INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

BIOPLASTIC FROM NATURE: MICROBIAL PRODUCTION OF THE BIOPLASTIC POLYHYDROXYBUTYRATE (PHB) FROM SOIL-DWELLING ISOLATED STRAINS AND THE FABRICATION OF BIODEGRADABLE THIN FILMS

Original Article

Arifa Sharif[1], Aqeel bhutto[1], Shahla Karim Baloch[2]*, Zohra Fatima Memon[2], Nighat Seema Soomro[3], Muharram Ali Qambrani[1], Allah Jurio Khaskheli[2], Bilqees Magsi[2], Nafeesa Baloch[4], Marvi zohaib[5]

[1] Department of Biotechnology & Genetic engineering, University of Sindh, Jamshoro, Pakistan.
[2]Department of Biotechnology, Sindh Agriculture University, Tando Jam, Pakistan.
[3]Department of Agronomy, Sindh Agriculture University, Tando Jam, Pakistan.
[4]Department of Soil Science, Sindh Agriculture University, Tando Jam, Pakistan.
[5]Department of Microbiology, University of Sindh, Jamshoro, Pakistan.

Corresponding Author: Shahla Karim Baloch, Department of Biotechnology, Sindh Agriculture University, Tando Jam, Pakistan, 70060. shahlabaloch@gmail.com

Conflict of Interest: None

Grant Support & Financial Support: None

Acknowledgment: The authors gratefully acknowledge the support of Sindh Agriculture University, Tandojam, for providing field and technical assistance during the study.

ABSTRACT

Background: The global dependency on synthetic plastics has resulted in significant environmental pollution due to their persistence and poor biodegradability. As an alternative, biodegradable bioplastics such as polyhydroxybutyrate (PHB) offer promising eco-friendly solutions. PHB, a bacterial polyester, can be synthesized and extracted from microbial sources using renewable substrates, providing a sustainable option to replace petroleum-derived plastics.

Objective: The objective of this study was to isolate PHB-producing bacteria from soil and characterize the extracted PHB both chemically and physically.

Methods: Soil samples were collected from the IBGE garden, and a total of 15 bacterial isolates were cultured on nutrient agar. PHB-producing strains were identified using 0.05% Sudan Black B staining, and five isolates showed positive results. These PHB-positive strains were cultivated in PHB production media containing glucose as the carbon source. PHB extraction was performed using sodium hypochlorite and chloroform, and the yield was calculated based on cell dry weight (CDW). Chemical characterization was conducted using Fourier Transform Infrared Spectroscopy (FTIR), and the physical morphology of electrospun PHB nanofibers was analyzed using Scanning Electron Microscopy (SEM).

Results: Among all isolates, 5AR yielded the highest PHB production with 0.53 g/L PHB and 1.04 g/L CDW, resulting in a 51.96% yield. Other isolates showed PHB yields as follows: 17AR (31.70%), 27AR (15.09%), 29AR (21.21%), and 45AR (11.76%). FTIR analysis revealed strong absorption peaks at 1723 cm^{-1}, 1720 cm^{-1}, 2946 cm^{-1}, and 1242 cm^{-1}, confirming PHB presence. SEM analysis showed successful fabrication of nanofibers from extracted PHB.

Conclusion: This study confirms the potential of soil bacteria in producing PHB and supports their use in bioplastic production. The extracted PHB showed comparable properties to standard PHB, indicating feasibility for eco-friendly applications.

Keywords: biodegradable plastics, cell dry weight, Fourier Transform Infrared Spectroscopy, nanofibers, polyhydroxybutyrate, scanning electron microscopy, Sudan Black B staining.

