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Study of Bioremediation Kinetic of Oil-Contaminated Water Utilizing Maize Bran as Nutrient Support

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Abstract - This study investigated the improvement of bioremediation kinetics of crude oil-contaminated water utilizing maize bran as nutrient support in combination with Aspergillus niger fungus. The proximate analysis revealed that maize bran contains essential macro- and micronutrients such as carbon, nitrogen, phosphorus, potassium, calcium, sodium, iron, cellulose, lignin, and proteins, which provide a rich nutrient matrix for microbial activity. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of functional groups that support microbial growth and hydrocarbon degradation. Bioremediation experiments were conducted by introducing Aspergillus niger and maize bran into crude oil-polluted water and monitoring parameters such as pH, microbial population, and total petroleum hydrocarbons (TPH) over 35 days. Results showed a significant reduction in TPH, demonstrating enhanced microbial degradation compared to the control setup without maize bran. Kinetic modeling revealed that the biodegradation process fitted well with both first-order and second-order models, with correlation coefficients (R^2) greater than 0.8, indicating reliable predictive performance. The rate constants further validated maize bran's efficiency in accelerating crude oil degradation. The findings suggest that organic biocarriers like maize bran offer a sustainable, cost-effective, and environmentally friendly alternative to inorganic fertilizers in hydrocarbon bioremediation by supplying nutrients that stimulate microbial growth.

Keywords - Maize bran, Kinetics, Bioremediation, Aspergillus Niger, Crude oil, first- order and second-order kinetic model.

I. INTRODUCTION

Petroleum hydrocarbons released into aquatic environments from oil spills, pipeline leaks, industrial discharges, and runoff pose serious ecological and public health risks [1];[2]. These pollutants reduce dissolved oxygen levels, cause toxicity to aquatic life, and can bioaccumulate through food chains [3]. Conventional remediation strategies such as physical recovery, chemical dispersants, or thermal treatments are often costly, energy-intensive, and may generate secondary pollution [3]. In contrast, bioremediation using microorganisms to biologically degrade contaminants into less harmful products is viewed as a more sustainable and eco-friendly approach (Das & Chandran, 2011). Among bioremediation strategies, fungal-based approaches (termed mycoremediation) are increasingly recognized as valuable due to fungi's versatile enzyme systems, ability to degrade complex organic molecules, and tolerance to environmental stressors [4].Fungi such as Aspergillus niger and Trichoderma harzianum secrete extracellular enzymes (e.g. laccases, peroxidases) that can oxidize and break down recalcitrant pollutants including polycyclic aromatic hydrocarbons (PAHs) and other high-molecular-weight fractions [4];[5]. Studies have demonstrated the ability of A. niger to grow in hydrocarbon-rich environments and degrade crude oil and kerosene in liquid media [5]. In one such investigation, A. niger was shown to remove hydrocarbons from liquid media, highlighting its potential in liquid-phase bioremediation [5]. Other research indicates that fungal strains can act both by adsorption (binding hydrocarbons at cell surfaces)

and metabolic degradation (uptake and enzymatic transformation) [6]. However, fungal bioremediation in aqueous systems often faces limitations in nutrient availability, microbial adhesion, biomass retention, and substrate accessibility.

To overcome these, employing carriers or support matrices is a common strategy to facilitate microbial immobilization, protect against washout, improve contact with pollutants, and provide nutrients [7], cited in Fungi for Bioremediation). Organic biocarriers especially agricultural residues are appealing because they can supply carbon, nitrogen, and micronutrients, and are low-cost and abundant Fungi for Bioremediation. Among such residues, maize bran (a byproduct of maize milling) stands out due to its composition and availability. Maize bran is rich in cellulose, hemicellulose, lignin, starch, proteins, and minerals, and has been studied extensively in food science and bioprocessing contexts [8];[9] Its structural matrix provides a porous scaffold that can support microbial colonization and maintain local microenvironments beneficial for biodegradation.

