

(RESEARCH ARTICLE)



INFLUENCE OF BIOREMEDIATION PARAMETERS ON TPH DEGRADATION AND MICROBIAL DYNAMICS IN CRUDE OIL-CONTAMINATED WATER TREATED WITH NATURAL BIOCARRIERS

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ABSTRACT

Crude oil contamination of water poses serious environmental and public health risks due to its persistence and toxicity. This study evaluated the effectiveness of natural biocarriers, cassava mash (CM), maize bran (MB), and soursop peel (SP) in enhancing the bioremediation of crude-oil-polluted water using *Aspergillus niger*. The objectives were to: (i) characterize the nutrient composition of the biocarriers, (ii) assess changes in water quality indicators before and after contamination, (iii) monitor bioremediation-indicating parameters during treatment, and (iv) determine the extent of total petroleum hydrocarbon (TPH) reduction over 35 days. Initial contamination significantly degraded water quality, with BOD increasing from non-detectable (ND) to 14.32 mg/L, COD rising from ND to 16.02 mg/L, DO decreasing from 7.2 to 3.16 mg/L, and TPH reaching 1000 mg/L, while pH remained at 7.14. Biocarrier characterization showed high nutrient levels: nitrogen 5.24-5.82%, total organic carbon 75.10-78.61%, phosphorus 18.652-19.898%, potassium 1.023-2.103 ppm, calcium 1.408-2.832 ppm, iron 0.376-0.4217 ppm, protein 6.300-9.102%, and carbohydrates 100.436-155.598 mg/L. During bioremediation, all treated reactors showed steady improvement across key parameters. DO increased progressively, while BOD and COD declined, indicating effective microbial degradation. Microbial population rose sharply, especially in reactors with maize bran and cassava mash. TPH reduction was substantial in all treatments, with maize bran achieving the highest degradation efficiency of 94%, followed by cassava mash 90% and soursop peel 85% over 35 days. Overall, the study demonstrates that nutrient-rich natural biocarriers significantly improve crude oil bioremediation performance. Maize bran proved most effective, followed by cassava mash and soursop peel, offering a low-cost, sustainable solution for treating oil-polluted water.

Keywords: Bioremediation, TPH Degradation, Natural Biocarrier, Microbial Community, Crude Oil Pollution, *Asperengiluis niger*

1 INTRODUCTION

Oil pollution remains a pressing environmental challenge in many parts of the world, especially in regions with intensive petroleum exploration, transportation and refining activities. Crude oil and its derivatives contain thousands of hydrocarbon compounds (aliphatic, aromatic, heterocyclic, resin and asphaltene fractions) that are released into water bodies and soils, posing significant risks to aquatic ecosystems, flora and fauna, and ultimately to human health [1]. In Bioremediation, the use of microorganisms and in some cases plants or biocarriers to degrade or transform pollutants has emerged as a cost-effective, environmentally sustainable remediation strategy compared with physical or chemical methods [2]. In aquatic systems, crude oil contamination depletes dissolved oxygen, increases biochemical oxygen demand, disrupts native microbial communities, and can persist for long periods due to its complex mixture of refractory hydrocarbons [1]. As such, enhancing indigenous microbial degradation via nutrient addition, aeration, biocarriers or co-substrates is increasingly regarded as a viable approach. One challenge, however, lies in ensuring that nutrient limitation, mass-transfer constraints, microbial access to the pollutant phase, and favorable physicochemical conditions (pH, temperature, and redox) are adequately addressed in the remediation design [2]. Bio-carriers (organic or inorganic materials that provide surfaces, nutrients or support for microbial colonization) have gained attention as a means to stimulate biodegradation. In particular within tropical regions, agricultural waste materials offer a dual benefit: low-cost feedstock and nutrient supply for hydrocarbon-degrading microbes [3]. For example, cassava peel waste has been shown to act as an effective biostimulant in soil-based crude oil remediation trials, achieving substantial reductions in total petroleum hydrocarbons (TPH) and total hydrocarbon content (THC) while improving soil microbial numbers and nutrient levels [4]. Building on that, more recent research emphasizes the potential of cassava-derived biocarriers not only to supply nutrients but also to provide porous matrices that enhance microbial habitat and pollutant-microbe contact. For instance, a recent study found that cassava mash as an organic biocarrier significantly improved TPH removal in contaminated water compared with traditional inorganic nutrient amendments [5]. Given the high level of oil pollution in many Nigerian aquatic systems and the strategic availability of cassava and its processing residues, it becomes imperative to investigate how cassava-based bio-carriers can be optimally applied for water remediation. The objectives of the present study were therefore: (i) to characterize cassava mash, maize bran and soursop peel as organic biocarriers (nutrient content, physical properties) for oil-polluted water; (ii) to monitor the temporal changes in key water quality parameters (pH, dissolved oxygen, BOD, COD, TPH, microbial population) during the bioremediation process; (iii) to assess how the cassava mash, maize bran and soursop peel biocarriers influences microbial growth and hydrocarbon degradation kinetics; and (iv) to evaluate the efficiency of TPH removal over a defined remediation period under controlled conditions. By focusing on such an organic biocarrier, this study also addresses the dual environmental challenge of oil contamination and agricultural-waste management. As observed by Kalia (2022), integrating waste biomass into bioremediation systems can improve sustainability and cost-effectiveness. Moreover, because many existing remediation studies are soil-based, applying natural biocarriers in an aqueous matrix fills a gap in knowledge, particularly for stagnant or slow-flowing water bodies where oil contamination persists. The findings are expected to provide insight into how nutrient supply, carrier structure and microbial dynamics interact to drive hydrocarbon removal, and may contribute to optimization of remediation strategies in the Niger Delta and similar regions globally.

