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Crude oil remediation using plant extract of Guava leaf (*Psidium guajava*) in soil environment

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A B S T R A C T

This study was carried out to evaluate the potential of fermented Guava Leaf (*Psidium guajava*) fluid extract in bioremediation of crude oil polluted soil. For this purpose, 6reactors were set up and labelled R_1 to R_6, with R_6 as the control. 2kg of soil sample was polluted with 150ml of crude oil and the remediant was added to each reactor. The volume of remediant were varied at increasing order of 20ml to 100ml from R_1 to R_5. R_6 contains no remediant. Samples were taken and analyzed at 4days interval for total petroleum hydrocarbon (TPH) and nutrient content. The study lasted for 20days. At the end of the study period, there was 75.65% and 91.95% decrease in the concentration of TPH in R_1 and R_5, with the lowest and the highest respectively, compared to 18.44% decrease in R_6. Similarly, there was a gradual decrease in the concentrations of total organic carbon, nitrogen, phosphorus, and potassium in all samples. The transient growth of the bacteria and fungi was monitored with respect to the degradation rate of the substrate in each batch bioreactor and the result obtained demonstrates the following phases, lag, progressive or exponential, stationery and death or decline. This reveals the theory of Monod's model indicating decline in a viable nutrient and substrate utilization in each bioreactor. This research has demonstrated that *psidium guajava* possess the characteristics of good biostimulant enhancing bioremediation of polluted soil environment.

Capsule Summary: Crude oil polluted soil remediation was performed using plant extract of Guava leaf (*Psidium guajava*) in soil environment and the total petroleum hydrocarbon was decreased significantly, which is one of promising treatment to manage the soil contaminated with hydrocarbons.

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INTRODUCTION

The dissertation addresses the role of remediant in treating contaminants in soil environment. The accident discharge of petroleum hydrocarbon into the soil influences the characteristics and the properties of the soil component negatively. However different techniques have been applied to enhance treatment of a contaminated site either biologically or chemically. The application of chemical approach has been used and the remediation or restoration of the polluted site restored to its integrity, but it was

discovered that adverse effect was recorded after some years. This reviewed that the chemical technique profound solution of restoration, but at the latter time the chemical induced the characteristics of the soil properties (Abslusalam et al., 2011; Danjuma et al., 2012; Karl and Krishna, 2003; Vangronsveld and Herzig, 2009).

The bio-remediant contain constituents that enhance bioremediation and those constituents act as nutrient to the organisms present in the soil environment. The nutrient catalyzed the reaction and facilitate the degradation of petroleum hydrocarbon, which leads to the formation of various metabolic pathway compounds that are further biodegraded and the process result to end products that are environmentally friendly. The bioremediant in most case contains elements such as nitrogen, phosphorous, potassium, high level of organic matter and other essential nutrients that can help catalyze the enzyme-substrate complex degradation to yield free enzyme and product.

The factors that induced the performance of bioremediation or degradation of petroleum hydrocarbon in a soil environment was considered in this investigation, which includes: pH, temperature, electrical conductivity, total hardness, chemical oxygen demand, etc. but, indeed, the roles played by these parameters has been monitored by various research groups and the mitigation to achieve a positive biostimulant and bio-argumentation (Ayotamuno et al., 2006; Dados et al., 2015; Gavrilescu, 2006; Ofoegbu, 2015).

However, this research was able to address the usefulness of the bioremediant used in this investigation and the meaningful contribution to enhance restoration of a polluted or contaminated soil. Several investigations on the performance of bioremediant to enhance restoration of contaminated soil environment have been reported in literatures. The constraints to achieve perfect remediation using plant extracts have been reported and the characteristics of various extracts have been demonstrated in different research conducted. Those research reveals positive solution at the end of the treatment illustrating the significance of the aim and objectives of their studies in terms of contaminants treatment. However, there is need to consider other plant extracts that has not been investigated as to ascertain the degree of efficiency in terms of performance to enhance bioremediation of contaminated soil. (Margesin et al., 2000; vidali, 2000).

