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Greener Technology of Producing Polyhydroxyalkanoate using Anthracene as Carbon Source

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Abstract. The study investigated the potential of *Bacillus cereus* AAR-1 (OQ999178) to simultaneously degrade anthracene, a toxic environmental pollutant, and produce polyhydroxyalkanoate (PHA), an eco-friendly and sustainable biopolymer. Using a Taguchi L16 (4*3) array for optimization, it was found that a 10% seed inoculum, grown for 8 days in a minimal salt medium containing 400 ppm of anthracene and 2 g/L of NH_4Cl as carbon and nitrogen sources, respectively, maximized anthracene degradation and PHA accumulation by *B. cereus* AAR-1. The bacterial biomass had a colony count of 1 x 10^6 cfu/ml and produced 286 mg/L of biopolymer, as extracted using a hypochlorite-chloroform solvent method. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed the biopolymer as PHA. This study identifies a key hydrocarbon-degrading bacterium at a municipal dumpsite, which plays a significant role in environmental biotechnology by supporting cleaner and greener technologies. This contribution aligns with the goals of SDG 12 and 14.

Keywords: *Bacillus cereus,* Bioplastic, Polyhydroxyalkanoate, Polycyclic aromatic hydrocarbon, Dumpsite, Chemicals and Waste management.

1. Introduction

The accumulation of polycyclic aromatic hydrocarbons (PAHs) in the environment poses significant ecological and health risks due to their carcinogenic and mutagenic properties [1]. Among these, anthracene is a notable PAH, commonly found in polluted environments such as municipal dumpsites, resulting from the incomplete combustion of organic materials.

Hydrocarbon-degrading bacteria have shown significant potential in bioremediation using PAHs as carbon and energy sources [2]. These species include *Bacillus, Acinetobacter, Pseudomonas, Comamonas, Coccobacillus, Burkholderia, Sphingomonas, Terrimonas* and *Fulvimonas* [3]. Besides breaking down pollutants, some bacteria can also produce polyhydroxyalkanoates (PHAs). PHAs are biodegradable polymers that are applicable as bioplastics, drug delivery molecules, medical suture, food and feeds, biofuels e.t.c. PHAs are synthesized by various microorganisms as intracellular carbon and energy storage materials and have gained considerable attention as eco-friendly alternatives to petrochemical-based plastics [4]. These biopolymers are typically produced with limited nutrients but abundant carbon. The ability of hydrocarbon-degrading bacteria to both degrade PAHs and produce PHAs offers a promising approach for combining bioremediation with biopolymer production [5].

2. Material and Methods

2.1 Identification of hydrocarbon-degrading bacteria with PHA potentials

Hydrocarbon-degrading bacteria were enriched using decomposed soil and leachate from Abule-egba municipal dumpsite in Lagos, Nigeria. Soil samples were mixed with a sterile growth medium composing in (g/L); 1.8 K_2PO_4, 1.2 KH_2PO_4, 4.0 NH_4Cl, 0.2 $MgSO_4.7H_2O$, 0.1 NaCl, and 0.01 $FeSO_4.7H_20$, supplemented with 1% crude oil and incubated at 30°C for 7 days. Sub-culturing was repeated every 7 days, followed by screening on oil agar plates to obtain pure isolates [6]. These isolates were then screened for PHA production on a sterilized PHA detecting agar medium (PDA) containing $(NH_4)_2SO_4$ – 2 g, $MgSO_4·7H_2O$ – 1.2 g, KH_2PO_4 – 13.3 g, citric acid – 1.7 g, and 10 ml of trace elements solution (TES), with 16 g of bacteriological agar in 1 liter of distill water [7]. Detection involved Nile Red and Blue dyes, with further confirmation using Sudan Black B staining and light microscopy [7]. The genomic identification of the bacterial strain was performed using 16S rRNA gene sequencing.

