MedCrave Online Journal of Applied Bionics and Biomechanics

Review article

Challenges for polyhydroxyalkanoates production: extremophilic bacteria, waste streams, and optimization strategies

Abstract

Polyhydroxyalkanoates (PHA) remain of high interest as a promising alternative to conventional plastics because they are biodegradable and biocompatible and have similar properties to common plastics. Despite their incredible potential, industrialization of PHA is hampered by the high production cost. To address this issue associated with PHA production cost, researchers have been exploring different approaches. In this review, we propose suggestions to overcome these challenges and highlight opportunities for future research such as producing PHA from sustainable waste streams as inexpensive renewable substrates, optimization strategies using experimental design to improve growth conditions. Furthermore, the uses of extremophilic microorganisms have gained significant attention to reduce the overall cost of PHA.

Volume 7 Issue 1 - 2023

Khouloud Hammami,[1] Yasmine Souissi,[2] Ameur Cherif,[1] Mohamed Neifar[3]
[1]BVBGR-LR11ES31, Higher Institute of Biotechnology of Sidi Thabet (ISBST), University of Manouba, Tunisia
[2]Department of Engineering, German University of Technology in Oman, Oman
[3]APVA-LR16ES20, National School of Engineers of Sfax (ENIS), University of Sfax, Tunisia

Correspondence: Mohamed Neifar, APVA-LR16ES20, National School of Engineers of Sfax (ENIS), University of Sfax, Sfax, Tunisia, Tel 21628762783, Email Mohamed.naifar@gmail.com

Received: June 13, 2023 | **Published:** July 7, 2023

Introduction

Polyhydroxyalkanoates (PHA) are biopolymers and promising alternative to commonly used petroleum-derived plastics. PHA has gained significant interest as eco-friendly bioplastics due to its biocompatibility and biodegradability (Figure 1).[1–3] They can be produced by various microorganisms as a response to unfavorable environmental conditions such as limited nutritional factors like oxygen, nitrogen, phosphorus and excess of carbon substrate and accumulated as intracellular carbon, and energy reserves in form of granules.[4–7] Despite the potential benefits of microbial bioplastics over synthetic plastics, production of PHA still faces limitations, including high production cost.[8,9] Consequently, industrialization of PHA is limited comparison to synthetic plastics.[10] The high production cost can be attributed to several factors such as low conversion efficiency of carbon substrates to PHA, slow growth rates of microorganisms used in the production, challenges associated with downstream separation processes, and high energy demand associated to with complex sterilization processes.[11] In this regard, challenges have been addressed for the development of cost-effective PHA production including engineering bacteria for enhanced PHA biosynthesis. Some studies have engineered strains for the robust production.[12–14] Furthermore, the optimization of fermentation parameters can further enhance PHA production in a cost-effective way. Optimization of PHA production through Response Surface Methodology (RSM) are required to enhance microbial growth and improve the efficiency of PHA production processes through optimization of different fermentation conditions such as substrate, time required for PHA synthesis, inoculum density.[15,16] Another practical strategy to reduce production cost is the use of robust production strains that can thrive in "extreme" environments where the majority of other organisms would not be able to multiply. It is a useful way to lower production cost via simplifying the production process.[17] Next-generation industrial biotechnology (NGIB) being the most competitive approach which involves the use of extremophilic bacteria. Overall, NGIB offers a robust and cost-effective approach to industrial biotechnology.[11,18] Another approach is exploring inexpensive feedstock from various waste streams are more suitable for increased PHA production.[19] Many microorganisms have been reported for the production of PHA using low-cost agricultural feed stock and surplus materials.

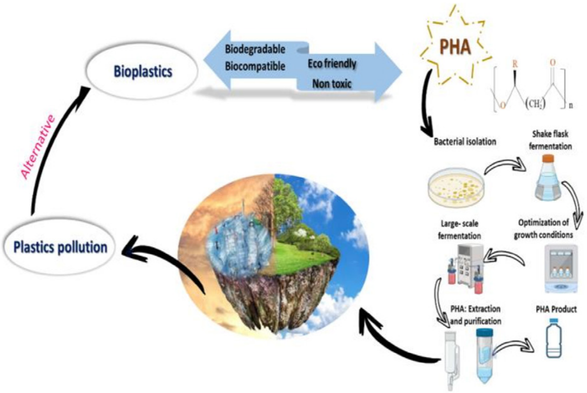

Figure 1 Sustainable alternative of petroleum derived plastics.

I. PHA: structure and properties

PHA are linear polyesters of hydroxyalkanoic acids. According to different monomer compositions, they can be divided into short-chain-length PHA (scl-PHA), consisting of monomers of C2–C5 carbon atoms, and medium-chain-length PHA (mcl-PHA), containing monomers of C6-C14.[3,20]

The physical and chemical properties of scl- and mcl-PHA vary due to differences in the chemical and structural composition of their monomeric units. Therefore, researchers have been exploring various strategies to optimize PHA synthesis pathways and modulate the composition and structure of these polymers to obtain desirable material properties for different applications.[4,21] Scl-PHA, constitute thermoplastic materials, they have characteristics of high hardness and low plasticity, high degree of crystallinity. While mcl-PHA are known as materials with elastomeric and latex-like properties, they have low crystallinity, low tensile strength, low glass transition temperature,

©2023 Hammami et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and build upon your work non-commercially.

and high elongation at break, high viscosity and high plasticity, which broadens the range of applications for mcl-PHA.[1,20–23]