This research was able to address the significance of the plant extracts used for the purpose of enhancing effectiveness in bioremediation in a polluted soil environment. The engineering principles were applied and the set-up experiment was monitored in accordance to the design concepts. (Shallu and Hardik, 2001; Bamforth and Singleton 2005).

The search for a suitable and more economical method to enhance cleanup and the process has been of worrisome task for the petroleum industries and the government. To this effect oxygen diffusion in petroleum hydrocarbon remediation using fermented Guava leaf (Psidumgujava) fluid in soil environment was carried out. The aim of this research is to evaluate petroleum hydrocarbon remediation with the aid of Guava leaf (*Psidium guajava*) as a bio-remediant. (Delate et al., 2012; Chukwuma, 2018). This study is of great importance to the oil producing regions which are most times faced with problems associated with oil spills, using Ogoni in Rivers State Nigeria as a case study, this research can come up with the best approach for the Ogoni land remediation.

However, this research will address the significance of Guava Leaf as a useful bioremediant to enhance bioremediation of a polluted soil environment. The plant extracts are easily prepared and the process is not harmful to mankind in terms of health challenges and the missio and vision of the purpose will be achieved with less stress. Therefore, it is recommended that this bioremediant should be used in treatment of contaminated soil with petroleum hydrocarbon.

MATERIAL AND METHODS

Materials

The materials used in this study includes: Reactor of plastic containers, soil–loamy soil, crude oil and Guava leaf (*Psidium guajava*).

Experimental set up

The various reactors used for this investigation were purchased at mile 3 market in Diobu Port Harcourt and then transported to the Department of Chemical/Petrochemical Engineering in faculty of Engineering, Rivers state University Port Harcourt. Crude oil sample was obtained from Chemical/Petrochemical Engineering laboratory of same university. Soil sample (loamy soil) was obtained from Afam community in Oyigbo Local Government Area of Rivers state and then transported to the Department of Chemical/Petrochemical Engineering. The remediant raw material was collected from Agricultural Development Program (ADP) located in Obio/Akpor Local Government Area of Rivers state and then transported to the same departmental laboratory for analysis.

Experimental procedure

A 2 kg of loamy soil was added to each reactor and a total of five (5) with additional one as control was used. 150ml of the crude oil sample was introduced into each reactor set-up and stirred to achieve uniformity in concentration. This process was conducted in each bioreactor set-up for this investigation. The mass of the remediant added are 20g for R1, 40 g for R2, 60 g for R3, 80 g for R4, 100 g for R5, with R6 as control. The remediant used was obtained from Guava leaf (*Psidium guajava*) and the powder form of the remediant was prepared ready for the investigation. The Guava leaf (*Psidium guajava*) was added into the various experimental set-up and then stirred to obtain uniformity in each reactor.

