Trends in Biotechnology

CellPress

Review

Grand Challenges for Industrializing Polyhydroxyalkanoates (PHAs)

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Polyhydroxyalkanoates (PHAs) are a diverse family of sustainable bioplastics synthesized by various bacteria, but their high production cost and unstable material properties make them challenging to use in commercial applications. Current industrial biotechnology (CIB) employs conventional microbial chassis, leading to high production costs. However, next-generation industrial biotechnology (NGIB) approaches, based on fast-growing and contamination-resistant extremophilic *Halomonas* spp., allow stable continuous processing and thus economical production of PHAs with stable properties. *Halomonas* spp. designed and constructed using synthetic biology not only produce low-cost intracellular PHAs but also secrete extracellular soluble products for improved process economics. Next-generation industrial biotechnology is expected to reduce the bioproduction cost and process complexity, leading to successful commercial production of PHAs.

Highlights

The high production cost, poor thermal and mechanical properties, and unstable quality of polyhydroxyalkanoates (PHAs) are the grand challenges to be addressed before industrialization.

Next-generation industrial biotechnology (NGIB) based on extremophiles is emerging to meet most of these challenges.

Fast-growing and contamination-resistant *Halomonas* spp. allow open, unsterile, and continuous fermentations to produce PHAs with low-cost and stable properties.

Halomonas spp. constructed by synthetic biology generate large sizes for PHA accumulation and gravity separation or coproduction of extracellular soluble products.

Challenges for Industrial Production of Polyhydroxyalkanoates

Plastic pollutions worldwide increase the demand for biodegradable bioplastics. **Polyhydroxyalkanoates (PHAs)** (see Glossary) are a family of environmentally friendly and sustainable polyesters produced by various microorganisms [1,2]. PHAs have been proposed to partially replace traditional chemical plastics, including polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), to solve pollution issues posed by nondegradable plastics [3]. However, although PHAs have been extensively studied for 30 years, their industrialization is limited due to high production cost. PHAs produced at industrial scale include poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P3HB4HB), and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) [4]. All of these PHAs have disadvantages, including high production cost, poor thermal mechanical properties, and unstable product quality associated with the **current industrial biotechnology (CIB)** process [5,6].

Many efforts have been made to address these challenges [6] and the most competitive one is to develop **next-generation industrial biotechnology (NGIB)**, using fast-growing extremophilic bacteria [7,8]. NGIB operates using low-cost mixed substrates, seawater, and less energy in long-lasting open continuous fermentation processes, aiming to overcome the disadvantages of CIB [8]. Engineered extremophilic ***Halomonas* spp.** grown under a high salt concentration and alkaline pH have been successfully exploited as an example of NGIB.

In addition to NGIB, some important parameters to push PHAs towards commercially competitive production include substrate choice, water and wastewater, oxygen utilization, energy consumption, automation, process complexity, continuity and reproducibility, substrate-to-PHA conversion efficiency, and so on.

This article reviews the production processes and applications of PHAs, current global commercialization companies and their technologies, and the challenges for PHAs industrialization, with

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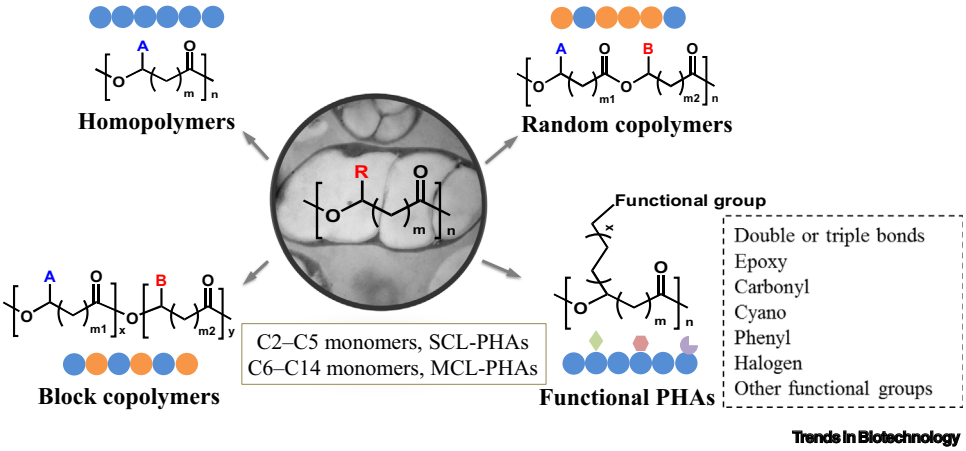

Figure 1. Diverse Polyhydroxyalkanoates (PHAs) Structures, Including Monomer Diversity, Homopolymers, Random and Block Copolymers, Functional Polymers, and Their Various Combinations. Abbreviations: MCL PHAs, medium-chain-length PHAs; SCL PHAs, short-chain-length PHAs.

the emphasis of NGIB strategy to partially overcome these grand challenges. This review provides industrial perspectives for competitive PHAs production.

