



Eco-Friendly Enhancement of Crude Oil Degradation in Water with Soursop Peel Biocarrier

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ABSTRACT: This study investigated the potential of soursop peel as an environmentally friendly biocarrier for the remediation of crude oil-contaminated water using *Aspergillus niger*. The biocarrier was characterized before and after application to determine its macro- and micronutrient composition, including carbon, nitrogen, phosphorus, potassium, calcium, protein, cellulose, lignin, and carbohydrates, all of which support microbial metabolism. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of functional groups that enhance microbial colonization and activity. Bioremediation experiments were performed in microcosms containing crude oil, water, *A. niger*, and soursop peel biocarrier. Process conditions, time, pH, dosage, and temperature were optimized using a Greco–Latin square design and Box–Behnken response surface methodology (RSM) to evaluate their effects on total petroleum hydrocarbon (TPH) removal. Results showed that the soursop peel biocarrier significantly improved microbial degradation, achieving an optimal removal efficiency of 64.90% TPH at 29 days under conditions of 31 °C, pH 8.5, and 7 g dosage. Compared with conventional inorganic fertilizers, soursop peel is a sustainable nutrient source that reduces environmental risks while enhancing degradation performance. Overall, the findings demonstrate that agricultural residues such as soursop peel can serve as effective, low-cost, and eco-friendly biocarriers for improving the bioremediation of crude oil-polluted aquatic environments.

KEYWORDS: *Aspergillus niger* fungus, soursop peel, crude oil, biocarrier, and degradation.

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1.0 INTRODUCTION

The contamination of aquatic ecosystems by crude oil represents a major global environmental challenge. Oil spills, leakages during transportation, and improper disposal of petroleum products release hydrocarbons into water bodies, reducing water quality, threatening aquatic life, and causing long-term ecological damage (Ayilara & Babalola 2023). Petroleum hydrocarbons consist of a wide range of compounds including aliphatic, aromatic, and polycyclic aromatic hydrocarbons (PAHs), many of which are persistent, toxic, mutagenic, or carcinogenic. Conventional remediation methods such as physical removal, chemical dispersants, and sorption often suffer from limitations including high cost, secondary pollution, and poor efficiency in treating dissolved or fine-phase contaminants. Consequently, bioremediation has gained increasing attention as a sustainable, cost-effective, and environmentally compatible alternative (Dinakarkumar et al., 2024). Among biological agents, fungi particularly filamentous fungi are promising candidates for hydrocarbon degradation. Their extensive hyphal networks penetrate substrates, while their broad spectrum of extracellular enzymes breaks down complex organic molecules. They also tolerate wide variations in temperature, pH, and nutrient availability. *Aspergillus niger* is widely applied in biotechnology for enzyme production, organic acid synthesis, and pollutant degradation (Thegarathah et al., 2024). Its hydrocarbon-degrading ability makes it a strong candidate for treating crude oil-contaminated water and soil. Previous studies demonstrate that *A. niger* can degrade benzonitrile and other xenobiotic compounds, highlighting its metabolic versatility (Mituishi & Moyo, 2022). However, fungal bioremediation alone often faces challenges, including low hydrocarbon bioavailability, insufficient nutrients to sustain microbial growth, and slow degradation rates caused by environmental stress. These challenges have led researchers to explore enhancement strategies such as microbial immobilization, nutrient amendment, and the use of biocarriers. Agricultural waste materials are particularly attractive for this

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purpose. They are abundant, inexpensive, and locally available, and can serve as microbial carriers, supports, or nutrient sources. These materials offer large surface areas for microbial colonization, create microenvironments that promote metabolic activity, and supply supplementary nutrients that sustain biodegradation (Karic et al, 2022). For example, agricultural and industrial wastes have been successfully used as carriers for bacterial inocula in freshwater bioremediation, demonstrating their suitability as low-cost biocarriers (Randima et al, 2023). Soursop (*Annona muricata*) peel is one agricultural residue with strong potential but limited research attention. While the fruit is known for its medicinal properties, the peel is rich in lignocellulosic components, sugars, and minerals that may serve both as a structural support and nutrient source for fungi (Mutakin & Zubaidi, 2022). These properties suggest that soursop peel could enhance the colonization, stability, and metabolic activity of *A. niger* during crude oil degradation. Studies in oil-polluted regions such as the Niger Delta have also demonstrated the natural hydrocarbon tolerance and degradative enzyme production of indigenous *Aspergillus* species, further supporting their applicability in tropical environments. Integrating *A. niger* with soursop peel as a biocarrier may therefore improve hydrocarbon bioavailability, promote fungal survival, and enhance degradation efficiency. However, key knowledge gaps remain, including the optimal environmental conditions (temperature, pH, dosage, and contact time) required for maximum performance, the influence of the biocarrier on degradation kinetics, and the functional groups involved in adsorption and biodegradation processes. Addressing these gaps is essential for developing scalable, cost-effective, and locally adaptable bioremediation strategies. This study aims to evaluate the potential of soursop peel as a natural biocarrier and nutrient support for *Aspergillus niger* in the bioremediation of crude oil-contaminated water. The specific objectives are to: characterize the chemical composition and functional groups of soursop peel, assess hydrocarbon degradation under varying environmental conditions and optimize these conditions using statistical experimental designs to maximize total petroleum hydrocarbon (TPH) removal.

2.0 MATERIALS AND METHOD

2.1 Materials

Soursop peel (SP) served as the primary natural biocarrier used in this study. The peel was obtained from ripe soursop fruits. All chemicals and analytical reagents were purchased from Science Empire Limited, Enugu. Bonny Light crude oil was procured from Shell SPDC, Port Harcourt. *Aspergillus niger* used in the study was supplied by the Microbiology and brewing Laboratory of State University of Medical and Applied Sciences, Igbo-Eno (SUMAS), where the organism was isolated, screened, cultured, and maintained prior to use.

