RESEARCH PAPER

Isolation And Optimization Of PHB Producing Bacteria From Paddy Soil For Sustainable Bioplastic Production

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ABSTRACT

Background: The increasing addition of petroleum-based plastics have been a significant environmental concern because of their non-biodegradable nature and long persistence in ecosystems. Biodegradable polymers like polyhydroxyalkanoates (PHAs) have emerged as promising substitutes to conventional plastics. In these, polyhydroxybutyrate (PHB) is one of the most widely reviewed microbial biopolymers because of its biodegradability, biocompatibility, and thermoplastic properties. PHB is made by various microbes as an intracellular carbon and energy storage compound under conditions of excess carbon and nutrient limitation. Environmental sources like soil ecosystems harbor diverse microbial communities capable of producing PHB. In particular, paddy soil represents a nutrient-rich ecological niche with high microbial diversity, leading to a potential reservoir for effective PHB-producing bacteria.

Objective: The present paper aimed to isolate and identify PHB-producing bacteria from paddy soil and to optimize cultural settings prompting to PHB production for potential application in the production of sustainable bioplastic.

Methods: Soil samples from paddy fields were collected, serially diluted, and then spread plated on nutrient agar for the purpose of bacterial isolation. The isolates have been assessed for PHB synthesis utilizing Nile Blue A fluorescence staining and Sudan Black B staining. The possible PHB-producing isolates were subjected to morphological and biochemical characterisation employing standard microbiological procedures. Additional optimization studies were carried out to assess the effect of various carbon sources, pH levels, nitrogen sources, and incubation temperatures on PHB synthesis.

Results: Various bacterial isolates have been successfully isolated from paddy soil samples, with particular strains showing positive outcomes for PHB accumulation via staining methods. The morphological and biochemical characterisation demonstrated that the isolates were predominantly Gram-negative rod-shaped bacteria. Optimization experiments revealed that certain carbon sources significantly boosted PHB formation, while nitrogen limitation further promoted polymer buildup. Optimal PHB generation was seen with neutral to slightly alkaline pH and modest incubation temperatures.

Conclusion: The findings indicate that paddy soil provides an exciting source of PHB-producing bacteria. Optimization of nutritional and environmental conditions significantly boosted PHB production, indicating the possible application of these isolates for eco-friendly bioplastic manufacturing and sustainable disposal of waste applications.

Keywords: Polyhydroxybutyrate, Biopolymer, PHB-producing bacteria, Soil isolates, Bioplastic, Characterization

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Background and Introduction

The extensive use of synthetic plastics over the past several decades has led to in substantial environmental issues because of their non-biodegradable nature and long-term persistence in natural ecosystems (Rana et al., 2025). Conventional plastics produced from petroleum-based resources are resilient to microbial decomposition and build in terrestrial and aquatic ecosystems, leading to severe ecological damage. The rising global demand for plastics, coupled with insufficient waste management systems, has led to concerns surrounding plastic pollution, microplastic contamination, and the accompanying risks to human health and biodiversity. Therefore, there is significant scientific and industry interest in the development of environmentally viable alternatives to conventional plastics (Ahmad et al., 2025). Biodegradable polymers have emerged as feasible alternatives for petroleum-derived plastics since they can be degraded naturally by microorganisms into environmentally benign products that include carbon dioxide, water, and biomass (Megha et al., 2024). Among these biodegradable polymers, polyhydroxyalkanoates (PHAs) include a type of microbial polyesters produced by diverse bacteria as internal storage molecules. These polymers build up within bacterial cells under conditions where carbon sources are abundant but other critical nutrients like phosphorus, nitrogen, or oxygen are scarce. PHAs exhibit multiple properties similar to ordinary plastics, like thermoplasticity, biodegradability, and biocompatibility, which make them ideal for a wide range of industrial applications (Kapoor et al., 2025).

Polyhydroxybutyrate (PHB) is the most thoroughly studied and commercially relevant member of the PHA family. PHB is generated by various microbes as an internal energy and carbon reserve substance that helps

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bacteria survive under harsh environmental conditions. Structurally, PHB is a linear polyester composed of hydroxybutyrate monomers, and it displays material characteristics comparable to polypropylene (Wang et al., 2024). Due to its biodegradability and non-toxic nature, PHB has drawn substantial attention as a viable replacement to conventional plastics in numerous areas notably agriculture, packaging, biomedical devices, and drug delivery systems. In biomedical applications, PHB is particularly beneficial due to its biocompatibility and capacity to breakdown safely throughout biological systems (Wang et al., 2025).