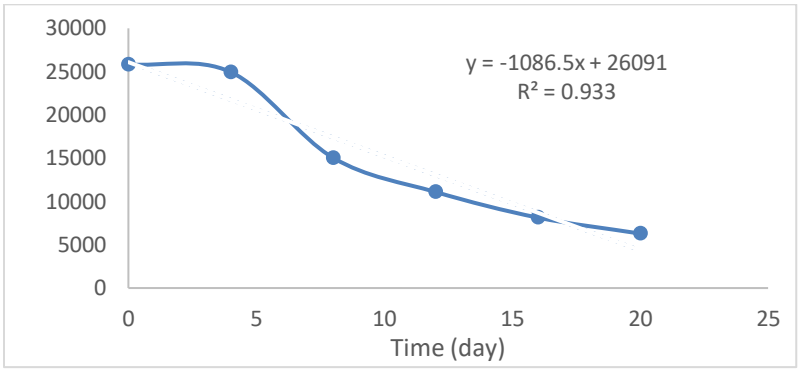

Fig. 1: TPH concentration versus time for R_1(20 g). A-axis = R1 TPH

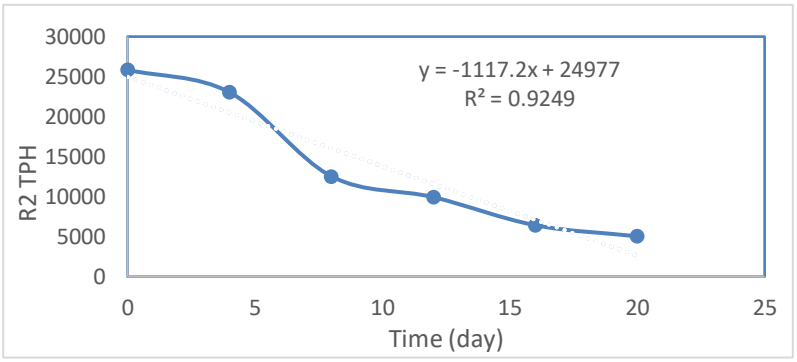

Fig. 2: TPH concentration versus time for R2 (40 g)

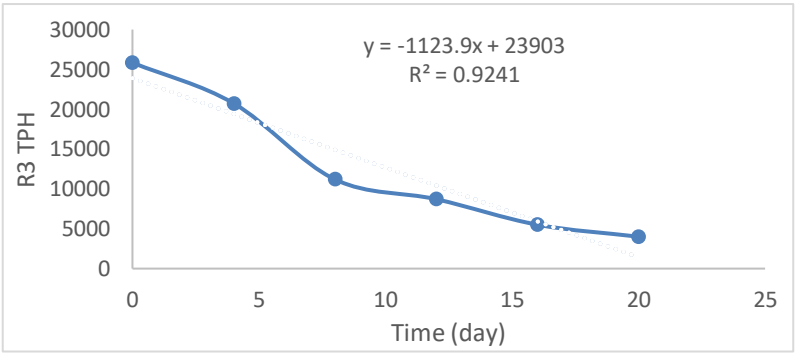

Fig. 3: TPH concentration versus time for R3 (60 g)

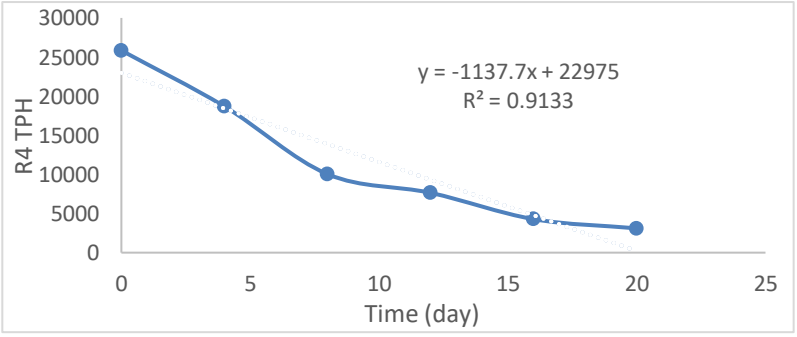

Fig. 4: TPH Concentration versus time for R4 (80 g)

Each of the reactors was allowed to interact with the microbes (bacteria and fungi) present in the crude oil which was isolated and identified. Analysis was conducted after four (4) days interval on the physicochemical properties of the polluted soil mixture, Total Petroleum Hydrocarbon (TPH) and microbial concentration.