2.2 Optimizing PHA Production from Anthracene Degradation

PHA production was carried out using a PHA mineral salt medium where anthracene was the sole carbon source. A 10% seed inoculum was introduced into the sterilized medium, which was then incubated at 35°C with continuous shaking at 150 rpm for 8 days. Bacterial growth, anthracene degradation, and PHA accumulation were regularly monitored throughout the incubation period. PHA extraction and residual anthracene measurements were performed using standard methods. The extracted PHA was qualitatively characterised by analyzing its functional groups using Fourier-transform infrared spectroscopy (FTIR Model; Bruker Alpha with Opus 7.5 software) in the transmittance wavenumber range between 4000 and 400 cm^{-1} with a spectral resolution of 4 cm^{-1}. Optimization of anthracene degradation was achieved using a Taguchi L16 (4x3) array, which involved 16 experimental runs.

3. Results and Discussion

A potential hydrocarbon-degrading bacterium isolated from a soil sample collected at the Abule-Egba dumpsite was identified as *Bacillus cereus* AAR-1, showing a close similarity to *B. cereus* NO7. Numerous studies have investigated the microbial communities residing in this landfill, examining both their potential risks to health and their possible industrial applications [8, 9]. Rajan and coworkers also isolated bacteria utilizing naphthalene ($C_{10}H_8$) and phenanthrene ($C_{14}H_{10}$) from soil samples of Perungudi and Kodungaiyur dumpsites in India [10]. One of the dominant microbial genus frequently encountered on dumpsites for the synthesis of PHA is *Bacillus*. Das *et al*. [11] employed *Bacillus cereus* RCL 02 for the synthesis of 7.8 g/l P(3HB-*co*-3HV) using sugarcane molasses as a carbon source. The bacterial growth in this study reached its peak colony-forming units on day 6 across the various experimental runs (Table 1). The maximum PHA yield of 286 mg/L was achieved with 2 g/L NH_4Cl and 400 ppm anthracene as the nitrogen and carbon sources, respectively (Table 2, Figure 1). Optimizing PHA accumulation is crucial for increasing the yield and quality of PHA produced by wild-type strains. The optimization process aims to develop strategies that enhance the microbe's uptake of available carbon substrates for bioconversion into PHA. The characteristic spectral peaks at 1736 cm^{-1} and 3384 cm^{-1}, corresponding to the PHA carbonyl (C=O) and hydroxyl (OH) functional groups, respectively, were observed from the FTIR analysis (Table 3).

Table 1: Bacterial colony counts on medium agar plates

Experimental Runs	Day 5 (CFU/ml) X 10^6	Day 6 (CFU/ml) X 10^6	Day 7 (CFU/ml) X 10^6	Day 8 (CFU/ml) X 10^6
1	2	21	14	1
2	9	16	10	9
3	1	44	4	3
4	31	33	44	21
5	2	37	9	8
6	3	13	1	5
7	24	10	2	1
8	58	70	35	31
9	2	15	13	13
10	4	3	2	2
11	5	4	1	1
12	42	40	21	15
13	10	9	2	1
14	1	8	1	1
15	6	6	1	1
16	28	18	3	1

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Table 2. Optimization of PHA production

Carbon concentration	Nitrogen source	Nitrogen concentration (g/L)	PHA yield (g/L)
100 ppm	NH_4Cl	0.5	0.018
100 ppm	NH_4NO_3	1.0	0.018
100 ppm	$(NH_4)_2SO_4$	1.5	0.084
100 ppm	Yeast extract	2.0	0.072
200 ppm	NH_4Cl	1.0	0.086
200 ppm	NH_4NO_3	0.5	0.068
200 ppm	$(NH_4)_2SO_4$	2.0	0.034
200 ppm	Yeast extract	1.5	0.028
300 ppm	NH_4Cl	1.5	0.124
300 ppm	NH_4NO_3	2.0	0.11
300 ppm	$(NH_4)_2SO_4$	0.5	0.034
300 ppm	Yeast extract	1.0	0.178
400 ppm	NH_4Cl	2.0	0.286
400 ppm	NH_4NO_3	1.5	0.01
400 ppm	$(NH_4)_2SO_4$	1.0	0.082
400 ppm	Yeast extract	0.5	0.24

Table 3. Spectral peaks of PHA functional groups

Functional group	Peaks wavelength (cm^{-1})	References
OH	3384	[12]
$-CH_2-CH_2-$	2920	[13]
$-CH_2-CH_3$	2853	[14]
C=O	1736	[15]
C-H	1134	[16]
$-(CH_2)_n$	711	[16, 17]