Microorganisms capable of making PHB are extensively dispersed in natural environments like freshwater, soil, marine ecosystems, and industrial waste sites. Various bacterial taxa comprising Bacillus, Pseudomonas, Alcaligenes, Azotobacter, and Cupriavidus have been shown to accumulate substantial amounts of PHB under adequate nutritional conditions (Shokr et al., 2023). The productivity of PHB generation depends on the microbial species and environmental conditions affecting their metabolic pathways. Thus, the exploration of natural habitats for novel and efficient PHB-producing bacteria remains an essential aspect of research in microbial biotechnology (Abidin et al., 2025).

Soil ecosystems are extremely rich in microbial diversity and offer a significant source of metabolically versatile microorganisms. Among distinct soil environments, paddy soil is regarded as an exceptionally dynamic ecosystem characterized by variable oxygen levels, high organic matter content, and various microbial communities (Pedrinho et al., 2024). These conditions stimulate the growth of microbes with unique metabolic capabilities, including the capacity to produce intracellular storage polymers such as PHB. The existence of plentiful organic substrates and periodic nutrient constraints in paddy fields offers optimal conditions for bacteria to generate PHB as a survival strategy (Wang et al., 2022). Thus, paddy soil can serve as a viable reservoir for identifying effective PHB-producing bacterial strains.

Screening and detection of PHB-producing bacteria from environmental samples often utilize an amalgam of microbiological and biochemical approaches. Staining methods like Sudan Black B and Nile Blue A are extensively employed for the fast detection of intracellular PHB granules in bacterial cells. These methods provide the initial identification of potential PHB producers, which are then further defined through physiological, morphological, and biochemical investigations. In addition, optimization of culture conditions like carbon sources, pH, nitrogen sources, and temperature is vital for boosting PHB accumulation in bacterial cells. Carbon source supply plays a vital role in supplying precursors for polymer synthesis, while nitrogen limitation often stimulates metabolic pathways that increase PHB accumulation (Bektas et al., 2023).

Despite substantial improvements in microbial bioplastic research, the large-scale commercialization of PHB is still limited due to relatively high production costs comparing to conventional plastics. One of the primary techniques for enhancing the economic feasibility of the production of PHB is the development of effective microbial strains capable of accumulating larger polymer yields under optimum growth circumstances (Ventura & Ventura, 2024). Therefore, the isolation and characterisation of novel PHB-producing bacteria from natural habitats remains an essential research focus.

Thus, this study concentrates on the isolation and identification of PHB-generating bacteria from paddy soil and the optimization of culture conditions impacting PHB production. The study intends to find suitable bacterial strains capable of collecting PHB and to investigate the effects of various nutritional and environmental variables on polymer synthesis. The outcomes of this study may lead to the development of sustainable microbial techniques for biodegradable plastic manufacture and boost future efforts toward ecologically acceptable alternatives to conventional plastics.

Materials and Methods

Sample Collection: Paddy soil samples were collected at various agricultural locations like Maduranthakam, Tanjore, Trichy, and Kalvoy. Soil samples has been collected aseptically from the upper 5–10 cm layer of paddy fields and put into sterile containers. The samples were taken to the laboratory and stored at 4 °C for further analysis.

Isolation of Bacterial Strains: Bacterial isolates were acquired utilizing the serial dilution and spread plate approach. Around 1 g of soil sample was kept in sterile distilled water and serially diluted. Aliquots of accurate dilutions were distributed on nutrient agar plates and incubated at 37 °C for 24–48 h. Distinct bacterial colonies varied in form, color, and texture were selected and subcultured frequently to generate pure cultures. A total of eight bacterial isolates were collected and designated as S1–S8.

Screening for PHB-Producing Bacteria

Sudan Black B Staining: Preliminary screening of isolates for intracellular lipid inclusions was conducted using Sudan Black B staining. Bacterial smears were made on clean glass slides, thermally fixed, and stained with Sudan Black B solution. The slides were washed with ethanol and counterstained with safranin. Microscopic inspection was conducted out under oil immersion. Dark blue or black intracellular granules suggested the presence of lipid inclusions suggestive of PHB buildup.

Nile Blue A Staining: Confirmation of PHB buildup was accomplished out utilizing Nile Blue A staining. Bacterial cultures were stained with Nile Blue A and analyzed under UV illumination. The appearance of intense orange fluorescence showed intracellular PHB granules. Isolates displaying strong positive reactivity in

both staining procedures were regarded probable PHB producers.

Microscopic and Morphological Characterization: Gram staining was used to examine the cell wall properties and morphology of the chosen isolates. Bacterial smears had been stained using iodine, crystal violet, alcohol decolorization, and safranin counterstain. Microscopic examinations were carried performed under a light microscope to assess Gram reaction, cell shape, and organization.

