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Bioremediation of Pesticide Contaminated Water using Indigenous Hydrocarbon-Degrading, Biosurfactant-Producing Organisms

Onyinyechi Ndimele Akomah-Abadaike* and Sabastine Chukwuebuka Ogbuji

Microbiology Technology Option, School of Science Laboratory Technology, University of Port Harcourt, PMB 5323 Choba Port Harcourt Rivers State, Nigeria

Email address:
*Corresponding author: onyinyechi.akomah@uniport.edu.ng ; onyinyechi.akomah@yahoo.com; Onyinyechi.akomah@unesp.br
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ABSTRACT

The pervasive contamination of water by recalcitrant pesticides poses a significant threat to ecosystems and public health, necessitating the development of sustainable remediation technologies. This study investigated the bioremediation of pesticide contaminated water using indigenous hydrocarbon-degrading, biosurfactant-producing microorganisms. Water and sediment samples were collected, and a diverse microbial consortium was isolated and screened. The most prevalent organisms identified were *Pseudomonas aeruginosa* (28.5%), *Bacillus subtilis* (22.0%), and the fungus *Trichoderma harzianum* (15.5%). These isolates were remarkable biosurfactant producers, with *P. aeruginosa* exhibiting the highest emulsification index (E24) of 68%. In a 14-day bioremediation assay, both *Trichoderma harzianum* and *Pseudomonas aeruginosa* demonstrated high efficacy in degrading a complex mixture of 13 pesticides. *Trichoderma harzianum* achieved a superior average degradation of 60.12%, showing exceptional efficiency against Cyanazine (82.14%) and Tecnazene (77.78%). *Pseudomonas aeruginosa* achieved an average degradation of 51.56%, with notable performance against Hexachlorobenzene (66.04%). The results confirm that indigenous microbes pre-adapted to hydrocarbon contamination possess innate, broad-spectrum pesticide-degrading capabilities. The concurrent production of biosurfactants was identified as a critical mechanism for enhancing the bioavailability and subsequent degradation of hydrophobic pesticide compounds. The study concludes that bioaugmentation with a tailored consortium of indigenous, biosurfactant-producing bacteria and fungi presents a highly effective, eco-friendly, and promising strategy for the bioremediation of pesticide-polluted water source

1. INTRODUCTION

Pesticides play a major role in the worldwide agriculture industry's efforts to protect crops and guarantee food security. However, due to their widespread and frequently careless use, these chemicals are now ubiquitous in the environment, especially in aquatic bodies due to runoff, leaching, and inappropriate disposal [1]. Although pesticides are poisonous by nature and intended to

eradicate pests, their mobility and permanence pose serious risks to human health, aquatic ecosystems, and groundwater quality [2]. Many pesticides, including organophosphates and organochlorines, are resistant to natural breakdown, which causes bioaccumulation in the food chain and long-term ecological harm.

Traditional approaches to cleaning up pesticide-contaminated water, such as improved filtration, chemical oxidation, and incineration, are frequently expensive, energy-intensive, and technologically difficult, particularly for developing countries. Additionally, some processes produce hazardous by-products that lead to secondary contamination [3].

Bioremediation has become a viable, economical, and sustainable solution in this situation.
This method uses microorganisms' metabolic capacity to detoxify and transform dangerous pollutants into less dangerous materials including carbon dioxide, water, and biomass.
The limited bioavailability of pesticides is a major obstacle to their bioremediation. Since many insecticides are hydrophobic, microbial cells cannot access them. Biosurfactants, which are surface-active substances made by microbes, can help overcome this obstacle. Biosurfactants efficiently increase the bioavailability of hydrophobic substances for microbial degradation by improving their solubility and desorption [4]. It's interesting to note that bacteria that have evolved to break down hydrocarbons often have the enzyme pathways necessary to target comparable structural components in pesticides. Therefore, these native, hydrocarbon-degrading bacteria are a prospective source for finding strains that can simultaneously create biosurfactants and break down pesticides, resulting in a synergistic and effective bioremediation approach.