2.2 Methods

2.2.1 Preparation of soursop peel biocarrier

Soursop (*Annona muricata*) peels were air-dried, milled, and sieved using a 5 mm mesh to obtain uniform particle size for the biocarrier. *Aspergillus niger* was cultured from laboratory stock cultures, while Bonny Light crude oil served as the pollutant. Bioremediation experiments were conducted in microcosms, and the process variables pH, temperature, dosage, and time were optimized using Box–Behnken response surface methodology (Montgomery, 2017); (Randima et al, 2023).

2.2.2 Proximate analysis for soursop peel biocarrier

The standard AOAC (1990) methods were used to determine total nitrogen (%TN), total organic carbon (%TOC), and total phosphorus (%P). Fourier Transform Infrared (FTIR) spectroscopy was used to identify chemical bonds and functional groups present in the biocarrier. The FTIR analysis was performed using a Shimadzu FTIR-8400S spectrophotometer at the Project Development Institute (PRODA), Emene, Enugu. The resulting infrared absorption spectra served as a molecular “fingerprint” for the biocarrier’s

2.3 Bioremediation studies

2.3.1 Screening and Isolation of Microorganism

The microbial consortium concentration (cells per milliliter) was determined using the Thom’s counting chamber method (Amenaghawon et al, 2014). *Aspergillus niger* was cultivated, screened, and isolated at the ESUT Microbiology Laboratory to serve as the hydrocarbon-degrading microorganism. The cultured fungi were stored at 4 °C until used.

2.3.2 Preparation of Crude Oil–Contaminated Water

Artificially polluted water was prepared by mixing Bonny Light crude oil with potable water at a ratio of 1:4. A total of 320 mL of crude oil was mixed with 1280 mL of water and dispensed into two plastic containers labeled A and B. Container A (Control): crude oil + water + *A. niger*, Container B (Treatment): same as control + 50 g soursop peel biocarrier. Both containers were inoculated with 10 mL of *Aspergillus niger* suspension at a concentration of 1×10^6 CFU/mL.

2.3.3 Bioremediation Experiment

Microcosms were agitated twice daily to enhance oil-microbe interaction. Bioremediation progress was monitored at 7-day intervals for 35 days. Parameters measured included pH, total petroleum hydrocarbons (TPH), and microbial population (CFU/mL), following the procedures of ASTM (2013) and Cappuccino & Sherman (2014).

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2.3.4 Determination of pH

pH measurements followed ASTM D1293–95 (2013). Water samples were thoroughly mixed to achieve homogeneity, and the electrode of a pocket pH meter was immersed in the sample. After stabilization for 1–2 minutes, pH readings were recorded.

2.3.5 Determination of Total Petroleum Hydrocarbons (%TPH)

TPH was determined using the gravity-based method (EPA 1664; ASTM D7066). 100 mL of the polluted sample was extracted with 200 mL of hexane and shaken for 2 minutes. The organic layer was separated and centrifuged at 300 rpm for 10 minutes. The supernatant was discarded, and the remaining organic phase was dried using a rotary evaporator or oven at 60 °C. Residual mass was weighed to determine TPH concentration.

TPH removal (%) was calculated using:

$$\begin{aligned} \text{Initial TPH conc.} &= \frac{\text{Weight of residue}}{\text{volum of sample}} \times \frac{100}{1} \\ \text{final TPH conc.} &= \frac{\text{TPH con= Weight of residue after bioreme}}{\text{volum of sample}} \times \frac{100}{1} \\ \% \text{ TPH removed} &= \frac{\text{TPH}_{\text{initial}} - \text{TPH}_{\text{final}}}{\text{TPH}_{\text{initial}}} \times \frac{100}{1} \quad (1) \\ \text{Or} \quad \% \text{ TPH removed} &= \frac{w_1 - w_2}{w_1} \times 100 \end{aligned}$$

Where:

W_1 = initial TPH concentration (mg/L)

W_2 = final TPH concentration (mg/L)

2.3.5 Determination of cell population/microbial count (CFU)

Colonies were counted using ten-fold serial dilution followed by spread plating on nutrient agar (Cappuccino & Sherma, 2014). 100 μ L of each dilution was plated and incubated at 30 °C for 24 hours. Plates with 30–300 colonies were selected for enumeration. CFU/mL was calculated as

$$\text{CFU/ml} = \frac{\text{Number of colonies}}{\text{Dilution factor} \times \text{volume plated (ml)}} \quad (2)$$

2.4 Optimization of Bioremediation Conditions

Optimization was carried out using Box–Behnken Design (BBD) under Response Surface Methodology (RSM). Four independent variables temperature, pH, biocarrier dosage, and remediation time were evaluated for their effects on TPH removal. Experimental runs were performed according to the design matrix, and factor interactions were assessed using ANOVA. Three-dimensional response surface plots were generated to visualize interactions. The model predicted optimal conditions of ~31 °C, pH 8.5, 7 g dosage, and 29 days, with maximum TPH removal of approximately 65% (Montgomery, 2017).