Table 1: Effect of time on TPH degradation using Guava leaf (*Psidium guajava*) plant extract as a remediant

	Total Petroleum Hydrocarbon Concentration					
Time (day)	R_1 (20) (ppm)	R_2 (40) (ppm)	R_3 (60) (ppm)	R_4 (80) (ppm)	R_5 (100) (ppm)	R_6 (control) (ppm)
0	25838.99	25838.99	25838.99	25838.99	25838.99	25838.99
4	24946.29	23041.06	20732.79	18686.30	17446.87	25838.99
8	15045.85	12538.01	11192.53	10032.19	8952.72	2458.26
12	11098.43	9936.77	8710.65	7644.91	6522.67	23470.37
16	8136.02	6427.50	5507.20	4281.53	3804.33	22181.74
20	6292.13	5046.47	4001.66	3104.92	2081.16	21073.50

Table 2: Specific rate of degradation and reciprocal of the specific rate and substrate for R1 (20 g)

Time (day)	Substrate (S) R_1 (ppm)	Rate of Degradation V (ppm/day)	$1/SR_1$ $(ppm)^{-1}$	$1/VR_1$ $(ppm)^{-1}$
0	25838.99	—	3.87×10^{-5}	—
4	24946.29	892.7	$4.01\times10^{\wedge-5}$	1.21×10^{-3}
8	15045.85	9900.44	6.64×10^{-5}	1.01×10^{-4}
12	11098.43	3947.42	9.01×10^{-5}	2.53×10^{-4}
16	8136.02	2962.41	1.23×10^{-4}	3.38×10^{-4}
20	6292.13	1843.89	1.59×10^{-4}	5.42×10^{-4}

Table 3: Specific rate of degradation and reciprocal of the specific rate and substrate for R2 (40 g)

Time(day)	Substrate (S) R_2 (ppm)	Rate of degradation V (ppm/day)	$1/SR_2$ $(ppm)^{-1}$	$1/VR_2$
0	25383.99	—	3.87×10^{-5}	—
4	23041.06	2797.93	4.34×10^{-5}	3.57×10^{-4}
8	12538.01	1053.05	7.98×10^{-5}	9.52×10^{-5}
12	9936.77	2601.24	1.00×10^{-4}	3.84×10^{-4}
16	6427.50	3509.27	1.56×10^{-4}	2.85×10^{-4}
20	5046.47	1381.03	1.98×10^{-4}	7.24×10^{-4}

Table 4: Specific rate of degradation and reciprocal of the specific rate and substrate for R_3 (60 g)

Time (day)	Substrate (S) R_3 (ppm)	Rate of degradation V (ppm/day)	$1/SR_3$ $(ppm)^{-1}$	$1/VR_3$
0	25383.99	—	3.87×10^{-5}	—
4	20732.79	5106.20	4.82×10^{-5}	1.96×10^{-4}
8	11192.53	9540.26	8.93×10^{-5}	1.05×10^{-4}
12	8710.65	2481.88	1.15×10^{-4}	4.03×10^{-4}
16	5507.20	3203.45	1.82×10^{-4}	3.12×10^{-4}
20	4001.66	1505.54	2.50×10^{-4}	6.64×10^{-4}

Table 5: Specific rate of degradation and reciprocal of the specific rate and substrate for R4 (80 g)

Time(day)	Substrate (S) R_4 (ppm)	Rate of degradation V (ppm/day)	$1/SR_4$	$1/VR_4$
0	25383.99	—	3.87×10^{-5}	—
4	18686.30	7152.69	5.35×10^{-5}	1.40×10^{-4}
8	10032.19	8654.11	1.00×10^{-4}	1.16×10^{-4}
12	7644.91	2387.28	1.31×10^{-4}	4.20×10^{-4}
16	4281.53	3363.38	2.34×10^{-4}	3.00×10^{-4}
20	3104.92	1176.61	3.22×10^{-4}	8.50×10^{-4}

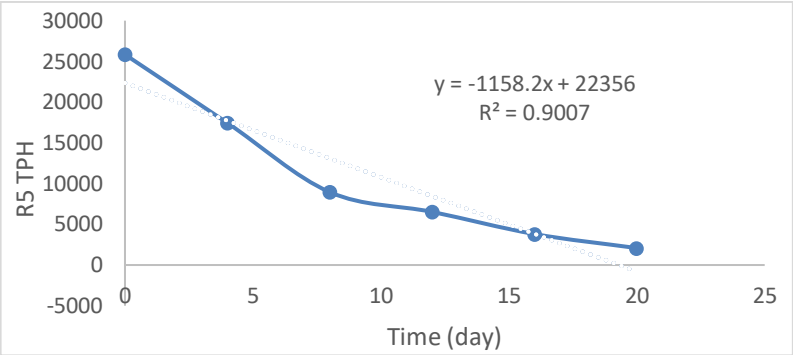

Fig. 5: TPH concentration versus time for R5 (100 g)