PHA biopolymers possess distinctive characteristics such as biodegradability, biocompatibility, non-toxicity and similar physicochemical properties to conventional petroleum-based plastics.[24,25] PHA can display a range of physicochemical properties, which vary based on the length of their chains and the composition of their monomers which make their material properties highly versatile.[21,23,26] Among scl-PHA, PHB and the copolyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) are best described.[23] The major obstacles that must be overcome before scl-PHA can be widely used are their slow crystallization rates, poor impact resistance. The slow crystallization rates of scl-PHA coupled with their poor impact resistance and rapid aging constitute the key challenges that need to be addressed prior to their widespread use.[21] PHB is a highly crystalline and brittle material with strong hardness and water resistance. The addition of 3-hydroxyvalerate (3HV) or 3-hydroxyhexanoate (3HHx) monomer to PHB significantly improve its unfavorable characteristics and shows better overall properties such as lower melting point, lower crystallinity, greater flexibility, and better tensile properties. Higher HV content in PHA also results in better water sensitivity and oxygen permeability. The proportion of HB and HV in PHA affects its final processing and mechanical properties, and by regulating the monomer composition of PHA, we can synthesize PHA with different processing requirements.[3,27,28]

II. Applications

The inherent properties make PHA an important material having diverse applications in numerous fields including medical and therapeutic applications, packaging, agriculture, food industry.[5,8,29] PHA is utilized as a packaging material in food industry to enhance the shelf life and quality of food products. Also, PHA are highly suitable for medical applications such as tissue engineering applications and even in drug delivery for biomedical and therapeutic purposes due their biodegradability, biocompatibility, and non-toxic nature.[29–32] PHA copolymers, such as P(3HB-HV) and P(3HB-4HB), have been used for implants and microspheres that can release antibiotics and hemoembolizing agents. PHB can be used as a supplement in various applications, including the development of artificial bones.[31]

Numerous companies are involved in industrial scale production of PHA in Germany, Brazil, China, Japan, USA. PHBV was firstly commercialized by Imperial Chemical Industries (ICI) and PHB by Industrial Brazil and Mitsubishi Gas Chemical Company.[33–35]

III. Biosynthesis pathways

Biosynthesis of PHA is highly dependent on the use of carbon source and the expression of the relevant genes involved in the biosynthetic pathways. Synthesis of scl-and mcl-PHA occurs via different metabolic pathways (Figure 2). Indeed, fatty acid oxidation, *de novo* fatty acid synthesis pathway, and glycolysis biosynthesis pathways are involved in the PHA precursors synthesis which is further polymerized into PHA polymer. The formation of PHA biopolymer involves a series of specific enzymatic reactions within the metabolic pathways. For scl-PHA biosynthesis, the enzyme β-ketoacyl CoA thiolase (PhaA) catalyzes the condensation of two acetyl-CoA molecules, obtained by glycolysis, to form acetoacetyl-CoA. Then, the reduction of acetoacetyl-CoA into 3-hydroxybutyryl-CoA takes place by the enzymatic activity of acetoacetyl-CoA reductase (PhaB). Afterwards, PHA synthase (PhaC) polymerize the 3 hydroxybutyryl-CoA to form PHB or PHBV.[24,30] On the other hand, the metabolic pathways involved in mcl-PHA biosynthesis are β-oxidation when using fatty acid carbon source or *de novo* synthesis pathway when using sugars as substrate. β-oxidation pathway generates intermediates such as trans-enoyl-CoA, (S)-3- hydroxyacyl-CoA, and 3-ketoacyl-CoA in which further converted into (R)- hydroxyacyl-CoA by the action of (R)-enoyl-CoA hydratase, 3-hydroxyacyl-CoA epimerase and ketoacyl-CoA reductase, respectively. 3-hydroacyl-CoA is polymerized by PHA synthase into mcl-PHA. Another pathway involved in mcl-PHA is *de novo* synthesis pathway. Indeed, 3-hydroxyacyl-Acyl (ACP), derived from the synthesis of fatty acids from sugars, converted to 3-hydroxyacyl by 3-hydroxyacyl-ACP-CoA transferase, followed by mcl-PHA synthesis by PHA synthase.[30,36]

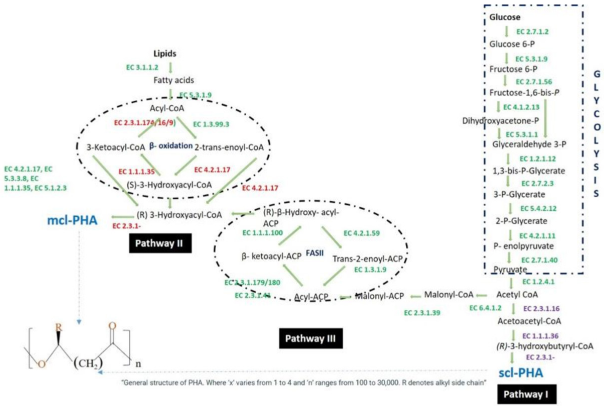

Figure 2 Putative metabolic pathways for scl-PHA and mcl-PHA biosynthesis.

