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Production and Application of Agar-based Slow-release Fertilizers, in the Bioremediation of Petroleum Hydrocarbon-impacted Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Author TS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CJO and GCO managed the analyses of the study. Author GCO managed all technical aspects of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to determine the potentials of novel slow-release fertilizers (SRF) in the bioremediation of petroleum hydrocarbon-impacted soil, in order to determine their potentials in the bioremediation of petroleum-impacted sites and as well evaluate the effect of nutrient concentration on the rate of bioremediation.
Study Design: A marine biopolymer (agar agar) was used as a coating for soluble NPK fertilizer in slow-release formulations (capsular and granular form).
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: The contaminated soil sample was recreated in four clean plastic containers and labeled A - D, as follows: Sample A = 300 g Soil + 20 g NPK Capsular SRF; Sample B = 300 g Soil + 20 g NPK Granular SRF; Sample C = 300 g Soil + 20 g Direct NPK; Sample D = 300 g Soil (without fertilizer - control). The determination of the effect of SRF on the population dynamics of

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total aerobic heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) was achieved through the use of nutrient agar (spread plate technique) and mineral salts agar (vapour phase transfer technique) in the enumeration of THB and HUB respectively.
Results: After a 42-day period, there was a significant difference, ($p < 0.05$) in the percentage loss of total petroleum hydrocarbon between the various treatment options. Sample D had the least percentage loss (33.6%) of total petroleum hydrocarbon, Sample A (50.5%), Sample B (73.1%) and Sample C had the highest percentage loss of 74.83%. The various bacterial counts (THB and HUB) increased progressively with increase in nutrient concentration.
Conclusion: The results revealed the applicability and effectiveness of slow release fertilizers in the bioremediation of hydrocarbon impacted soil. These novel SRFs are also recommended for their applicability in the bioremediation of water and sediments.

Keywords: Slow-release fertilizers; bioremediation; hydrocarbon; marine biopolymer; nutrient release.

1. INTRODUCTION

Bioremediation of hydrocarbon polluted sites has been the main focus of current research issues. This is due to the increasing activities of petroleum industries which inundate the environment with hydrocarbon through various activities. Consequently, much research has targeted the application of microorganisms or their product to clean up contaminated sites [1]. Notable among them were the bacterial species of *Arthrobacter* [2], *Sphingomonas* (a novel *Pseudomonas* sp) [3] and *Pseudomonas* spp. [4]. The ability of *Nitrosomonas and Nitrobacter* species to degrade crude oil was found to be plasmid–mediated, through curing experiment and electrophoresis [5].

The rate of bioremediation is a function of the bacterial community composition and the physicochemical properties of the contaminated site, including availability of nutrients. The use of fertilizers as sources of nutrient to biostimulate microbial population explores the principle that fertilizers are mainly composed of nitrogen, phosphorus, potassium and other micro and macro elements/nutrient required by microorganisms to carry out the metabolic activities. The activities of these microbial groups help in degrading hydrocarbon, which are utilized as a carbon source by microorganisms [6].

Also, the use of fertilizers in bioremediation seems indispensable. Fertilizer application help to bring about a balanced carbon-to-nitrogen (C:N) ratio thereby supporting microbial growth and a concomitant hydrocarbon microbial biodegradation. However, underground water pollution following leaching, eutrophication, nutrient imbalance are some of the major problems associated with the use of fertilizer.

Regarding the nutrient supply, the controlled-release substrate with optimal ratio can provide the microorganisms a slow and constant nutrient supply under the controlled conditions. Previous studies have displayed that the addition of essential metabolic nutrients, N and P, to oil contaminated beaches is an effective approach for stimulating bioremediation of oil pollutants by indigenous microbial biomass [7]. The use of slow-release nutrients with an appropriate rate may provide a continuous nutrient supply by maintaining a sufficient nutrient status for the perpetuation of microbial metabolic activities without causing environmental concerns and save cost [8].

To improve the biodegradation efficiency, integrating various components, such as microbial strains in consortium, solid oxygen source, appropriate rate of nutrients with controlled-release pattern, into a granule formulation with an oleophilic matrix, may provide an ideal approach to improving bioremediation of crude oil pollutants.

Several factors influence the behavior of slow release fertilizers (SRFs). They include the nature of the binding matrix or polymer being used. Tomaszewska [9] was able to discover that the porosity of the polymer was largely influenced by the concentration of the polymer used in the preparation of the SRF. Researcher observed that the porosity of the polymer was inversely proportional to the concentration of the polymer used. Researcher opined that the porosity of the polymer was very crucial in the performance of the SRF, as it is a function of the amount of water that can permeate through the coating. Also, Zhao et al. [10] carried out similar work on slow-release mechanism of SRFs and were able to produce a novel macromolecular

SRF (MSF) composed of nitrogen, potassium and phosphorus. They observed that the slow release behavior of the fertilizer which contained nitrogen, phosphorus and potassium was attributable to the decomposition of the macromolecular coating. The fertilizer was able to release 39.5%, 91.8% and 98.9% of nitrogen, phosphorus and potassium, respectively after thirty days of investigation.

Many attempts have been made to design nutrient delivery systems that overcome the leaching problems associated with fertilizer application. SRFs are normally in solid forms that consist of inorganic nutrients coated with hydrophobic materials like paraffin or vegetable oils. This approach may cost less than adding water-soluble nutrients due to frequent applications. From oil bioremediation studies, SRFs have shown some promising potentials and applicability in the bioremediation of petroleum hydrocarbon impacted sites [11,12]. SRFs, like the customblem (vegetable oil coated calcium phosphate, ammonium phosphate, and ammonium nitrate) was reported to have performed well on some of the shore lines of Prince William sound, particularly in combination with an oleophilic fertilizer [13,14].