Structure and Diversity of PHAs

PHAs are structurally diverse polymers of at least 150 hydroxyalkanoates with molecular weights ranging from 5×10^4 to 2×10^7 Da [4,9] (Figure 1). Based on their monomer sizes, PHAs can be divided into **short-chain-length PHAs (SCL PHAs)**, consisting of monomers of two to five carbon atoms (C2–C5), and **medium-chain-length PHAs (MCL PHAs)**, containing monomers from C6 to C14. PHAs can also be synthesized into homopolymers, random copolymers, block copolymers, or functional polymers, containing double or triple bonds, epoxy, carbonyl, cyano, phenyl, and/or halogen groups on the polymer side chains [6,10]. Combinations of these microstructures provide endless variations of structures, functions, and properties, not to mention further chemical modifications [2,11], resulting in a variety of PHAs with advantages and disadvantages compared with traditional chemical plastics (Table 1).

Applications of PHAs

The diversity of PHAs in structures and properties has been explored for a variety of applications, including disposable bioplastics, animal feed, biofuels, 3D printing and smart materials usages, as well as biomedical applications [12,13].

Table 1. Advantages and Disadvantages of PHAs Compared with Traditional Chemical Plastics

	Advantages of PHAs	Disadvantages of PHAs	Refs
Properties	Biodegradability, biocompatibility, diverse structures and properties, edibility, nontoxic degraded products including oligomers and monomers	Poor thermal and mechanical properties, wide molecular weight (Mw) distribution, difficulty to control precise Mw, post-crystallization	[14,17,77,78]
Production process	Sustainable production process using agriculture raw materials dissolved in aqueous solutions, raw material purity not required, room temperature and normal pressure	Complex production process, high energy and freshwater demanding, discontinuous process, inhomogeneous product quality, high biological and chemical oxygen demand (BOD and COD) in wastewater	[8,79,80]

[7]http://life.tsinghua.edu.cn/lifeen/info/1034/1088.htm
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PHAs have been extensively studied as disposable bioplastics for a few decades. The hydrophobicity, gas barrier properties, and nontoxicity make PHAs more attractive than traditional plastics for packaging purposes and disposable consumer goods, especially for food packaging [12]. PHAs are also developed as high-quality textiles and agricultural mulch films, which have been manufactured by Ningbo Tianan Biologic Material in China and Medpha Co. Ltd., respectively. The huge market and increasing demand for disposable bioplastics worldwide facilitates their rapid commercial use, while the major concern is to reduce the production cost to increase their competitiveness with traditional packaging materials.

Recently, one PHA family member, PHB, has been studied and confirmed as a safe and weight-increasing feed additive for marine large yellow croaker fish and land-based weaned piglets [14]. Thus, PHA as a feed additive has promise to reduce microplastic pollution harmful to both marine and land animals.

PHA monomers can be turned into methyl esters such as 3-hydroxybutyrate methyl ester (3HBME) and 3-hydroxyalkanoate methyl ester (3HAME) and developed as biofuels [15]. They can be directly used or mixed with other fuels as a good alternative for petroleum-based fuels.

PHAs are advantageous as 3D printing implants for humans compared with the most commonly used acrylonitrile butadiene styrene (ABS) or polylactide (PLA) due to their favorable thermal and mechanical properties, nontoxicity, and biocompatibility [16]. Chemically modified functional PHAs could be developed as smart materials responding to external stimuli, including temperature, pH, light, and moisture [10,17,18]. A thermal responsive PHA copolymer, polyhydroxyalkanoate-*g*-poly (N-isopropylacrylamide), has been shown to have thermally responsive hydrophilicities and biocompatibilities at different temperatures [19]. Rare-earth (Eu^{3+} and Tb^{3+}) modified fluorescent PHAs exhibit intense photoluminescence properties under UV laser excitation combined with enhanced hydrophilicity and superior biocompatibility [20]. These fields are quite promising to extend diverse applications of PHAs, while it is also challenging to achieve desirable structures and properties of these smart PHAs.