2 LITERATURE REVIEW

Bioremediation is widely accepted as an economical and environmentally sustainable strategy for treating petroleum-contaminated water and soils, because it harnesses indigenous or introduced microorganisms to transform hydrocarbons into less harmful products [6]. Success depends on microbial community composition, bioavailability of hydrocarbons, and environmental controls such as dissolved oxygen (DO), nutrient availability (N, P), pH, temperature, and contact between microbes and pollutants [7]. Recent reviews emphasize integrated approaches combining bioaugmentation, biostimulation and physical supports (carriers or amendments) to overcome mass-transfer and nutrient-limitation challenges that limit biodegradation in aqueous systems. The use of agricultural wastes as natural biocarriers is receiving growing attention because they simultaneously supply nutrients, sorptive surfaces, and habitat for microbial colonization while valorizing biomass residues [2]; [8]. Cassava residues (peel, mash, or fermented liquid) have been repeatedly tested as biostimulants: studies report that cassava by-products increase soil N and organic carbon, enhance hydrocarbon-degrader abundance, and produce statistically significant TPH reductions compared with untreated controls [4]; research reports 2023–2025). These materials are rich in labile carbon and micronutrients, which stimulate heterotrophic hydrocarbon degraders and can improve enzymatic breakdown of aliphatics and some aromatics. Maize bran and other cereal processing wastes are likewise effective as low-cost nutrient matrices and porous substrates that increase surface area for biofilm formation [9]. Comparative work suggests that different agricultural carriers vary in nutrient profile (e.g., C: N: P ratio), porosity, and release rates of soluble organics factors which influence both the growth rate of degraders and the kinetics of TPH removal. Thus, carrier selection must be

matched to the pollutant mixture and local environmental conditions to optimize performance. Fungi particularly species of *Aspergillus* and *Penicillium* have gained attention for hydrocarbon biodegradation because they produce extracellular oxidative enzymes and biosurfactants that increase the bioavailability of hydrophobic fractions [10]; fungal reviews 2022–2024). *Aspergillus niger*, used in the present study, has been shown in laboratory and field trials to degrade significant proportions of TPH under aerobic conditions, often complementing bacterial consortia by attacking high-molecular-weight and aromatic components that are less accessible to bacteria. Fungal-mediated biosurfactant production can also enhance desorption of oil from carrier surfaces and promote co-metabolism. Less conventional carriers such as fruit residues (e.g., soursop peel) have useful properties beyond nutrient content: high fiber and porosity give them sorptive capacities for dyes, heavy metals and some organics, and they may act as short-term adsorbents that concentrate hydrocarbons near attached microbes [11]. However, the dual role of adsorption and biodegradation must be monitored carefully because strong adsorption can reduce bioavailability unless carriers subsequently release hydrocarbons at rates that sustain microbial metabolism. Recent experimental and modelling studies underline that operational variables carrier dosage, particle size, inoculum type and density, aeration regime, and temperature drive outcomes as much as carrier identity. Optimization studies using cassava mash report improved TPH removal under moderate temperatures ($\sim 25\text{--}30^\circ\text{C}$), near-neutral pH, and sufficient aeration, with diminishing returns above certain dosages due to oxygen depletion and excessive biochemical oxygen demand (Ugwuoke M.O. et al, 2025). These findings motivate systematic optimization (dosage $\times$ inoculum $\times$ aeration) when translating lab results to field applications. In summary, the literature supports using locally available agricultural wastes (cassava mash, maize bran and soursop peels) as cost-effective biocarriers that supply nutrients, surface area and ecological niches for hydrocarbon-degrading microbes. Yet variability in carrier chemistry, pollutant composition and environmental constraints means that site-specific testing and parameter optimization remain essential steps before field deployment. The present study contributes to this evidence base by comparing cassava mash, maize bran and soursop peel under controlled aqueous remediation trials and by linking carrier chemistry with microbial dynamics and TPH degradation.

3 MATERIALS AND METHODS

3.1 Materials

Cassava mash (CM), maize bran (MB), and soursop peel (SP) were selected as natural biocarriers due to their high organic nutrient content and proven ability to enhance microbial activity in bioremediation systems [3]. Bonny Light crude oil was obtained from Total Energies, Port Harcourt, while all analytical-grade reagents were purchased from Chemical Line in Ogbete Market, Enugu. *Aspergillus niger* used for inoculation was isolated and supplied by the Microbiology/ brewery department of State University of Medical and Applied Sciences Igbo- Eno following standard microbial preparation procedures [12].

3.2 Methods

3.2.1 Preparation of raw materials

Cassava tubers were peeled, washed, and chopped into small pieces, then submerged in clean water and allowed to ferment for four days. The fermented mixture was sieved to obtain cassava mash (CM), following procedures commonly applied in organic biocarrier preparation [13]. Maize seeds were soaked in water for three days, ground, and sieved to produce maize bran (MB), consistent with methods described for cereal-based biostimulants (Ani&Chukwuma, 2020). Fresh soursop fruits were allowed to ripen, peeled, and the peels were sun-dried for two weeks before being ground and sieved to 5 mm uniformity. Sun-drying of fruit residues is recognized as an effective method for preserving nutrient-rich biomass used in microbial enhancement [11]. All processed materials were stored in airtight containers until use.

3.3 Chemical and biological analysis of processed water before and after contamination with crude oil

Key water quality parameters including pH, DO, BOD, COD, and TPH were analyzed before and after crude oil contamination to assess pollutant impact and remediation potential. These parameters are standard indicators for evaluating hydrocarbon-polluted water systems [6];[15].

3.3.1 Determination of dissolved oxygen(DO)

The Winkler method (2005) was used to measure the dissolved oxygen. In order to oxidize Manganese Oxide, which reacts with Potassium iodide to release iodine (I₂), 20 ml of polluted samples were collected and placed to a BOD bottle together with manganese (ii) sulfate (M₂SO₄) and alkaline potassium iodide (KI). Afterwards, a solution of Sodium thiosulfate (Na₂S₂O₃) was added to dissolve the precipitate and release iodine into the solution. The color was then titrated with sulfuric acid (H₂SO₄) until it became pale yellow. A few drops of starch indicator were then added to turn the color blue. The titration was continued until the color disappeared.