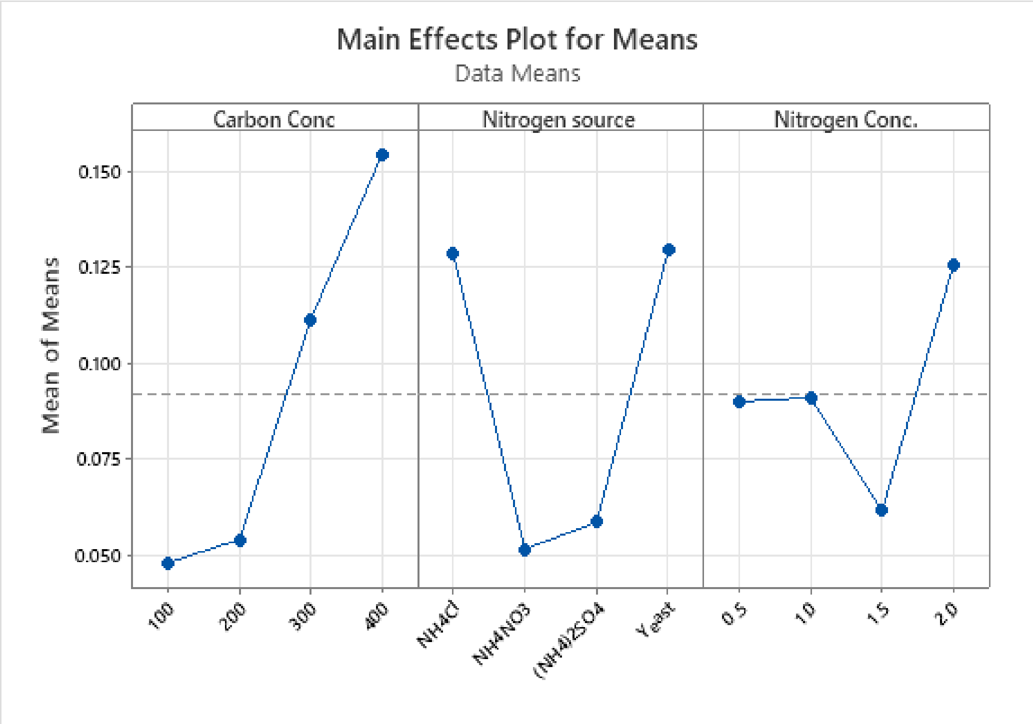

Figure 1. Optimal conditions to increase PHA yield from *B. cereus* AAR-1

4. Conclusion and Recommendation

Remediating hydrocarbon contaminants to synthesize biopolymer is crucial for mitigating their adverse effects. Leveraging and optimizing the metabolic capabilities of hydrocarbon-degrading bacterium, *B.cereus* AAR-1, isolated from municipal dumpsite offers a sustainable and efficient approach to pollutant detoxification, thereby increasing environmentally safe materials. The idea of using anthracene, a chemical waste (pollutant) from the environment to produce eco-friendly bioplastic, supports the bio-circular economy and helps achieve SDGs 12 and 14. Hence, further research should focus on genetically modifying potential biocatalysts to increase the yield of PHA using these pollutants.

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REFERENCES

[1]. Isibor P.O., Imoobe T.O.T., Enuneku A.A., Akinduti P.A., Dedeke G.A., Adagunodo T.A., Obafemi D.Y. (2020). Principal Components and Hierarchical Cluster Analyses of Trace Metals and Total Hydrocarbons in Gills, Intestines and Muscles of *Clarias gariepinus* (Burchell, 1822). Scientific Reports, 10: 5180.

[2]. Sakshi, Singh, S. K., & Haritash, A. K. (2022). Evolutionary relationship of polycyclic aromatic hydrocarbons degrading bacteria with strains isolated from petroleum contaminated soil based on 16S rRNA diversity. *Polycyclic Aromatic Compounds*, *42*(5), 2045–2058.

[3]. Miri, S., Naghdi, M., Rouissi, T., Kaur Brar, S., & Martel, R. (2019). Recent biotechnological advances in petroleum hydrocarbons degradation under cold climate conditions: A review. *Critical Reviews in Environmental Science and Technology*, *49*(7), 553-586.