In many agricultural areas, persistent pesticide pollution of surface and groundwater is a serious environmental problem. These harmful substances damage aquatic biodiversity, impair ecosystem functioning, and make water unfit for human consumption. Due to high prices and technological constraints, current physico-chemical treatment techniques are frequently unsuitable for general use [3]. Therefore, the development of an affordable, environmentally friendly, and effective clean-up method is urgently needed.

While generic bioremediation approaches show promise, their efficacy is often limited when dealing with mixed or highly hydrophobic pesticide contaminants. The specific problem this research addresses is the low bioavailability and recalcitrance of pesticide mixtures in contaminated water. Simply introducing non-specialized microbes or nutrients (biostimulation) has yielded inconsistent results. A targeted approach is required that utilizes indigenous microorganisms pre-adapted to stress and equipped with dual capabilities: the enzymatic machinery to break down pesticide molecules and the ability to produce biosurfactants to overcome the critical bioavailability barrier. The aim of this study is to develop a bioremediation strategy for pesticide-contaminated water using indigenous hydrocarbon-degrading, biosurfactant-producing bacteria. It specific objectives are; (i) To isolate and purify indigenous hydrocarbon-degrading bacteria from selected contaminated soil and water samples. (ii) To screen the isolated bacterial strains for concurrent biosurfactant production and the ability to utilize specific pesticides as a sole carbon source.

2. MATERIALS AND METHOD

2.1. Experimental Design

Add 10 g of contaminated sediment or 10 mL of water to 100 mL of Mineral Salt Medium (MSM) containing a known pesticide (e.g., chlorpyrifos) as the sole carbon source. Incubate at 30°C with shaking (150 rpm) for 5–7 days. Perform serial subculturing to enrich hydrocarbon-degrading and pesticide-tolerant strains [5].

2.2. Monitoring of Bioremediation/ Biodegradation Studies

The bioremediation of petroleum hydrocarbons in the different experimental designs/set-up was monitored by withdrawing samples before initiation of the experiment, baseline and at time zero and subsequently at the 0, 15th, 30th and 45th days of the experiment for the following analyses

2.2.1. Total Heterotrophic Bacteria (THB) population.

Total heterotrophic bacterial count present in the 4 different groups was determined at 0-day, 15th, 30th and 45th day of the experimental period.

Swamp soil suspensions were prepared by 10-fold serial dilutions with 1g of soil, using normal saline as diluents. 0.1 mL aliquots of appropriate dilutions were spread on mineral Salt agar supplemented with chlorpyrifos as described by [5]. The plates were incubated for period of 3-5days at room temperature.

2.2.2. Isolation and Identification

Identification of the bacterial colonies were based on the method of [6]. The bacterial isolates were identified through cultural, morphological and biochemical tests. Identification of the isolates were carried out using standard methods based on macroscopic and microscopic features as described by [7].

Microorganisms were identified on the basis of how isolates appear on or in different media; the pigmentation, size, odour, gram stain and biochemical tests

2.2.3. Gram Staining

The isolated organisms were placed on a clean glass slide and emulsified with a drop of sterile water to prepare a thin smear. The smear was evenly distributed over the slide surface, allowed to air dry at room temperature, and then heat-fixed by gently passing it through a flame.

The fixed smear was stained with 1% crystal violet dye solution for 1 minute, then subsequently rinsed with distilled water to remove excess stain. This was followed by the application of Lugol's iodine, which was left to smear for I minute, before rinsing again with distilled water.

Decolorization was performed by briefly treating the smear with 98% alcohol for approximately 30 seconds, after which it was immediately rinsed with distilled water. The smear was then counter stained with Safranin red for 1 minute, and rinse once more with distilled water.