Lee et al. [15] also showed that oil biodegradation rates increased with the use of a SRF (sulphur coated urea) compared to water soluble fertilizers.

SRFs have an advantage of ready supply of nutrient for a long period of time. The problem of ground and surface water contamination is mitigated by the use of SRFs due to low application rate as against conventional fertilizers which require frequent application.

In this work, agar agar was used as a binding matrix to reduce the solubility of both NPK and urea fertilizers. The agar was also used to prepare capsular form of SRF, to evaluate the relationship between nutrient availability and bioremediation rate.

2. MATERIALS AND METHODS

2.1 Preparation of SRFs

A commercial NPK fertilizer (20:10:10) was purchased from Creek road market, Port Harcourt, Rivers State and used as the active ingredient. Agar agar (oxoid) was applied as a material for preparation of the polymer coating. The film forming solution was prepared by dissolution of the polymer in water at a concentration of 1.5%. The capsular SRF was prepared using a **casting method** which allows the active ingredient to permeate through the capsular coats while the granular SRF was prepared using a **homogenization method** in which the nutrient is released as the granules disintegrate or decompose.

2.1.1 Capusular SRF

Three grams (3 g) agar powder was mixed in 200 ml distilled water and autoclaved at 121℃ for 15 minutes. The molten agar was then poured into the Petri dishes to a depth of 2 mm at reduced temperature of about 37 - 42℃ to form a primary layer after solidification. Five gram (5 g) dry NPK granules were placed/laid on the primary layer and a molten agar was again poured over the NPK – primary layer structure to sandwich/encapsulate the fertilizer after solidification of the molten agar. The final structure or coating was further cut into capsular forms as shown in Plate 1.

2.1.2 Granular SRF

The granular SRF was prepared by mixing 3 g agar powder with 20 g granular NPK fertilizer in 200 ml distilled water and autoclaved at 121℃ for 15 minutes. The molten agar – NPK mixture was then poured into plastic Petri dishes at reduced temperature of about 37- 42℃. The mixture was allowed to solidify and then cut into granules (Plate 2). The SRFs were wrapped in sterile aluminum foil papers prior to use.

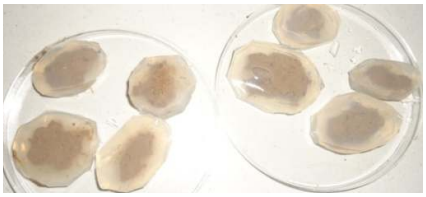

Plate 1. Capsular SRF

Plate 2. Granular SRF

2.2 Assessing the Rate of NPK Release

The dynamic preliminary test was conducted under a laboratory condition. The coated granules were placed in distilled water, and the amount of nutrients released over time was measured. The amount of nutrient: nitrogen (ammonium nitrogen), phosphorus (phosphate) and potassium released over time were determined by introducing 5 g each of the SRF preparations into 200 ml of distilled water under a laboratory condition. A pure agar preparation was used as control. The release rate was assessed at weekly intervals by sampling 20 ml solution over a 35-day period and additional 20 ml de-ionized water was injected into the bottles to maintain a constant amount of solvent. The nitrogen was analyzed using special analysis instruments like electro-thermal heater, distillation flask and Liebig condenser while and phosphorus was estimated by an element analysis instrument such as thermospectronic spectrophotometer and potassium was also determined by the use of a spectrophotometer.

2.3 Experimental Setup

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

The crude oil impacted soil sample was amended as follows and kept under a controlled environmental condition.

Sample A = 300 g soil + 20 g NPK Capsular SRF
Sample B = 300 g soil + 20 g NPK Granular SRF
Sample C = 300 g Soil + 20 g Direct NPK fertilizer
Sample D = 300 g unamended Soil: Control.

2.4 Growth Dynamics of Total Heterotrophic Bacteria and Hydrocarbon Utilizing Bacteria

The population of heterotrophic bacteria was enumerated using the spread plate technique, at fourteen (14) days interval. A 10-fold serial dilution of the soil sample was carried out by weighing 1 g of the soil 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.

A vapour phase transfer technique was employed in the enumeration of the hydrocarbon utilizing bacteria. This was achieved by culturing the diluted soil samples on mineral salts agar. A Whatman's filter paper was saturated with crude oil and placed on the lid of each glass Petri dish using sterile forceps. The crude oil served as the sole source of hydrocarbon (that is carbon and energy source for the hydrocarbon utilizers). The inoculated mineral salts agar plates were inverted and placed over the lid containing the saturated filter paper, and incubated at room temperature for seven (7) days. Isolated colonies were further purified by sub-culturing and identified using biochemical tests and microscopy.

2.5 Determination of Physicochemical Parameters of Soil

Parameters such as pH, moisture content, total nitrogen, phosphate and total organic carbon (TOC) were determined using the methods from APHA [16].

2.6 Chromatographic Analysis

Residual total petroleum hydrocarbons (TPH) was extracted from the soil samples and quantified using gas chromatograph - flame ionization detector (GC-FID) according to the methods of ASTDM 3921 and USEPA 8270B [17].

2.7 Statistical Analysis

One sample students T-test was used to check for significant difference in the values of the various treatment options and correlation analysis was as well used to determine degrees of relationship between the various parameters.