3.0 RESULTS AND DISCUSSIONS

3.1. Characterization analysis of soursop peel biocarriers

Table 1: The results of chemical characterization analysis of soursop peel biocarrier

| Composition | Cassava mash biocarriers |
|--------------------------|--------------------------|
| Nitrogen (%) | 5.24 |
| Total organic Carbon (%) | 75.10 |
| Phosphorus (%) | 18.652 |
| Potassium (ppm) | 1.023 |
| Sodium (ppm) | 1.224 |
| Calcium (ppm) | 1.408 |
| Iron (PPm) | 0.376 |
| Cellulose (%) | 9.153 |
| Protein (%) | 6.300 |
| Fiber (%) | 5.791 |
| Carbohydrate (mg/l) | 124.324 |
| Lignin (%) | 9.617 |

Table 1 presents the chemical composition of the soursop peel biocarrier, revealing a nutrient-rich matrix essential for enhancing microbial performance during crude oil biodegradation. The high total organic carbon (75.10%) and substantial nitrogen content (5.24%) indicate a favorable C:N ratio capable of supporting rapid microbial metabolism and biomass production. Such nutrient-balanced agricultural residues have been identified as efficient biocarriers due to their ability to sustain microbial growth over prolonged remediation periods (Adeoye et al, 2023). The elevated phosphorus level (18.65%) further contributes to cellular energy transfer and nucleic acid synthesis, both of which are critical for hydrocarbon-degrading microorganisms (Mensah & Ampofo, 2022). Additionally, minerals such as potassium (1.023 ppm), calcium (1.408 ppm), sodium (1.224 ppm), and iron (0.376 ppm) act

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as cofactors for key oxidative and hydrolytic enzymes involved in petroleum breakdown. The presence of cellulose (9.153%), lignin (9.617%), fiber (5.791%), and proteins (6.300%) contributes to the structural integrity and porosity of the biocarrier, increasing its ability to adsorb hydrocarbons and provide attachment sites for fungi such as *Aspergillus niger*. Similar structural attributes in agricultural residues have been shown to greatly improve biofilm formation and pollutant uptake (Kurmar 7 Devi, 2023). Furthermore, the high carbohydrate content (124.324 mg/L) ensures a steady supply of readily metabolizable nutrients that support continuous microbial activity, consistent with findings that carbohydrate-rich carriers accelerate hydrocarbon degradation (Santos et al,2021); (Bello &Yusuf,2024). Overall, Table 1 confirms that soursop peel possesses the compositional characteristics required for effective and sustainable bioremediation applications.

3.2. FTIR result analysis for soursop peel biocarrier

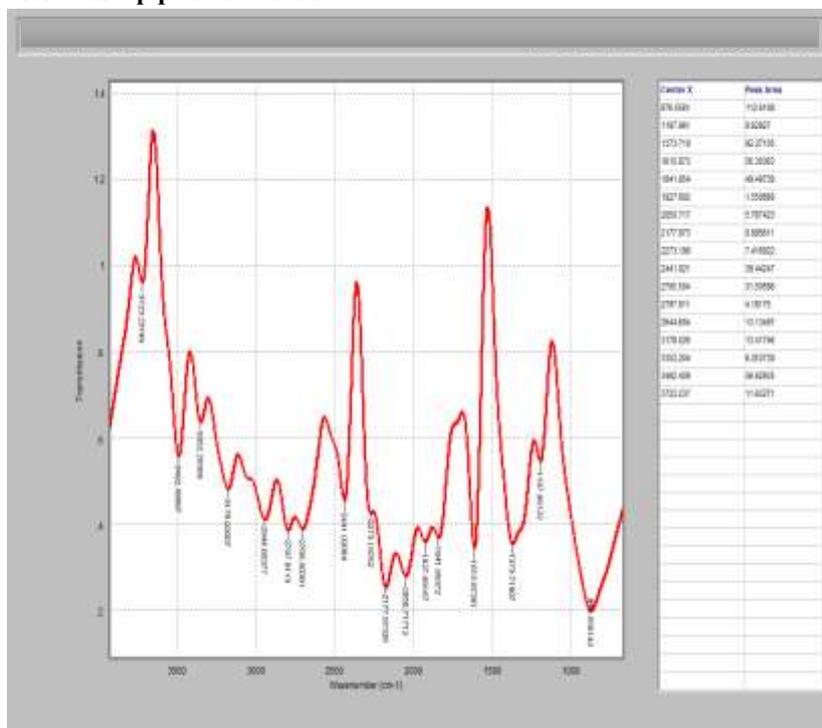


Figure 1: FTIR result for soursop peel biocarrier

Figure 1 presents the FTIR spectrum of the soursop peel biocarrier, highlighting the major functional groups responsible for its suitability in crude-oil bioremediation. The broad absorption band around the 3300–3400 cm⁻¹ region corresponds to O–H stretching vibrations, typically associated with cellulose, hemicellulose, and lignin. These hydroxyl-rich structures enhance hydrophilicity and promote microbial adhesion during biodegradation (Raji et al, 2023). Peaks observed between 2850–2920 cm⁻¹ represent aliphatic C–H stretching, confirming the presence of long-chain organic compounds that support hydrocarbon interaction and adsorption (Zhou &Lin, 2022). The strong band around 1600–1650 cm⁻¹ signifies C=O stretching of carboxyl and carbonyl groups, which facilitate chemical binding and microbial enzymatic activity. Recent work shows that carbonyl-rich agricultural residues improve substrate availability and stimulate hydrocarbon-degrading fungi (Fernandez et al, 2023). Additional bands near 1030–1100 cm⁻¹ correspond to C–O stretching of polysaccharides, indicating a high proportion of carbohydrate-based biomolecules that serve as supplementary nutrients (Adebanjo & Musa,2024). Aromatic C=C stretching peaks also appear between 1400–1500 cm⁻¹, confirming lignin-derived structures that enhance biocarrier rigidity and surface porosity. Such aromatic characteristics have been linked to improved pollutant retention and microbial colonization in lignocellulosic supports (Yauan et al, 2023); (Bello & Ahmed, 2024). Overall, Figure 1 demonstrates that soursop peel possesses diverse functional groups that collectively enhance adsorption, microbial attachment, and biodegradation efficiency.