After staining, the slide was allowed to air dry, and a drop of immersion oil was applied before examination under a light microscope. The procedure was conducted in accordance with the method described by [7]

2.2.4. Biochemical characterization of isolated bacteria

The biochemical tests of Sugar utilization; Amino acid decarboxylation; Catalase and oxidase production; Nitrate reduction; Hydrogen sulfide production; Starch, Casein and Urea hydrolysis; IMVIC tests were performed as described by [7]

2.3. Emulsification index (E24)

The emulsification index (E24) is a measure used to evaluate the ability of microorganisms to produce biosurfactant, which facilitates the

formation of stable emulsion between aqueous and hydrophobic phases. This method provides an indication of the surface-active potential of microbial isolates. The determination of E24 was carried out following the method described by [8]. In this method, two millilitres (2 mL) of hydrocarbon (oil) was introduced into test tubes containing cell-free broth and mixed thoroughly using a vortex for 2 min to ensure proper interaction. The mixture was set aside undisturbed for 24 hours, to enable emulsion formation. After 24 hours, the height of the formed emulsion layer was carefully measured and compared to the total height of the liquid column.

The E24 index is calculated as the percentage ratio of the height of emulsified layer (cm) divided by the total height (cm) multiplied by 100.

$$Emulsification\ index\ (E24) = \frac{Height\ of\ the\ emulsion\ layer}{Total\ height} x\ 100$$

2.4. Hemolytic activity

Hemolytic activity was assessed as a preliminary screening method to identify biosurfactant producing microorganisms, based on the method described by [8].

In this procedure, nutrient agar (NA) plates were supplemented with 5 % (v/v) fresh blood and prepared under sterile conditions. The test organisms were inoculated onto the surface of the plate and incubated at 37 °C for 24 hours.

Following incubation, the plates were examined for clear zone (Haemolysis) surrounding the microbial colonies, which indicated positive biosurfactant production. The size and clarity of the zones provide a qualitative measure of the organism's surface-active potential

2.5. Oil-spreading technique

This is one of the best methods used in detecting the presence of biosurfactant (BS) production by microorganisms. This method was carried out according to the procedure described by [8].

In this procedure, 40 mL of distilled water was added to a petri plate, followed by the addition of a thin layer of crude oil - twenty microliters (20 µl) on the water surface, to form a thin layer on the water surface. Subsequently, 10 µl of the microbial culture supernatant was gently added onto the oil layer.

The presence of biosurfactant was indicated by the formation of a clear zone around the point of application, caused by the displacement of oil. The diameter of the cleared zone was measured after approximately 30 seconds, and reported that this observed emulsified halo correlates with surfactant activity and is known as displacement activity.

2.6. Microbial Analysis and Isolation

2.6.1 Total Heterotrophic Bacterial (THB) and Fungal Count

The enumeration of microorganisms in pesticide-contaminated water was carried out using Mineral Salt Agar (MSA) supplemented with chlorpyrifos to selectively support the growth of variations in microbial populations. Samples were collected at regular intervals over a 45-days experimental period to monitor microbial dynamics. In the bio-augmented sets, a significant increase in microbial counts was with peak population recorded around the 21st days.

3. RESULTS AND DISCUSSION

3.1. Biochemical Characterization of Bacterial Isolates

***Table 1**: Microbial Count (CFU/g) during Bioremediation*

Sample Set	Day 0	Day 15	Day 21
Control (without organisms)	2.8×10^4	3.1×10^4	1.9×10^4
Test + *Trichoderma*	3.0×10^4	1.2×10^6	5.2×10^6
Test + *Pseudomonas*	3.2×10^4	2.5×10^6	9.8×10^6

The biochemical profiles of the predominant bacterial isolates are summarized in Table 2.

***Table 2:** Biochemical Characteristics of Predominant Bacterial Isolates*

Biochemical Test	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Acinetobacter calcoaceticus*
Gram Reaction	Gram-negative rods	Gram-positive rods	Gram-negative coccobacilli
Catalase	+	+	+
Oxidase	+	-	-
Citrate	+	+	+
Indole	-	-	-
Methyl Red	-	-	-
Voges-Proskauer	-	+	-

***Table 3.** Macroscopic and Microscopic Features of Fungal Isolates*

Feature	*Trichoderma harzianum*	*Aspergillus niger*
Growth Speed	Very Rapid	Moderate to Rapid
Colony Color	White mycelium turning green in patches	Jet-black

Feature	*Trichoderma harzianum*	*Aspergillus niger*
Colony Texture	Cottony, tufted	Velvety, powdery
Hyphae	Septate, hyaline	Septate, hyaline
Conidia	Smooth, green, in slimy heads	Rough, black, in dry cha

3.2. Screening for Biosurfactant Production

Pseudomonas aeruginosa exhibited the strongest biosurfactant activity, as evidenced by the highest emulsification index and largest oil displacement zone. *Bacillus subtilis* and *Trichoderma harzianum* also showed significant activity, making them promising candidates for enhancing the bioavailability of hydrophobic pesticides. *Aspergillus niger* showed relatively weaker biosurfactant production. The isolated microorganisms were screened for biosurfactant production. The results are presented in Table 4.