3. RESULTS AND DISCUSSION

The physicochemical and microbiological parameters of the polluted soil were assayed prior to amendment as shown in Table 1.

Table 1. Baseline properties of soil prior to amendment

Parameter	Measurement
Total heterotrophic bacterial count -THB (cfu/g)	$3.10\pm0.8 \times 10^6$
Hydrocarbon utilizing bacterial count - HUB (cfu/g)	$3.2\pm0.5 \times 10^5$
Total petroleum hydrocarbons - TPH (mg/kg)	3113.38
pH	6.64±0.2
Moisture (%)	20.42±0.4
Total nitrogen (%)	0.273±0.005
Phosphate (mg/kg)	3.3±0.01
Total organic carbon - TOC (%)	3.159±0.002

Agar agar has been used in this work to demonstrate the potentials of SRFs in hydrocarbon bioremediation. Agar, a marine biopolymer is soluble in water at a concentration of 1.5%. This has made it a good polymer of choice when cost is being put into consideration, as very little quantity of the polymer powder can be used in the preparation of large amounts of SRFs. The knowledge of the fact that agar is sparingly soluble in water (hydrophobic) when solidified formed the basis for the integration of agar in the production of these SRFs. Agar exhibits hysteresis, melting at 85℃ (358 K, 185℉) and solidifies from 32-40℃ (305-313 K, 90-104℉) [18]. This property lends a suitable balance between easy melting and good gel stability at relatively high temperatures.

Preliminary investigations from this work revealed that the granular SRF released the active ingredients at rates faster than that of the capsular version. After a period of one week, the granular SRF released 37.07 mg/L of ammonium nitrogen, while after a two week period; an increase was observed (73.15 mg/L). A slight increase also followed after three weeks of the investigation when the highest nitrogen release rate of 77.3 mg/L was observed. The fourth and fifth weeks showed gradual decrease of 52.6 mg/L and 49.29 mg/L, respectively (Fig. 1).

Same pattern was observed for the capsular SRF. The average rate of nitrogen release was 8.37 mg/L, 10.55 mg/L, 12.35 mg/L, 7.82 mg/L, and 5.73 mg/L for weeks 1, 2, 3, 4 and 5, respectively (Fig. 1). This trend was the same for both the rate of phosphorus (Fig. 2) and potassium release (Fig.3).

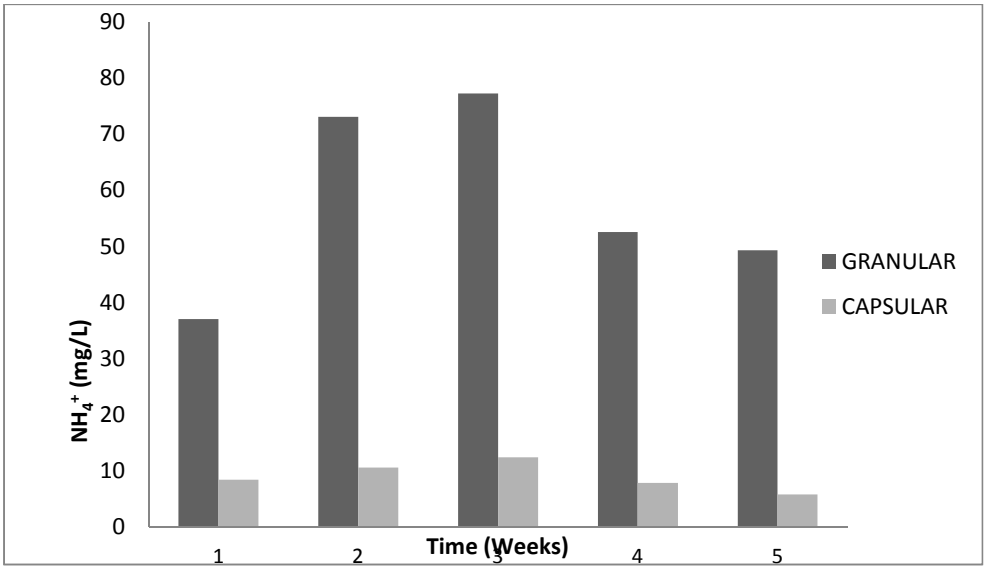

Fig. 1. Rate of release of nitrogen in distilled water (mg/L)