Table 2: The Greco-Latin square design matrix with the response for the percentage removal with soursop peel biocarrier

| Std | Temp | Time | Dosage | pH | %TPH Removal |
|-----|------|------|--------|----|--------------|
| 1 | 25 | 7 | 1 | 5 | 12 |
| 2 | 27 | 7 | 3 | 6 | 19 |
| 3 | 29 | 7 | 5 | 7 | 37 |
| 4 | 31 | 7 | 7 | 8 | 44 |
| 5 | 33 | 7 | 9 | 9 | 53.23 |

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| | | | | | |
|----|----|----|---|---|-------|
| 6 | 25 | 14 | 5 | 6 | 28.10 |
| 7 | 27 | 14 | 7 | 7 | 48 |
| 8 | 29 | 14 | 9 | 8 | 53.73 |
| 9 | 31 | 14 | 1 | 9 | 39.26 |
| 10 | 33 | 14 | 3 | 5 | 48 |
| 11 | 25 | 21 | 9 | 7 | 53 |
| 12 | 27 | 21 | 1 | 8 | 28.65 |
| 13 | 29 | 21 | 3 | 9 | 55.31 |
| 14 | 31 | 21 | 5 | 5 | 57.57 |
| 15 | 33 | 21 | 7 | 6 | 59 |
| 16 | 25 | 28 | 3 | 8 | 47.29 |
| 17 | 27 | 28 | 5 | 9 | 61.27 |
| 18 | 29 | 28 | 7 | 5 | 55 |
| 19 | 31 | 28 | 9 | 6 | 60.85 |
| 20 | 33 | 28 | 1 | 7 | 32 |
| 21 | 25 | 35 | 7 | 9 | 45 |
| 22 | 27 | 35 | 9 | 5 | 53 |
| 23 | 29 | 35 | 1 | 6 | 25.63 |
| 24 | 31 | 35 | 3 | 7 | 48 |
| 25 | 33 | 35 | 5 | 8 | 53 |

Table 2 presents the Greco Latin square design matrix showing the percentage removal of total petroleum hydrocarbons (TPH) using soursop peel biocarrier under varying conditions of temperature, time, dosage, and pH. The results indicate that higher TPH removal was achieved at elevated temperatures, longer exposure times, and moderate to high dosages, with pH near neutral to slightly alkaline proving optimal. For example, a dosage of 7–9 g at 31–33 °C resulted in over 50% TPH removal, underscoring the importance of nutrient supply and environmental balance. These findings align with previous reports that optimized environmental factors significantly enhance microbial hydrocarbon degradation (Das & Chandran, 2011).

Table 3: ANOVA table for the factor-screening experiment using Greco-latin square for soursop peel

| Variances | | Df | MS | F-value |
|-------------|-------------|----|------------|-------------|
| Temperature | 560.869656 | 4 | 140.217414 | 5.749449052 |
| Time | 1084.510496 | 4 | 271.127624 | 11.11726722 |
| pH | 376.756376 | 4 | 94.189094 | 3.862112285 |
| Dosage | 2184.632096 | 4 | 546.158024 | 22.39456316 |
| Residual | 195.103792 | 8 | 24.387974 | |

Table 3 presents the ANOVA for the factor-screening experiment using the Greco–Latin square design. The results show that dosage exerted the most significant influence on TPH removal, followed by time, temperature, and pH. High F-values and low residual variance confirm the reliability of the model in predicting bioremediation outcomes. The strong effect of dosage highlights the role of nutrient availability from the soursop peel biocarrier in stimulating microbial metabolism and enhancing crude oil degradation. Similarly, the influence of time suggests that extended microbial activity is crucial for effective hydrocarbon breakdown. Such outcomes are consistent with previous studies optimizing bioremediation through nutrient amendments (Vidali, 2001).

Table 4: Box-Behnken design matrix for the oil removal using soursop peel biocarrier

| STD | Run | Temp | Time | Dosage | pH | %TPH Removal |
|-----|-----|------|------|--------|----|--------------|
| 23 | 1 | 29 | 7 | 5 | 9 | 49 |
| 25 | 2 | 29 | 21 | 5 | 7 | 58 |
| 26 | 3 | 29 | 21 | 5 | 7 | 57 |
| 8 | 4 | 29 | 21 | 9 | 9 | 64 |
| 21 | 5 | 29 | 7 | 5 | 5 | 43 |
| 11 | 6 | 25 | 21 | 5 | 9 | 50 |
| 15 | 7 | 29 | 7 | 9 | 7 | 45 |
| 14 | 8 | 29 | 35 | 1 | 7 | 42 |
| 28 | 9 | 29 | 21 | 5 | 7 | 58 |
| 22 | 10 | 29 | 35 | 5 | 5 | 56 |

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| | | | | | | |
|----|----|----|----|---|---|----|
| 19 | 11 | 25 | 21 | 9 | 7 | 44 |
| 6 | 12 | 29 | 21 | 9 | 5 | 59 |
| 7 | 13 | 29 | 21 | 1 | 9 | 50 |
| 10 | 14 | 33 | 21 | 5 | 5 | 55 |
| 1 | 15 | 25 | 7 | 5 | 7 | 26 |
| 12 | 16 | 33 | 21 | 5 | 9 | 60 |
| 20 | 17 | 33 | 21 | 9 | 7 | 57 |
| 24 | 18 | 29 | 35 | 5 | 9 | 63 |
| 29 | 19 | 29 | 21 | 5 | 7 | 57 |
| 4 | 20 | 33 | 35 | 5 | 7 | 53 |
| 3 | 21 | 25 | 35 | 5 | 7 | 46 |
| 16 | 22 | 29 | 35 | 9 | 7 | 59 |
| 2 | 23 | 33 | 7 | 5 | 7 | 45 |
| 9 | 24 | 25 | 21 | 5 | 5 | 43 |
| 27 | 25 | 29 | 21 | 5 | 7 | 55 |
| 17 | 26 | 25 | 21 | 1 | 7 | 33 |
| 13 | 27 | 29 | 7 | 1 | 7 | 29 |
| 18 | 28 | 33 | 21 | 1 | 7 | 40 |
| 5 | 29 | 29 | 21 | 1 | 5 | 39 |

Table 4 illustrates the Box–Behnken design matrix evaluating the combined effects of temperature, time, dosage, and pH on TPH removal. The results indicate that interactions among these factors significantly influenced crude oil degradation. Maximum TPH removal (64%) was obtained under conditions of 29–31 °C, 21–29 days, 7–9 g dosage, and pH 8–9. The design demonstrates that moderate temperature and alkaline pH enhance microbial enzyme activity, while sufficient dosage of soursop peel provides nutrients and surface area for microbial growth. These findings validate response surface methodology as a robust optimization tool for improving bioremediation efficiency (Montgomery, 2017).