PHA synthases can be classified into four classes depending on their in vivo substrate specificities, subunit composition, and primary amino acid sequences. PhaC I, PhaC III and PhaC IV use precursors to produce scl-PHA and PhaC II use alkane precursors to produce mcl-PHA.[37–39]

IV. PHA Production: challenges and prospects

PHA are synthesized by numerous microorganisms particularly Gram-negative bacteria. Capabilities of bacteria to produce different types of PHA can vary due to their differential sensitivities towards production factors.[30,35] Several microorganisms are responsible for producing PHA as bioplastics, including *Ralstonia eutropha*, *Halomonas* sp., *Bacillus* sp, and *Pseudomonas* sp.[40–43]

However, due to the relatively high production cost in comparison to petrochemical plastics, large-scale production and commercial applications of PHA is limited. In order to reduce the overall expense of PHA, there are various attempts. This includes enhancing microbial strains, optimizing cultivation parameters, use of renewable biomass as well as use of extremophilic bacterium (Figure 3). Genetic engineering strategies have been used to enhance PHA production.[44] The overexpression of related genes can further increase the conversion efficiency of substrates to PHA. The successful knockout of *Halomonas* TD01 resulted in a significant enhancement of the conversion rate of propionic acid into the 3HV fraction within the copolymer which leading to a reduction in the substrate cost.[45] Also, an engineered strain of *Cupriavidus necator* has been shown to accumulate PHA.[46] Furthermore, developing approaches that permit the recovery of PHA by a simple, efficient and less polluting process participate in reducing the cost of downstream processes.[17]

1) Inexpensive feedstocks

The primary factor related to the high production cost is the use of a simple and pure carbon source. In which, carbon source accounts around 40-50% of the total production cost of PHA. The use of waste and renewable biomass contributes to the reduction of high production

Citation: Hammami K, Souissi Y, Cherif A, et al. Challenges for polyhydroxyalkanoates production: extremophilic bacteria, waste streams, and optimization strategies. *MOJ App Bio Biomech.* 2023;7(1):101‒107. DOI: 10.15406/mojabb.2023.07.00181

cost of PHA for large-scale PHA synthesis and also helps in reducing of organic waste in landfills associated with waste disposal.[10,15,42,47–50] Research endeavors are being carried out to use inexpensive feedstocks as suitable substrate for cost-effective production of PHA (Table 1), Among such inexpensive feedstocks, non-edible biomass, by-products, and agro-waste generated from various industrial processes, was already investigated for PHA production such as lignocellulosic biomass,[42] fruit pomace,[51] rice mill effluent,[52] waste frying oils (WFO) from fast food industries[51,53,54] PHA can also be synthesized using plastics waste.[55–58] By using these wastes as carbon sources, PHA production becomes more cost-efficient and resolves waste management challenges and addresses waste management issues.[16,59] A recent study exploits the use of discarded fish scale waste for PHA production by *Bacillus megaterium* NCDC0679 with a maximum PHA yield of 6.33 g/L.[60] Moreover, Allegue et al.[61] 42% of PHA using urban organic waste.

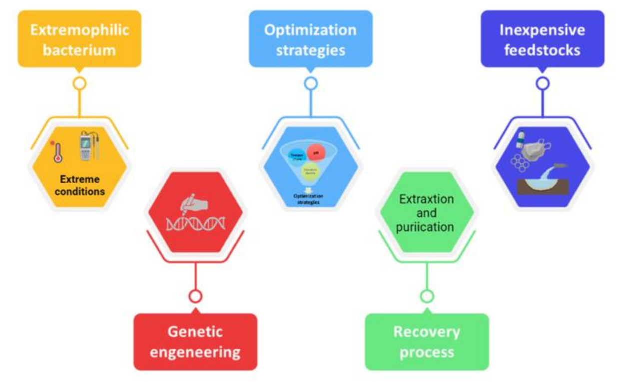

Figure 3 Factors affecting PHA production cost.

2) Extremophilic microorganisms

Another approach has been addressed to reduce cost of biotechnological PHA production process is the use of extremophilic bacteria.[11,18,62] Indeed, microorganisms that are able to thrive in extreme environmental conditions, such as high temperatures, salinity, heavy metal exposure, or acidic/alkaline pH values, are becoming more prominent as potential candidates for PHA biosynthesis in which they reduce the risk of microbial contamination.[39,63] Extremophiles microorganisms capable of producing PHA in an open and unsterile fermentation process, which eliminates the need for sterilization procedures, results in energy savings and enables the use of low-cost materials.[17,18] For instance, a study by Kouřilová et al.[64] has investigated PHA accumulation by two thermophilic species, *Rubrobacter xylanophilus* and *Rubrobacter spartanus*. Furthermore, a halophilic *Haloferax mediterranei* is capable of synthesizing PHA from various inexpensive substrates.[65–67] Halophiles, which are currently considered as potential candidates and best studied cases, possess the remarkable capability to accumulate PHA.[68] Their adaptation to extreme conditions provides them with unique advantages, including reduced extraction cost, lower contamination risks, and the ability to produce PHA using unconventional carbon sources such as food waste, agricultural residues, or industrial by-products.[69] The majority of halophilic microorganisms exhibit characteristics of both alkaliphilic and halophilic properties, which provide dual barriers to effectively prevent microbial contamination.[18] Haloalkalophilic microorganisms offers several advantages in in the production of PHA, easy recovery, and lower cost compared to other extremophiles.[39] For instance, the production of PHB was carried out using an alkaliphilic strain, *Bacillus marmarensis* DSM 21297,[70] and an acidophilic bacterium was used in another study.[71] Halophiles PHA-producer such as *Haloferax mediterannei*[72] and halophilic marine bacteria *Vibrio proteolyticus*[73] were operated in unsterilized conditions. Among halophiles microorganisms, *Halomonas* species has been widely studied. *Halomonas* TD01, a potential PHA producer, exhibits contamination-free growth under unsterile and continuous processes at high pH and high salt concentration. It has been successfully developed as a cost-effective platform for the unsterile and continuous production of chemicals.[12,74] Additionally, *H. campaniensis* LS21 was reported to produce PHA under open and continuous conditions.[75]