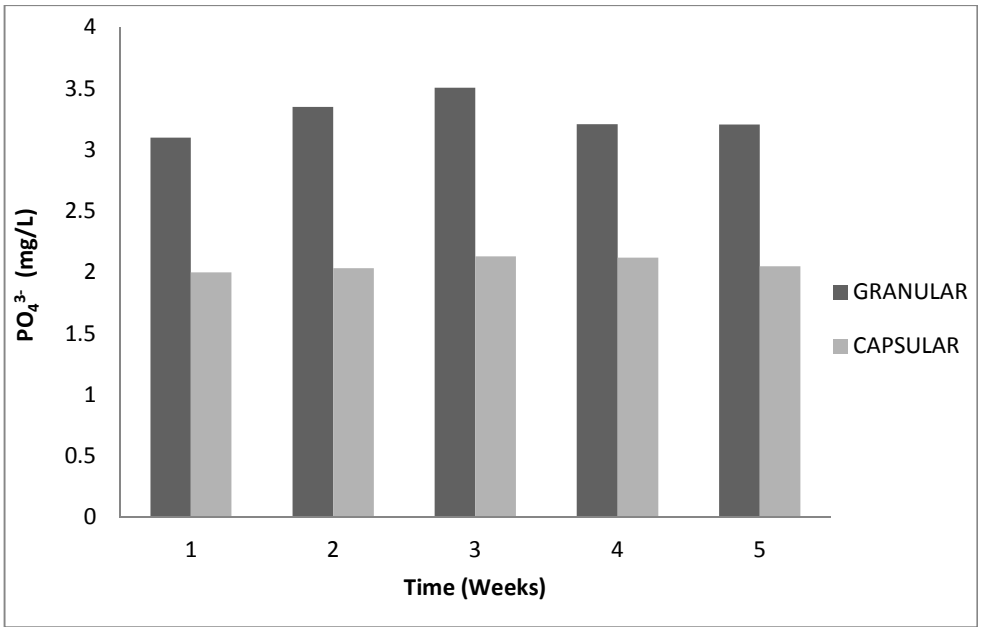

Fig. 2. Rate of release of phosphorus in distilled water (mg/L).

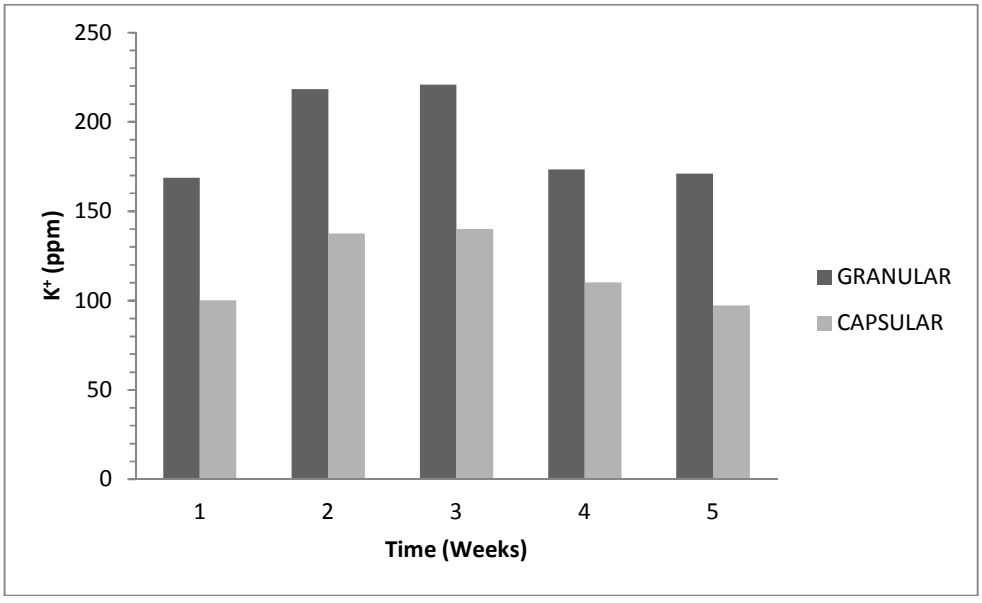

Fig. 3. Rate of release of potassium in distilled water (ppm)

The difference in the rate of nutrient release can be tied to surface area difference and capillary action. The granular SRF had larger surface area compared to the capsular SRF. Hence, more nutrients were released over time.

The capsular SRF released nutrient due to capillary action which was dependent on the diffusion rate. And it can be noted that the rate of diffusion was a function of the polymer concentration and thickness. Therefore, the diffusion as well as nutrient release rate can be engineered or controlled by adjusting the polymer thickness and balancing the polymer concentration. The coating acted as a barrier which reduced the rate of intra-granular diffusion of water, the dissolution of ingredients and fertilizer transfer out of the granule.

Tomaszewska [9] observed that the polymer concentration in the coating solution influenced the porosity of the prepared coating. The coatings formed from solutions with a higher polymer concentration exhibited lower porosity. It was also discovered that the amount of nitrogen and potassium released over time was always higher than that of phosphorus. This difference can be attributed to the difference in solubility, as nitrogen is more soluble than phosphorus [19].

Nearly all N, K, and S fertilizers are completely water soluble. Meaning all of the available nutrients are soluble. The solubility of P fertilizers, on the other hand, varies between carriers and is dependent upon manufacturing processes and composition [19].

The changes in soil pH following soil amendment with NPK SRF fertilizer was revealed in this study [Fig. 4]. It was discovered that the initial pH of the polluted soil was 6.64±0.02. After soil amendment with NPK SRF, the pH values increased slightly towards neutrality and alkalinity and ranged from 6.34±1.0 to 8.83±0.01. This is in conformity with the pH range observed by Okpokwasili and Oton [20]. The experimental setup with the capsular SRF had pH values lower than that of the granular and direct fertilizer application. However it is known that fertilizer application has varying effect on soil pH [19]. The changes in soil pH are also a function of other intrinsic factors relating to microbial activities (metabolites).

The total organic carbon of the experimental soil samples was analyzed over time. It was discovered that the total organic carbon decreased as time progressed. The total organic carbon content of the pristine soil was observed to be 3.159%. However, after a 42-day period, the changes in the total organic carbon (TOC) content of the various experimental set-ups were recorded as shown in Fig. 5. The control experimental set-up had the highest TOC values of 2.874%, 2.744% and 2.09% for day 14, 28 and day 42, respectively. Experimental set-up 'A' (capsular SRF) was next and had lower TOC values of 2.619, 2.705, and 1.12% for days 14, 28 and 42, respectively. Experimental set-up B (granular SRF) had values that ranged between 1.14% (at day 42) to 2.73% (at day 14). The sample with direct fertilizer application (Experimental set-up C) had the least TOC values which ranged between 1.1% (at day 42) to 2.559% (at day 14) (Fig. 5). The decrease observed could be associated with the rate of hydrocarbon loss. This is on the premise that petroleum hydrocarbon also forms integral part of the soil total organic carbon. The rate of loss of petroleum hydrocarbon will therefore, have a concomitant effect on the total organic carbon.