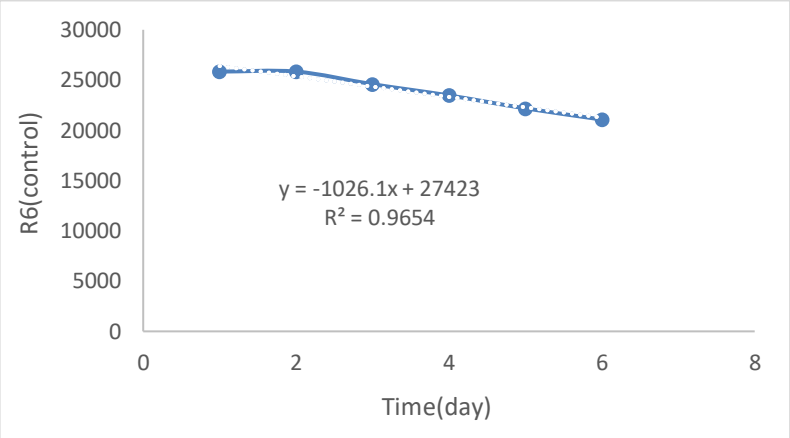

Fig. 6: TPH concentration versus time for R6 (control)

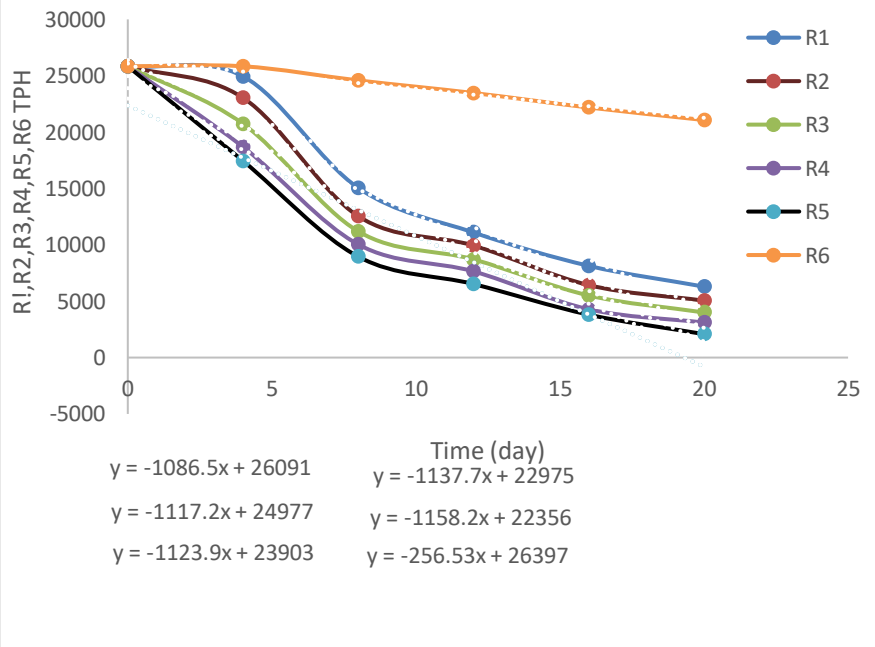

Fig. 7: Comparison of TPH concentration versus time for all reactors

The physicochemical parameters examined are pH, temperature, electrical conductivity, nitrogen, phosphorus and potassium in each and the trend of the behaviour recorded. The total petroleum hydrocarbon was analyzed using the gas chromatography (GC) analyzer and the individual concentration of the TPH was obtained but the total petroleum hydrocarbon concentration was used in the plotting of graph to examine the effect time in TPH degradation in each reactor as presented in results and discussion.