DO concentration was calculated by the formula:

$$DO \text{ (mg/l)} = \frac{V_{\text{thiosulfate}} \times N \times 8}{Vol. \text{ of sample}} \quad (1)$$

Where: $V_{\text{thiosulfate}}$ = Volume of sodium thiosulfate used

N = Normality of thiosulphatution

V_{sample} = Volume of the water sample

3.3.2 Determination of %TPH

The gravity-based method EPA 1664 of ASTM D7066 was used to determine the percentage hydrocarbon (TPH). 200 milliliters of hexane was added to 100 milliliters of pollutant oil sample taken from the bioremediation setup, and the mixture was shook violently for two minutes to facilitate phase separation. Once the phases had separated, the organic phase containing TPH was collected and spun at 300 rpm for 10 minutes in a centrifuge. The supernatant was then discarded. A rotary evaporator or oven set to 60 °C was used to dry the filtrate, which was collected from the remaining organic phase following centrifugation. The weight of the remaining residue was then reordered. % TPH was determined using the formula below:

$$\% \text{ TPH} = \frac{W_1 - W_2}{W_1} \times \frac{100}{1} \quad (2)$$

Where W_1 = TPH_{initial} (mg/l) or (g/ml), W_2 = TPH_{final} (mg/l) or (g/ml)

3.3.3 Determination of chemical oxygen demand (COD)

COD was calculated using AOAC 1999 method 973.4. The following steps were taken: a clean cylinder was used to measure one liter of oil-contaminated water; 2–5 milliliters of this sample was transferred to the COD digestion vessel; 2 milliliters of COD reagent and 1–2 milliliters of sulfuric acid were added; mixed thoroughly. Following two hours of heating to a temperature of 150°C ± 2°C, the organic matter was allowed to digest and oxidize in the digestion vessel. When the digestion vessel cooled down to room temperature, the absorbance of the digested sample was measured using a COD meter. This is the formula that was used to determine the COD in the water sample:

$$COD \left(\frac{\text{mg}}{\text{L}} \right) = (\text{absorbance of sample} - \text{absorbance of blank}) \times \left(\frac{\text{COD standard solution conc}}{\text{absorbance of COD standard solution}} \right) \quad (3)$$

3.3.4 Determination of pH

The pH of the contaminated and treated water samples was measured using a calibrated digital pH meter following the standard procedure recommended by ASTM D1293 [16]. Prior to measurement, the pH meter was standardized with buffer solutions of pH 4.0, 7.0, and 10.0 to ensure accuracy. The electrode was rinsed with distilled water and immersed in each sample until readings stabilized. pH monitoring is essential in bioremediation studies because microbial degradation efficiency is strongly influenced by acidity or alkalinity conditions [6].

3.3.5 Determination of BOD

Eaton et al. (2005) technique was used to estimate BOD. To dilute the oil-contaminated sample water, it was collected, sealed in a BOD bottle so that oxygen couldn't dissolve or escape, and then mixed with aerated dilution water. To prevent photosynthesis, incubate the sealed BOD bottle in a dark place at 20°C for 5 days. Starting at the beginning of the incubation period and again after 5 days, the initial dissolve oxygen (DO) was determined using the Winkler DO method to track the amount of oxygen eaten. To determine BOD, the difference between the starting and ending DO concentrations was used.

$$BOD_5 = DO_{\text{initial}} - DO_{\text{final}} \quad (4)$$

3.4 Proximate analysis of natural biocarriers

The official method of analytical chemistry (AOAC 1990) was used to determine the chemical constituents of the natural biocarriers such as nitrogen content (%N), carbon (% TOC), phosphorus (%P) etc.

3.5 Preparation of crude oil for bioremediation experiment

A small amount of Bonny light crude oil (BLCO) was spilled upon treated portable water to create the artificially polluted water used in this investigation. An oil refining company in Port-Harcourt River State was the source of the oil recovery. At 41 °C, the crude oil sample had a specific gravity of 0.85, a sulfur concentration of 0.15 wt %, a viscosity of 3.28 cp, and an API gravity of 35.2%. A mixture of 320 milliliters of crude oil and 1280 milliliters of portable water was artificially

produced in four plastic containers at a ratio of one to four [17]. There were four plastic reactors (1, 2, 3, and 4) into which these were introduced; the control reactor (1) contained crude oil, water, and microorganisms; the second reactor (2) contained the same, plus cassava mash; the third reactor (3) contained the same, plus soursop peels; and the fourth reactor (4) contained the same, plus maize bran. Inoculated into the several vessels for the bioremediation study procedures were 50 grams of biocarrier and 10 milliliters of *Aspergillus Niger* microorganisms at a concentration of 1×10^6 CFU/mL.

3.5.1 Bioremediation experiment

Bioremediation of water polluted with crude oil was conducted according to the protocol laid out by [17]. The plastic reactors 1, 2, 3, and 4 were mixed and agitated twice a day using an Vortex 3000 mixer to bring the oil-contaminated water, microorganisms, and nutrients (biocarriers) into closer contact with one another. After that, on day zero and then every seven days for 35 days, bioremediation indicating parameters in the polluted water were measured, such as biochemical oxygen demand (BOD), pH, dissolve oxygen (DO), microbial population (C), and total hydrocarbon (TPH).

3.5.2 Determination of microbial population (MP)

Microbial population or total microbial count in CFU was determined by Cappuccino and Sherman procedure (2014). A known sample of contaminated oil water containing the microorganisms were weighed into a conical flask and diluted to a 10-fold serial dilution for easy colony counting.