INTRODUCTION

The rising urgency of global environmental challenges has significantly influenced the development of novel materials and sustainable processes, particularly in the context of reducing ecological footprints and promoting green chemistry (1). The widespread persistence of conventional plastics in the environment, their resistance to degradation, and the release of toxic gases upon incineration pose serious threats to both ecosystems and human health. Furthermore, the reliance on finite petroleum resources and the increasing limitations in landfill capacity have heightened the demand for biodegradable alternatives derived from renewable sources (2). Among these, the pursuit of eco-friendly polymers has garnered significant momentum, especially in addressing the limitations of synthetic plastics, which contribute substantially to greenhouse gas emissions and long-term environmental pollution (3). In response to these concerns, biopolymers such as polyhydroxybutyrate (PHB) have emerged as promising alternatives. PHB is a thermoplastic polyester classified under the broader group of polyhydroxyalkanoates (PHAs), which are intracellularly accumulated by various bacterial species as energy and carbon reserves, particularly under nutrient-limiting conditions involving nitrogen, phosphorus, and carbon (4). Despite their environmental advantages, the production of PHB via microbial fermentation remains cost-prohibitive when compared to conventional petrochemical-based plastics. However, advancements in fermentation and downstream processing technologies have significantly improved the cost-effectiveness and yield of PHB production, thus making it more feasible for large-scale applications (5-8).

Bioplastics like PHB possess several desirable properties, including biodegradability, thermoplasticity, water resistance, and most notably, biocompatibility. These properties render PHB not only suitable for general environmental applications but also highly favorable in the biomedical field. PHB molecules are naturally present across a broad spectrum of biological cells and are non-toxic, immunologically inert, and capable of integrating into both aqueous and hydrophobic cellular environments. As a result, PHB has potential applications in a wide array of medical devices such as temporary stents, bone plates, sutures, scaffolds for tissue regeneration, drug delivery systems, and other implantable materials (9-12). Unlike traditional plastics, which present considerable challenges in disposal and recycling, PHB-based materials degrade naturally without releasing harmful byproducts, thus offering an environmentally responsible solution to plastic waste management (13). Their ability to decompose through microbial action eliminates the need for elaborate sorting or incineration, further emphasizing their sustainability. PHAs, especially PHB, not only represent a promising alternative to synthetic plastics but also contribute toward integrated waste management strategies and reduced environmental burden. Given the growing interest in sustainable biomaterials and the need for clinically safe, biodegradable, and biocompatible alternatives to petroleum-derived plastics, this study aims to explore the microbial synthesis, properties, and biomedical potential of PHB. The objective is to rationalize its role as a versatile biomaterial with particular emphasis on its applications in the medical and pharmaceutical domains.

METHODS

STERILIZATION OF MEDIA AND GLASSWARE: All culture media and distilled water utilized during this study were sterilized using an autoclave at 121°C under 15 lbs/in² pressure for 15–20 minutes. Glassware, including Petri dishes, culture tubes, and flasks, was thoroughly cleaned and subsequently autoclaved to prevent contamination. In addition, instruments such as forceps, inoculating loops, and spreaders were sterilized by flaming before each use to ensure aseptic conditions.

ACCUMULATION OF SAMPLES: Soil samples were selected as the primary source for isolating polyhydroxybutyrate (PHB)-producing bacterial strains. Approximately 15–20 grams of soil were collected from various regions of the IBGE garden using sterile spatulas and transferred into sterilized centrifuge tubes. Each sample was clearly labeled and immediately stored at –4°C until further processing.

DILUTION OF SOIL SAMPLES: To isolate specific bacterial strains efficiently, a serial dilution method was employed. Initially, 1 gram of soil was suspended in 99 ml of sterilized distilled water and placed in a water bath at 37°C for 5 minutes. From this, 1 ml was transferred to a tube containing 9 ml sterile water, labeled "A." Without allowing soil particles to settle, 1 ml from tube A was transferred to another 9 ml sterile water tube labeled "B." This serial dilution process was continued for three additional tubes to decrease microbial concentration gradually. The final dilution tube was used to inoculate agar plates for isolating PHB-producing bacteria.