As for high-value-added biomedical applications, PHAs have widespread studies in tissue implants, including artificial heart valves, blood vessels, cartilage or tendons, nerve conduits, esophagus replacements, bone replacements [13,21,22], surgical sutures, porous microspherical implant-scaffolds for microsurgery [23], and organogels/hydrogels [24]. In addition, the major PHA degradation product 3-hydroxybutyrate (3HB) has been proven to exhibit anti-osteoporosis effects, which is promising as a treatment for osteoporosis [25]. PHAs can also be turned into nanoparticles for targeted and controllable drug delivery to the desirable locations [26], for surface display of various target proteins and protein purification [27], or as PHA nano-vaccines [28].

More and more applications of PHAs are under intensive studies [4]. New technologies are required to reduce the PHAs production cost for their bulk applications as packaging material, while for the high-value-added biomedical applications, 3D printing, and smart materials, new PHAs with controllable functional properties are required.

PHA Production

Current Industrial Biotechnology for PHA Production

Several major steps, including strain development, shake flask optimization, laboratory and pilot fermenter studies, followed by industrial scale-up, are involved in the scale-up of PHA production from laboratory to industrial scale for cost control [29] (Figure 2).

Glossary

Current industrial biotechnology (CIB): an industrial biotechnology based on conventional microbial chassis, which produces chemicals, biofuels, and materials using traditional fermentation technology.

***Halomonas* spp.:** halophiles with the ability to rapidly grow under high salt concentration and alkaline pH.

Medium-chain-length PHAs (MCL PHAs): contain monomers of six to fourteen carbon atoms (C6–C14).

Next-generation industrial biotechnology (NGIB): a newly developed industrial biotechnology based on extremophiles, which allows long-lasting, open, and continuous, energy-saving fermentation processes under artificial intelligence control for production of PHAs with stable properties.

Polyhydroxyalkanoates (PHAs): a family of biopolyesters produced by various microorganisms.

Short-chain-length PHAs (SCL PHAs): consist of monomers of two to five carbon atoms (C2–C5).

Standard European Vector Architecture (SEVA): a free database of formatted vector platform, providing a user-friendly, web-based resource of scaffold plasmids for the public (http://seva.cnb.csic.es).

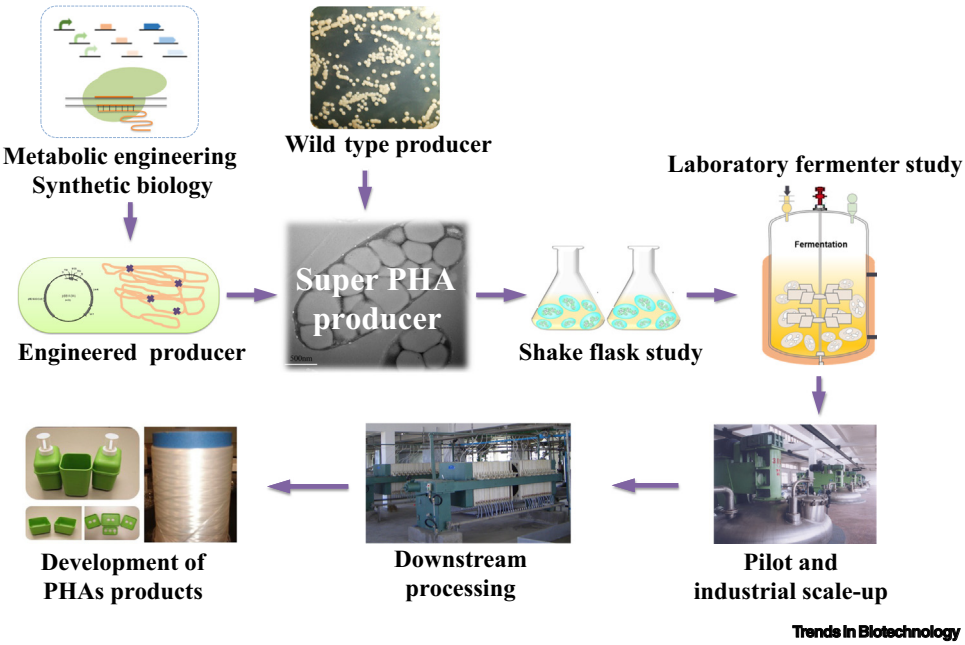

Figure 2. Polyhydroxyalkanoates (PHAs) Production from Laboratory to Industrial Scale and Commercial Exploration. Strain development, shake flask optimization, laboratory and pilot fermenter studies, and then industrial scale-up are involved in the process of PHA industrialization.