The rate of nutrient release in soil was evaluated in this study. Nitrogen (total nitrogen) and phosphorus (phosphate) were among the active ingredients assayed to monitor the rate of nutrient release and their effect on the process of bioremediation. The pristine soil had total nitrogen value of 0.273±0.005. After a 42-day period of investigation it was observed that the total nitrogen of the control experimental setup reduced with respect to time. This reduction is attributable to the microbial utilization of nitrogen for biomass production. However the release rate of nitrogen was revealed as total nitrogen (%). It was seen that setup C, direct fertilizer application had the highest amount of nitrogen at day 14, compared to setup A and B. After a 28- and 42-day period of time, sharp decrease was noted for the direct fertilizer application, whereas, the SRF application witnessed a gradual increase and later, a gradual decrease was observed. These values were subjected to statistical analysis and showed a significant difference ($p<0.05$, $t = 11.142$, PV, 0.008) in the mean total nitrogen of setups A, B, and C, when compared to the control experiment, D.

This observed trend has revealed the need for frequent application of fertilizer due to rapid loss, following microbial utilization and washout by rain and other meteorological factors. This is a potential disadvantage associated with direct fertilizer application as it will eventually lead to eutrophication, underground water pollution (due to leaching) and higher cost implication due to frequency of application.

The capsular and granular SRFs released nitrogen at a gradual and controlled rate. Hence the amount of available nitrogen was higher after days 28 and 42, compared to direct fertilizer application [Fig. 6]. This has revealed the promising potentials of SRFs in mitigating the negative implications associated with over dosing, and high cost implications of direct fertilizer application. It has shown the ability of SRFs to guarantee supply of nutrients at a steady and readily available rate. These nutrients are released gradually and are utilized by the soil biota.

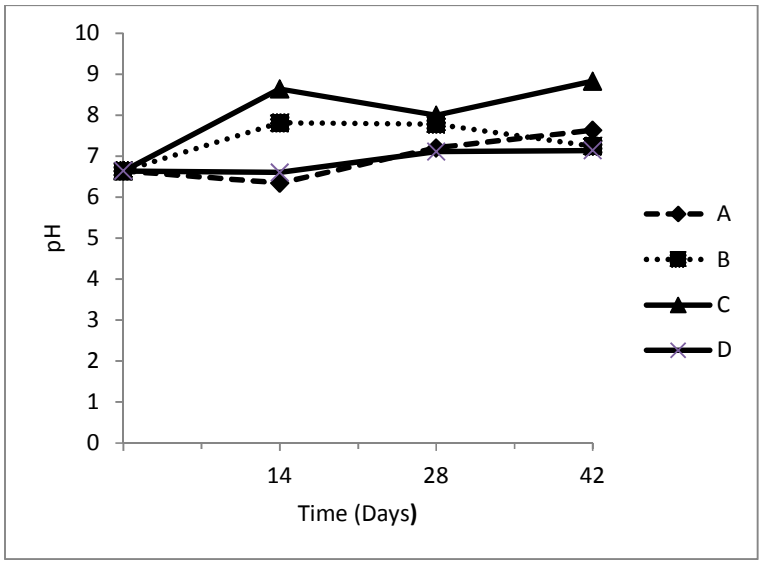

Fig. 4. Changes in soil pH

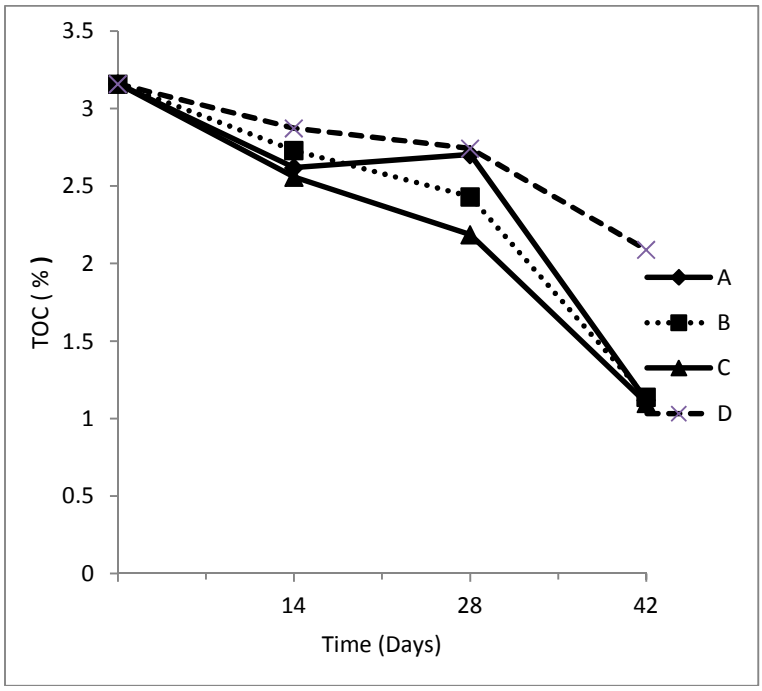

Fig. 5. Soil total organic carbon

A similar trend was observed with the release of phosphorus. In this case, however, the direct fertilizer application maintained the highest level of phosphorus (phosphate) between days 14 and 28. After a 42-day period it had the least value compared to setup A and B (Fig. 7). A one-sample T-test showed that there was a significant difference in the mean phosphate in experimental setups A, B, and C when compared to the control 'D' ($p<0.05$, $t = 6.503$. PV = 0.023). This difference can be tied to the solubility of phosphorus [21] which eventually affected the release rate and time. Hence the solubility of phosphorus should be taken into cognizance during the preparation of SRFs.