Table 1 PHA production by a diverse range of microbial strains involves using various renewable resources

Strain	Substrate	PHA g/L	Polymer type	References
Pseudomonas putida CA-3	Oil styrene from polystyrene pyrolysis	NM	mcl-PHA	55
Bacillus siamensis PD- A10	Orange peel	2.16	PHA	84
Bacillus subtilis JCM 1465	Orange peel	2.93	PHA	84
Halomonas taeanenisis YLGW01	Crude glycerol	10.5	PHB	85
Microbial mixed culture	Olive Oil Mill Waste water	NM	PHBV	86
Pseudomonas resinovorans	Spent coffee grounds	1.6	mcl-PHA	87
Zobellellae tiwanensis DD5	Banana peels	1.13	PHB	43
Pandoraea sp.	Crude glycerol	2.12, 0.21	PHB PHBV	28
Ralstonia eutropha and *Bacillus megaterium*	Carob pods	12.2	PHB	47
Bacillus cereus VK92 *Bacillus cereus* VK98	Rice straw	2.96, 2.51	PHA	88
Acinetobacter junii BP25	Rice mill effluent	3.04	PHB	52
Pseudomonas resinovorans	Fruit pomace	1.4	mcl-PHA	51
Pseudomonas aeruginosa CWS2020	Poultry (chicken feather) waste	4.8	PHB	89
Lysinibacillus sp. RGS	Sugarcane bagasse	5.31	PHB	9
Bacillus siamensis PD-A10	Orange peel	2.16		84
Paraburkholeria sacchari IPT 101	Wood hydrolysate	NM	PHB	90
Bacillus cereus 64-INS	Raw potato starch	2.78	PHB	49
Pseudomonas putida GO1	PET derived terephtalic acid	2.61	mcl-PHA	58
Ideonella sakaiensis 201-F6	PET	0.75	PHB	91

Citation: Hammami K, Souissi Y, Cherif A, et al. Challenges for polyhydroxyalkanoates production: extremophilic bacteria, waste streams, and optimization strategies. *MOJ App Bio Biomech.* 2023;7(1):101–107. DOI: 10.15406/mojabb.2023.07.00181

3) Optimisation strategies

Optimization strategies play a crucial role in enhancing the yield of PHA and improving the cost-effectiveness. Therefore, various mathematical models and statistical tools, such as Response Surface Methodology (RSM), Central Composite Design (CCD), and Plackett-Burman Design (PBD) have been used to optimize growth conditions and maximize PHA production. Indeed, optimizing fermentation parameters enables the identification of optimal conditions for the growth of PHA-producing microorganisms, leading to increased PHA yield and reduced cost of production.[76] RSM is a statistical optimization approach that has emerged as a widely employed experimental design method for describing and optimizing the response.[77] Many microorganisms have reported the effectiveness of RSM in enhancing the biosynthesis of PHA.[78] A study by Ray et al.[79] carried out the optimization of PHA production by *Pannonibacter phragmitetus* ERC8 using glycerol waste, also a genetically modified strain *C. necator* was investigated to produce P(3HB-co-3HHx) copolymers under statistical optimized conditions.[77] Furthermore, Experimental designs carried out to select the best conditions for obtaining PHA by *H. boliviensis*.[69] Studies have used Box-Behnken design (BBD) for optimization of process parameters for higher PHA yield from microorganisms such as *Acinetobacter junii* BP 25 using rice mill effluent,[52] *Burkholderia* sp. ISTR5,[80] marine *Pichia kudriavzevii* VIT-NN02 using banana peels and chicken feather hydrolysate,[81] *Halomonas* sp. YLGW01 using volatile fatty acids.[82] A Plackett Burman Design (PBD) was also used for RSM analysis for screening the significant parameters on PHA production. *Priestia megaterium* POD1 produce 1.431±0.06 g/L PHA employing PBD,[78] the halophilic bacterium *H. boliviensis* employ PBD for optimize variables, agitation, temperature, pH, initial concentration of glucose, nitrogen and KH_2PO_4.[69] *Bacillus endophyticus* is capable of accumulating a maximum yield of 46.57% PHA under optimized conditions.[83] A large number of optimization studies have been conducted, and it has been observed that the optimization is generally done on the various factors such as nutrient composition, inoculum size, and incubation time.[84–91]

Conclusion

(PHA), a biodegradable and biocompatible polymer that is a used in various applications due to their functionality, physico-chemical properties. The high cost of production is still an issue which poses a significant constraint on large-scale PHA production. Future research should prioritize the use of sustainable waste streams as cost-effective carbon substrates, optimize fermentation conditions, and focus on engineering microbes for efficient substrate utilization to enhance PHA production potential. Additionally, the emphasis should be placed on engineering PHA-producing microorganisms, particularly extremophiles that are resistant to contamination, and developing specialized engineering techniques for these extremophiles.