PHA production by wild type and engineered bacteria has been extensively investigated. *Alcaligenes latus* and *Ralstonia eutropha* have been employed for SCL PHA production [30], while *R. eutropha* and *Aeromonas hydrophila* have been used to produce PHBHHx [31,32]. *Pseudomonas* spp. are commonly used for MCL PHA production [33]. By knocking out several key genes in the β-oxidation pathway of *Pseudomonas* spp., a fatty acid fed to the bacteria will maintain its original chain length and structure when incorporated into the PHA chains; thus, PHA homopolymers, random or block copolymers, and functional polymers can be produced in the β-oxidation-weakened *Pseudomonas* spp. when related fatty acids are added to their cultures [34]. *Escherichia coli* is also frequently engineered to produce various types of PHAs and has achieved some success [35,36]. All of these super producers of PHAs rely on current industrial biotechnology (CIB), which has led to a new wave of PHAs production and development since the 1980s [37] (Table 2). Among them, *R. eutropha* and recombinant *E. coli* are widely used for PHA production in industrial scales based on CIB and the highest cell density was achieved in *R. eutropha*, with cell dry weight of 232 g/l, while the highest volumetric productivity of 4.63 g/l/h was obtained in recombinant *E. coli* harboring the *phaCAB* operon from *A. latus* for the production of PHB, as indicated in Table 2 [38–40].

Despite the successful industrial scale production, these strains require full sterilization and complicated contamination prevention procedures, making the process quite difficult. In addition, the limited market success of CIB is also attributed to the high cost of glucose substrate with low substrate-to-PHA conversion efficiency, large amount of freshwater consumption, high cost of wastewater treatment, batch or fed-batch cultivation and the associated low productivity, energy intensive aeration, unstable processes and PHA quality, and complicated and energy intensive downstream processes, among others [8]. Thus PHAs produced by CIB still suffers a high cost, which has resulted in a high price of US$4–6/kg, fivefold to sixfold that of petroleum-based plastics [4].

Table 2. Comparison of PHA Productivity in CIB and NGIB Using Different Microbial Producers

Strains	PHA products	Production strategy	Substrate	CDW (g/l)	PHA content (wt%)	Highest volumetric productivity (g/l/h)	Refs
Escherichia coli	Various PHAs	CIB	Glucose	141.6	73	4.63	[39]
Ralstonia eutropha	SCL-PHAs, MCL-PHAs, PHBHHx	CIB	Glucose, fatty acids	232	80	3.14	[40]
Aeromonas hydrophila	PHBHHx	CIB	Fatty acids	43.3	45.2	1.01	[32]
Pseudomonas spp.	MCL-PHAs	CIB	Fatty acids	72.6	51.4	1.91	[81]
Halomonas spp.	SCL-PHAs	NGIB	Glucose	100	60–92	1.67–3.2	[57,66,70]

Recently, extremophilic bacteria, especially halophilic *Halomonas* spp., have become rising stars for cost-reduction and bulk production of PHAs. And NGIB based on extremophiles has been developed to meet the grand challenges for PHAs commercialization associated with CIB [7].

Next-Generation Industrial Biotechnology for PHA Production

NGIB employs contamination-resistant extremophilic bacteria to allow long-lasting, open and continuous, energy-saving bioprocessing, which uses low-cost substrates and less freshwater under artificial intelligence (AI) control (Figure 3, Key Figure) [7,8].