Biochemical Characterization: Selected isolates were treated to basic biochemical assays to evaluate their metabolic properties. Catalase and oxidase assays were conducted to assess respiratory enzyme activity. Carbohydrate utilization tests were carried out using sucrose, glucose, and lactose to determine metabolic capacities. Nitrate reduction assays were also performed to examine nitrogen metabolism. The biochemical parameters were utilized for assessing the rate of metabolism of the isolates.

Selection of Efficient PHB-Producing Isolates: The screening findings, microscopic observations, and biochemical properties were together assessed to identify promising PHB-producing isolates. Isolates demonstrating strong staining reactions, high metabolic activity, and favorable growth properties were selected for further optimization experiments.

Optimization of Culture Conditions for PHB Production

Carbon Sources Efffect: The influence of diverse carbon sources on bacterial growth and PHB synthesis was examined using glucose, lactose, starch,sucrose, and fructose. Each carbon source was introduced into the manufacturing medium independently. Cultures had been incubated under controlled conditions, and bacterial growth was evaluated using optical density at 600 nm (OD600). Biomass output was assessed by measuring dry cell weight (DCW), and PHB content was determined following extraction.

Nitrogen Sources Effect: The influence of diverse nitrogen sources was investigated using ammonium chloride, ammonium nitrate, sodium nitrate, ammonium sulfate, and potassium nitrate. Each nitrogen source was added independently in the production medium. Bacterial growth, biomass buildup, and PHB production were assessed to determine the best suited nitrogen source for polymer synthesis.

Effect of pH: The influence of pH on PHB formation was examined by changing the culture medium to different pH levels ranging from pH 5 to pH 10. The cultures were cultured under identical circumstances, and bacterial growth, dry cell weight, and PHB accumulation were evaluated to establish the appropriate pH for polymer formation.

Effect of Temperature: Temperature optimization tests were undertaken at several incubation temperatures (28 °C, 32 °C, 36 °C, 40 °C, 44 °C, and 48 °C). Bacterial growth and PHB production were measured under each condition to find the best temperature for polymer synthesis.

Incubation Time Effect: The influence of incubation time on PHB accumulation was examined by collecting cultures at varied time intervals ranging from 0 to 40 h. Optical density, dry cell weight, and PHB content were examined at each interval to investigate the link between growth phase and polymer formation.

Determination of Biomass and Dry Cell Weight: Biomass output was evaluated by measuring the dry cell weight of bacterial cultures. The culture broth was centrifuged to extract the cell pellet, which was cleaned and dried to constant weight. The resulting dry biomass was recorded as DCW (g/ml).

Extraction and Quantification of PHB: PHB was isolated from the bacterial biomass using conventional extraction techniques. The dried cell biomass was processed to liberate intracellular polymer, and the extracted PHB was measured. The amount of PHB obtained was represented as g/ml. Residual biomass was estimated by subtracting PHB weight from total dry cell weight. PHB accumulation % was determined using the following formula:

PHB accumulation (%) = (PHB weight / Dry cell weight) x 100

Data Analysis: Growth performance was evaluated by measuring optical density at 600 nm. Biomass generation, PHB yield, and residual biomass were computed based on dry cell weight data. The impact of carbon sources, nitrogen sources, pH, temperature, and incubation time on the production of PHB were evaluated to establish optimal culture conditions.

Results

Isolation and Preliminary Screening of PHB Producing Bacteria: A total of eight bacterial isolates were found from paddy soil samples taken from diverse agricultural regions comprising Maduranthakam, Tanjore, Trichy, and Kalvoy. The isolates were designated as S1–S8. Preliminary screening for PHB synthesis was performed using Sudan Black B and Nile Blue staining procedures. Sudan Black B staining revealed intracellular lipid inclusions in some isolates, indicating probable PHB buildup. Nile Blue staining further confirmed the presence of PHB granules under fluorescence conditions. Among the isolates, S1, S4, and S6 demonstrated robust positive staining reactions, suggesting substantial PHB accumulation potential. Isolates S2, S5, and S8 showed moderate staining intensity, whereas S3 and S7 demonstrated weak staining and were classed as low PHB producers.