Table 4: Biosurfactant Production by the Isolated Microorganisms

Screening Test	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Trichoderma harzianum*	*Aspergillus niger*
Tilted Glass Technique	Positive (Rapid collapse)	Positive (Collapse)	Positive (Collapse)	Weakly Positive
Emulsification Index (E_{24}%)	68%	58%	55%	45%
Haemolytic Activity	Positive (Clear zone)	Positive (Clear zone)	Positive (Partial zone)	Negative
Oil-Spreading Technique (cm)	4.5 cm	3.8 cm	3.2 cm	2.1 cm

3.3. Bioremediation Efficiency: Reduction of Pesticide Compounds

The efficacy of the two most prominent isolates in remediating the pesticide-contaminated water was evaluated over 14 days. The results demonstrate that both *Trichoderma harzianum* and *Pseudomonas aeruginosa* were highly effective in degrading a wide spectrum of pesticide compounds within 14 days. On average, *Trichoderma harzianum* showed a slightly higher degradation efficiency (60.12%) compared to *Pseudomonas aeruginosa* (51.56%). The presence of a diverse consortium of other organisms like *Bacillus* and *Aspergillus* likely contributed to a synergistic effect, creating a more robust degradative environment, though the two tested organisms were the most effective as single agents. The percentage reduction of various pesticide compounds is summarized in Table 5.

Table 5: Percentage Reduction of Pesticides after 14 Days of Bioremediation

Compound	*Trichoderma* Conc. (mg/l)	% Reduction by *Trichoderma*	*Pseudomonas* Conc. (mg/l)	% Reduction by *Pseudomonas*
Captan	0.73	63.79%	0.73	47.95%
Chlordane, alpha (cis)	1.96	47.06%	1.96	42.35%
Chlorothalonil	0.64	60.47%	0.64	57.81%
DDT, o,p'-	0.78	62.75%	0.78	46.15%
DDT, p,p'-	1.35	42.72%	1.35	35.56%
Endosulfan-alpha	1.64	65.17%	1.64	37.20%
Endosulfan-sulfate	0.87	55.56%	0.87	58.62%
Hexachlorobenzene	0.53	60.71%	0.53	66.04%
Methoxychlor	0.90	56.76%	0.90	52.22%
Tecnazene	0.83	77.78%	0.83	59.52%
Cyanazine	0.77	82.14%	0.77	68.85%
O	2.38	7%	2.38	14%
DO	0.42	18%	0.42	15%
Average % Reduction	0.86	**60.12%**	0.86	**51.56%**

Based on the hypothesis that organisms naturally selected for one trait (hydrocarbon degradation) in a contaminated environment may also exhibit complementary bioremediation abilities, this study highlights the effectiveness of indigenous microbial isolates in reducing pesticide contamination. The results of this study strongly validate this premise. The isolation of a diverse consortium (Table 1), dominated by renowned hydrocarbon-degrading genera like *Pseudomonas* and *Bacillus*, aligns with findings from [9] , who reported the dominance of these genera in soils with high level of both pesticides and hydrocarbons contaminated. The significant presence of fungi like *Trichoderma harzianum* and *Aspergillus niger* further strengthens the consortium's degradative potential.

The role of fungi in bioremediation has gained attention due to their versatile enzymatic

machinery, such as cytochrome P450 monppxygenases and lignin-modifying enzymes, which are particularly effective at breaking down persistent aromatic structures present in many pesticides compounds [10]. Therefore, the indigenous microbial community we isolated was not a random assembly but a pre-adapted, synergistic consortium ideally suited for the project's goal.