A known volume of (100 μ l) were placed on each dilution onto nutrient agar plate, spread evenly on a sterile glass spreader and incubated at temperature of 30°C (for 24-48 hours) or until colonies visible. After, the incubation, the number of colonies was counted on the plates with 30-300 colonies. The number of microbial population (cfus) was calculated by:

$$\text{MP (cfu/ml)} = \frac{\text{Number of colonies}}{\text{Dilution factor} \times \text{volume plated (ml)}} \quad (5)$$

4 RESULTS AND DISCUSSIONS

Table 1 show that crude oil contamination caused significant deterioration in water quality. BOD and COD increased from non-detectable levels to 14.32 mg/L and 16.02 mg/L, indicating a high organic load and increased oxygen demand, which is typical of hydrocarbon pollution [6]. Dissolved oxygen dropped from 7.2 to 3.16 mg/L, reflecting oxygen depletion due to microbial oxidation of oil components. TPH also rose to 1000 mg/L, confirming heavy petroleum contamination. Although pH remained within the WHO acceptable range (6.5-8.5), overall results show clear ecological stress associated with crude oil presence [15].

Table 1: Result of chemical and biological analysis of processed water before and after contamination with crude oil.

Parameters analyzed	WHO	Result Before Analysis	Result After Analysis
pH	6.5-8.5	7.14	7.14
BOD (mg/l)	5	ND	14.32
COD (mg/l)	10	ND	16.02
DO (mg/l)	5	7.2	3.16
TPH (mg/l)	-	ND	1000

(World Health Organization (2017); NIS- SON: 554-2015)

Table 2: The results of characterization analysis of natural biocarriers

Composition	Soursop Peel Biocarrier	Cassava Mash Biocarrier	Maize Bran Biocarrier
Nitrogen (%)	5.24	5.56	5.82
Total organic Carbon (%)	75.10	75.42	78.61
Phosphorus (%)	18.652	19.748	19.898
Potassium (ppm)	1.023	1.114	2.103

Calcium (ppm)	1.408	1.447	2.832
Iron (PPm)	0.376	0.387	0.4217
Protein (%)	6.300	8.92	9.102
Carbohydrate (mg/l)	124.324	155.598	100.436

Table 2 shows that the natural biocarriers soursop peel, cassava mash, and maize bran contain substantial levels of nitrogen, phosphorus, and organic carbon, all essential for supporting microbial activity during bioremediation. Maize bran exhibited the highest nutrient composition, particularly nitrogen (5.82%) and total organic carbon (78.61%), indicating stronger potential for stimulating hydrocarbon-degrading microbes. High carbon and moderate protein levels across all biocarriers suggest adequate energy sources for microbial metabolism [8]. These nutrient-rich profiles align with studies showing agricultural residues as effective low-cost biostimulants in hydrocarbon remediation [4].

4.1 Influence of DO, BOD, pH, MP AND TPH during bioremediation

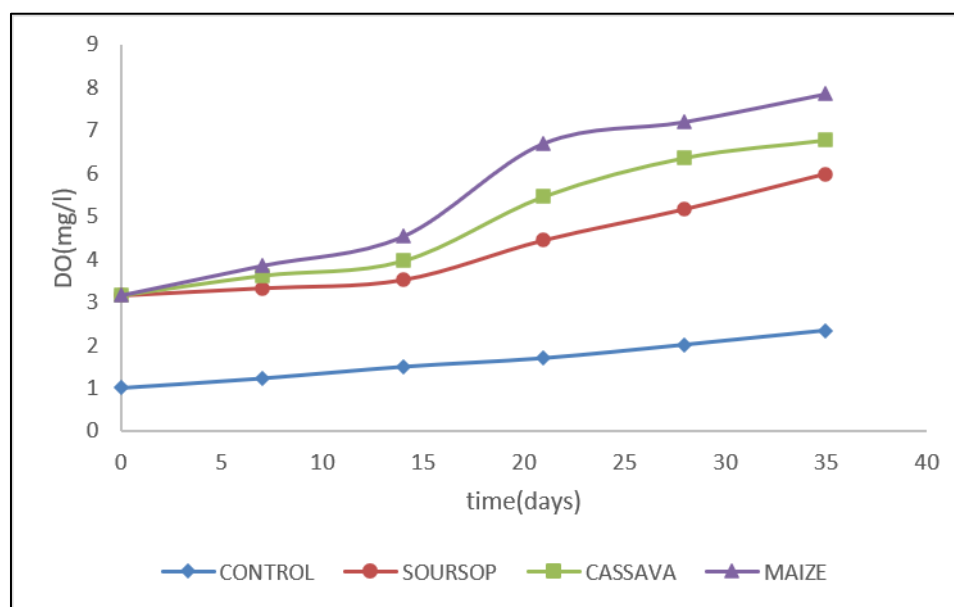


Figure 1: Variation of biochemical oxygen demand (DO) with remediation time

Figure 1 illustrates the variation of dissolved oxygen (DO) during the bioremediation of crude-oil-contaminated water across all treatment reactors. At the start (Day 0), DO levels were relatively low due to the immediate oxygen-depleting effect of crude oil, which limits gas exchange and stimulates rapid microbial oxygen consumption [6]. As remediation progressed, reactors amended with natural biocarriers cassava mash, maize bran, and soursop peel showed a gradual increase in DO levels. This improvement is likely due to enhanced microbial degradation supported by the additional nutrients in the biocarriers, which promote balanced microbial growth and increase oxygen regeneration through improved system aeration and mixing [8]. The control reactor, which lacked biocarrier support, showed weaker DO recovery, demonstrating the importance of nutrient-rich substrates in sustaining microbial activity and preventing oxygen collapse. The steady rise in DO in the amended reactors suggests that hydrocarbons were progressively degraded, reducing oxygen demand and allowing oxygen to accumulate over time, a trend widely reported in effective hydrocarbon bioremediation systems [7].

