by genome edited *Pseudomonas entomophila*, Sci. China Life Sci. 57 (2014) 4–10. https://link.springer.com/article/10.1007/s11427-013-4596-8.

[70] Y.M. Ma, H. Yao, D.X. Wei, L.P. Wu, G.Q. Chen, Synthesis, characterization and application of comb-like polyhydroxyalkanoate-graft-poly(N-isopropylacrylamide), Biomacromolecules 17 (2016) 2680–2690. https://pubs.acs.org/doi/10.1021/acs.biomac.6b00724.

[71] H. Yao, D.X. Wei, Y.M. Ma, L.W. Cai, L. Tao, L. Liu, L.P. Wu, G.Q. Chen, Comb-like temperature-responsive polyhydroxyalkanoate-graft-poly(2-dimethylaminoethylmethacrylate) for controllable protein adsorption, Polym. Chem. 7 (2016) 5957–5965. https://pubs.rsc.org/en/content/articlelanding/2016/PY/C6PY01235C#!divAbstract.

[72] L.P. Yu, X. Zhang, D.X. Wei, Q. Wu, X.R. Jiang, G.Q. Chen, Highly efficient fluorescent material based on rare-earth-modified polyhydroxyalkanoates, Biomacromolecules (2019), https://doi.org/10.1021/acs.biomac.8b01722. https://pubs.acs.org/doi/10.1021/acs.biomac.8b01722.

[73] X. Zhang, Z.H. Li, X.M. Che, L.P. Yu, W.Y. Jia, R. Shen, J.C. Chen, Y.M. Ma, G.Q. Chen, Synthesis and characterization of polyhydroxyalkanoate organo/hydrogels, Biomacromolecules (2019), https://doi.org/10.1021/acs.biomac.9b00479. https://pubs.acs.org/doi/10.1021/acs.biomac.9b00479.

[74] H. Yao, L.P. Wu, G.Q. Chen, Synthesis and characterization of electroconductive PHA-graft-graphene nanocomposites, Biomacromolecules 2 (2019) 645–652. https://pubs.acs.org/doi/10.1021/acs.biomac.8b01257.

[75] X.M. Che, D.X. Wei, G.Q. Chen, Superhydrophobic polyhydroxyalkanoates: preparation and applications, Biomacromolecules 109 (2018) 141–150. https://pubs.acs.org/doi/10.1021/acs.biomac.8b01176.