In nutritional profiling, maize bran has been shown to contribute water absorption, retention capacity, and matrix support [8]. Although primarily studied in the context of bioactive food components [9], these traits suggest maize bran has latent potentia
l as a microbial carrier in environmental biotechnology.In microbial fermentation research, maize bran residues have been used as carriers or substrates for biofertilizer formulations, where their water-holding capacity, swelling behavior, and structural integrity support bacterial inoculants [10]. This indicates that maize bran can serve dual roles: as a physical support and nutrient reservoir. In spontaneous fermentation studies, maize bran's microbiological and chemical characteristics evolve over time, supporting diverse microbial communities evaluation of microbial consortia . Combined, these observations underscore its suitability as a biocarrier. Despite this promising background, there is a gap in knowledge regarding the kinetics of hydrocarbon degradation when a fungus like A. niger is immobilized or supported by maize bran, especially in aqueous systems. Kinetic modeling is essential for predicting degradation rates, designing reactors, and scaling processes. Previous kinetic studies on hydrocarbon bioremediation have largely focused on bacterial systems or free fungal cultures; for instance, in one study, Aspergillus niger degradation data conformed to a first-order kinetic model with a rate constant of 0.422 day^{-1} [11].

The same study compared fungal and bacterial systems, observing that Pseudomonas aeruginosa outperformed A. niger in degrading oil and grease, and that the fungal system fit a first-order model while the bacterial system was better described by a pseudo-first-order model [11]. Yet, these findings do not directly address the role of a solid carrier like maize bran in modulating the kinetics of degradation in aqueous media. Moreover, in natural contaminated environments such as in the Niger Delta, fungal species including Aspergillus oryzae and Mucor irregularis have been isolated from aged crude oil polluted sites and shown enzymatic hydrocarbon degradation activity [12].

This underscores the indigenous relevance of fungal bioremediation in real-world settings. However, field-level or bench-scale studies integrating organic carriers, fungal inoculation, and kinetic modeling remain scarce. Therefore, this study aims to fill this gap by investigating the kinetics of crude oil degradation in contaminated water when Aspergillus niger is supported by maize bran as an organic biocarrier. Specific objectives include: (1) characterizing maize bran (proximate analysis, structural features, nutrient content); (2) immobilizing or introducing A. niger with maize bran into oil-contaminated water and monitoring degradation; (3) measuring key environmental parameters such as pH, total petroleum hydrocarbons (TPH), and microbial population over time; and (4) fitting kinetic models (first-order, and second-order) to the degradation data to derive rate constants and evaluate model suitability.

II. MATERIALS AND METHODS

Materials.

Maize bran (MB), obtained as agro-waste, was processed, air-dried, ground, and sieved to uniform particle size following standard procedures [13]. Aspergillus niger was isolated and cultured at Enugu State University science and technology microbiology & brewing laboratory. Bonny Light crude oil (BLCO) was procured from Shell SPDC. Bioremediation experiments involved artificially polluted water inoculated with 10 mL of A. niger (1×10^6 CFU/mL) and 50 g maize bran, monitored for pH, microbial count, and total petroleum hydrocarbons (TPH) over 35 days 1];[14].

2.1.1 Proximate Analysis of maize bran biocarrier

The official method of analytical chemistry (AOAC 1990) was used to determine the total percentage organic carbon (% OC), total percentage nitrogen content (%N) and total percentage phosphorous content (%P) Fourier transform infra - red (FTIR) analysis was used to determine the types of chemical bonds (functional groups) in maize bran biocarrier molecule by creating an infra-red absorption spectrum, which is similar to a molecule's "finger print," using an FTIR-8400S Fourier Transform Spectrophotometer, manufactured by Shimadzu and installed at the Project development institute Enugu (PRODA).

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 410C, 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 two plastic containers at a ratio of 1:4 [15]. There were two plastic containers (B and C) into which these were introduced; the control container (B) contained crude oil, water, and microorganisms; the second container (C) 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 1x106 CFU/mL.

Bioremediation experiment

Bioremediation of water polluted with crude oil was conducted according to the protocol laid out by (Anih et al 2019). The plastic containers B and C were mixed and agitated twice a day using an orbital mixer H-Z 200 model 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, we measured bioremediation indicating parameters in the polluted water, such as pH, cell population (TMC), and total hydrocarbon (TPH).

Determination of cell population/microbial count (CFU)

Using the method developed by Cappuccino and Sherman (2014), the total microbial count (cfu) or cell population was calculated. The microbes were found in a known sample of polluted oil water.