The effect of nutrient concentration on the dynamics of hydrocarbon utilizing bacterial population was investigated in this work. The available nutrient was a function of the nutrient release rate. Statistical analysis showed a strong positive and significant relationship between the rate of nutrient release and bacterial counts (total heterotrophic bacteria and hydrocarbon utilizing bacteria). This was analyzed at a 95% confidence level ($P < 0.05$ for all correlations). This has established that the amount of nutrient released or available has a significant effect on the growth rate of hydrocarbon utilizing bacterial population. The initial cell count of hydrocarbon utilizing bacteria and total heterotrophic bacteria in the contaminated soil was $3.2\pm0.5 \times 10^5$ cfu/g and $3.10\pm0.8 \times 10^6$ cfu/g, respectively. The unamended soil had the least bacterial count for both the total heterotrophic and hydrocarbon utilizing bacteria. This has shown that the addition of nutrients supported proliferation of bacteria following the metabolism of the crude oil contaminants.

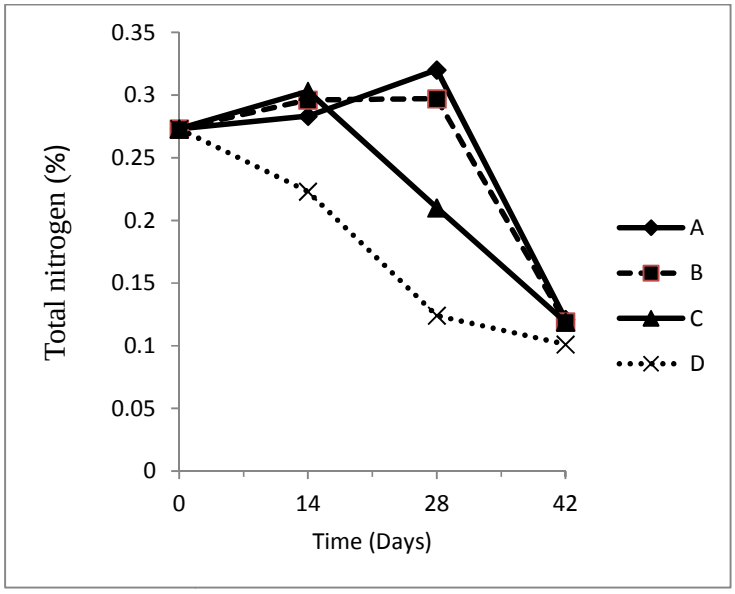

Fig. 6. Rate of nitrogen release - total nitrogen (%)

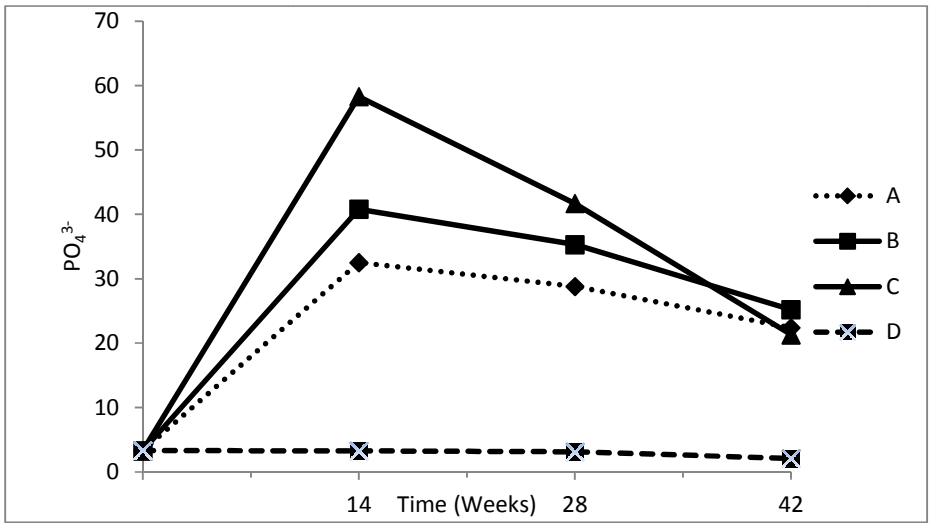

Fig. 7. Rate of phosphorus (phosphate) release in soil media

The increases in biomass of hydrocarbon utilizing bacterial group for experimental setups A,B,C and D were 12.2±0.6 x10^5, 11.7±0.5 x10^5, 18.8±0.4 x10^5, and 5.3±0.2 x10^5 cfu/g, respectively, after a 42-day period of study (Fig. 9). Same trend was observed for the total heterotrophic bacterial count. After 42 days of study, 14.6±0.7 x10^6, 15.4±0.8 x10^6, 17.7±0.6 x10^6, and 4.8±0.2 x10^6 cfu/g (total heterotrophic bacterial counts) was recorded for experimental setups A, B, C and D, respectively (Fig. 8).

The differences in the cell counts observed are attributable to the differences in the concentrations of nutrients in the experimental media. This is in conformity with the fact that nitrogen and phosphorus are limiting nutrients.