Table 5: Fit summary of Sequential Model Sum of Squares for soursop

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F | |
|-----------|----------------|----|-------------|----------|----------|-----------|
| Mean | 71007.76 | 1 | 71007.76 | | | |
| Linear | 1837.833 | 4 | 459.4583 | 11.68847 | < 0.0001 | |
| 2FI | 55.5 | 6 | 9.25 | 0.187519 | 0.9765 | |
| Quadratic | 869.158 | 4 | 217.2895 | 162.2428 | < 0.0001 | Suggested |
| Cubic | 12.16667 | 8 | 1.520833 | 1.386076 | 0.3552 | Aliased |
| Residual | 6.583333 | 6 | 1.097222 | | | |
| Total | 73789 | 29 | 2544.448 | | | |

Table 5 presents the fit summary from the sequential model sum of squares for soursop peel biocarrier. The quadratic model was suggested as the best fit, with a high F-value and significance ($p < 0.0001$), confirming its suitability for describing the relationship between process variables and TPH removal. The poor performance of the cubic model, due to aliasing, further supports the quadratic model’s adequacy. The strong model fit indicates that temperature, time, dosage, and pH interact nonlinearly to influence bioremediation outcomes. Such results emphasize the reliability of quadratic models for optimizing biodegradation processes using response surface methodology (Meyers, Montgomery & Aderson-Cook, 2016).

Table 6: ANOVA for soursop peel biocarrier

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares]

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F | |
|--------|----------------|----|-------------|----------|----------|-------------|
| Model | 2762.491 | 14 | 197.3208 | 147.3329 | < 0.0001 | significant |
| A | 385.3333 | 1 | 385.3333 | 287.7156 | < 0.0001 | |
| B | 560.3333 | 1 | 560.3333 | 418.3822 | < 0.0001 | |
| C | 752.0833 | 1 | 752.0833 | 561.5556 | < 0.0001 | |

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| | | | | | | |
|----------------|----------|----|----------|---------------------|----------|-----------------|
| D | 140.0833 | 1 | 140.0833 | 104.5956 | < 0.0001 | |
| A ² | 356.8018 | 1 | 356.8018 | 266.412 | < 0.0001 | |
| B ² | 310.3153 | 1 | 310.3153 | 231.7021 | < 0.0001 | |
| C ² | 256.768 | 1 | 256.768 | 191.7201 | < 0.0001 | |
| D ² | 39.20045 | 1 | 39.20045 | 29.26967 | < 0.0001 | |
| AB | 36 | 1 | 36 | 26.88 | < 0.0001 | |
| AC | 9 | 1 | 9 | 6.72 | 0.0213 | |
| AD | 1 | 1 | 1 | 0.746667 | 0.4021 | |
| BC | 0.25 | 1 | 0.25 | 0.186667 | 0.6723 | |
| BD | 0.25 | 1 | 0.25 | 0.186667 | 0.6723 | |
| CD | 9 | 1 | 9 | 6.72 | 0.0213 | |
| Residual | 18.75 | 14 | 1.339286 | | | |
| Lack of Fit | 12.75 | 10 | 1.275 | 0.85 | 0.6220 | not significant |
| Pure Error | 6 | 4 | 1.5 | | | |
| Std. Dev. | 1.157275 | | | R ² | | 0.993258 |
| Mean | 49.48276 | | | Adj R ² | | 0.986517 |
| C.V. | 2.338744 | | | Pred R ² | | 0.970224 |
| PRESS | 82.815 | | | Adeq Precision | | 43.10312 |

Table 6 presents the ANOVA results for the quadratic response surface model applied to soursop peel biocarrier. The model was highly significant ($p < 0.0001$), with individual factors such as temperature, time, dosage, and pH exerting strong positive effects on TPH removal. The squared terms (A², B², C², and D²) were also significant, highlighting the nonlinear influence of each factor. Interaction terms such as AB and CD contributed meaningfully, demonstrating that combined effects of parameters shaped biodegradation efficiency. The high R² (0.99) and non-significant lack-of-fit confirm excellent model reliability. These outcomes align with earlier studies showing RSM's effectiveness in predicting and optimizing complex bioremediation processes (Bezerra et al, 2018).