INOCULATION: A volume of 0.5 ml from the diluted soil samples was aseptically inoculated on three different culture media: nutrient agar, Luria-Bertani (LB) agar, and nutrient-rich agar. Each sample was prepared in triplicates and incubated at 37°C for 48 hours. All plates were clearly labeled to ensure traceability.

SUB-CULTURING: Following incubation, bacterial colonies were assessed based on morphological features such as size, color, and edge characteristics. Distinct colonies were selected and sub-cultured on fresh sterile media to obtain pure isolates. This streaking procedure was performed under a laminar airflow cabinet to maintain sterility and prevent airborne contamination.

BACTERIAL SCREENING: Preliminary screening of biopolymer-producing bacteria was conducted using Sudan III staining. Two solutions were prepared for this purpose. Solution A comprised 0.5 g of Sudan III dissolved in 75 ml of 95% ethanol, heated in a water bath, and then diluted to 100 ml with distilled water. After filtration, it served as a stock solution. Solution B (0.05%) was prepared by mixing 0.05 ml of Solution A with 9.95 ml of ethanol. This working solution was applied directly to bacterial colonies on culture plates and allowed to react for 30 minutes. Excess dye was rinsed with 96% ethanol. Colonies producing PHB appeared dark blue, while non-PHB producers remained white. Additional microscopic screening was performed on heat-fixed smears counterstained with safranin, with granules visualized at 10x and 40x magnifications.

PRODUCTION OF PHB PRODUCING BACTERIA AND SEED INOCULUM

SEED INOCULUM: Isolates identified as PHB-positive were used for seed inoculum preparation. A loopful of each PHB-positive isolate was inoculated into 5 ml of sterile broth media in labeled test tubes and incubated at 37°C for 72 hours to allow sufficient bacterial growth for downstream applications.

MEDIUM FOR PRODUCTION OF PHB: PHB fermentation was carried out using a defined production medium containing (g/L): Glucose 20.0, $Na_2HPO_4 \cdot 12H_2O$ 4.8, KH_2PO_4 2.65, $(NH_4)_2SO_4$ 4.0, $MgSO_4 \cdot 7H_2O$ 0.3, $CaCl_2 \cdot 2H_2O$ 0.01, $FeSO_4 \cdot 7H_2O$ 0.01. The pH was adjusted to 7.0 ± 0.2 using NaOH or HCl. The medium was sterilized at 121°C for 15 minutes and transferred into 250 ml Erlenmeyer flasks. Each flask was inoculated with seed inoculum post-sterilization and incubated at 37°C for 72 hours.

DRY WEIGHT OF CELLS: After the incubation period, cultures were harvested by centrifugation at 7000 rpm for 30 minutes at 4°C. The resulting cell pellets were washed with phosphate-buffered saline (PBS) and centrifuged again to obtain a purified cell mass. The biomass was allowed to dry at room temperature until a constant dry weight was achieved.

EXTRACTION PROCESS: PHB extraction was performed using sodium hypochlorite and chloroform. The dried biomass pellet was treated with sodium hypochlorite and heated in a water bath at 80°C for 60 minutes to lyse cells and eliminate non-PHB materials. The lysate was washed with acetone:alcohol (v/v) and re-centrifuged multiple times to ensure purity. The final dried pellet was dissolved in near-boiling chloroform in a 1:5 ratio. The chloroform solution was filtered and left at room temperature for solvent evaporation, leaving purified PHB. The yield was quantified using the formula:
% PHB = (Dry weight of extracted PHB / Cell dry weight) × 100 (14–17)

CHARACTERIZATION OF PHB BY FTIR: The extracted PHB was characterized chemically using Fourier-transform infrared spectroscopy (FTIR). The dried PHB sample was ground into a fine powder and scanned over a wavelength range of 400–4000 cm^{-1}. Absorption peaks corresponding to functional groups were analyzed and recorded using the software connected to the FTIR instrument.