Acknowledgements

None

Funding

None

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Cha D, Ha HS, Lee SK. Metabolic engineering of Pseudomonas putida for the production of various types of short-chain-length polyhydroxyalkanoates from levulinic acid. *Bioresour Technol*. 2020;309:123332.
2. Che L, Jin W, Zhou X, et al. Current status and future perspectives on the biological production of polyhydroxyalkanoates. *Asia-Pac J Chem Eng*. 2023;18(3):e2899.
3. Wei T, Fang Q. Regulating the monomer of polyhydroxyalkanoate from mixed microbial culture: with particular emphasis on substrate composition: A review. *Environmental Engineering Research*. 2022;27(5):210333.
4. Kumar M, Rathour R, Singh R, et al. Bacterial polyhydroxyalkanoates: Opportunities, challenges, and prospects. *Journal of Cleaner Production*. 2020;263:121500.
5. Coppola G, Gaudio MT, Lopresto CG, et al. Bioplastic from renewable biomass: a facile solution for a greener environment. *Earth systems and environment*. 2021;5:231–251.
6. Sabapathy PC, Devaraj S, Meixner K, et al. Recent developments in Polyhydroxyalkanoates (PHAs) production–A review. *Bioresource technology*. 2020;306:123132.
7. Mitra R, Xu T, Chen GQ, et al. An updated overview on the regulatory circuits of polyhydroxyalkanoates synthesis. *Microbial Biotechnol*. 2022;15(5):1446–1470.
8. Zhang X, Lin Y, Wu Q, et al. Synthetic biology and genome-editing tools for improving PHA metabolic engineering. *Trends in biotechnology*. 2020;38(7):689–700.
9. Saratale RC, Si-Kyung S, Ganesh D, et al. A comprehensive overview and recent advances on polyhydroxyalkanoates (PHA) production using various organic waste streams. *Bioresource technology*. 2021;325:124685.
10. Bhatia SK, Otari SV, Jeon JM, et al. Biowaste-to-bioplastic (polyhydroxyalkanoates): Conversion technologies, strategies, challenges, and perspective. *Bioresource Technology*. 2021;326:124733.
11. Chen GQ, Jiang XR. Engineering microorganisms for improving polyhydroxyalkanoate biosynthesis. *Current opinion in biotechnology*. 2018;53:20–25.
12. Tan D, Wu Q, Chen JC, et al. Engineering *Halomonas* TD01 for the low-cost production of polyhydroxyalkanoates. *Metabolic engineering*. 2014;26:34–47.
13. Brojanigo S, Gronchi N, Cazzorla T, et al. Engineering *Cupriavidus necator* DSM 545 for the one-step conversion of starchy waste into polyhydroxyalkanoates. *Bioresource technology*. 2022;347:126383.
14. Beckers V, Poblete-Castro I, Tomasch J, et al. Integrated analysis of gene expression and metabolic fluxes in PHA-producing *Pseudomonas putida* grown on glycerol. *Microbial Cell Factories*. 2016;15(73):1–18.
15. Sabapathy PC, Devaraj S, Parthipan A, et al. Polyhydroxyalkanoate production from statistically optimized media using rice mill effluent as sustainable substrate with an analysis on the biopolymer's degradation potential. *International journal of biological macromolecules*. 2019;126:977–986.
16. Mahato RP, Kumar S, Singh P. Production of polyhydroxyalkanoates from renewable resources: a review on prospects, challenges and applications. *Archives of Microbiology*. 2023;205(5):172.
17. Zheng Y, Chen JC, Ma YM, et al. Engineering biosynthesis of polyhydroxyalkanoates (PHA) for diversity and cost reduction. *Metabolic engineering*. 2020;58:82–93.
18. Tan D, Wang Y, Tong Y, et al. Grand challenges for industrializing polyhydroxyalkanoates (PHAs). *Trends in biotechnology*. 2021;39(9):953–963.