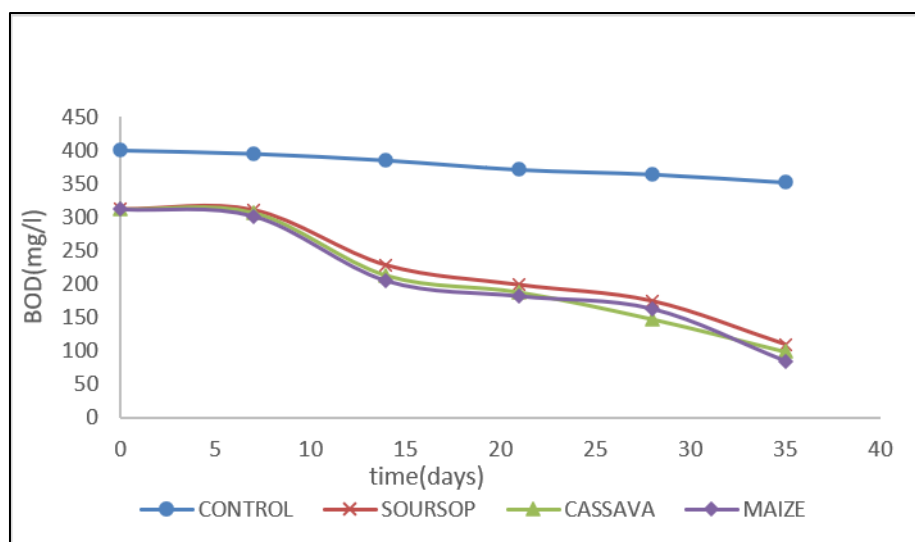


Figure 2: Variation of biochemical oxygen demand (BOD) with remediation time

Figure 2 illustrates the change in Biochemical Oxygen Demand (BOD) during bioremediation of crude-oil-contaminated water. At Day 0, BOD values were high, reflecting the presence of large amounts of degradable organic matter from crude oil. As remediation progressed, reactors amended with cassava mash, maize bran, and soursop peel demonstrated a continuous decrease in BOD, indicating effective microbial degradation of hydrocarbons. This decline suggests that microbial communities utilized available organic pollutants as substrates, reducing the oxygen required for decomposition [6]. Maize bran showed the fastest BOD reduction, likely due to its rich nutrient composition, which enhances microbial metabolism and accelerates breakdown of petroleum compounds. The cassava mash and soursop peel reactors also showed steady BOD reduction, though at slightly slower rates. In contrast, the control reactor exhibited minimal improvement, emphasizing that natural attenuation alone is insufficient in nutrient-limited environments. Similar trends have been reported in recent bioremediation studies where organic substrates significantly lower BOD through enhanced microbial activity [8];[7]. Overall, Figure 2 confirms that natural biocarriers effectively reduce organic pollution stress, improving water quality and supporting successful bioremediation.

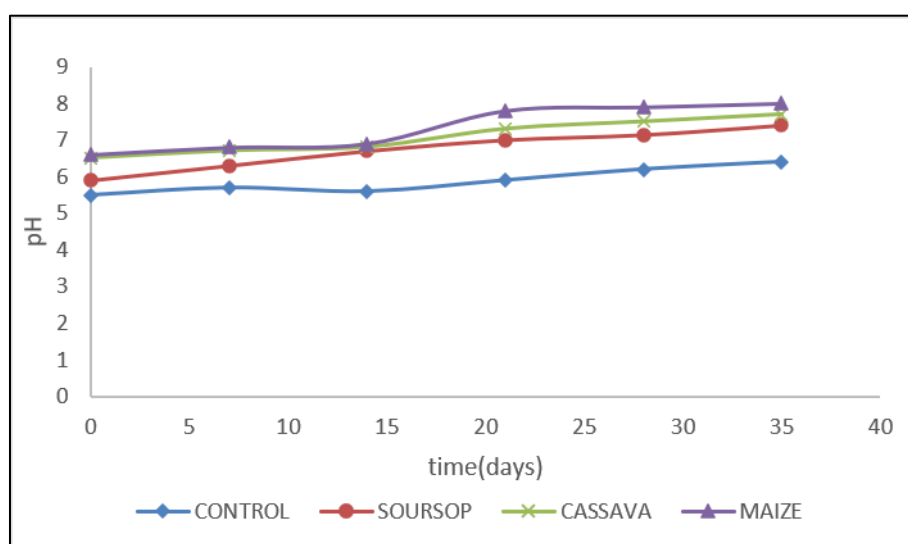


Figure 3: Variation of pH with remediation time

Figure 3 presents the variation of pH during the bioremediation of crude-oil-contaminated water across the different treatment reactors. At the beginning of the experiment, pH values were slightly neutral, reflecting the initial condition of the water before significant microbial activity commenced. As remediation progressed, reactors amended with cassava mash, maize bran, and soursop peel showed moderate fluctuations in pH, generally remaining within the optimal range (6.5-8.0) suitable for hydrocarbon-degrading microorganisms [6]. These slight shifts in pH can be attributed to microbial metabolic processes, including the production of organic acids during hydrocarbon breakdown and the release of ammonium or other basic compounds from nutrient-rich biocarriers [8]. Reactors with biocarriers maintained more stable pH levels compared to the control, indicating that the organic substrates provided buffering

capacity and promoted balanced microbial activity. A stable near-neutral pH is crucial because extreme acidity or alkalinity can inhibit enzyme function and slow the biodegradation of petroleum hydrocarbons [7]. The overall pattern in Figure 3 suggests that natural biocarriers not only enhance microbial growth but also help maintain favorable pH conditions throughout the remediation period. This stability contributes significantly to improved bioremediation efficiency.