ELECTROSPINNING

PREPARATION OF ELECTROSPINNING SOLUTION: For the fabrication of PHB nanofibers, 1 gram of purified PHB was dissolved in 10 ml of chloroform to make a 10% (w/v) solution. The mixture was stirred overnight on a magnetic stirrer to ensure complete homogenization.

ELECTROSPINNING PROCESS: The electrospinning setup consisted of a high-voltage power supply, a 10 ml syringe with an 18-gauge stainless steel needle connected to a syringe pump, and an aluminum foil-wrapped hardboard collector. Optimized parameters included a 6 ml PHB solution volume, a 15 cm needle-to-collector distance, a flow rate of 1 ml/hr, and an applied voltage of 20 kV. Electrospinning was performed until the entire solution was converted into nanofibers, which deposited as a white membrane on the collector. The membrane was air-dried for 48 hours before removal with sterile forceps and stored in airtight, sterile bags.

CHARACTERIZATION OF NANOFIBERS: Scanning electron microscopy (SEM) was used to evaluate the morphology and diameter of the fabricated nanofibers. The acquired images were further analyzed using ImageJ software to calculate fiber diameter. A histogram was plotted using Origin software, showing the distribution along with mean and standard deviation. The study was conducted following biosafety and ethical standards approved by the Institutional Review Board (IRB).

RESULTS

ISOALTION OF PHBP RODUCING BACTERIA FROM SOIL: Soil served as a diverse microbial reservoir, allowing the successful isolation of 50 morphologically distinct bacterial colonies after 48 hours of incubation on nutrient media. Each colony was sub-cultured individually to obtain pure cultures for further characterization. The isolates exhibited a range of colony morphologies, confirming the diversity of soil microbiota capable of growing on simple nutrient formulations (1,2).

SCREENING OF PHB PRODUCING BACTERIA: Sudan Black B dye was used to screen for PHB-producing bacterial strains based on intracellular lipid accumulation. Out of the 15 isolates selected from garden soil samples, only 5 developed dark black pigmentation upon staining, indicating the presence of PHB granules. These results were further validated by microscopic examination, where intracellular granules were distinctly visualized at both 10x and 40x magnifications. These five isolates were subsequently used for downstream PHB production experiments.

BIOPOLYMER PRODUCTION: Quantitative analysis of PHB production revealed that isolate 5AR produced the highest amount of PHB with a cell dry weight (CDW) of 1.04 g/L and a PHB yield of 0.53 g/L, resulting in a conversion efficiency of 51.96%. Isolate 17AR exhibited a CDW of 0.41 g/L and a PHB yield of 0.13 g/L with 31.70% efficiency. Other isolates, including 27AR, 29AR, and 45AR, demonstrated comparatively lower yields at 15.09%, 21.21%, and 11.76%, respectively. Notably, there was no significant variation in cell dry weight across all isolates, suggesting that PHB yield is more influenced by metabolic capacity than biomass accumulation. These findings are consistent with earlier reports on PHB production by Bacillus species in glucose-enriched media (3).

FTIR: Fourier-transform infrared spectroscopy (FTIR) analysis confirmed the chemical identity of the extracted PHB biopolymer. Prominent peaks were observed at 1727 cm^{-1} and 1281 cm^{-1}, indicative of ester carbonyl functional groups. Additional bands at 1380 cm^{-1} and 1455 cm^{-1} corresponded to methyl (CH_3) and methylene (CH_2) deformations, while the range of 1228–1286 cm^{-1} was associated with C–O–C and C–O bond vibrations. The appearance of these functional groups confirmed the successful extraction and structural fidelity of PHB (4).

ELECTROSPINING: Electrospinning of the extracted PHB produced uniform nanofibrous membranes. The average diameter of the PHB nanofibers was calculated to be 180.5 ± 57.8 nm using SEM images analyzed via ImageJ software. The nanofiber morphology was smooth and continuous. It was noted that polymer concentration had a direct influence on fiber diameter, and further optimization may allow tailoring of fiber dimensions for specific biomedical or industrial applications.