Substrates are major costs in PHA production. If low-cost substrates, such as treated kitchen waste, cellulose hydrolyses, heat-treated activated sludge, syngas or shale gas could be used, PHA production cost would be significantly reduced [41]. Additionally, a high substrate-to-PHA conversion efficiency is important for reducing substrate consumption. Seawater, as a widely available and inexhaustible water source, could be a sustainable alternative to replace limited freshwater in NGIB. Furthermore, an open and unsterile fermentation process without a sterilization procedure saves sterilization energy, allowing the use of low-cost plastic, ceramic, or even cement fermenters instead of the common stainless steel for fermenter construction (Figure 3). Since extremophiles are usually robust and more stable, they permit the growth processes in NGIB to be conducted under a long-lasting continuous condition, with stability maintained by AI controls instead of labor-intensive handling and monitoring processes. AI controls can be achieved by big data collection of bioprocessing parameters followed by machine learning of the controller, which can be equipped in the key points of the production line [42]. The automatic processing by AI controls especially allows a stable product quality, avoiding the inhomogeneity from batch-to-batch fermentation processes that frequently occurs in CIB.

Extremophilic bacteria like acidophiles, alkalophiles, psychrophiles, thermophiles, methanotrophs, xerophiles, gaseous substrate utilizers, halophiles [43–48], and their recombinants are suitable chassis for NGIB [7]. Among these extremophiles, most halophilic microorganisms are both alkaliphilic and halophilic, providing double barriers to prevent microbial contamination [44,49]. *Halomonas bluephagenesis* [50] and *Halomonas campaniensis* [51] are the best studied NGIB chassis due to their contamination resistance, rapid growth, and feasibility for molecular engineering [45,52,53] (Figure 3). For example, in one study, *H. campaniensis* was reported to maintain 65 days of contamination-free growth in artificial seawater under open and continuous conditions. *Halomonas* spp. appear to be suitable chassis to meet these challenges.

Key Figure

Next-Generation Industrial Biotechnology (NGIB) Based on Reprogrammed *Halomonas* spp. for Low-Cost Polyhydroxyalkanoates (PHAs) Production