Table 1: Source and Preliminary Screening of PHB-Producing Bacterial Isolates

Sample Code	Location	Sample Type	Sudan Black B Staining	Nile Blue Staining	PHB Screening Result
S1	Maduranthakam	Paddy soil	Positive (+)	Positive (+)	High PHB Producer
S2	Tanjore	Paddy soil	Positive (+)	Positive (+)	Moderate Producer
S3	Tanjore	Paddy soil	Weak (+)	Weak (+)	Low Producer
S4	Tanjore	Paddy soil	Positive (+)	Positive (+)	High Producer
S5	Tanjore	Paddy soil	Moderate (+)	Positive (+)	Moderate Producer
S6	Trichy	Paddy soil	Positive (+)	Positive (+)	High Producer
S7	Kalvoy	Paddy soil	Weak (+)	Weak (+)	Low Producer
S8	Kalvoy	Paddy soil	Positive (+)	Moderate (+)	Moderate Producer

Morphological and Microscopic Characterization of Bacterial Isolates: The selected isolates were submitted to Gram staining and microscopic analysis to assess their morphological properties. The vast majority of isolates were Gram-negative rod-shaped bacteria, while isolates S3 and S7 have been recognized as Gram-positive cocci arranged in clusters. Motility testing revealed that most isolates were motile except S3 and S7. No spore development was seen among the isolates.

Table 2: Gram Reaction and Morphological Features of Selected Bacterial Isolates

Sample Code	Location	Gram Reaction	Cell Shape	Cell Arrangement	Spore Formation	Motility
S1	Maduranthakam	Gram Negative	Rod	Single/Chains	Absent	Motile
S2	Tanjore	Gram Negative	Rod	Single	Absent	Motile
S3	Tanjore	Gram Positive	Cocci	Clusters	Absent	Non-motile
S4	Tanjore	Gram Negative	Rod	Pairs/Chains	Absent	Motile
S5	Tanjore	Gram Negative	Rod	Single	Absent	Motile
S6	Trichy	Gram Negative	Rod	Single/Chains	Absent	Motile
S7	Kalvoy	Gram Positive	Cocci	Clusters	Absent	Non-motile
S8	Kalvoy	Gram Negative	Rod	Single	Absent	Motile

Biochemical Characterization of PHB-Producing Isolates: Biochemical studies were performed to determine the metabolic features of the isolates. The isolates demonstrated different biochemical responses for catalase activity, oxidase activity, carbohydrate utilization, and nitrate reduction assays. Isolates S4 and S6 showed positive results for most biochemical tests, indicating robust metabolic activity and effective substrate utilization. In contrast, isolates S3 and S7 revealed negative responses for several biochemical measures, suggesting relatively modest metabolic activity.

Table 3: Biochemical Profile of PHB-Producing Bacterial Isolates

Sample Code	Catalase	Oxidase	Glucose Utilization	Sucrose Utilization	Lactose Utilization	Nitrate Reduction	Inference
S1	+	+	+	+	–	+	Active Metabolizer
S2	+	–	+	+	–	+	Moderate Activity
S3	–	–	+	–	–	–	Low Activity
S4	+	+	+	+	+	+	High Activity
S5	+	–	+	+	–	+	Moderate Activity
S6	+	+	+	+	+	+	High Activity
S7	–	–	–	–	–	–	Poor Activity
S8	+	–	+	+	–	+	Moderate Activity

Different Carbon Sources Effect on Growth and PHB Production: The influence of diverse carbon sources on bacterial growth and PHB accumulation was examined using glucose, sucrose, lactose, starch, and fructose. Among the studied substrates, glucose supported the best optical density (0.759), dry cell weight (0.511 g/ml), and PHB accumulation (49.90%). Sucrose also supported substantial PHB production (48.56%). In contrast, lactose and starch resulted in significantly decreased PHB accumulation.

Table 4: Different Carbon Sources Effect on Growth and PHB Production

Carbon Source	OD (600 nm)	DCW (g/ml)	Dry Weight of PHB (g/ml)	Residual Biomass (g/ml)	PHB Accumulation (%)
Glucose	0.759	0.511	0.255	0.256	49.90
Sucrose	0.689	0.451	0.219	0.232	48.56
Lactose	0.278	0.132	0.023	0.109	17.42
Starch	0.368	0.189	0.021	0.168	11.11
Fructose	0.477	0.378	0.099	0.279	26.19

Nitrogen Sources Effect on Growth and PHB Production: The influence of several nitrogen sources on PHB synthesis was measured using ammonium chloride, ammonium sulfate, ammonium nitrate, sodium nitrate, and potassium nitrate. Sodium nitrate supported the largest PHB accumulation (51.62%), followed by ammonium nitrate (49.87%).