Table 1: The yield % of biopolymer (PHB) extracted from soil bacteria

ISOLATES NO	CDW g/L	PHB g/L	YIELD %
Isolate no: 5AR	1.04	0.53	51.96%
Isolate no: 17AR	0.41	0.13	31.70%
Isolate no: 27AR	0.53	0.08	15.09%
Isolate no: 29AR	0.33	0.07	21.21%
Isolate no: 45AR	0.51	0.06	11.76%

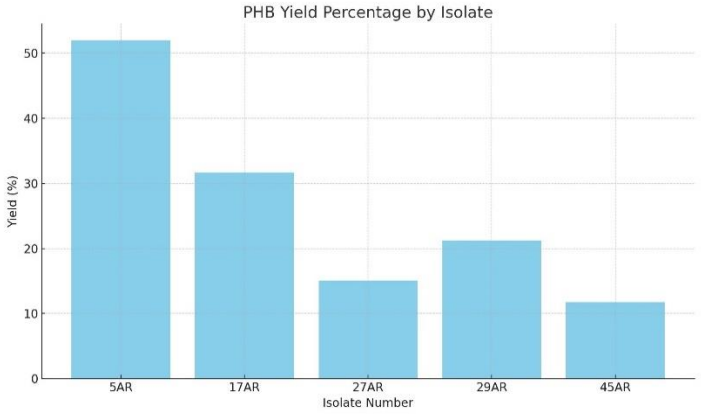


Figure 1PHB Yield Percentage by Isolate

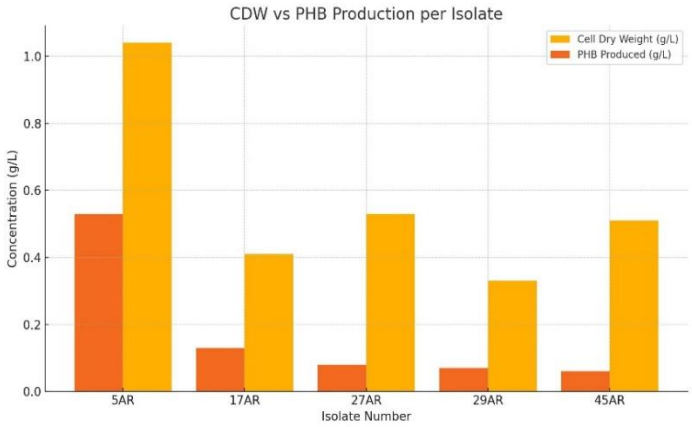
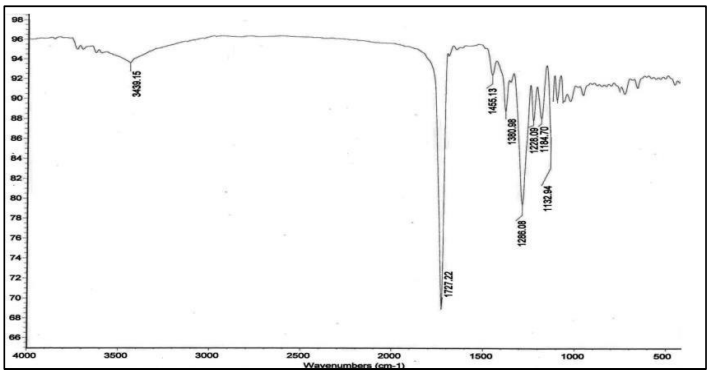


