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Simulating Biodegradation of Hydrocarbon Pollutants under Slow Nutrient Delivery Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. The work was designed and carried out by author TS under the close supervision and technical support of authors CJO and GCO. The manuscript was prepared by author TS while authors CJO and GCO aided in intellectual contributions as well as restructuring. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Bacterial growth and petroleum hydrocarbon degradation kinetics was studied under controlled laboratory conditions, to determine and numerically simulate the fate of hydrocarbon pollutants.
Study Design: A 35-day study was setup to investigate crude oil degradation and bacterial growth dynamics in water, using slow-release fertilizer formulations.
Place and Duration of Study: This study was carried out in the Environmental Microbiology Laboratory, University of Port Harcourt, Nigeria, between January and June 2015.
Methodology: Crude oil degradation studies were carried out in six (6) 500 ml capacity conical flask containing 200 ml mineral salt solution and 0.25% crude oil. The setups were inoculated with suspensions of 24-h old pure cultures of bacterial isolates (*Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp.).
Results: At the end of the study, the crude-oil concentration (S) was reduced from an initial value of 11250 ppm to 6360.6 ppm and 1471.3 ppm at weeks 5 and 10, respectively. The bacterial biomass on the other hand increased from 4.69 at the start of the experiment, to 19.36 and 34.03 (Log_{10} cfu/ml) at weeks 5 and 10, respectively. Results from the numerical simulations showed that a 50%

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variation (reduction) on the maximum degradation rate (q_{max}) led to 332.32% biodiversity gain in hydrocarbon substrate (S) and a concomitant 43.11% biodiversity loss on bacterial biomass (X) after a ten-week period of simulation. Also, an 80% variation (reduction) on q_{max} led to a 132.93% biodiversity gain on hydrocarbon substrate and a 17.24% biodiversity loss on bacterial biomass.
Conclusion: This study revealed that lower rates of crude oil utilization leads to increased volume of petroleum hydrocarbon in the environment as well as a concomitant loss in species diversity. Nutrient amendment as well as seeding with bacteria consortium is recommended for faster rates of crude oil degradation.

Keywords: Bioremediation; biodiversity; simulation models; slow-release formulations; natural attenuation.

1. INTRODUCTION

Various terminologies are associated with the definition of bioremediation. However, it is the act of exploring the potentials of microbes/ microorganisms including their products to breakdown organic as well as inorganic compounds through a detoxification or mineralization process which usually results in the conversion of these products to less harmful products [1]. It relies on the ability of microorganisms to convert these organic and inorganic materials, making them to become nontoxic and restore the environment to its pristine state [2]. This technology results in the terminal destruction of hazardous materials to harmless end-products.

Bioremediation is a cost effective technology. The soil ecosystem has microorganisms which interact with crude oil, depending on the physicochemical properties of the hydrocarbon. The lighter crude is more susceptible than the heavier crude. Also, the compounds less complex are broken down faster than those compounds that are more complex. The fate of hydrocarbon pollutant is also dependent on other factors inherent in the contaminated environment. The presence of inhibitors of microbial growth is an important factor. Also the concentration of the contaminant in the spill affected area determines the effectiveness of bioremediation technology. These factors affect microbial activities in the polluted environment and therefore monitoring the progress of crude oil bioremediation involves biological, chemical and physical approach to ensure the goal of clean up of contaminant or removal of pollutants in the environment is achieved [3].

Fertilizers from agrochemical industries pose some environmental challenges and also have some deleterious effect on the degrading population. This has served as a major setback in the direct application of conventional fertilizers on spill sites. Recent advancement has been towards strategies to improve on the application of fertilizer in bioremediation. This has led to the use of slow-delivery fertilizers. Slow-release fertilizer (SRF) is prepared by using coatings or binding matrix to reduce the rate of dissolution of fertilizers [4].

Bioremediation is rather a slow process and monitoring the progress of a bioremediation process is time consuming making most investigations to be conducted within a short period of time. Mathematical models and simulations are important in evaluating bioremediation process monitoring and evaluation. And many researchers have made progress in this area [5,6].

There is paucity of information regarding the kinetics model of microorganisms; to elucidate the ability of these organisms to utilise petroleum hydrocarbon [7,8] under slow release conditions.

Presently, there is uncertainty associated with the prediction of remediation time at contaminated sites. Models can be used to investigate the rate limiting factors influencing the remediation time for bioremediation of hydrocarbon pollutants in soil and water. Although numerous models are available in literatures, the potentials of indigenous microorganisms to clean up pollutants, under slow-nutrient release conditions was described, using different equations and kinetics parameters.