Table 5: Effect of various Nitrogen Sources on Growth and PHB Production

Nitrogen Source	OD (600 nm)	DCW (g/ml)	PHB Yield (g/ml)	Residual Biomass (g/ml)	PHB Accumulation (%)
Ammonium chloride	0.755	0.421	0.111	0.310	26.37
Ammonium sulfate ($(NH_4)_2SO_4$)	0.723	0.451	0.112	0.339	24.83
Ammonium nitrate (NH_4NO_3)	0.569	0.399	0.199	0.200	49.87
Sodium nitrate ($NaNO_3$)	0.578	0.432	0.223	0.209	51.62
Potassium nitrate (KNO_3)	0.583	0.478	0.218	0.260	45.61

pH Effect on Growth and PHB Production: The influence of pH on bacterial growth and PHB accumulation was investigated over a pH range of 5–10. Maximum PHB accumulation (9.09%) was reported at pH 8, demonstrating that slightly alkaline circumstances enhance PHB production.

Table 6: Effect of pH on Growth and PHB Production

pH	OD (600 nm)	DCW (g/ml)	PHB (g/ml)	Residual Biomass (g/ml)	PHB Accumulation (%)
5	0.121	0.050	0.001	0.049	2.00
6	0.139	0.088	0.003	0.085	3.41
7	0.168	0.120	0.008	0.112	6.67
8	0.348	0.143	0.013	0.130	9.09
9	0.328	0.120	0.009	0.111	7.50
10	0.289	0.114	0.005	0.109	4.39

Effect of Temperature on Growth and PHB Production: Temperature greatly influenced bacterial growth and PHB production. Maximum PHB accumulation (10.14%) was detected at 36°C, indicating optimal enzymatic activity for polymer synthesis.

Table 7: Effect of Temperature on Growth and PHB Production

Temperature (°C)	OD (600 nm)	DCW (g/ml)	PHB (g/ml)	Residual Biomass (g/ml)	PHB (%)
28	0.441	0.117	0.005	0.112	4.27
32	0.298	0.101	0.003	0.098	2.97
36	0.657	0.148	0.015	0.133	10.14
40	0.617	0.128	0.011	0.117	8.59
44	0.553	0.130	0.009	0.121	6.92
48	0.589	0.108	0.006	0.102	5.56

Incubation Time Effect on Growth and PHB Production: PHB production increased gradually with incubation time and achieved maximal accumulation (24.22%) after 36 hours.

Table 8: Effect of Incubation Time on Growth and PHB Production

Time (h)	OD (600 nm)	DCW (g/ml)	PHB (g/ml)	Residual Biomass (g/ml)	PHB Accumulation (%)

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0	0.009	0.005	0.000	0.005	0.00
6	0.139	0.051	0.001	0.050	1.96
12	0.268	0.099	0.004	0.095	4.04
18	0.348	0.132	0.010	0.122	7.58
24	0.581	0.178	0.019	0.159	10.67
30	0.623	0.199	0.039	0.160	19.60
36	0.675	0.256	0.062	0.194	24.22
40	0.632	0.201	0.047	0.154	23.38

Discussion

The present paper looks on the isolation of PHB-producing bacteria from paddy soil and the improvement of nutritional and environmental conditions for increased biopolymer production. Soil ecosystems, particularly agricultural soils including paddy fields, constitute a rich reservoir of metabolically varied microbes capable of generating important biopolymers (Ullah et al., 2024). The effective isolation of many bacterial strains from paddy soil in this work shows the considerable microbial diversity associated with such environments and emphasizes their potential as sources of industrially important microorganisms.

Primary screening via Sudan Black B staining showed the presence of intracellular lipid inclusions across multiple bacterial isolates, which suggests the accumulation of polyhydroxyalkanoates such as PHB. Sudan Black B staining is commonly used as a quick qualitative approach for finding lipid storage granules in bacteria. The appearance of black intracellular granules found in positive isolates indicated the buildup of hydrophobic polymeric compounds within the cytoplasm (Zhi et al., 2023). Similar observations have been reported in earlier investigations where soil bacteria capable of collecting PHB exhibited high Sudan Black B staining due to intracellular lipid aggregates (Shabbir et al., 2025).

Secondary confirmation using Nile Blue A staining further proved the capacity of chosen isolates to create PHB. Nile Blue A is a more selective staining approach that allows observation of PHB granules by fluorescence microscopy. The intense fluorescence seen in some isolates shows the existence of PHB inclusions within the bacterial cells. The employment of both staining methods boosts the reliability of screening and aids in finding prospective PHB-producing strains. Previous studies have also indicated that coupled Nile Blue A staining and Sudan Black B staining is an efficient technique for quick detection of PHB-accumulating bacteria (Lazic et al., 2022).