To make colony counting easier, the mixture was weighed into a conical flask and diluted to a 10-fold serial dilution. Then, 100µl of each dilution was distributed uniformly onto nutrient agar plates using a sterile glass spreader. The plates were then incubated at 30°C for at least 24 hours, or until colonies were visible. Upon completion of incubation, 30-300 colonies were counted on each plate. A total of cfus were determined by:

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

Determination of %TPH

The percentage of total petroleum hydrocarbons (TPH) was determined following the EPA 1664 method with slight modifications [16]. One hundred milliliters of polluted water sample from the bioremediation setup was extracted with 200 mL of n-hexane by vigorous shaking for two minutes to ensure efficient phase separation. The organic phase was collected and centrifuged at 300 rpm for 10 minutes to remove suspended particles. The supernatant was evaporated at 60 °C using a rotary evaporator until dryness. The remaining residue, representing petroleum hydrocarbons, was carefully

weighed. The concentration of TPH was calculated from the weight difference, and percentage removal was determined using:

$$\%\text{TPH} = \frac{(W1-W2)\times 100}{W1}$$

Where W_1 = initial TPH (mg/L) and W_2 = final TPH (mg/L). (Ezenwoko et al., 2023).

Determination of pH

The pH of the water was determined using the standard test technique outlined in ASTM D1293-95 (2013). After collecting the water sample, it was agitated to make sure it was stable. Then, it was immersed with the electrode of a pocket pH meter. After waiting for the sample to stabilize for 1-2 minutes, the pH values were recorded appropriately.

Bioremediation kinetics

The kinetics experiments were carried out to determine the percentage (TPH) of crude oil removed. Three different concentrations of crude oil and water were prepared: 200g/2000ml, 300g/3000ml, and 400g/4000ml. To each of these solutions, 10ml of 1x10-6CFU/mL of microorganisms and 10g of maize bran were added at 300c and pH 7.5. The mixture was then stirred in a shaker for contact times of 5, 10, 15, 20, and 25 days. At the end the bioremediation kinetics was acquired using the decreasing concentration of the oil. The order of reaction of bioremediation of hydrocarbon obtained was fitted to the first order and second order kinetic models.

III. RESULTS AND DISCUSSIONS

Table 1: Results of Proximate analysis of maize bran biocarrier

Composition	Maize bean
Nitrogen (%)	5.82
Total organic Carbon (%)	78.61
Phosphorus (%)	19.90
Potassium (ppm)	2.10
Sodium (ppm)	1.83
Calcium (ppm)	2.83
Iron (PPm)	0.42
Cellulose (%)	17.80
Protein (%)	9.10
Fiber (%)	10.55
Carbohydrate (mg/l)	100.44
Lignin (%)	4.06

Proximate analysis of maize bran biocarrier

The proximate analysis indicated that maize bran is nutrient-rich, containing high organic carbon (78.61%), nitrogen (5.82%), phosphorus (19.90%), fiber, cellulose, lignin, and proteins. These nutrients are crucial for microbial growth and hydrocarbon degradation, as they support enzyme production and metabolic pathways (Hussain et al., 2021). The presence of cellulose and lignin also enhances structural stability, providing attachment sites for microbial colonization [10]. Such composition makes maize bran a valuable organic biocarrier, capable of stimulating microbial activity and improving bioremediation efficiency in hydrocarbon-polluted environments [17].

Table 2: FTIR Result for maize bran biocarrier

S/N	Peaks	Functional Groups
1	768.67394	C-Cl
2	1001,57394	C-O-C
3	1208.90935	C-O, C-N
4	1317.16755	C-O,C-N
5	1423.73326	C-H, C=C
6	14612.279	C-H
7	1612,27954	C=C, C=O
8	1745.8498	C=O

9	18855.57931	C=O
10	1924.87466	C=O
11	2025.26143	C≡C & C≡N
12	2223.05103	C≡N
13	2459.87834	C≡N , C≡C & O≡C
14	2743.71866	O-H
15	2869.71713	C-H
16	3050.22324	C-H
17	3184.57238	N-H
18	3265.35975	N-H
19	3393.2171	O-H
20	3615.51985	O-H
21	3803.672	O-H

Table 2 shows the FTIR spectra of maize bran, confirming the presence of functional groups such as hydroxyl (O–H), carbonyl (C=O), amide (C–N), and aromatic (C=C) bonds. These groups are characteristic of cellulose, hemicellulose, lignin, and proteins, which dominate maize bran composition [9]. The strong absorption peaks of O–H and C=O suggest high carbohydrate and lignin content, providing carbon sources for microbial metabolism. Amide groups reflect protein presence, supplying nitrogen crucial for microbial growth and hydrocarbon degradation [17].