2. MATERIALS AND METHODS

2.1 Sources and Preparation of Materials

2.1.1 Soil sample

A 0-10 cm depth, hydrocarbon contaminated soil sample was collected from Etelebuo-Ogboloma, flow station in Yenagoa L.G.A. of Bayelsa State.

2.1.2 Fertilizer

Commercial NPK fertilizer (15:15:15) and Urea (46% Nitrogen) was used.

2.1.3 The polymer

Agar-agar was applied as a material for preparation of slow-release fertilizers (SRFs). The film forming solution was prepared by dissolving 3 g agar powder in 200 ml distilled water and autoclaved at 121℃.

2.2 Experimental SETUP for Kinetics Study

Crude-oil concentration: A 0.25% concentration of crude was achieved by introducing 0.5 ml crude oil into a 500 ml capacity Erlenmeyer flask containing 200 ml mineral salt solution.

Nutrient concentration: Each SRF contained 2 g of respective fertilizer (NPK, Urea).

Preparation of bacterial consortium: The bacterial species used in this study were isolated from hydrocarbon polluted soil by plating decimal dilutions of the soil sample on a mineral salt agar plate through a vapour phase transfer technique. Suspensions of 24-h old pure cultures of bacterial isolates identified as *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. were used to inoculate the setups as shown below. This was achieved by adjusting the turbidity of each suspension using McFarland's standard. The setup was however seeded with 0.5 ml of each bacterial suspension in a 500 ml capacity Erlenmeyer flask containing 200 ml mineral salt solution and 0.5 ml contaminant (crude oil).

Sample Identity

Sample A = 200 ml MSS + 2 g NPK Capsular SRF + biomass

Sample B = 200 ml MSS + 2 g NPK Granular SRF + biomass

Sample C = 200 ml MSS + 2 g Urea capsular SRF + biomass

Sample D = 200 ml MSS + 2 g Urea granular SRF + biomass

Sample E = 200 ml MSS - No fertilizer + biomass: Control 1

Sample F = 200 ml MSS - No fertilizer, no biomass: Control 2

*MSS = Mineral salt solution

Study duration: The experimental setup was monitored for a 35-day period during which changes in bacterial biomass, nutrient (nitrate) concentration and total hydrocarbons content (THC) was investigated at seven (7) days interval.

Precautions taken: In order to avoid sampling error, suicidal sampling technique was employed: each setup was sacrificed after a particular period (day) of analyses. Also, the results were taken in triplicates.

2.3 Growth Dynamics of Bacterial Consortium

The population of the bacteria was enumerated using the spread plate technique, at seven (7) days interval. A 10-fold serial dilution of the solution (sample) was carried out by weighing 1 ml of the water sample into a sterile test tube containing 9 ml of sterile physiological saline. From here, a ten-fold serial dilution was performed to a dilution of 10^{-5}.

From each dilution, 0.1 ml was inoculated on nutrient agar plates (Petri dishes). However, a triplicate plating of each dilution was employed. A sterile glass rod (spreader) was used to spread the inoculums over the media. The plates were incubated in an incubator at room temperature (25℃) for 24 hours.

2.4 Determination of Physicochemical Parameters and Residual Hydrocarbon

Parameters such as pH, total nitrogen, and phosphate were determined using the methods from APHA [9] while the total hydrocarbon content (THC) was determined using the methods of ASTDM 3921 and USEPA 8270B [10,11].

2.5 Statistical Analysis

One-way Analysis of variance (ANOVA) was used to check for significant difference in the values of the various treatment options and Pearson's Moment Correlation coefficient ρ was used to determine degrees of relationship between the various parameters.

2.6 Modeling

2.6.1 Formulation of the model

Differential equation models for single – substrate limited process (Okpokwasili and Nweke) [11] based on Monod's Kinetics were adopted.

2.6.2 Numerical simulations

Numerical simulations used in this study are based on MathLab implementation software which was programmed to solve the post system differential equations that describe substrate – bacteria interaction. In this study, the simulation was done for a ten-week period.

2.6.3 Change in biodiversity during bioremediation process

The change in biodiversity resulting in loss or gain was determined as;

$$PG\ (\%) = BG\ (\%) = \{(S_{new} - S_{old}) / S_{old}\}*100 = \{(x_{new} - x_{old}) / x_{old}\}*100$$

Where;

S = Substrate concentration
X = Bacterial biomass
PG = Population Gain and BG = Biodiversity Gain.
S_{old} and x_{old} are values at time (t)
S_{new} and x_{new} are simulated values at time (t) based on variations on kinetics parameters.