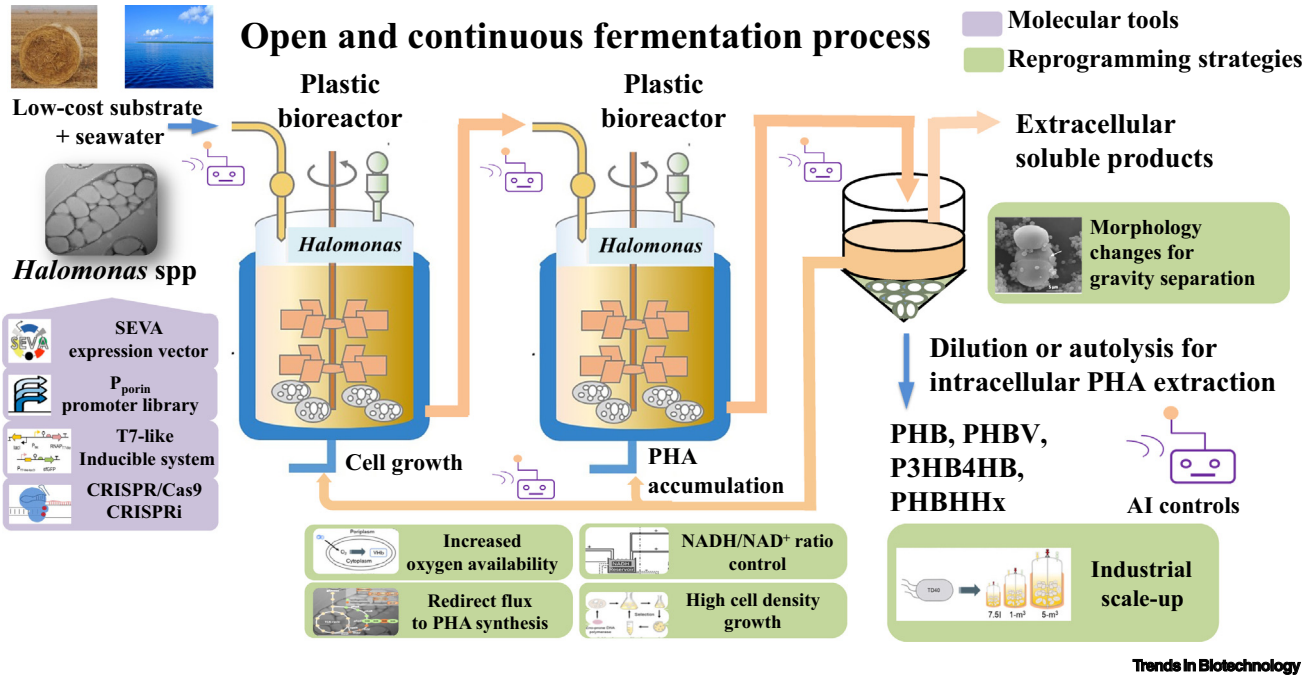

Figure 3. NGIB based on engineered extremophilic *Halomonas* spp. grown on low-cost substrates and seawater under open and continuous process with recycling of culture broth and inducible gravity separation of cells during downstream processing. Bioprocessing is controlled by artificial intelligence (AI), which helps to stabilize the product quality. The coproduction of intracellular PHAs with extracellular products further improves the economics of NGIB. Abbreviations: PHB, poly(3-hydroxybutyrate); P3HB4HB, poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate); PHBHHx, poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate); PHBV, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); SEVA, Standard European Vector Architecture.

A series of molecular engineering tools for *Halomonas* spp. have been developed based on **Standard European Vector Architecture (SEVA)** plasmids for gene expression [54], conjugation [55], a strong constitutive promoter P_{porin} with tunable variants [56], a powerful inducible T7-like expression system [57], and the genome editing tools CRISPR/Cas9 and CRISPRi [58] (Figure 3). *H. bluephagenesis* has been engineered to redirect metabolic flux to PHAs synthesis [59], reduce PHA synthase specificity for PHA diversification [60], increase oxygen uptake via periplasmic expression of *Vitreoscilla* hemoglobin VHb under a strong low-oxygen inducible promoter [61], control the NADH/NAD^+ ratio to enhance PHA accumulation by deleting electron transfer flavoprotein subunits α and β [62], increase cell growth density [63], and change cell morphology for easy downstream processing via overexpressing the cell division inhibitors SulA or MinCD and/or deleting the cytoskeletal protein MreB [64,65]. Combining the strategies of morphology engineering, self-flocculation [66], autolysis [67], and enlarged PHA granular size [68], large cells of *H. bluephagenesis* accumulating lager PHA granules were capable of self-flocculation with gravity precipitation and autolysis, reducing the complexity and cost of downstream processing (Figure 3).

Halomonas spp. have been reprogrammed for production of PHB, PHBV, P3HB4HB, PHBHHx, or functional poly(3-hydroxybutyrate-*co*-3-hydroxyhex-5-enoate) (PHB3HHxE)

[69–71]. Coproduction of PHAs with high-value-added extracellular 5-aminolevulinic acid (ALA) or ectoine is a good option to improve process economics [72–74]. Recombinant *H. bluephagenesis* has been scaled up from 1 l fermenter to 1000 and 5000 l industrial fermenters for PHA production [70], and cells were grown to a high cell density of about 100 g/l cell dry weight (CDW) with stable P3HB4HB content of 60 wt% in the 5000 l bioreactors under open nonsterile conditions [70,75], demonstrating good potential for industrial production.

The current volumetric productivity of *Halomonas* spp.-based NGIB is lower than that of CIB using *R. eutropha* and recombinant *E. coli* (Table 2), which may be attributed to the competitive synthesis of essential ectoine in *Halomonas* spp. and the recycling of culture broth that contains possible toxic byproducts in NGIB process. The comparison indicates the space for productivity improvement of NGIB by efficient supernatant recovery, as well as a high conversion efficiency from substrate to PHAs. Despite all this, PHA production cost will be reduced to US$1.68/kg using NGIB [70], which is lower than that of CIB of US$2.3/kg when recombinant *E. coli* was used [5].

Commercializing PHAs

PHAs have become popular bioplastics for fighting plastic pollution worldwide [3]. Efforts have been made to industrially produce PHB, PHBV, P3HB4HB, and PHBHHx, with various degrees of successes, and companies have come and gone. Recently, several companies have been established to promote the industrialization of PHAs (Table 3).