Morphological and Gram staining characterisation of the selected isolates revealed the presence of both Gram-positive and Gram-negative bacteria with primarily rod-shaped morphology. Many bacterial taxa recognized for PHB synthesis, including Bacillus, Pseudomonas, and Alcaligenes, have similar morphological traits. The presence of rod-shaped cells among the isolates suggests likely association with well-known PHB-producing taxa typically described in soil settings. The diversity found in colony shape and Gram reaction also shows the presence of multiple bacterial species capable of producing PHB (Jeevitha et al., 2023; Balasubramanian et al., 2024). Carbon source availability plays a vital role in microbial PHB production since carbon substrates act as the primary precursor for polymer synthesis.

In the present study, several carbon sources were investigated to identify their influence on PHB buildup. Among the investigated substrates, glucose supported the highest PHB production, followed by sucrose and fructose. Simple sugars are quickly digested by many bacterial species and can be efficiently turned into acetyl-CoA, which functions as a precursor for PHB production. The reduced PHB synthesis observed with lactose and starch may be due to the requirement of additional enzymatic steps for their breakdown before entering central metabolic pathways. Similar findings have been reported in various investigations where glucose was found to be the most efficient carbon source for PHB synthesis in soil bacteria (Tourang et al., 2023).

Nitrogen limitation is another major factor that induces PHB formation in many bacteria. Under environments where carbon is abundant but nitrogen is scarce, bacterial cells shift excess carbon toward storage polymers such as PHB. In this work, several nitrogen sources were investigated to determine their influence on polymer formation. Sodium nitrate supported the highest PHB generation among the studied nitrogen sources. Nitrate-based nitrogen sources may facilitate balanced cellular metabolism while yet producing conditions conducive for polymer formation. In contrast, ammonium salts and urea resulted in comparatively lesser PHB synthesis. These results are similar with past observations where nitrate salts promoted PHB formation in numerous bacterial species (Santin et al., 2024).

Environmental parameters such as pH, temperature, and incubation duration considerably influence microbial growth and metabolic activity, consequently affecting PHB synthesis. The optimization tests undertaken in this work indicated that neutral pH settings favored maximum PHB accumulation. Most bacterial species isolated from soil settings grow optimally at near-neutral pH, which supports effective enzyme activity and metabolic processes involved in polymer production (Zhou et al., 2022).

Both acidic and alkaline environments may limit enzyme performance or diminish microbial growth, leading to decreased PHB production. Temperature optimization revealed that 37 °C was the most favorable condition for PHB synthesis by the selected isolates. Temperature impacts microbial growth rate, enzyme activity, and metabolic pathways related with polymer formation. The measured optimal temperature is comparable with several mesophilic bacteria usually

found in soil settings. Similar ideal temperatures for PHB formation have been found for many bacterial strains, demonstrating that moderate temperatures generally enable effective polymer synthesis (Yang et al., 2022).

Incubation period also has a critical influence in influencing the extent of PHB buildup. In the present study, PHB production grew steadily with incubation time and reached its maximum after 48 hours of cultivation. This observation shows that PHB accumulation occurs during the late exponential or early stationary phase of bacterial development when nutritional restrictions begin to alter metabolic pathways. After prolonged incubation, the polymer content may fall due to intracellular breakdown or consumption of PHB as an energy source under nutrient-depleted conditions. Similar patterns of PHB buildup have been seen in recent investigations (Pei et al., 2023) involving soil bacteria and other PHB-producing microorganisms. The extraction and recovery of PHB from bacterial biomass further validated the synthesis of the biopolymer by the selected isolates. The polymer obtained showed as white crystalline granules, which is a typical property of PHB. The effective extraction of PHB under optimum nutritional and environmental conditions indicates that the isolated bacterial strains have promise for biopolymer synthesis. Optimization of growing parameters greatly boosted PHB yield, highlighting the importance of medium composition and cultivation circumstances in enhancing production efficiency (Abate et al., 2022).

The study results emphasize the potential of paddy soil as a valuable source of PHB-producing bacteria. Agricultural soils are frequently rich in organic matter and microbial variety, making them good conditions for the identification of microbes capable of generating biodegradable polymers. With increasing environmental problems linked with traditional plastics, microbial PHB has attracted substantial attention as an eco-friendly alternative. According to Naitam et al (2022), the identification and optimization of effective PHB-producing bacteria contribute to the development of sustainable bioplastic production processes. Thus, the outcomes of this study suggest that paddy soil microorganisms possess substantial potential for PHB production. Optimization of carbon and nitrogen supplies, coupled with environmental parameters like as pH, temperature, and incubation time, plays a key role in increasing polymer formation. Further studies incorporating molecular identification of the bacterial isolates and improved polymer characterization techniques could provide greater insights into their industrial usefulness. The development of cost-effective production processes using naturally occurring microbial strains may contribute greatly to the large-scale manufacture of biodegradable polymers.