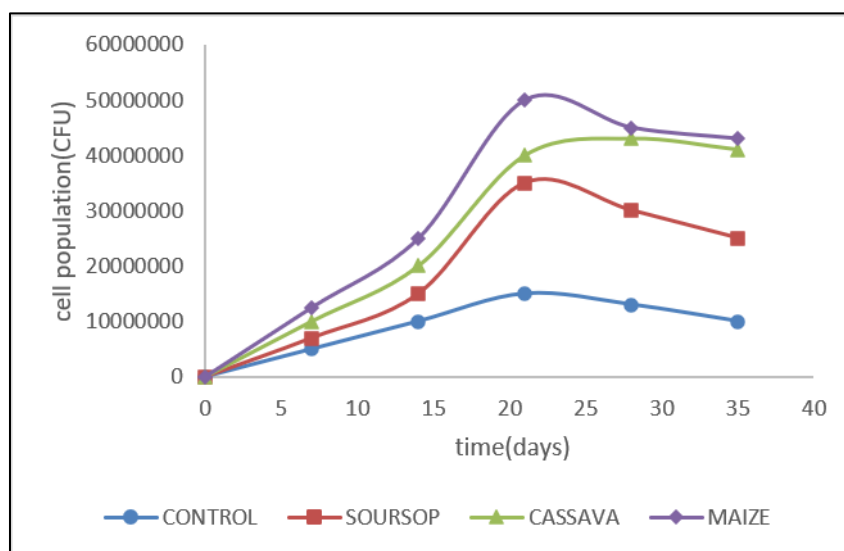


Figure 4: Variation of total microbial growth (TMC) with remediation time

Figure 4 shows the trend in total microbial count (TMC) during the bioremediation of crude-oil-contaminated water across all treatment reactors. At Day 0, microbial populations were relatively low, reflecting the initial stress caused by hydrocarbon toxicity. However, reactors amended with cassava mash, maize bran, and soursop peel demonstrated a steady and significant increase in microbial growth throughout the remediation period. This rapid increase is attributed to the high nutrient content of the biocarriers, which supply essential carbon, nitrogen, and phosphorus required for microbial metabolism and proliferation [8]. Among the biocarriers, maize bran supported the highest microbial growth, likely due to its superior nutrient composition, as shown in Table 2. The control reactor, lacking biocarrier supplementation, exhibited minimal microbial increase, highlighting the inhibitory effect of crude oil in nutrient-poor conditions. Enhanced microbial growth in amended reactors aligns with findings from recent bioremediation studies showing that organic substrates stimulate hydrocarbon-degrading consortia and accelerate TPH reduction [6];[7]. Overall, Figure 4 demonstrates that natural biocarriers substantially enhance microbial proliferation, which is a key driver of efficient hydrocarbon degradation and improved bioremediation outcomes.

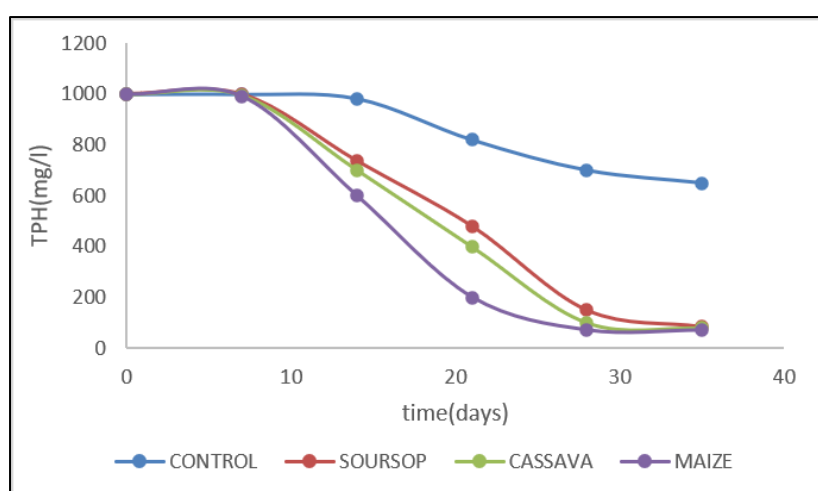


Figure 5: Variation of total petroleum hydrocarbon (%TPH) with remediation time

Figure 5 illustrates the variation of Total Petroleum Hydrocarbon (TPH) concentration over the 35-day bioremediation period. At day 0, all reactors exhibited high TPH levels due to the initial crude oil contamination. As remediation progressed, reactors amended with natural biocarriers, cassava mash, maize bran, and soursop peel showed a steady

and significant decline in TPH, indicating active hydrocarbon degradation. Maize bran demonstrated the highest TPH reduction rate, consistent with its superior nutrient content and ability to stimulate rapid microbial growth [8]. The cassava mash and soursop peel reactors also showed notable reductions, though at slightly slower rates. In contrast, the control reactor exhibited only minor TPH decrease, suggesting that natural attenuation alone was insufficient to drive substantial degradation. This difference reinforces evidence from recent studies showing that nutrient-rich organic biocarriers enhance microbial enzymatic activity and accelerate hydrocarbon breakdown [6];[7]. The overall decreasing trend in Figure 5 aligns with typical bioremediation behavior, where increased microbial biomass correlates with enhanced degradation of petroleum fractions. Figure 5 confirms that natural biocarriers markedly improve TPH removal efficiency and are effective low-cost options for bioremediation of oil-contaminated water