3. RESULTS AND DISCUSSION

Models: Differential models for single – substrate limited process [11] based on Monod's Kinetics were adopted.

$$S(t) = -(q_{max} * SX) / (K_s + S) \quad (1)$$

$$X(t) = -Y(ds / dt) = (Yq_{max} * SX) / (K_s + S) \quad (2)$$

$$Y_{x/s} = dx/ds \quad (3)$$

$$q_{max} = = \{q(K_s + S)\} / S \quad (4)$$

Where;

Y (yield coefficient) = actual bacterial growth yield = $(\frac{dx}{ds})$

dx = mass of new cells produced

ds = mass of substrate consumed

S = concentration of substrate in aqueous phase

X = bacterial biomass

t = time (weeks)

K_s = affinity constant for substrate.

From the experimental setup, the initial crude-oil concentration and bacterial biomass was 11250.0 ppm and 4.69 ($Log._{10}$ cfu/ml), respectively. The above model parameters were evaluated based on the experimental results; q_{max} was determined as 0.23; μ_{max} = 0.69; Y = 3, while k_s was 1155.0 ppm. However, the hydrocarbon degradation was simulated numerically using special MATHLAB software. The software is a power tool that helps to simulate both linear and non-linear differential equations. This was useful in predicting crude-oil degradation as well as change in bacterial biomass at a given period and condition. In this research, hydrocarbon degradation was experimentally monitored for a five-week period. However, with the aid of MATHLAB simulation software, a ten-week period of degradation was simulated under varying kinetics parameters such as changes in crude oil degradation rate and initial bacterial biomass, as shown in Tables 1 – 3. These Tables 1 – 3 show that the hydrocarbon substrate was degraded from an initial concentration of 11250.0 ppm to 1471.3 ppm (S_{old}) at week 10. It follows that the bacterial biomass increased from an initial value of 4.9 x 10^4 cfu/ml, i.e 4.69 ($Log._{10}$ cfu/ml) to 1.07 x 10^{34} cfu / ml, i.e 34.03 ($Log._{10}$ cfu / ml) (X_{old}) at week 10. These results were simulated using equations 1 - 4, above.

At q_{max}, the rate of substrate removal or utilization is at maximum level due to favourable substrates-species interactions. Also, at u_{max} bacterial growth is at maximum rate. The impact of changes in these parameters (with respect to time) were simulated and tabulated as S_{new} and X_{new}, for substrate concentration and bacterial biomass, respectively. The percentage (%) gain or loss in biodiversity was therefore calculated as $\{(S_{new} - S_{old}) / S_{old}\}$ *100 and $\{(x_{new} - x_{old}) / x_{old}\}$ *100, for hydrocarbon substrate and bacterial biomass, respectively. The impact analysis was done by varying q_{max} at 50% and 80%. This

involves reduction of q_{max} from an experimental value of 0.23 to 0.12 and 0.18, at 50% and 80% variations, respectively. From the numerical simulations, it was observed that a 50% variation (reduction) on q_{max} led to a 332.32% biodiversity gain on hydrocarbon substrate (S) and a 43.11% biodiversity loss on bacterial biomass (X) (Table 1), while an 80% variation (reduction) on q_{max} led to a 132.93% biodiversity gain on hydrocarbon substrate (S) as well as a 17.24% biodiversity loss on bacterial biomass (X) (Table 2), at the end of a 10-week period of simulation. This shows that lower rates of substrate (hydrocarbon) metabolism leads to increased concentrations of the contaminant and a decrease in bacterial population.

Also, numerical simulations involving a 50% variation (reduction) on the initial biomass concentration (from 4.69 to 2.35 $Log._{10}$ cfu / ml), showed a constant 50% loss in bacterial diversity and a 332.32% biodiversity gain on hydrocarbon substrate (S), at week 10 (Table 3).