The core concept of using "biosurfactant-producing organisms" was a key determinant of the study's success. Table 1 demonstrate that the leading isolates possess strong capabilities for surface-active agents production. *Pseudomonas aeruginosa* exhibited the highest emulsification index (E24: 68%), a finding consistent with a recent study by [8], who reported a *P. aeruginosa* strain with an E24 of 72% capable of enhancing phenanthrene degradation by over 70%. Biosurfactants function by reducing surface and interfacial tension, thereby emulsifying hydrophobic pollutants and increasing their surface area for microbial attachment and enzymatic attack [11].

This mechanism directly addresses the primary bottleneck in the bioremediation of pesticides and hydrocarbons: their low aqueous solubility and bioavailability. The positive results in the haemolytic and oil-spreading tests further confirm the production of potent surfactants. The fact that *Trichoderma harzianum*, a fungus, also showed significant biosurfactant activity (E24: 55%) is a notable finding. While bacterial surfactants are well-studied, fungal biosurfactants are an emerging frontier. This study result corroborates the work of [12], who highlighted the potential of *Trichoderma* spp. in producing glycolipid biosurfactants that enhance the removal of hydrophobic contaminants. This biosurfactant production is the critical "bridge" that connects the organism's inherent degradative capacity to the target pollutant, making the process efficient and effective.

The bioremediation efficacy data (Table 4) reveals a remarkable degradation profile, with an average reduction of 60.12% and 51.56% by *Trichoderma* and *Pseudomonas*, respectively, over 14 days. This performance is comparable, and in some cases superior, to recent studies. For instance [13], using a consortium of *Bacillus* and *Acinetobacter* achieved a 65% reduction of chlorpyrifos in 15 days. achieving similar efficiency with a different mix of pesticides, demonstrate the robustness of the indigenous isolates.

The compound-specific degradation patterns provide insight into the underlying microbial mechanisms. The exceptional degradation of Cyanazine (82.14%) and Tecnazene (77.78%) by *Trichoderma* suggests the presence of highly efficient dealkylation and dechlorination pathways. This aligns with the work of [14], who demonstrated *Trichoderma viride's* ability to degrade atrazine via sequential dechlorination and dealkylation. Conversely, the superior performance of *Pseudomonas* against Hexachlorobenzene (66.04%) points to a strong reductive dechlorination capability, a well-documented trait in *Pseudomonas* species for treating chlorinated solvents and pesticides [15].

The lower degradation rates observed for persistent organochlorines like DDT, p,p'- (35.56-42.72%) and Chlordane (42.35-47.06%) are expected due to their high chemical stability and toxicity. However, the fact that significant degradation occurred at all is promising. This is likely a result of co-metabolism, where the microbes, while utilizing the more labile TPH and other pesticides as primary substrates, fortuitously degraded these recalcitrant compounds. This phenomenon underscores the advantage of using a

mixed-waste adapted consortium, as highlighted in the title.

A pivotal finding of this study is the slightly higher overall degradation efficiency of the fungus *Trichoderma harzianum* compared to the bacterium *Pseudomonas aeruginosa*. This can be attributed to the unique biology of fungi. Their filamentous hyphal networks physically penetrate the soil/water matrix, accessing even sequestered contaminants [16]. Furthermore, their enzymatic systems, particularly extracellular laccases and peroxidases, are non-specific and powerful enough to oxidize a vast range of xenobiotics. A recent meta-analysis by [16] concluded that fungal-based bioremediation often outperforms bacterial systems for complex, multi-component pesticide mixtures, which directly supports our findings.

4. CONCLUSION

Trichoderma harzianum showed a slightly higher degradation efficiency compared to *Pseudomonas aeruginosa*. While this study tested the organisms individually, the presence of a diverse consortium suggests that a synergistic effect likely occurred in the microcosms. Bacteria and fungi often work in concert; fungi can break down complex polymers into smaller intermediates that bacteria can then mineralize. This partnership, combined with the biosurfactant production from both kingdoms, creates a highly efficient and self-sustaining bioremediation system.

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Conflict of Interest Declaration

The authors declare no conflict of interest.

ORCID ID:
https://orcid.org/0000-0001-7043-3643

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