Furthermore, the combination of hydrophilic (O–H) and structural (C–C, C=O) groups enhances microbial adhesion and enzymatic activity, as reported in similar studies on agro-waste carriers [10]. These findings indicate that maize bran offers both nutritional and structural support, making it a sustainable organic biocarrier for enhancing microbial remediation of hydrocarbon-contaminated environments.

Table 3: Raw data for first- order and second- order kinetic model for bioremediation of crude oil using Maize bran at constant 7g/100ml of oil

At 298K			
Time	Conc	$\ln C_t$	$1/C_t$
0	100	4.60517	0.01
5	97.01	4.574814	0.010308
10	84.42	4.435804	0.011846
15	72.33	4.281239	0.013826
20	61.27	4.11529	0.016321
25	48.15	3.874321	0.020768

At 300K			
Time	Conc	$\ln C_t$	$1/C_t$
0	100	4.60517	0.01
5	94.61	4.549763	0.01057
10	83.26	4.421968	0.012011

15	72.23	4.279855	0.013845
20	55.16	4.010238	0.018129
25	43.18	3.765377	0.023159

At 302K			
Time	Conc	lnC_t	$1/C_t$
0	100	4.60517	0.01
5	95.2	4.55598	0.010504
10	80.53	4.38863	0.012418
15	63.28	4.147569	0.015803
20	44.33	3.791662	0.022558
25	28.42	3.347093	0.035186

At 304K			
Time	Conc	lnC_t	$1/C_t$
0	100	4.60517	0.01
5	94.3	4.546481	0.010604
10	82.3	4.410371	0.012151
15	60.23	4.098171	0.016603
20	40.24	3.694862	0.024851
25	25.26	3.229222	0.039588

At 306K			
Time	Conc	lnC_t	$1/C_t$
0	100	4.60517	0.01
5	98.88	4.593907	0.010113
10	81.72	4.403299	0.012237
15	60.9	4.109233	0.01642
20	46.23	3.833629	0.021631
25	26.86	3.290638	0.03723

First-order kinetic plot for TPH degradation using maize bran as a biocarrier

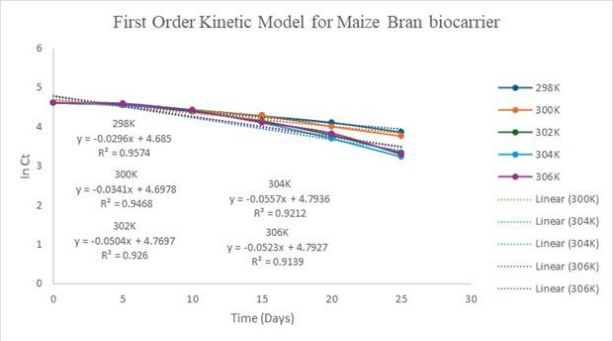

Figure: 1. First order kinetic model for maize bran biocarrier

Figure 1 illustrates the first-order kinetic plot for TPH degradation using maize bran as a biocarrier. The linearity of the plots across different temperatures confirms that crude oil degradation follows first-order kinetics, with R^2 values exceeding 0.80. This suggests that degradation primarily depends on pollutant concentration, consistent with classical biodegradation models [1].

The steeper slopes observed at higher temperatures indicate increased degradation rates due to enhanced microbial enzymatic activity, aligning with [10], who reported that moderate temperature elevation accelerates hydrocarbon metabolism. The performance of maize bran in supporting microbial activity is comparable to other organic amendments like compost and potato peels, which also improved hydrocarbon degradation under first-order kinetics [17]. Thus, Figure 1 reinforces the suitability of maize bran as a natural biocarrier for efficient and predictable hydrocarbon remediation.

Table 4. Calculated first order kinetic parameter for %TPH removal from contaminated water using maize bran biocarriers.

First order Kinetic model	298K	300K	302K	304K	306K
Maize bran K (days)	0.0296	0.0341	0.504	0.556	0.001
R^2	0.9574	0.9468	0.9268	0.828	0.8069

Table 4 presents the first-order kinetic parameters for total petroleum hydrocarbon (TPH) removal at different temperatures. The results show that maize bran-supported bioremediation followed first-order kinetics, with rate constants (k) increasing as temperature rose, while correlation coefficients (R^2) remained above 0.80.