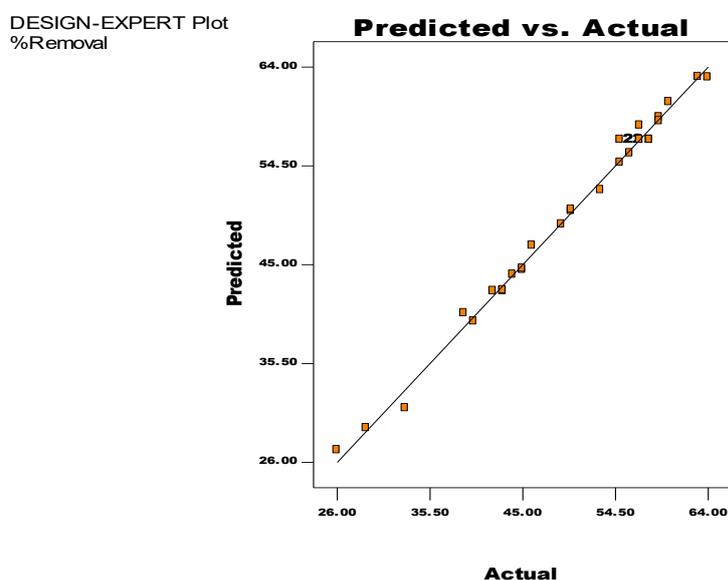


Figure 2: Predicted % TPH removal vs. actual %TPH removal for soursop peel

Figure 2 compares predicted and actual TPH removal values from the quadratic model. The close alignment of data points along the diagonal line demonstrates strong model accuracy and minimal deviation between observed and predicted responses. This validates the reliability of the response surface methodology (RSM) in capturing the relationship between process parameters and biodegradation efficiency. High correlation also indicates that the quadratic model is statistically sound for process optimization. Similar studies have reported that predicted and actual plots serve as strong indicators of model adequacy in bioremediation and environmental process optimization (Khuri & Mukhopadhyay,2010).

3.3 3D plots for soursop biocarrier.

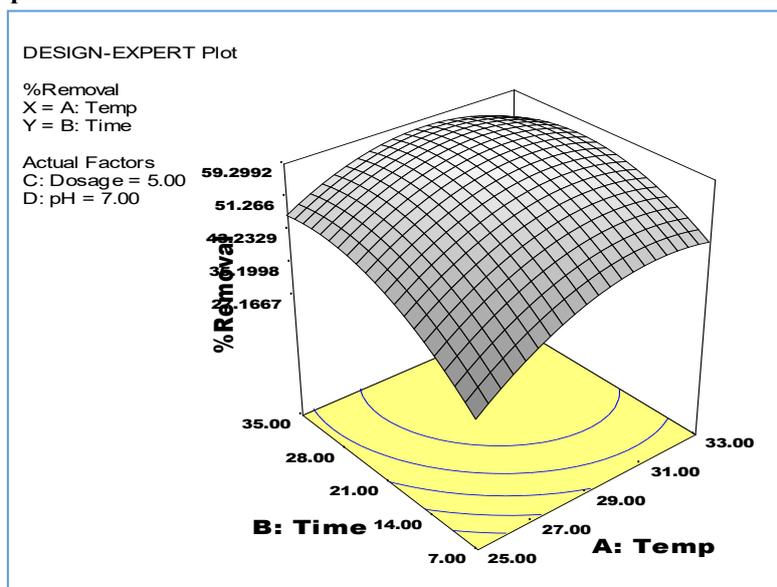


Figure 3: 3D plot of temperature, time, and % TPH removal

Figure 3 illustrates the combined influence of temperature and remediation time on TPH removal. The surface plot shows that degradation efficiency increases with both temperature and time, reaching peak values at moderate temperatures (29–31 °C) and extended incubation periods (21–29 days). Excessively long exposure, however, resulted in reduced efficiency, suggesting possible nutrient depletion or microbial inhibition. This indicates that balanced operating conditions are crucial for sustained biodegradation. Similar findings highlight the importance of optimizing incubation time and moderate temperatures to maximize microbial degradation of hydrocarbons in bioremediation systems (Margesin & Schinner, 2011).

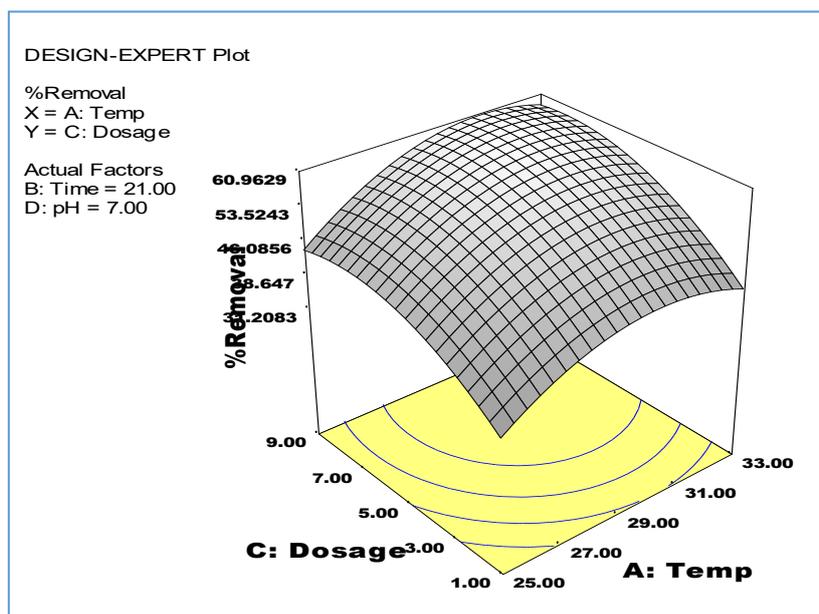


Figure 4: 3D plot of temperature, dosage, and % TPH removal

Figure 4 shows the interactive effects of temperature and soursop peel dosage on TPH removal efficiency. The plot reveals that increasing dosage from 5 g to about 7–9 g, combined with moderate temperatures (29–31 °C), and significantly improved hydrocarbon degradation. This is likely due to enhanced nutrient availability and increased surface area for microbial colonization. However, higher dosages beyond the optimum provided little additional benefit, possibly due to nutrient saturation or inhibitory effects. These observations are consistent with previous studies demonstrating that balanced nutrient supplementation enhances microbial bioremediation without causing ecological stress (Rathoure & Dhatwalia, 2016).