Figure 2 CDW vs PHB Production per Isolate



FTIR results of extracted PHB

DISCUSSION

The present study reaffirmed the potential of soil as a prolific source of polyhydroxybutyrate (PHB)-producing bacteria, aligning well with previous findings that highlighted the richness of soil microbial communities in synthesizing biopolymers and other valuable biomolecules (16). Consistent with earlier studies, the use of Sudan Black B dye for primary screening proved to be an efficient and selective approach in identifying lipid-accumulating bacterial colonies. From 15 soil isolates, five were confirmed to produce PHB based on black pigmentation and subsequent microscopic evaluation, demonstrating a comparable yield of PHB-positive isolates as previously reported in literature involving larger isolate pools (17). Among the screened isolates, isolate 5AR emerged as the most productive,

yielding 0.53 g/L of PHB from a total biomass of 1.04 g/L, reflecting a conversion efficiency of 51.96%. This yield falls within the spectrum reported for similar strains under optimized fermentation conditions and reinforces the feasibility of using simple fermentation media supplemented with glucose as a carbon source to support efficient PHB biosynthesis. The observed variation in yield among isolates, despite relatively uniform cell dry weights, indicated strain-specific metabolic capabilities rather than biomass-driven production, a trend that has also been previously reported (17,18).

The extraction methodology employed in this study combined sodium hypochlorite digestion with chloroform solubilization, a widely accepted approach known for producing PHB of relatively high purity. While the use of chloroform provides cleaner yields compared to sodium hypochlorite alone, it remains a chemically intensive process with environmental and health risks. Nonetheless, the dual-phase method used here has previously demonstrated recovery rates above 90% in similar studies (19,20). Although alternative greener solvents are being explored in recent literature, chloroform remains a benchmark for comparative PHB recovery due to its efficacy (21). Chemical characterization of the extracted biopolymer via FTIR spectroscopy showed definitive absorption peaks in the 1723–1281 cm^{-1} range, corresponding to ester carbonyl and C–O functional groups. These spectral features were in strong agreement with published IR spectra for PHB, confirming the structural identity of the extracted material (22). This provided validation for both the extraction and the fermentation protocols used.

Further, the application of electrospinning to the extracted PHB successfully resulted in the fabrication of nanofibrous membranes, with morphological assessment revealing smooth fiber surfaces and an average diameter of 180.5 ± 57.8 nm. While the study used a 10% (w/v) chloroform-PHB solution, the resulting fiber dimensions and uniformity suggest a high-quality polymer suitable for biomedical or packaging applications. The reproducibility and controllability of fiber morphology via electrospinning were supported by literature that associates polymer concentration and electrospinning parameters with fiber diameter and distribution (23). However, optimization of solution properties and electrospinning conditions could further enhance the mechanical and functional properties of the fabricated nanomembranes. One of the strengths of this study was the integration of microbial isolation, polymer extraction, structural characterization, and material fabrication within a single workflow, demonstrating the practical conversion of a bacterial bioproduct into a functional material. Additionally, the use of glucose as a cost-effective and accessible carbon source added economic feasibility to the fermentation process.

Despite these strengths, the study faced limitations that warrant consideration. First, the number of bacterial isolates screened was relatively limited, potentially overlooking other high-yielding strains. Expanding the screening pool could improve the likelihood of identifying more efficient producers. Secondly, no statistical analyses were performed to compare yields or fiber characteristics, which limits the rigor of quantitative comparisons. Furthermore, the study did not assess the mechanical properties, degradation behavior, or cytocompatibility of the nanofibrous membrane, which are crucial for validating its application in biomedical or environmental contexts. Future studies should focus on optimizing fermentation parameters such as pH, temperature, and incubation duration to enhance PHB yield. Comparative analysis using alternative, greener solvents for extraction may also help mitigate environmental concerns. Additionally, evaluating the nanomaterial for mechanical strength, biocompatibility, and degradation kinetics would extend its translational value for medical and industrial use. In conclusion, the study demonstrated the potential of soil-derived bacterial strains for PHB production and confirmed the usability of extracted biopolymer in advanced fabrication processes. However, broader screening, process optimization, and application-level validation remain necessary to fully harness the potential of biopolymer-producing soil bacteria in scalable, eco-friendly manufacturing systems.