19. Kannah R, Yukesh K, Dinesh M, et al. Production and recovery of polyhydroxyalkanoates (PHA) from waste streams- A review. *Bioresource Technology*. 2022;366:128203.
20. Bedade DK, Edson CB, Gross RA. Emergent approaches to efficient and sustainable polyhydroxyalkanoate production. *Molecules*. 2021;26(11):3463.
21. Choi TR, Park YL, Song HS, et al. Fructose-based production of short-chain-length and medium-chain-length polyhydroxyalkanoate copolymer by arctic *Pseudomonas* sp. B14-6. *Polymers*. 2021;13(9):1398.
22. Yang S, Li S, Jia X. Production of medium chain length polyhydroxyalkanoate from acetate by engineered *Pseudomonas putida* KT2440. *Journal of Industrial Microbiology and Biotechnology.* 2019;46(6):793–800.
23. Koller M. Polyhydroxyalkanoate biosynthesis at the edge of water activitiy-haloarchaea as biopolyester factories. *Bioengineering*. 2019;6(2):34.
24. Kaur L, Khajuria R, Parihar L, et al. Polyhydroxyalkanoates: Biosynthesis to commercial production- A review. *Journal of Microbiology, biotechnology and Food sciences*. 2021;6(4):1098–1106.
25. Ponnusamy S, Viswanathan S, Periyasamy A, et al. Production and characterization of PHB-HV copolymer by *Bacillus thuringiensis* isolated from Eisenia foetida. *Biotechnology and Applied Biochemistry*. 2019;66(3):340–352.
26. Reddy VUN, Ramanaiah SV, Reddy MV, et al. Review of the developments of bacterial medium-chain-length polyhydroxyalkanoates (mcl-PHAs). *Bioengineering*. 2022;9(5):225.
27. Grigore ME, Grigorescu RM, Iancu L, et al. Methods of synthesis, properties and biomedical applications of polyhydroxyalkanoates: a review. *Journal of Biomaterials Science, Polymer Edition*. 2019;30(9):695–712.
28. De Paula FC, Kakazu S, de Paula CBC, et al. Polyhydroxyalkanoate production from crude glycerol by newly isolated *Pandoraea* sp. *Journal of King Saud University-Science*. 2017;29(2):166–173.
29. Kalia VC, Patel SKS, Shanmugam R, et al. Polyhydroxyalkanoates: Trends and advances toward biotechnological applications. *Bioresource Technology*. 2021;326:124737.
30. Prajapati K, Nayak R, Shukla A, et al. Polyhydroxyalkanoates: An exotic gleam in the gloomy tale of plastics. *Journal of Polymers and the Environment.* 2021;29(7):2013–2032.
31. Vermeer CM, Rossi E, Tamis J, al. From waste to self-healing concrete: A proof-of-concept of a new application for polyhydroxyalkanoate. *Resources, Conservation and Recycling*. 2021;164:105206.
32. Elmowafy E, Abdal-Hay A, Skouras A, et al. Polyhydroxyalkanoate (PHA): Applications in drug delivery and tissue engineering. *Expert review of medical devices*. 2019;16(6):467–482.
33. Endres, Hans-Josef, Andrea Siebert-Raths. “Basics of PHA.” *Bioplastics magazine* 6.5. 2011:43–45.
34. Du C, Sabirova J, Soetaert W, et al. Polyhydroxyalkanoates production from low-cost sustainable raw materials. *Current Chemical Biology*. 2012;6(1):14–25.
35. Yadav B, Chavan S, Atmakuri A, et al. A review on recovery of proteins from industrial wastewaters with special emphasis on PHA production process: sustainable circular bioeconomy process development. *Bioresource technology*. 2020;317:124006.
36. Muhammadi S, Afzal M, Hameed S. Bacterial polyhydroxyalkanoates-eco-friendly next generation plastic: production, biocompatibility, biodegradation, physical properties and applications. *Green Chemistry Letters and Reviews*. 2015;8(3–4):56–77.
37. Jain R, Tiwari A. Bioplastics for use in medical industry. *Asian Journal of Pharmaceutics*. 2014:139.
38. Mezzolla V, D’Urso OF, Poltronieri P. Role of PhaC type I and type II enzymes during PHA biosynthesis. *Polymers*. 2018;10(8):910.
39. Obulisamy PK, Mehariya S. Polyhydroxyalkanoates from extremophiles: a review. *Bioresource Technology*. 2021;325:124653.
40. Riaz S, Rhee KY, Park SJ. Polyhydroxyalkanoates (PHAs): biopolymers for biofuel and biorefineries. *Polymers*. 2021;13(2):253.
41. Sen KY, Baidurah S. Renewable biomass feedstocks for production of sustainable biodegradable polymer. *Current Opinion in Green and Sustainable Chemistry*. 2021;27:100412.
42. Al-Battashi HS, Annamalai N, Sivakumar N, et al. Lignocellulosic biomass (LCB): a potential alternative biorefinery feedstock for polyhydroxyalkanoates production. *Reviews in Environmental Science and Bio/Technology*. 2019;18:183–205.
43. Maity S, Das S, Mohapatra S, et al. Growth associated polyhydroxybutyrate production by the novel *Zobellellae tiwanensis* strain DD5 from banana peels under submerged fermentation. *International journal of biological macromolecules*. 2020;153:461–469.
44. Wang J, Liu S, Huang J, et al. Genetic engineering strategies for sustainable polyhydroxyalkanoate (PHA) production from carbon-rich wastes. *Environmental Technology & Innovation*. 2023;30:103069.
45. Fu XZ, Tan D, Aibaidula G, et al. Development of *Halomonas* TD01 as a host for open production of chemicals. *Metabolic Engineering.* 2014;23:78–91.
46. Bhatia SK, Kim JH, Kim MS, et al. Production of (3-hydroxybutyrate-co-3-hydroxyhexanoate) copolymer from coffee waste oil using engineered *Ralstonia eutropha*. *Bioprocess and biosystems engineering*. 2018;41(2):229–235.
47. Manikandan NA, Pakshirajan K, Pugazhenthi G. A closed-loop biorefinery approach for polyhydroxybutyrate (PHB) production using sugars from carob pods as the sole raw material and downstream processing using the co-product lignin. *Bioresource technology*. 2020;307:123247.
48. Bhola S, Arora K, Kulshrestha S, et al. Established and emerging producers of PHA: Redefining the possibility. *Applied Biochemistry and Biotechnology*. 2021;193(11):3812–3854.
49. Ali I, Jamil N. Enhanced biosynthesis of poly (3-hydroxybutyrate) from potato starch by *Bacillus cereus* strain 64-INS in a laboratory-scale fermenter. *Preparative Biochemistry and Biotechnology*. 2014;44(8):822–833.
50. Altun M, Çelebi M, Şen S, et al. Characterization of polyhydroxyalkanoate-based composites derived from waste cooking oil and agricultural surplus. *Polymer Composites*. 2023;44(7):4055–4068.
51. Follonier S, Goyder MS, Silvestri AC, et al. Fruit pomace and waste frying oil as sustainable resources for the bioproduction of medium-chain-length polyhydroxyalkanoates. *International journal of biological macromolecules*. 2014;71:42–52.
52. Sabapathy PC, Devaraj S, Parthipan A, et al. Polyhydroxyalkanoate production from statistically optimized media using rice mill effluent as sustainable substrate with an analysis on the biopolymer’s degradation potential. *International journal of biological macromolecules*. 2019;126:977–986.
53. Vastano M, Corrado I, Sannia G, et al. Conversion of no/low value waste frying oils into biodiesel and polyhydroxyalkanoates. *Scientific reports*. 2019;9(1):13751.
54. Gamal RF, Abdelhady HM, Khodair TA, et al. Semi-scale production of PHAs from waste frying oil by *Pseudomonas fluorescens* S48. *Brazilian Journal of Microbiology*. 2013;44(2):539–549.