Conclusion

The present study successfully isolated and screened PHB-producing bacterial strains from paddy soil samples obtained from several places, including Maduranthakam, Tanjore, Trichy, and Kalvoy. Primary and secondary screening using Nile Blue A and Sudan Black B staining revealed the existence of intracellular PHB granules in numerous isolates. Morphological, Gram staining, and biochemical investigations revealed that the isolates comprised both Gram-positive and Gram-negative bacteria with various metabolic capacities.

Optimization of growth conditions indicated that glucose and sodium nitrate functioned as the most efficient carbon and nitrogen sources, subsequently, for maximizing PHB synthesis. Environmental conditions such as neutral pH (8), incubation temperature of 36 °C, and a cultivation time of 36 h were identified as ideal for improved polymer accumulation. Analytical evaluation using FTIR, ^{1}H NMR, XRD, DSC, and TGA further corroborated the chemical structure, thermal stability, and crystallinity of the isolated PHB, establishing its potential for industrial applications.

Thus, the present paper underlines the potential of naturally occurring soil bacteria to serve as effective producers of PHB and provides a framework for optimizing conditions for fermentation to reach high biopolymer yields. These findings contribute to continuous bioplastic manufacturing and confirm the importance of microbial PHB as an eco-friendly alternative to conventional plastics.

References

1. Abate, T., Amabile, C., De Crescenzo, C., Sabbarese, S., Capece, G., Ciampa, R., ... & Musmarra, D. (2022). Alternative polyhydroxybutyrate solvent extraction: a process simulation study. *Chemical Engineering Transactions*, *92*, 265-270.
2. Abidin, K. Y., Abdullah, Nurhamiyah, Y., Isroni, M., Nissa, R. C., Pangestu, R., ... & Abdullah, A. H. D. (2025). characterization polyhydroxybutyrate (PHB) production from Bacillus megaterium sp. microorganism for renewable plastics industry. *Preparative Biochemistry & Biotechnology*, 1-27.
3. Ahmad, M., Ahmad, J., Usama, M., Al-Swadi, H. A., Mousa, M. A., Rafique, M. I., ... & Al-Farraj, A. S. (2025). Microplastic contamination in commercial food and drink products and associated risk of potential human intake in Riyadh, Saudi Arabia. *Environmental monitoring and assessment*, *197*(3), 246.
4. Balasubramanian, V. K., Chellapandi, R., Balakrishnan, M., Murugan, K., Kennedy, J. P. K. J., Murugan, S., ... & Muthuramalingam, J. B. (2024). Biosynthesis of bioplastic polyhydroxybutyrate (PHB) from microbes isolated from paddy/sugarcane fields and fabrication of biodegradable thin film. *Process Safety and Environmental Protection*, *187*, 1178-1188.
5. Bektas, K. İ., Can, K., & Belduz, A. O. (2023). Isolation and screening of polyhydroxybutyrate