[4]. Akinwumi, A. R., Nwinyi, O. C., Ayeni, A. O., Ahuekwe, E. F., & Chukwu, M. N. (2022). An overview of the production and prospect of polyhydroxyalkanote (PHA)-based biofuels: Opportunities and limitations. *Scientific African*, *16*, e01233.

[5]. Crisafi, F., Valentino, F., Micolucci, F., & Denaro, R. (2022). From organic wastes and hydrocarbons pollutants to polyhydroxyalkanoates: bioconversion by terrestrial and marine bacteria. *Sustainability*, *14*(14), 8241.

[6]. Nwinyi, O. C., Kanu, I. A., Tunde, A., & Ajanaku, K. O. (2014). Characterization of diesel degrading bacterial species from contaminated tropical ecosystem. *Brazilian Archives of Biology and Technology*, *57*(5), 789–796.

[7]. Fadipe, T. O., Jamil, N., & Lawal, A. K. (2021). Biosynthesis and characterization of poly-(3)-hydroxyalkanoic acid by *Bacillus megaterium* SF4 using different carbohydrates. *Microbial Polymers: Applications and Ecological Perspectives*, 109–129.

[8]. Salami, L. & Susu, A.A. (2019). A comprehensive study of leachate characteristics from three soluos dumpsites in Igando Area of Lagos State, Nigeria. *Greener Journal of Environmental Management and Public Safety*. 8 (1), 1–14.

[9]. Emmanuel-Akerele, H. & Peter, F. (2021). Microbial and physico-chemical assessment of soil and water around waste dumpsites. *International Journal of Applied Biology*. 5 (1), 73–82.

[10]. Rajan, S., Geethu, V. & Chakraborty, P. (2022). Bioremediation of polycyclic aromatic hydrocarbons from contaminated dumpsite soil in Chennai city, India. In *Biological Approaches to Controlling Pollutants*. Woodhead Publishing, pp. 241–258.

[11]. Das, R., Pal, A. & Paul, A.K. (2019). Bioconversion of sugarcane molasses to poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by endophytic *Bacillus cereus* RCL 02. *Journal of Applied Biology and Biotechnology*. 7 (2), 20–24.

[12]. Pramanik, N., Mukherjee, K., Nandy, A., Mukherjee, S., & Kundu, P. P. (2014). Comparative analysis of different properties of polyhydroxyalkanoates isolated from two different bacterial strains: *Alkaliphilus oremlandii* OhILAs and recombinant Escherichia coli XL1B. *Journal of Applied Polymer Science*, *131*(22).

[13]. Joyline, M., & Aruna, K. (2019). Production and characterization of polyhydroxyalkanoates (PHA) by *Bacillus megaterium* strain JHA using inexpensive agro-industrial wastes. *Int. J. Rec. Sci. Res.*, *10*(7), 33359-74.

[14]. Masood, F., Aslam, A., Perveen, K., Berger, M. R., & Hameed, A. (2024). Characterization of folic acid-grafted poly (3-hydroxybutyrate) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) nanoparticles as carriers for sustained release of epirubicin. *Journal of Molecular Structure*, *1304*, 137631.
[15]. Mostafa, Y. S., Alrumman, S. A., Otaif, K. A., Alamri, S. A., Mostafa, M. S., & Sahlabji, T. (2020). Production and characterization of bioplastic by polyhydroxybutyrate accumulating *Erythrobacter aquimaris* isolated from mangrove rhizosphere. *Molecules*, *25*(1), 179.
[16]. Mojaveryazdi, F. S., Muhamad, I. I., Rezania, S., & Benham, H. (2014). Importance of glucose and Pseudomonas in producing degradable plastics. *Jurnal Teknologi*, *69*(5).
[17]. Akinwumi, A. R., Nwinyi, O. C., Ayeni, A. O., & Mohan, S. V. (2024). Influence of synthetic carbon grade on the metabolic flux of polyhydroxyalkanoate monomeric constitution synthesized by *Bacillus cereus* AAR-1. *Bioresource Technology Reports*, 101958.