China started nationwide plastic bans in 2008, with the prohibition of the production, sale, and use of plastic shopping bags with thickness less than 0.025 mm. The plastic ban was taken further in 2020 with nondegradable plastic tableware, hotel supplies, delivery packaging, and microplastic in cosmetics becoming forbidden. The situation promotes the development of PHAs industrialization in China. Seven PHAs companies have been established in China, including TianAn, which has existed for over 20 years, making it the longest existing PHAs producing company. GreenBio and Ecomann are barely surviving in tough economic conditions. PhaBuilder, Medpha, COFCO, and Bluepha have been recently set up to explore NGIB for low-cost PHAs production. So far, five PHA producers based in the USA have been established, although Metabolix was sold to the Korean company CJ. RWDC and Danimer employed recombinant *R. eutropha* grown in fatty acids for PHBHHx production, and Newlight and Full Cycle utilize greenhouse gas and mixed organic waste, respectively, for low-cost production by their unique PHA producers. Most other PHAs producers are trying different ways to exploit various low-cost waste substrates for PHAs production, including kitchen waste, forest waste, shale gas, and agriculture wastes, aiming to reduce production costs. Fortunately, a global organization called Go!PHA has been established to coordinate the promotion of PHAs as a carbon-neutral green bioplastic to address the global plastic pollution issue.

Some producers do not disclose their PHAs production strains, but most of the companies who disclose their strains use recombinant *E. coli*, *R. eutropha*, or *Halomonas* spp. (Table 3). Most companies employ CIB for PHAs production; only three use *Halomonas* spp.-based NGIB for production. Large production facilities have been constructed to produce PHAs in large quantities. The global PHAs production is expected to constantly expand to meet the increasing market demand. However, studies on improving the properties of PHAs are increasingly remaining confidential, as fewer publications are available as public information.

Concluding Remarks and Future Perspectives

Grand challenges for PHA industrialization include their high production cost, unstable material composition and molecular weights, and poor thermal and mechanical properties. These are

Outstanding Questions

How can PHAs with stable properties, including a constant monomer ratio and molecular weight, be obtained from various microbial production processes?

How can intracellular PHAs be coproduced with extracellular products to improve their production economics?

What are the effective strategies to enhance conversion efficiency from substrates to PHAs?

How do we achieve downstream purification of PHAs polymers that is efficient, low-cost, and environmentally friendly?

How can the PHA titer (g/l) and productivity (g/l/h) of NGIB be improved?

Table 3. PHA Commercial Companies

Company	PHA type	Technology	Scale (ton/year)	Websites
Go!PHA, The Netherlands	All types	PHA global promotion	Unknown	gopha.org
PhaBuilder, China	All types	*Halomonas* spp. (NGIB[a])	1000–10 000	www.phabuilder.com
Medpha, China	P3HB4HB	*Halomonas* spp. (NGIB)	100	www.medpha.com.cn
COFCO, China	PHB	*Halomonas* spp. (NGIB)	1000	www.cofco.com
Bluepha, China	PHBHHx	*Ralstonia eutropha* and NGIB	1000	www.bluepha.com
TianAn Biopolymer, China	PHBV	*R. eutropha*	2000	www.tianan-enmat.com
GreenBio, Tianjin, China	P3HB4HB	*Escherichia coli*	10 000	www.tjgreenbio.com
Ecomann, Shenzhen, China	P3HB4HB	*E. coli*	10 000	ecomannbruce.plasway.com
RWDC, Singapore and USA	PHBHHx	*R. eutropha*	Unknown	www.rwdc-industries.com
Danimer Scientific, USA	PHBHHx	*R. eutropha*	10 000	danimerscientific.com
Newlight, USA	PHB	Ocean microbes grown on greenhouse gas	Unknown	www.newlight.com
Full Cycle, USA	PHA[b]	Non-genetically modified bacteria	Unknown	fullcyclebioplastics.com
Metabolix, USA	P3HB4HB	*E. coli*	5000	IP sold to CJ, Korea
BOSK Bioproducts, Canada	PHA[b]	Forest wastes for PHA production	Unknown	www.bosk-bioproducts.com
Genecis, Canada	PHBV	Unknown	Unknown	genecis.co
TerraVerdae Bioworks, Canada	PHA[b]	Unknown	Unknown	terraverdae.com
Kaneka, Japan	PHBHHx	*R. eutropha*	5000	www.kaneka.be
Nafigate, France	PHB	Toxic waste as substrates	Unknown	www.nafigate.com
CJ, Korea	P3HB4HB	*E. coli*	Unknown	www.cj.co.kr
Helian Polymers, The Netherlands	PHB/PHBV	Non-genetically modified bacteria	Unknown	helianpolymers.com
Biocycle, Brazil	PHB	*Bacillus* spp.	100	fapesp.br
Biomer, Germany	PHB	*Alcaligenes latus*	Unknown	biomer.de
Bioextrax, Sweden	PHA[b]	Bioextrax DSP method	Unknown	bioextrax.com
SABIO srl, Italy	PHA[b]	Organic wastes for PHA production	Unknown	www.bio-on.it

[a]Next-generation industrial biotechnology.
[b]Unknown PHA type.

mainly associated with CIB, which suffers from complicated sterilization and unstable batch processes. NGIB based on fast-growing *Halomonas* spp. has been developed to at least partially overcome the challenges for PHA industrialization, which makes the bioprocess more cost-effective and operation friendly, while maintaining stable molecular weights and compositions.