CONCLUSION

This study successfully demonstrated the potential of soil as a rich source of PHB-producing bacteria and highlighted the effectiveness of using simple screening, fermentation, and extraction methods to obtain biopolymers suitable for material fabrication. Among the isolates examined, certain strains exhibited promising capabilities for PHB production when provided with a glucose-based medium, validating the objective of identifying efficient microbial candidates. The extracted biopolymer was not only confirmed through chemical characterization but was also effectively utilized to fabricate nanofibrous membranes via electrospinning, suggesting its practical applicability in various industrial and biomedical domains. Overall, the findings contribute to the ongoing pursuit of sustainable, biodegradable alternatives to conventional plastics and present a feasible foundation for future scale-up and functional testing of PHB-based materials.

AUTHOR CONTRIBUTION

Author	Contribution
Arifa Sharif	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Aqeel bhutto	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Shahla Karim Baloch*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Zohra Fatima Memon	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Nighat Seema Soomro	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muharram Ali Qambrani	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Allah Jurio Khaskheli	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Bilqees Magsi	Writing - Review & Editing, Assistance with Data Curation
Nafeesa Baloch	Writing - Review & Editing, Assistance with Data Curation
Marvi zohaib	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Miao J, Zhu Y, Li W, Che R, Zong X, Li J, et al. Reductive soil disinfestation influences microbial aging of low-density polyethylene and polyhydroxyalkanoate microplastics and microbial communities in plastispheres. J Environ Manage. 2024;372:123331.
2. Pattnaik S, Dash D, Mohapatra S, Pati S, Devadarshini D, Samal S, et al. Reclamation of chromium-contaminated soil by native Cr(VI)-reducing and PHA-accumulating Bacillus aryabhattai CTSI-07. Int Microbiol. 2024;27(3):731-42.
3. Ibrahim R, Aranjani JM, Prasanna N, Biswas A, Gayam PKR. Production, isolation, optimization, and characterization of microbial PHA from Bacillus australimaris. Sci Rep. 2025;15(1):8395.
4. Shah S, Kumar A. Production and characterization of polyhydroxyalkanoates from industrial waste using soil bacterial isolates. Braz J Microbiol. 2021;52(2):715-26.
5. Sullivan KP, Werner AZ, Ramirez KJ, Ellis LD, Bussard JR, Black BA, et al. Mixed plastics waste valorization through tandem chemical oxidation and biological funneling. Science. 2022;378(6616):207-11.
6. Vannini C, Rossi A, Vallerini F, Menicagli V, Seggiani M, Cinelli P, et al. Microbial communities of polyhydroxyalkanoate (PHA)-based biodegradable composites plastisphere and of surrounding environmental matrix: a comparison between marine (seabed) and coastal sediments (dune sand) over a long-time scale. Sci Total Environ. 2021;764:142814.
7. Tyubaeva PM, Varyan IA, Gasparyan KG, Romanov RR, Yurina LV, Vasilyeva AD, et al. Life Cycle of Functional All-Green Biocompatible Fibrous Materials Based on Biodegradable Polyhydroxybutyrate and Hemin: Synthesis, Service Life, and the End-of-Life via Biodegradation. ACS Appl Bio Mater. 2024;7(4):2325-37.
8. Venu Gopala Kumari S, Pakshirajan K, Pugazhenthi G. Key insights into mechanism and kinetics of biodegradation of poly (3-hydroxybutyrate)-based nanocomposite films in natural soil and river water environments. Bioresour Technol. 2024;409:131238.
9. Chhetri G, Kim HJ, Jeon JM, Yoon JJ. Isolation of Massilia species capable of degrading Poly(3-hydroxybutyrate) isolated from eggplant (Solanum melongena L.) field. Chemosphere. 2024;368:143776.
10. Fang J, Sheng Z, Liu J, Li C, Lyu T, Wang Z, et al. Interference of microplastics on autotrophic microbiome in paddy soils: Shifts in carbon fixation rate, structure, abundance, co-occurrence, and assembly process. J Hazard Mater. 2024;474:134783.