It has been observed from this study that the rate of hydrocarbon removal has a strong positive relationship with nutrient availability and microbial growth rate and population. It is desired for SRF to bring about more efficient rates of bioremediation or better still, rates equivalent to that of direct fertilizer application. However, it was discovered from this research that the direct fertilizer application brought about faster rates of hydrocarbon removal when compared to the granular and capsular SRF, where the control setup maintained the least rate of removal.

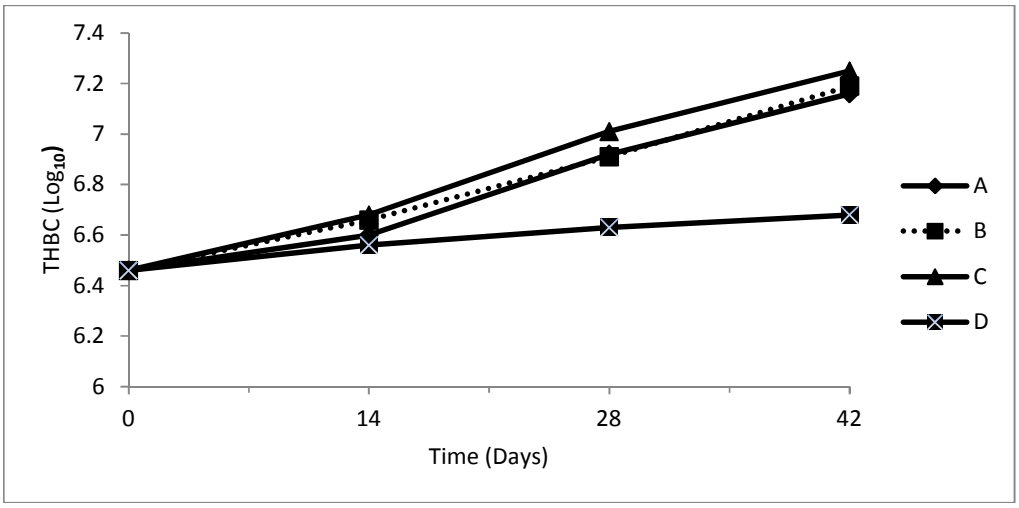

Fig. 8. Total Heterotrophic Bacterial Count (THBC)