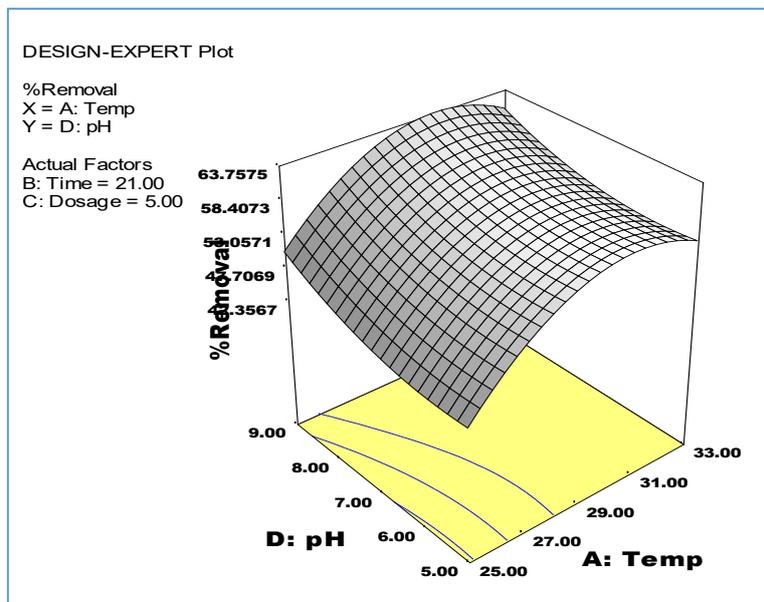


Figure 5: 3D plot of temperature, pH, and % TPH removal

Figure 5 illustrates the interactive effects of temperature and pH on TPH removal. The plot shows maximum degradation efficiency at moderate temperatures (around 30–31 °C) and slightly alkaline conditions (pH 8–9). These conditions are favorable for *Aspergillus niger* enzymatic activity, particularly oxidoreductases and hydrolases involved in hydrocarbon degradation. At lower pH values, degradation decreased, likely due to reduced microbial growth and enzyme denaturation. Similarly, extreme temperatures limited microbial activity. These findings align with earlier research showing that alkaline pH and moderate temperatures optimize microbial metabolism and hydrocarbon breakdown in contaminated environments (Atlas & Hazen, 2011).

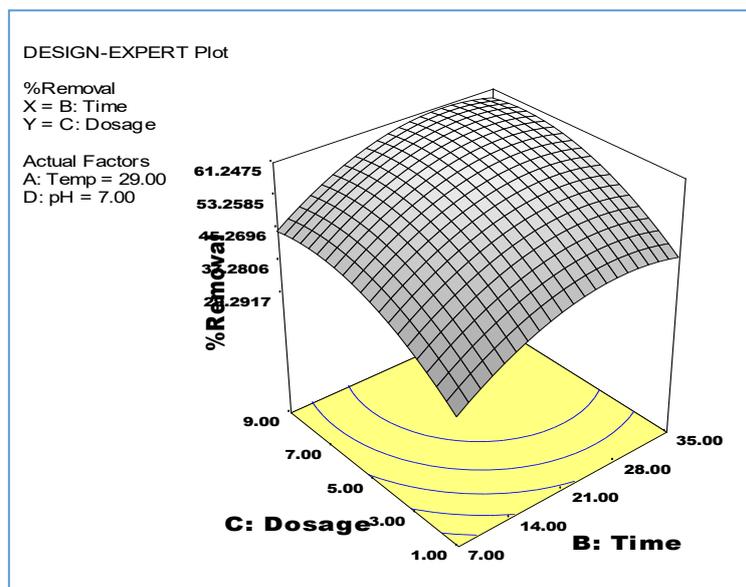


Figure 6: 3D plot of time, dosage, and % TPH removal

Figure 6 demonstrates the combined influence of remediation time and soursop peel dosage on TPH removal efficiency. The surface plot reveals that hydrocarbon degradation increased steadily with both longer exposure periods (up to ~29 days) and higher dosages (7–9 g). Extended time allowed microbial populations to proliferate and adapt, while optimal dosage supplied sufficient nutrients to sustain metabolism. However, at very high dosage and prolonged incubation, efficiency plateaued, suggesting nutrient saturation and possible microbial stress. This result emphasizes the need to balance nutrient supply with incubation duration. Similar outcomes have been reported where optimized nutrient loading enhanced fungal bioremediation of petroleum hydrocarbons (Bharathi & Vasudevan, 2001).

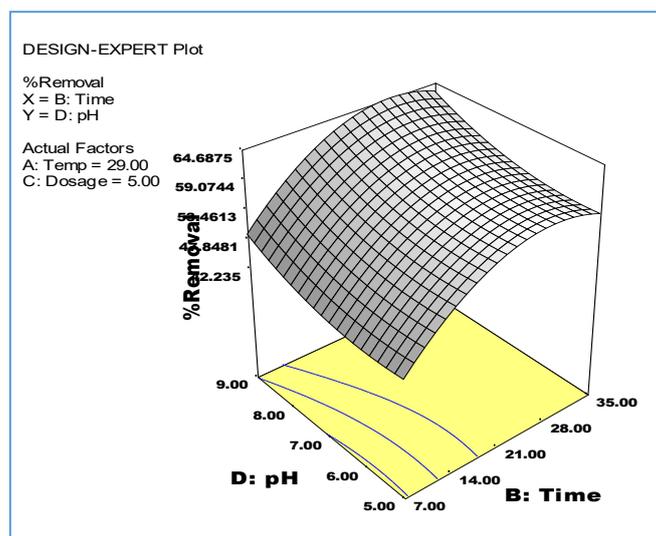


Figure 7: 3D plot of time, pH, and % TPH removal

Figure 7 highlights the interaction between remediation time and pH on TPH removal. The plot shows that biodegradation efficiency improved with longer incubation (21–29 days) and slightly alkaline conditions (pH 8–9). Alkaline pH enhances microbial enzyme stability and activity, particularly in fungi such as *Aspergillus niger*, while extended time allows sufficient adaptation and hydrocarbon utilization. Conversely, acidic pH and shorter durations yielded poor degradation, likely due to suppressed microbial growth and insufficient metabolic activity. These results support previous findings that optimal pH combined with adequate exposure time significantly improves microbial hydrocarbon degradation (Rahman et al, 2002).