55. Ward PG, Goff M, Donner M, et al. A two step chemo-biotechnological conversion of polystyrene to a biodegradable thermoplastic. *Environmental science & technology*. 2006;40(7):2433–2437.

56. Goff M, Ward PG, Oconnor K. Improvement of the conversion of polystyrene to polyhydroxyalkanoate through the manipulation of the microbial aspect of the process: a nitrogen feeding strategy for bacterial cells in a stirred tank reactor. *Journal of biotechnology*. 2007;132(3):283–286.

57. Kenny ST, Runic JN, Kaminsky W, et al. Up-cycling of PET (polyethylene terephthalate) to the biodegradable plastic PHA (polyhydroxyalkanoate). *Environmental science & technology*. 2008;42(20):7696–7701.

58. Kenny ST, Runic JN, Kaminsky W, et al. Development of a bioprocess to convert PET derived terephthalic acid and biodiesel derived glycerol to medium chain length polyhydroxyalkanoate. *Applied microbiology and biotechnology*. 2012;95(3):623–633.

59. Altun M. Polyhydroxyalkanoate production using waste vegetable oil and filtered digestate liquor of chicken manure. *Prep Biochem Biotech.* 2019;49(5):493–500.

60. Umesh M, Suresh S, Sarojini S, et al. A sustainable approach for fish waste valorization through polyhydroxyalkanoate production by *Bacillus megaterium* NCDC0679 and its optimization studies. *Biomass Conversion and Biorefinery*. 2022:1–13.

61. Allegue LD, Ventura M, Melero JA, et al. Unraveling PHA production from urban organic waste with purple phototrophic bacteria via organic overload. *Renewable and Sustainable Energy Reviews*. 2022;166:112687.

62. Pernicova I, Novackova I, Sedlacek P, et al. Introducing the newly isolated bacterium *Aneurinibacillus* sp. H1 as an auspicious thermophilic producer of various polyhydroxyalkanoates (PHA) copolymers–1. Isolation and Characterization of the Bacterium. *Polymers*. 2020;12(6):1235.

63. Koller M, Mukherjee A. A new wave of industrialization of PHA biopolyesters. *Bioengineering*. 2022;9(2):74.

64. Kouřilová X, Schwarzerová J, Pernicová I, et al. The first insight into polyhydroxyalkanoates accumulation in multi-extremophilic *Rubrobacter xylanophilus* and *Rubrobacter spartanus*. *Microorganisms*. 2021;9(5):909.

65. Cui Y-W, Zhang H-Y, Ji S-Y, et al. Kinetic analysis of the temperature effect on polyhydroxyalkanoate production by Haloferax mediterranei in synthetic molasses wastewater. *J Polym Environ*. 2017;25:277–285.

66. Ghosh S, Gnaim R, Greiserman S, et al. Macroalgal biomass subcritical hydrolysates for the production of polyhydroxyalkanoate (PHA) by *Haloferax mediterranei*. *Bioresour Technol*. 2019;271:166–173.

67. Wang K, Zhang R. Production of polyhydroxyalkanoates (PHA) by *Haloferax mediterranei* from food waste derived nutrients for biodegradable plastic applications. *J Microbiol Biotechnol*. 2021;31(2):338–347.

68. Koller M. Production of polyhydroxyalkanoate (PHA) biopolyesters by extremophiles? *MOJ Polymer Science*. 2017;1(2):69–85.

69. Arcila-Echavarría DC, Lu-Chau TA, Gómez-Vanegas NA, et al. Optimization of nutritional and operational conditions for producing PHA by the halophilic bacterium *halomonas boliviensis* from oil palm empty fruit bunch and gluten hydrolysates. *Waste and Biomass Valorization*. 2022;13:1589–1597.

70. Atakav Y, Pinar O, Kazan D. Investigation of the Physiology of the Obligate Alkaliphilic *Bacillus Marmarensis* GMBE 72T Considering its Alkaline Adaptation Mechanism for Poly (3-Hydroxybutyrate) Synthesis. *Microorganisms*. 2021;9(2):462.

71. Mieszkin S, Pouder E, Uroz S, et al. Acidisoma silvae sp. nov. and *Acidisoma cellulosilytica* sp. nov., two acidophilic bacteria isolated from decaying wood, hydrolyzing cellulose and producing poly-3-hydroxybutyrate. *Microorganisms*. 2021;9(10):2053.