4.2 Effect of process parameters on microbial community

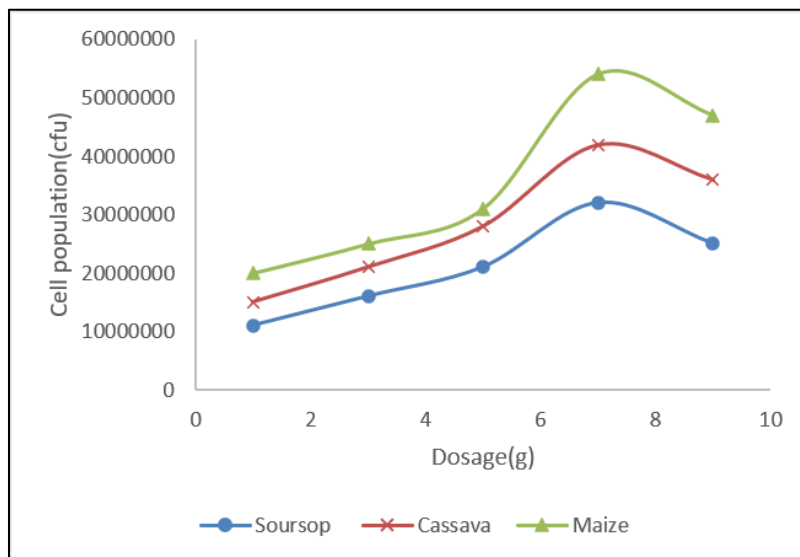


Figure 6: Effect of dosage of biocarriers on microbial growth

Figure 6 shows the influence of biocarrier dosage on microbial population during remediation. Microbial growth increased significantly as biocarrier dosage increased across all treatments. Higher dosages supplied larger amounts of carbon, nitrogen, and phosphorus nutrients essential for microbial metabolism and enzyme production [8]. The trend supports the principle of biostimulation, where nutrient enrichment enhances microbial degradation of petroleum hydrocarbons [6]. Maize bran exhibited the strongest positive response to increased dosage, consistent with its high nutrient density shown in Table 2. Cassava mash and soursop peel also supported increased microbial growth, though with slightly lower intensities. However, extremely high dosages in bioremediation systems may cause oxygen depletion or excess organic load, although such effects were not evident in this study. The overall pattern agrees with recent findings that optimal nutrient supplementation increases microbial biomass and accelerates TPH degradation in contaminated water [7].

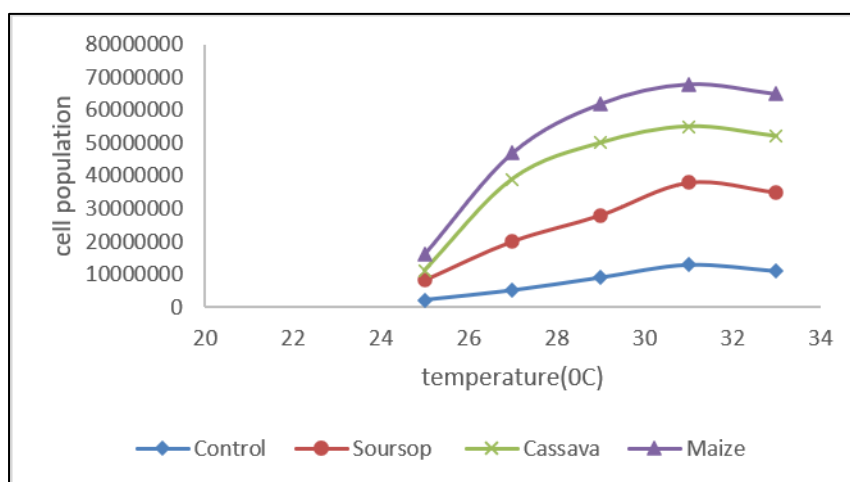


Figure 7: Effect of temperature on microbial growth using biocarriers

Figure 7 presents the effect of temperature on microbial growth during bioremediation. Microbial counts increased substantially at moderate temperatures (25-35°C), indicating optimal growth conditions for hydrocarbon-degrading microorganisms such as *Aspergillus niger*. This range supports enzyme activity and cellular metabolism required for effective hydrocarbon breakdown [6]. At lower temperatures, microbial activity declined due to reduced enzymatic rates and slower nutrient uptake. At temperatures above the optimum, growth also decreased, likely because elevated heat can denature enzymes or inhibit cell membrane stability [7]. Maize bran-supported microbes showed the highest temperature responsiveness, again linked to its superior nutrient profile. These results are consistent with recent research showing that bioremediation efficiency increases at moderate tropical temperatures where microbial metabolic rates peak [7]. Finally, Figure 7 reinforces temperature as a critical operational factor that must be optimized for successful hydrocarbon bioremediation.