Although the production cost is expected to be remarkably reduced by NGIB, further breakthroughs need to be addressed to eventually realize a strong PHA industrial value chain from upstream production to downstream applications. Efforts have been made to meet these requirements one by one (Table 4).

Table 4. Grand Challenges of CIB Met by NGIB and Further Developments

Items	CIB	NGIB	Further strategies	Refs
Substrates	Expensive substrates	Kitchen waste, cellulose, or activated sludge hydrolysates	Constructions of microorganisms capable of utilizing multiple or mixed substrates	[41,46,51,82,83]
Water	Freshwater	Seawater	Construction of halophiles or robust recombinants	[48–51,82]
Process	Batch process	Continuous process	Contamination-resistant strains	[45]
Energy for sterilization	121°C and high-pressure steam	Unsterile and open process	Contamination-resistant and rapid growing strains	[5]
Energy for aeration	Poor O_2 uptake	Enhanced O_2 uptake	Periplasmic expression of hemoglobin	[61]
Facility	Equipment built by expensive stainless steels	Plastic, ceramic, or cement bioreactors	Development of contamination-resistant strains	[7,8]
Downstream	Continuous centrifugal or filter separation and cell disruption using organic solvents	Gravity separation process, autolysis, and aqueous PHA purification	Morphology engineering for larger cells and larger PHA granules, self-flocculation, and autolysis cells	[65–68]
Wastewater	Expensive wastewater treatment	Recycling wastewater	Self-flocculating strains to conveniently remove cells and reuse broths	[66]
Labor cost	Experienced engineers with high pay	AI control based on big data and deep learning	Robust microorganisms allow wide control errors, data collection, and analysis	[7,42]
Substrate-to-PHA ratio	Low	High	Redirecting and enhancing metabolic flux to the synthesis of target products	[59]
Growth	Slow and low cell density	Fast and high cell density	Changing growth patterns for rapid proliferation, enhancing cell resistance to high-density growth	[63,84]
Product diversity	One strain for one product	One strain for multiple products	Construction of multiple product synthesis pathways, intra- and extracellular products	[45,72,74]
Product quality	Unstable quality	Stable quality, including purity and molecular weight	Stable continuous fermentation and downstream controlled by AI	[5,42]
Product properties	Poor	Flexible	Screening and construction of microorganisms capable of utilizing multiple precursors with various functional groups	[17]

Synthetic biology and morphology engineering will facilitate high cell density and high volumetric productivity and cells can be induced to grow to over 200 g/l CDW containing more than 80 wt% PHAs in large granule sizes with a very high conversion efficiency from substrate to PHA (P/S ratio) of 50% for competitive process economics and convenient downstream development. Recycling the fermentation broth will not only reduce the cost of wastewater treatment but also green the PHA production process, and improved recycling strategies are needed for higher productivity of PHAs (see Outstanding Questions).

Poor thermal and mechanical properties limit the widespread high-value-added applications of PHAs. Microorganisms capable of utilizing multiple precursors with various functional groups can be screened or constructed by synthetic biology approaches to yield functional PHAs with diverse structure and properties.

The automation of industrial production is promising to maintain product stability based on AI controls, which mainly rely on big data collection, analysis, and deep learning procedures. It requires multidisciplinary knowledge and controls throughout the whole production line. Robust microorganisms are also needed to allow wide control errors.

Other methods to improve PHA production economics include coproduction of intracellular PHAs with extracellular products such as the small molecular chemicals ectoine, inositol, or

amino acids [72,76]. This will allow the treatment of wastewater supernatants accompanied by recovery of valuable products with PHAs in the solid biomass.

Furthermore, the unique properties and enzymes of other extremophilic microorganisms besides *Halomonas* spp., can also be further explored for developing NGIB. NGIB offers extensive opportunities for competitive bioproduction and NGIB-based fully automatic bioprocessing plants can be expected.

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