Table 1. 50 Percent change in q_{max} on S (t) and X (t)

Time (week)	$S_{old\ (t)}$	$S_{new\ (t)}$	PG (%)	$X_{old\ (t)}$	$X_{new\ (t)}$	PG (%)
0	11250.0	11250.0	0.0000	4.6900	4.6900	0.0000
1	10272.1	10761.1	4.7598	7.6236	6.1568	-19.2403
2	9294.3	10272.1	10.5212	10.5572	7.6236	-27.7877
3	8316.4	9783.2	17.6375	13.4908	9.0904	-32.6178
4	7338.5	9294.3	26.6504	16.4244	10.5572	-35.7225
5	6360.6	8805.3	38.4344	19.3581	12.0240	-37.8862
6	5382.8	8316.4	63.5833	22.2917	13.4908	-39.4804
7	4404.9	7827.5	77.6985	25.2253	14.9576	-40.7038
8	3427.0	7338.5	114.1361	28.1589	16.4244	-41.6723
9	2449.2	6849.6	179.6702	31.0925	17.8913	-42.4580
10	1471.3	6360.6	332.3167	34.0261	19.3581	-43.1082

Table 2. 80 Percent change in q_{max} on $S_{(t)}$ and $X_{(t)}$

Time (week)	$S_{old\ (t)}$	$S_{new\ (t)}$	PG (%)	$X_{old\ (t)}$	$X_{new\ (t)}$	PG (%)
0	11250.0	11250.0	0.0000	4.6900	4.6900	0.0000
1	10272.1	10467.7	1.9039	7.6236	7.0369	-7.6961
2	9294.3	9685.4	4.2085	10.5572	9.3838	-11.1151
3	8316.4	8903.1	7.0550	13.4908	11.7300	-13.0471
4	7338.5	8120.8	10.6601	16.4244	14.0776	-14.2890
5	6360.6	7338.5	15.3738	19.3581	16.4244	-15.1545
6	5382.8	6556.2	21.8000	22.2917	18.7713	-15.7922
7	4404.9	5773.9	31.0794	25.2253	21.1182	-16.2815
8	3427.0	4991.6	45.6544	28.1589	23.4651	-16.6689
9	2449.2	4209.3	71.8681	31.0925	25.8120	-16.9832
10	1471.3	3427.0	132.9267	34.0261	28.1589	-17.2433

Table 3. 50 Percent change in initial biomass on S (t) and X (t)

Time (week)	$S_{old\ (t)}$	$S_{new\ (t)}$	PG (%)	$X_{old\ (t)}$	$X_{new\ (t)}$	PG (%)
0	11250.0	11250.0	0.0000	4.6900	2.3450	-50.0000
1	10272.1	10761.1	4.7598	7.6236	3.8118	-50.0000
2	9294.3	10272.1	10.5212	10.5572	5.2786	-50.0000
3	8316.4	9783.2	17.6375	13.4908	6.7454	-50.0000
4	7338.5	9294.3	26.6504	16.4244	8.2122	-50.0000
5	6360.6	8805.3	38.4344	19.3581	9.6790	-50.0000
6	5382.8	8316.4	54.5000	22.2917	11.1458	-50.0000
7	3427.0	7338.5	114.1361	28.1589	14.0794	-50.0000
9	2449.2	6849.6	179.6702	31.0925	15.5463	-50.0000
10	1471.3	6360.6	332.3167	34.0261	17.0131	-50.0000

The influence of nutrient and bacterial growth on crude oil degradation was investigated. Nutrients (in slow release formulations) and bacterial suspensions were added to experimental set-ups A – D while set-up E (control 1) was seeded with bacterial suspension, without nutrient addition). Neither nutrient nor bacteria was added to set-up F which served as control 2. The result indicated that set-ups E and F recorded the least hydrocarbon removal rates, which imply that crude-oil degradation is greatly influenced by bacterial growth and nutrient availability. However, the control experiment F had the least rate of hydrocarbon removal.

It has been well documented that the fate of crude oil pollutants is dependent on physical, chemical and biological factors. Where the rate of hydrocarbon removal was influenced by nutrient concentration in treatments A, B, C, and D, a 37.3% loss of hydrocarbon (day 35) was noticed in the control set-up (without fertilizer). This is probably due to natural attenuation taking place in the unfertilized soil. Chikere et al. [12] observed a hydrocarbon loss in the heat-killed control, signifying that abiotic factors could as well contribute to hydrocarbon attenuation in the environment.

However, the difference in the rate of biodegradation of crude oil observed between the fertilized setup and the unfertilized accounts for the role of fertilizers in hydrocarbon bioremediation. Monitoring the process and progress of bioremediation is very crucial. Most monitoring involves analyzing for changes in physicochemical parameters as well as microbiological parameters such as changes in cell biomass and community diversity. Several researchers have evaluated bioremediation reports on model basis. However, there is paucity of information on kinetics models of crude oil degradation under slow nutrient delivery condition.