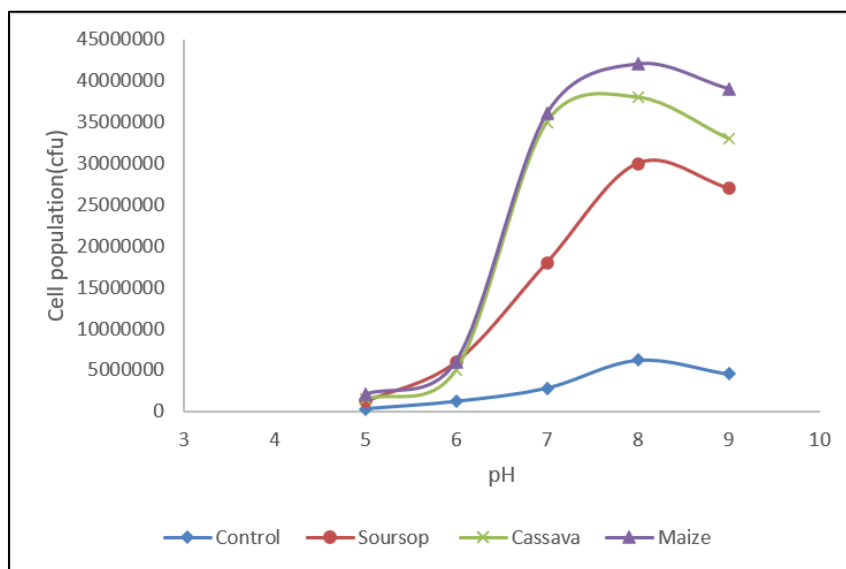


Figure 8: Effect of pH on the microbial growth using biocarriers

Figure 8 shows the influence of pH on microbial growth. Microbial populations peaked at near-neutral pH values (6.5-7.5), which support optimal enzymatic processes and cellular function in hydrocarbon degraders [6]. At acidic or alkaline extremes, the microbial population declined, indicating that non-neutral conditions inhibit cell activity and reduce degradation efficiency. Biocarrier-amended reactors demonstrated stronger microbial growth across the optimal pH range due to their buffering capacity and nutrient release. Cassava mash and maize bran, in particular, stabilized pH conditions and supported elevated microbial counts. Similar findings have been reported in studies showing that slightly neutral pH enhances the biodegradation of petroleum hydrocarbons by promoting enzyme stability [8]. Figure 8, highlights the critical role of pH management in maintaining microbial activity and maximizing remediation performance.

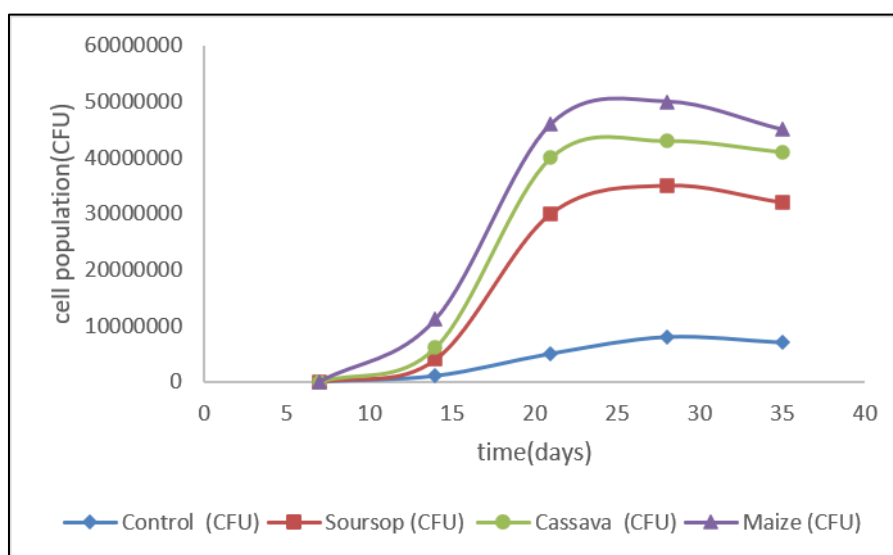


Figure 9: Effect of time on the microbial growth

Figure 9 shows the effect of remediation time on microbial population. Microbial growth increased steadily throughout the experiment, peaking around Day 21-28, indicating active hydrocarbon degradation supported by available nutrients from the biocarriers. This growth pattern aligns with typical microbial degradation cycles: lag phase, exponential growth, and stabilization [7]. Growth was highest in maize bran and cassava mash reactors due to their high nutrient content. After Day 28, microbial activity began to level off, likely due to reduced hydrocarbon availability or nutrient depletion. Such trends are consistent with bioremediation studies reporting that microbial populations are highest during active degradation phases [6]. Figure 9 ultimately demonstrates that time-dependent microbial growth is a strong indicator of hydrocarbon breakdown and confirms that natural biocarriers effectively support sustained microbial activity during remediation.

5 CONCLUSION

This study investigated the bioremediation of crude oil-contaminated water using three natural biocarriers, cassava mash (CM), maize bran (MB), and soursop peel (SP) supported by *Aspergillus niger* inoculation. The results demonstrated that crude oil contamination significantly deteriorated water quality, with BOD rising from ND to 14.32 mg/L, COD increasing from ND to 16.02 mg/L, DO decreasing from 7.2 mg/L to 3.16 mg/L, and TPH reaching 1000 mg/L. Although pH remained at 7.14, within the acceptable range, other parameters confirmed severe pollution. Characterization of the natural biocarriers showed that they contained substantial nutrient profiles capable of supporting microbial growth. Nitrogen ranged from 5.24-5.82%, total organic carbon from 75.10-78.61%, phosphorus from 18.652-19.898%, potassium from 1.023-2.103 ppm, calcium from 1.408-2.832 ppm, iron from 0.376-0.4217 ppm, protein from 6.300-9.102%, and carbohydrates from 100.436-155.598 mg/L. Maize bran consistently showed the highest nutrient content. Across Figures 1-5, all biocarriers improved water quality indicators throughout the 35-day remediation period. DO increased steadily in biocarrier-amended reactors, BOD and COD decreased consistently, and TPH exhibited a continuous decline, with maize bran achieving the fastest degradation from 1000mg/l - 70.3mg/l, cassava mash 1000mg/l - 101mg/l and soursop peel 1000mg/l - 150mg/l. Microbial population also rose sharply, especially in reactors treated with maize bran and cassava mash. Figures 6-9 demonstrated that microbial growth was strongly influenced by biocarrier dosage, temperature (optimum around 25-35 °C), pH (6.5-7.5), and remediation time, with populations peaking between Days 21-28 before stabilizing. Overall, the study concludes that natural biocarriers significantly enhanced the bioremediation performance, with maize bran giving the best results, followed by cassava mash and soursop peel. Their rich nutrient composition, combined with *Aspergillus niger* activity, accelerated hydrocarbon degradation, restored water quality parameters, and effectively reduced TPH levels. These findings demonstrate that agricultural wastes provide a low-cost, sustainable, and highly effective approach for remediating crude-oil-polluted water.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

I declared that there is no conflict of interests exist.

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