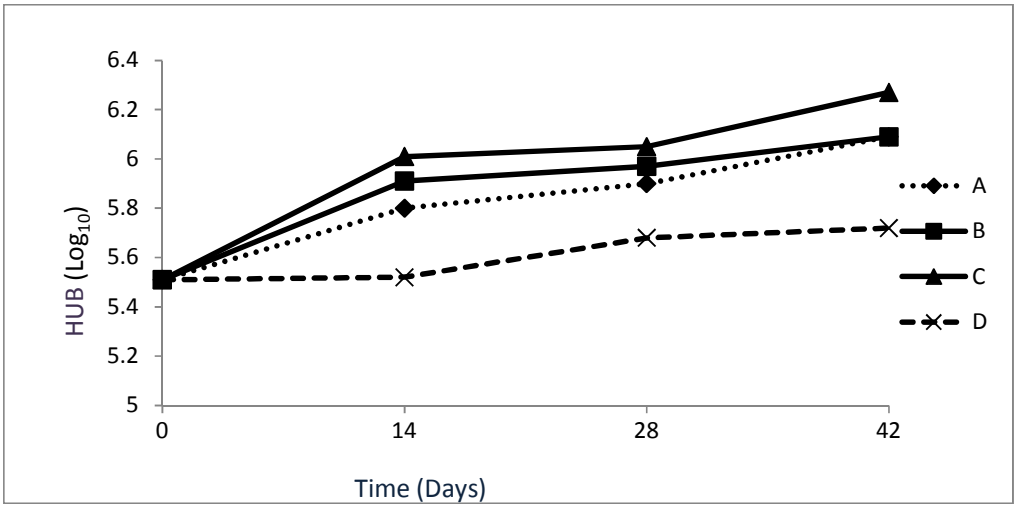

Fig. 9. Changes in hydrocarbon utilizing bacterial count

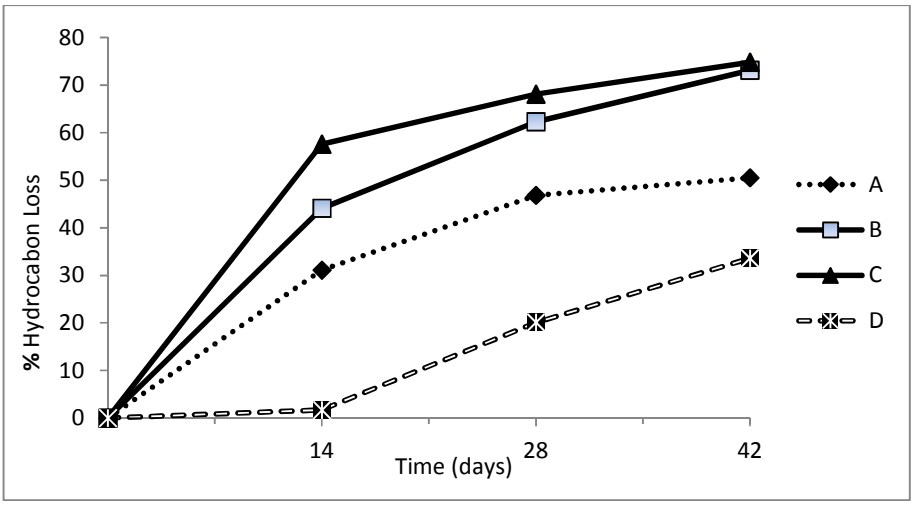

Fig. 10. Percentage hydrocarbon loss

The reason for the direct fertilizer performing better than the slow release formulations is attributable to an "early stage nutrient deficiency phenomenon" observed in this work. It follows that at the early state the nutrients are released at rates that do not support much microbial growth necessary for high microbial proliferation, compared to the direct fertilizer application. Hence the direct fertilizer gave rise to an early microbial activity that caused a reduction in the concentration of the hydrocarbon. The late starters (granular and capsular SRF) experienced more rapid rates of removal after 28- and 42-day periods of investigation. This was partly responsible for the statistical difference (t = -5.323, PV = 0.034) observed in the mean total petroleum hydrocarbon (TPH). At day 14, much difference was observed in the residual hydrocarbon between the direct fertilizer and that of the granular SRF. The percentage residual hydrocarbons for setups A, B, C and D were 68.9, 55.9, 42.4, and 98.3% respectively, at day 14 (Fig. 10). It follows that at day 14, the difference in the percentage residual TPH between the direct fertilizer and the granular SRF was 13.5%. After 42 days of investigation, 49.5, 26.9, 25.17 and 66.4% residual total petroleum hydrocarbons were observed in treatment options A, B, C and D, respectively. It also follows that the difference in the percentage residual TPH between the direct fertilizer and the granular SRF was 1.73%. This observation can be linked to the fact that the rate of bioremediation is dependent on the amount of available nutrient. This is in agreement with the findings of Okpokwasili and Oton [20]. Their findings on the remediation efficiencies of NPK fertilizer in a locally fabricated bioreactor system and a shake-flask system, using samples of oily sludge is in conformity with the results presented in this research. The rate of crude-oil removal or degradation is therefore a function of nutrient concentration and other physicochemical conditions [20].

Gas chromatographic studies showed the peak heights and base widths of the constituent petroleum hydrocarbons. The total petroleum hydrocarbon of the unamended soil was seen to be 3113.38 ppm. The total petroleum hydrocarbons of experimental setups A, B, C and D were 1541.92, 838.6, 783.65, and 2066.06, respectively after a period of 42 days. This represented a 50.5%, 73.1%, 74.83%, 74.83% and 33.6% loss of hydrocarbon observed for treatment options A, B, C and D, respectively (Fig. 10).

Where the rate of hydrocarbon removal was influenced by nutrient concentration in treatments A, B and C, a 33.6% loss of hydrocarbons (day 42) was noticed in the control setup, without fertilizer. This is probably due to natural attenuation taking place in the unfertilized soil. It has been well documented that the fate of crude oil pollutants is dependent on physical, chemical and biological factors. Chikere et al. [22] observed a hydrocarbon loss in the heat-killed control signifying that abiotic factors could as well contribute to hydrocarbon attenuation in the environment.

An NPK fertilizer (20:10:10) was used in this study. Other researchers have also this

formulation of fertilizer in bioremediation studies [23,24]. NPK fertilizer is readily soluble in water and hence was the fertilizer of choice in this study involving nutrient release potentials. The bioremediation efficiency of NPK fertilizer has been shown. These remediation potentials of NPK fertilizer is attributed to its chemical composition compared to other sources of nutrient. In a tropical crude oil polluted soil undergoing bioremediation, Chikere et al. [23] observed that the use of NPK fertilizer, urea fertilizer and poultry droppings effectively stimulated bacteria into utilization of crude oil. However, NPK fertilizer [20:10:10) was a more effective option as it reduced TPH from 3666.0 mg/kg to 89.68 mg/kg for 57 days whereas, urea fertilizer option reduced TPH from 3666 mg/kg to 162 mg/kg for 57 days while the poultry droppings option was reduced TPH from 3666.0 mg/kg of soil to 135.01 mg/kg of soil. Also, Chikere et al. [24] carried out a similar study on the phylogenetic diversity of dominant bacterial communities during bioremediation of crude oil-polluted soil and came up with the conclusion that NPK fertilizer seems to be the best nutrient for the biostimulation of indigenous bacterial community in crude oil-polluted soil and concomitant degradation of hydrocarbons. These findings [23,24] have justified the choice of NPK fertilizer in the production of SRFs for the bioremediation of petroleum hydrocarbons.

Different slow-release formulations have been reported to include sulphur coated SRF, paraffin supported SRF, Osmocote etc. Xu et al. [25] found out that an addition of 0.8% of SRF, Osmocote^{TM} consisting of 18, 4.8, and 8.3% N-P-K (w/w) to oil polluted sediments was sufficient to maximize metabolic activity of the microbial biomass and the biodegradation of straight-chain alkanes (C10-C33); and application of 1.5% rate resulted in optimal biodegradation of recalcitrant branched-chain alkanes, such as pristane and phytane. But no publication has been made with respect to the use of biopolymers (agar agar). Hence this work is innovative.

4. CONCLUSION

SRFs have promising potentials in bioremediation. However, the “early stage nutrient deficiency phenomenon” has been proposed in this work. The noteworthy results from this investigation have confirmed the theoretical information base that had already been established by previous scientific studies. The addition of nutrients in the form of fertilizer (directly or in slow-release formulations) to indigenous microorganisms has proved to be effective in enhancing biodegradation and environmentally safe at the same time.

These novel SRFs are recommended for their applicability in the bioremediation of water and sediments. They are best suited for use in the Niger Delta wet lands and Marine environment. The capsular SRF is strongly recommended for treatment options that will not involve tilling.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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