The results from this study conform to the fact that the rate of hydrocarbon removal is inversely related to the rate of increase in biomass. It follows that a change in bacterial growth rate gives a concomitant reduction in the total hydrocarbon content with respect to time. Differential models for single – substrate limited process [11] based on Monod's Kinetics were therefore adopted and evaluated.

The results from the simulation models revealed that decreasing the rate; at maximum level of substrate utilization/removal (q_{max}) leads to S-biodiversity gain resulting in increased volume of petroleum hydrocarbon substrate in the environment and a concomitant loss in species diversity due to loss in biomass (X). This implies that if the rate of bacterial metabolism of crude oil hydrocarbon is below q_{max}, the volume of crude oil lost will be low, thereby having more volume of hydrocarbon in the environment when compared to the volume lost at q_{max}. Also, a reduction in the rate of hydrocarbon removal (q_{max}) will cause loss in biodiversity or decrease in colonial count. Therefore, the application of slow-release fertilizers at optimal rates may be very vital in correcting nutrient imbalance and supply nutrients for sustained microbial activities in the environment [13].

The rate of hydrocarbon removal can be below q_{max} when the bacterial biomass is reduced and vice versa. Table 3 showed the effect of initial biomass on biodiversity. It was revealed that when the initial biomass was reduced by 50% variation, the bacterial biomass witnessed a constant 50% loss throughout the ten (10) weeks period of simulation which gave rise to a gain in substrate concentration. It was also observed that a 50% regressive change on bacterial biomass resulted in the same degree of gain in hydrocarbon substrate at 50% variation on q_{max} (Table 1). This depicts that the rate of hydrocarbon removal is a function of the microbial population in the media (soil or water). Therefore, the rate of hydrocarbon removal is dependent on the initial biomass. Hence, it is important to determine the initial conditions of the impacted media prior to the start of a bioremediation project. This will help to initiate an optimal condition for bioremediation. This will involve bioaugmentation at the first instance where the count is low or biostimulation with nutrient formulation; oxygen, etc, as the case may be. It also connotes the fact that 50% reduction leads to loss in bacterial diversity and gain in total hydrocarbons content. The reverse been the case, a 50% increase will lead to a gain in bacterial biomass and loss in crude oil hydrocarbon.

Crude oil pollution reduces bacterial population in the affected media, leading to loss in species diversity. This is due to nutrient imbalance (high C:N ratio) as well as the physicochemical properties of the oil. The degree of impact is dependent on the volume of spill/contamination. On the other hand, nutrient enrichment and seeding with exogenous sources of bacteria are ways of increasing bacterial biomass. These

techniques should however target an increase well above 50%, in order to achieve an effective bioremediation process. Chikere et al. [14] observed that some bacteria are strongly and rapidly selected when hydrocarbon degradation is simulated by addition of organic and inorganic nutrients. In this study, the experimental set-ups were inoculated with bacterial consortium (*Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp.) and further enriched with nutrients which caused an appreciable increase in bacterial biomass as well as a concomitant loss in the hydrocarbon substrate. This has provided an easy framework for field application and the simulation models from this study are very important for future researches about petroleum hydrocarbon pollutants and their degradation.This observation is at par with the principle of bioremediation and environmental biotechnology which involves engineering and harnessing the potentials of microorganisms for the uptake of crude oil in the environment.

4. CONCLUSION

The results from the simulation models revealed that decreasing the rate at maximum level of substrate utilization/removal (q_{max}) leads to increased volume of petroleum hydrocarbon substrate in the environment as well as a concomitant decrease in species diversity (bacterial biomass). These models are very important for future researches about petroleum hydrocarbon pollutants and their degradation.

Bioremediation techniques such as seeding with bacterial populations capable of metabolizing crude oil hydrocarbon as well as nutrient enrichment should be optimized to achieve an increase in bacterial biomass above 50%, for effective rate of crude oil uptake by the degrading species. Also, molecular screening should be performed to identify and characterize other species of bacteria possessing the ability (genes) to rapidly degrade crude oil. Bioengineering and application of such species to contaminated sites will bring about a more rapid rate of hydrocarbon metabolizing. The contribution of abiotic factors was noted. Also natural attenuation was observed to have taken place in the unfertilized set-up. Therefore, addition of exogenous sources of bacteria as well as nutrient enrichment will aid/enhance natural attenuation.

Controlling biodiversity loss or gain is therefore critical in hydrocarbon bioremediation. Variations on the determinants of biodegradation rate should be monitored to ensure efficient remediation conditions. This study has shown that SRFs are promising in hydrocarbon bioremediation. However, field applications as well as a model based evaluation of these processes are therefore recommended for simulation and prediction.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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