11. Gęsicka A, Gutowska N, Palaniappan S, Oleskowicz-Popiel P, Łężyk M. Enrichment of mixed methanotrophic cultures producing polyhydroxyalkanoates (PHAs) from various environmental sources. Sci Total Environ. 2024;912:168844.
12. Brtnicky M, Kucerik J, Skarpa P, Mustafa A, Siddiqui MH, Hammerschmiedt T, et al. Dose-dependent effects of poly-3-hydroxybutyrate on soil quality and maize development: A trade-off between soil quality and crop productivity. Ecotoxicol Environ Saf. 2025;295:118131.
13. Palucha N, Fojt J, Holátko J, Hammerschmiedt T, Kintl A, Brtnický M, et al. Does poly-3-hydroxybutyrate biodegradation affect the quality of soil organic matter? Chemosphere. 2024;352:141300.
14. Afshar SV, Boldrin A, Christensen TH, Corami F, Daugaard AE, Rosso B, et al. Disintegration of commercial biodegradable plastic products under simulated industrial composting conditions. Sci Rep. 2025;15(1):8569.
15. Lascu I, Mereuță I, Chiciudean I, Hansen H, Avramescu SM, Tănase AM, et al. Complete genome sequence of Photobacterium ganghwense C2.2: A new polyhydroxyalkanoate production candidate. Microbiologyopen. 2021;10(2):e1182.
16. Chang YC, Venkateswar Reddy M, Suzuki H, Terayama T, Mawatari Y, Seki C, et al. Characterization of Ralstonia insidiosa C1 isolated from Alpine regions: Capability in polyhydroxyalkanoates degradation and production. J Hazard Mater. 2024;471:134348.
17. Parada-Pinilla MP, Ferreira MA, Roncallo JC, Santos SN, Melo IS, Assef ANB, et al. Biopolymer production by halotolerant bacteria isolated from Caatinga biome. Braz J Microbiol. 2021;52(2):547-59.
18. Rogala MM, Gawor J, Gromadka R, Kowalczyk M, Grzesiak J. Biodiversity and Habitats of Polar Region Polyhydroxyalkanoic Acid-Producing Bacteria: Bioprospection by Popular Screening Methods. Genes (Basel). 2020;11(8).
19. Oliveira PR, Mendoza PX, Crespo JDS, Daitx TDS, Carli LN. Biodegradation study of poly(hydroxybutyrate-co-hydroxyvalerate)/halloysite/oregano essential oil compositions in simulated soil conditions. Int J Biol Macromol. 2024;277(Pt 1):133768.
20. Kim J, Gupta NS, Bezek LB, Linn J, Bejagam KK, Banerjee S, et al. Biodegradation Studies of Polyhydroxybutyrate and Polyhydroxybutyrate-co-Polyhydroxyvalerate Films in Soil. Int J Mol Sci. 2023;24(8).
21. Brtnicky M, Pecina V, Kucerik J, Hammerschmiedt T, Mustafa A, Kintl A, et al. Biodegradation of poly-3-hydroxybutyrate after soil inoculation with microbial consortium: Soil microbiome and plant responses to the changed environment. Sci Total Environ. 2024;946:174328.
22. Fernandes M, Salvador AF, Vicente AA. Biodegradation of PHB/PBAT films and isolation of novel PBAT biodegraders from soil microbiomes. Chemosphere. 2024;362:142696.
23. Khumthong I, Siripornadulsil W, Siripornadulsil S. Bacteria isolated from soil degrade low-density polyethylene for growth and polyhydroxyalkanoate synthesis. J Environ Manage. 2025;380:125072.