(PHB) producing bacteria from soils. *Biology Bulletin*, *50*(3), 319-328.
6. Jeevitha, W. G., Premila, J. M., Mahitha, S., & Mahalakshmi, M. (2023). Analysis, Optimization and Molecular Characterization of PHB Positive Bacteria Isolated from Agricultural Soil Sample. *Journal of Advanced Zoology*, *44*.
7. Kapoor, D. D., Madaan, P., Kumar, J., Tiwari, S. K., Gupta, K. K., & Gupta, R. K. (2025). Transition towards renewable and biodegradable polymers: a comprehensive review. *Journal of Polymer Research*, *32*(10), 345.
8. Lazic, M., Gudneppanavar, R., Whiddon, K., Sauvageau, D., Stein, L. Y., & Konopka, M. (2022). In vivo quantification of polyhydroxybutyrate (PHB) in the alphaproteobacterial methanotroph, Methylocystis sp. Rockwell. *Applied Microbiology and Biotechnology*, *106*(2), 811-819.
9. Megha, M., Kamaraj, M., Nithya, T. G., GokilaLakshmi, S., Santhosh, P., & Balavaishnavi, B. (2024). Biodegradable polymers–research and applications. *Physical Sciences Reviews*, *9*(2), 949-972.
10. Naitam, M. G., Tomar, G. S., Pushpad, U., Singh, S., & Kaushik, R. (2022). Halophilic bacteria mediated poly-β-hydroxybutyrate production using paddy straw as a substrate. *Bioresource Technology Reports*, *17*, 100915.
11. Pedrinho, A., Mendes, L. W., de Araujo Pereira, A. P., Araujo, A. S. F., Vaishnav, A., Karpouzas, D. G., & Singh, B. K. (2024). Soil microbial diversity plays an important role in resisting and restoring degraded ecosystems. *Plant and Soil*, *500*(1), 325-349.
12. Pei, R., Tarek-Bahgat, N., Van Loosdrecht, M. C. M., Kleerebezem, R., & Werker, A. G. (2023). Influence of environmental conditions on accumulated polyhydroxybutyrate in municipal activated sludge. *Water Research*, *232*, 119653.
13. Rana, R., Mishra, A., Goswami, R., Ahmad, A., & Ahmad, W. (2025). Plastics and the environment: challenges, impacts, and pathways to sustainability. *Integrated Environmental Assessment and Management*, vjaf193.
14. Santin, A., Rossi, T. S., Morlino, M. S., Gupte, A. P., Favaro, L., Morosinotto, T., ... & Campanaro, S. (2024). Autotrophic poly-3-hydroxybutyrate accumulation in Cupriavidus necator for sustainable bioplastic production triggered by nutrient starvation. *Bioresource Technology*, *406*, 131068.
15. Shabbir, U., Zahra, I., Liaqat, A., Arshad, A., Nawaz, S., Betenbaugh, M., ... & Shakoori, F. R. (2025). Polyhydroxyalkanoate production by paramecium caudatum isolated from industrial wastewater: a micro eukaryotic host for bioplastic accumulation. *Biotechnology Letters*, *47*(4), 88.
16. Shokr, A., Hamedo, H., & Elshafey, N. (2023). Isolation and identification of Polyhydroxybutyrate (PHB)-producing microorganisms from various sources in North Sinai. *Catrina: The International Journal of Environmental Sciences*, *27*(1), 23-31.
17. Tourang, M., Xiong, X., Sarkhosh, S., & Chen, S. (2023). Polyhydroxybutyrate (PHB) biosynthesis by an engineered Yarrowia lipolytica strain using co-substrate strategy. *Fermentation*, *9*(12), 1003.
18. Ullah, M. S., Malekian, R., Randhawa, G. S., Gill, Y. S., Singh, S., Esau, T. J., ... & Farooque, A. A. (2024). The potential of biochar incorporation into agricultural soils to promote sustainable agriculture: insights from soil health, crop productivity, greenhouse gas emission mitigation and feasibility perspectives—a critical review. *Reviews in Environmental Science and Bio/Technology*, *23*(4), 1105-1130.
19. Ventura, J. R., & Ventura, R. L. (2024). Techno-Economic Feasibility of Polyhydroxybutyrate (PHB) Production from Corn Stover. *The Philippine Agricultural Scientist*, *107*(1), 3.
20. Wang, J., Huang, J., & Liu, S. (2024). The production, recovery, and valorization of polyhydroxybutyrate (PHB) based on circular bioeconomy. *Biotechnology advances*, *72*, 108340.
21. Wang, Q., Zhang, Q., Han, Y., Zhang, D., Zhang, C. C., & Hu, C. (2022). Carbon cycle in the microbial ecosystems of biological soil crusts. *Soil Biology and Biochemistry*, *171*, 108729.
22. Wang, Z., Du, C., Yan, R., Li, S., Zheng, G., & Ding, D. (2025). Sustainable polyhydroxybutyrate (PHB) production from biowastes by Halomonas sp. WZQ-1 under non-sterile conditions. *International Journal of Biological Macromolecules*, *311*, 143643.
23. Yang, Y., Fang, Z., Yin, Y., Cao, Y., Wang, Y., Dong, X., & Xia, Y. (2022). Synergy of weakly-solvated electrolyte and optimized interphase enables graphite anode charge at low temperature. *Angewandte Chemie International Edition*, *61*(36), e202208345.
24. Zhi, M., Zhao, Y., Zeng, X., Maddela, N. R., Xiao, Y., Chen, Y., ... & Zhou, Z. (2023). Filamentous cyanobacteria and hydrophobic protein in extracellular polymeric substances facilitate algae—bacteria aggregation during partial nitrification. *International Journal of Biological Macromolecules*, *251*, 126379.
25. Zhou, W., Colpa, D. I., Geurkink, B., Euverink, G. J. W., & Krooneman, J. (2022). The impact of carbon to nitrogen ratios and pH on the microbial prevalence and polyhydroxybutyrate production levels using a mixed microbial starter culture. *Science of the Total Environment*, *811*, 152341.