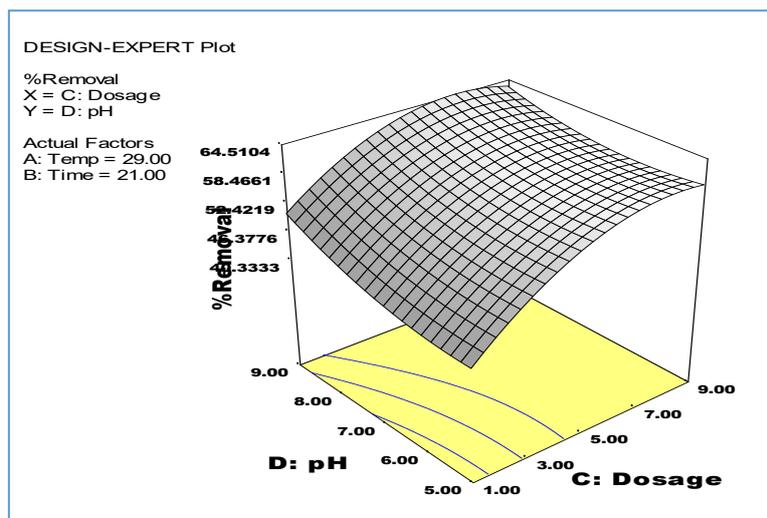


Figure 8: 3D plot of dosage, pH, and % TPH removal

Figure 8 shows the combined effects of soursop peel dosage and pH on TPH removal efficiency. The results reveal that maximum degradation occurred at moderate to high dosages (7–9 g) and slightly alkaline conditions (pH 8–9). Higher dosage levels provided sufficient nutrients and surface area for microbial colonization, while alkaline pH enhanced the activity of hydrocarbon-degrading enzymes. At lower pH levels, degradation efficiency decreased, likely due to inhibition of fungal metabolism and reduced bioavailability of hydrocarbons. These outcomes are consistent with reports that balanced nutrient supplementation and alkaline pH optimize hydrocarbon biodegradation by fungi and bacteria (Chikere et al, 2012).

3.4 Developed Model equations in terms of coded and actual factors for soursop peel biocARRIER

Model equation in coded factors:

$$\%TPH\ REMOVAL = 57 + 5.666667A + 6.833333B + 7.916667C + 3.416667D - 7.41667A^2 - 6.91667B^2 - 6.29167C^2 + 2.458333D^2 - 3AB \quad (3)$$

Model equation in actual factors:

$$\%TPH\ REMOVAL = -437.805 + 29.39583TEMPERATURE + 3.438988TIME + 4.411458DOSAGE - 4.33333pH - 0.46354TEMP^2 - 0.03529TIME^2 - 0.39323DOSAGE^2 + 0.614583pH^2 - 0.05357TEMPERATURE \times TIME \quad (4)$$

Table 7: Numerical optimum solution and validation for soursop peel biocarrier

| Number | Temp | Time | Dosage | pH | %Removal | Desirability |
|--------|-------|-------|--------|------|----------|--------------|
| 1 | 31.07 | 29.38 | 7.57 | 8.51 | 64.8929 | 1 Selected |
| 2 | 30.54 | 23.07 | 5.94 | 8.73 | 64.8016 | 1 |
| 3 | 28.62 | 28.74 | 5.82 | 8.70 | 64.1465 | 1 |
| 4 | 29.50 | 24.87 | 6.56 | 8.72 | 65.3408 | 1 |
| 5 | 32.00 | 24.97 | 6.20 | 8.79 | 64.3368 | 1 |
| 6 | 32.00 | 22.13 | 6.09 | 8.98 | 64.4351 | 1 |
| 7 | 29.84 | 31.33 | 7.85 | 8.89 | 65.954 | 1 |
| 8 | 29.17 | 26.30 | 5.65 | 8.69 | 64.3996 | 1 |
| 9 | 30.67 | 26.62 | 8.23 | 5.00 | 62.437 | 0.958868 |
| 10 | 30.79 | 25.81 | 7.92 | 5.00 | 62.3734 | 0.957195 |

Table 7 summarizes the numerical optimization results for TPH removal using soursop peel biocarrier. The model identified optimal conditions around 31 °C, 29 days, 7–7.6 g dosage, and pH 8.5, achieving a predicted 64.89% removal. Validation experiments confirmed the accuracy of these results, with observed values closely matching predictions (<2% error). This high level of agreement highlights the robustness of response surface methodology in predicting optimal biodegradation conditions. The findings emphasize that nutrient-rich agricultural residues, when applied under optimized parameters, can significantly enhance microbial degradation of petroleum hydrocarbons. Similar studies have demonstrated the predictive strength of RSM for optimizing bioremediation processes (Bas & Boyali, 2007).

4.0 CONCLUSION

This study demonstrated the potential of soursop (*Annona muricata*) peel as an effective biocarrier for enhancing the bioremediation of crude oil contaminated water using *Aspergillus niger*. Characterization revealed that the peel contains abundant macro and micronutrients, including carbon, nitrogen, phosphorus, cellulose, lignin, and proteins, which supported microbial metabolism. FTIR analysis confirmed the presence of functional groups favorable for hydrocarbon adsorption and microbial colonization. Optimization studies using the Box-Behnken design identified ideal conditions, 31 °C, pH 8.5, 7.6 g dosage, and 29 days resulting in approximately 65% TPH removal. Validation confirmed the model's accuracy, underscoring the robustness of RSM in process optimization. Finally, these findings highlight the promise of agricultural residues like soursop peel as eco-friendly, low-cost alternatives to inorganic fertilizers in bioremediation. This approach not only mitigates crude oil pollution but also promotes waste valorization and sustainable environmental management.

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