This indicates a strong fit of the degradation data to the first-order model, consistent with previous reports that hydrocarbon biodegradation often follows first-order reaction patterns under nutrient-enriched conditions (Das & Chandran, 2011). The higher k values at elevated temperatures suggest enhanced microbial enzymatic activity and faster degradation rates, in agreement with findings that moderate temperature increases accelerate biodegradation by improving microbial metabolism [10].

Similar studies using organic amendments also reported high R^2 values, confirming the reliability of first-order modeling in predicting hydrocarbon degradation [17].Thus, maize bran effectively supports microbial activity, making it a robust organic biocarrier for hydrocarbon remediation.

Second-order kinetic plot for TPH degradation using maize bran as a biocarrier

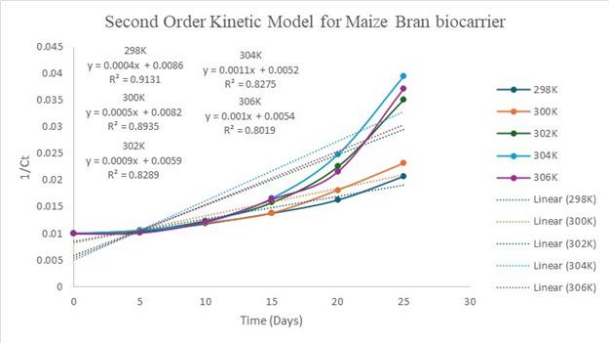

Figure: 2: Second order kinetic model for maize bran biocarriers

Figure 2 presents the second-order kinetic plot for TPH degradation with maize bran. The non-linear but strong correlation (R^2 = 0.80–0.91) suggests that degradation was influenced not only by pollutant concentration but also by microbial growth dynamics and nutrient availability [18]. The results demonstrate that second-order kinetics can capture interactions between microbial populations and hydrocarbon substrates, particularly when nutrient-rich carriers like maize bran are introduced. Similar findings were reported by [19], where microbial consortia degrading crude oil in nutrient-amended systems followed second-order kinetics. The role of maize bran as both a nutrient source and structural support likely enhanced microbial colonization and enzymatic efficiency, thereby improving model predictability.

These observations suggest that second-order modeling provides valuable insights into biodegradation dynamics under nutrient-enriched conditions, complementing first-order models.

Table 5. Calculated second order kinetic parameter for %TPH removal from oil contaminated water using maize bran biocarriers.

Second order Kinetic model	298K	300K	302K	304K	306K
Maize bran					
K(days)	0.0004	0.0005	0.0009	0.00011	0.0001
R^2	0.9131	0.8935	0.8289	0.8275	0.8019

Table 5 shows the second-order kinetic parameters for TPH removal using maize bran biocarriers. The results indicate that biodegradation data also fitted the second-order model, with correlation coefficients (R^2) ranging from 0.80 to 0.91. Although slightly lower than the first-order model, these values still demonstrate a reliable kinetic relationship. The rate constants (k) increased with temperature, confirming that higher thermal conditions enhance microbial metabolism and enzymatic activity, thus accelerating hydrocarbon degradation [10].The good fit of the second-order model suggests that bioremediation may be influenced not only by pollutant concentration but also by microbial population growth and nutrient availability [18]. This agrees with findings from organic amendment studies, where nutrient-rich carriers enhanced degradation efficiency and supported model predictability [17]. Therefore, maize bran provides dual benefits, nutrient enrichment and microbial support by allowing second-order kinetics to capture the biodegradation dynamics effectively.

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IV. CONCLUSION

This study demonstrated that maize bran is an effective organic biocarrier for enhancing the bioremediation of crude oil contaminated water using Aspergillus niger. Proximate and FTIR analyses confirmed that maize bran is rich in essential nutrients such as carbon, nitrogen, phosphorus, proteins, and lignin, alongside functional groups (O–H, C=O, C–N) that support microbial adhesion and enzymatic activity. These properties facilitated microbial growth and accelerated hydrocarbon degradation. Kinetic studies revealed that TPH degradation followed both first-order and second-order models, with correlation coefficients (R^2) above 0.80, confirming reliable predictive performance. First-order kinetics highlighted pollutant concentration as the primary driver of degradation, while second-order kinetics emphasized microbial substrate interactions under nutrient-enriched conditions.

The findings align with recent research demonstrating that organic biocarriers and agro waste amendments enhance microbial activity and hydrocarbon biodegradation compared to inorganic fertilizers . Thus, maize bran provides a cost-effective, eco-friendly, and sustainable alternative for bioremediation, particularly in regions prone to oil pollution.

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