72. Alsafadi D, Alhesan JSA, Mansoura A, et al. Production of polyhydroxyalkanoate from sesame seed wastewater by sequencing batch reactor cultivation process of *Haloferax mediterranei*. *Arabian Journal of Chemistry*. 2023;16(4):104584.

73. Hong JW, Song HS, Moon YM, et al. Polyhydroxybutyrate production in halophilic marine bacteria Vibrio proteolyticus isolated from the Korean peninsula. *Bioprocess and biosystems engineering*. 2019;42(4):603–610.

74. Tan D, Xue YS, Aibaidula G, et al. Unsterile and continuous production of polyhydroxybutyrate by *Halomonas* TD01. *Bioresource technology*. 2011;102(17):8130–8136.

75. Yue H, Ling C, Yang T, et al. A seawater-based open and continuous process for polyhydroxyalkanoates production by recombinant *Halomonas campaniensis* LS21 grown in mixed substrates. *Biotechnology for biofuels*. 2014;7(1):1–12.

76. Lhamo P, Behera SK, Mahanty B. Process optimization, metabolic engineering interventions and commercialization of microbial polyhydroxyalkanoates production–A state-of-the art review. *Biotechnology Journal*. 2020;16(9):e2100136.

77. Trakunjae C, Boondaeng A, Apiwatanapiwat W, et al. Statistical optimization of P (3HB-co-3HHx) copolymers production by *Cupriavidus necator* PHB− 4/pBBR_CnPro-phaC Rp and its properties characterization. *Scientific reports*. 2023;13(1):9005.

78. Sehgal R, Kumar A, Gupta R. Bioconversion of Rice Husk as a Potential Feedstock for Fermentation by *Priestia megaterium* POD1 for the Production of Polyhydroxyalkanoate. *Waste and Biomass Valorization*. 2023:1–14.

79. Ray S, Prajapati V, Patel K, et al. Optimization and characterization of PHA from isolate *Pannonibacter phragmitetus* ERC8 using glycerol waste. *International journal of biological macromolecules*. 2016;86:741–749.

80. Morya R, Sharma A, Kumar M, et al. Polyhydroxyalkanoate synthesis and characterization: A proteogenomic and process optimization study for biovalorization of industrial lignin. *Bioresource Technology*. 2021;320(Part B):124439.

81. Ojha N, Das N. Process optimization and characterization of polyhydroxyalkanoate copolymers produced by marine *Pichia kudriavzevii* VIT-NN02 using banana peels and chicken feather hydrolysate. *Biocatalysis and Agricultural Biotechnology.* 2020;27:101616.

82. Jeon JM, Son YS, Chang L, et al. Polyhydroxyalkanoate production by *Halomonas* sp. YLGW01 using volatile fatty acids: a statistical approach to apply for food-waste water. *Biomass Conversion and Biorefinery*. 2022:1–11.

83. Geethu M, Chandrashekar HR, Divyashree MS. Statistical optimisation of polyhydroxyalkanoate production in *Bacillus endophyticus* using sucrose as sole source of carbon. *Archives of Microbiology*. 2021;203(10):5993–6005.

84. Vijay R, Tarika K. Microbial production of polyhydroxyalkanoates (PHAs) using kitchen waste as an inexpensive carbon source. *Biosciences Biotechnology Research Asia*. 2019;16(1):155–166.

85. Kim B, Oh SJ, Hwang JH, et al. Polyhydroxybutyrate production from crude glycerol using a highly robust bacterial strain *Halomonas* sp. YLGW01. *International Journal of Biological Macromolecules*. 2023;236:123997.

86. Gameiro T, Sousa F, Silva FC, et al. Olive oil mill wastewater to volatile fatty acids: statistical study of the acidogenic process. *Water, Air, & Soil Pollution*. 2015;226(115):1–13.

87. Kang BJ, Jeon JM, Bhatia SK, et al. Two-Stage Bio-Hydrogen and Polyhydroxyalkanoate Production: Upcycling of Spent Coffee Grounds. *Polymers*. 2023;15(3):681.

88. Van Thuoc D, Chung NT, Hatti-Kaul R. Polyhydroxyalkanoate production from rice straw hydrolysate obtained by alkaline pretreatment and enzymatic hydrolysis using *Bacillus* strains isolated from decomposing straw. *Bioresources and Bioprocessing*. 2021;8(1):1–11.

89. Murugan S, Duraisamy S, Balakrishnan S, et al. Production of eco-friendly PHB-based bioplastics by *Pseudomonas aeruginosa* CWS2020 isolate using poultry (chicken feather) waste. *Biologia Futura*. 2021;72(4):497–508.

90. Dietrich K, Dumont MJ, Orsat V, et al. Consumption of sugars and inhibitors of softwood hemicellulose hydrolysates as carbon sources for polyhydroxybutyrate (PHB) production with *Paraburkholderia sacchari* IPT 101. *Cellulose*. 2019;26:7939–7952.

91. Fujiwara R, Sanuki R, Ajiro H, et al. Direct fermentative conversion of poly (ethylene terephthalate) into poly (hydroxyalkanoate) by *Ideonella sakaiensis*. *Scientific Reports*. 2021;11(1):19991.

Citation: Hammami K, Souissi Y, Cherif A, et al. Challenges for polyhydroxyalkanoates production: extremophilic bacteria, waste streams, and optimization strategies. *MOJ App Bio Biomech.* 2023;7(1):101–107. DOI: 10.15406/mojabb.2023.07.00181