Table 6: Specific rate of degradation and reciprocal of the specific rate and substrate for R5 (100 g)

Time(day)	Substrate(S) R_5(ppm)	Rate of degradation V(ppm/day)	$1/SR_5$	$1/VR_5$
0	25383.99	—	3.87×10^{-5}	—
4	17446.87	8392.12	5.73×10^{-5}	1.19×10^{-4}
8	8952.72	8494.15	1.12×10^{-4}	1.18×10^{-4}
12	6522.67	2430.05	1.53×10^{-4}	4.12×10^{-4}
16	3804.33	2718.34	2.63×10^{-4}	3.68×10^{-4}
20	2081.16	1723.17	4.81×10^{-4}	5.80×10^{-4}

The microbial analysis was conducted by the microbiology department of the same university, as part of the samples after collection were submitted for isolation, identification and characterization of the organisms as a bacteria and fungi. The analysis was carried out for a period of 20days and the physicochemical properties, microbial analysis and TPH examined in each of the reactor.

Microbial analysis

Bacteria and fungi organisms were isolated and identified and then cultured for the purpose of inoculation into the different reactors set up for the remediation of crude oil. The organism was cultured by introducing 500ml of sterile nutrient broth medium and the obtained solution was incubated at temperature of 37^0C for a period of 24hours.

Inoculation of microbes into reactors

The cultured microbes were inoculated with the aim of microbes feeding on the Total Petroleum Hydrocarbon. The samples were collected after 4days interaction and the collected samples were subjected to microbial analysis using Brennan and Withgott (2005) methods.

Mathematical model of Micheal's Menten and Monod application

The model of Monod`s equation and Michael`s Menten equation was adopted in this research work (Eq. 1). However, the two models are not new, thus.

$$V = \frac{V_{max}\, S}{K_S + S} \quad (1)$$

Where, V is the specific rate of TPH degradation (ppm/day), V_{max} is the maximum specific rate TPH degradation $(ppm/day)^{-1}$, S is the TPH concentration (ppm) and K_s is the dissociation or equilibrium constant.

Equation (1) express the Michael`s Menten equation whereas the Monod`s model is expressed as demonstrated Eq. 2.

$$\mu = \frac{\mu_{max}}{k_m + s} \quad (2)$$

Where, μ is the specific growth rate of microbes (cfu/ml/day), μ_{max} is the maximum specific growth rate of microbes (cfu/ml/day), K_m is the dissociation or equilibrium constant and S is the substrate concentration (ppm)

LineWeaver Burk plot

The equation 3-4 can be translated to the Line Weaver Burk Plot. In terms of Michael`s Menten equation or model. But in terms of the Monod`s model, is shown in Eq. 4.

$$\frac{1}{V} = \frac{K_s}{V_{max}} S + \frac{1}{V_{max}} \quad (3)$$

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} S + \frac{1}{\mu_{max}} \quad (4)$$

RESULTS AND DISCUSSION

Results of total petroleum hydrocarbon concentration

Table 1 predicts the TPH degradation upon the action of the microbes (bacteria and fungi) inoculated in each bioreactor in the presence of various mass of the remediant (*psidium guajava*). Decrease in TPH concentration was experienced with increase in time as well as increase in the concentration of the remediant.

Specific rate and degradation of TPH

The specific rate of the TPH degradation and the reciprocal of the substrate as well as the specific rate of TPH was evaluated as presented in Table 2 to 6 for reactors of $R_1, R_2, R_3, R_4\ R_5$.

Results of Total Petroleum Hydrocarbon Degradation

The result of the total Petroleum Hydrocarbon degradation for each bioreactor is presented in Figure 1 to 6. The results reveal decrease in TPH concentration with increase in contact time and the detail description is demonstrated after each figure as shown in this research. Figure 1 predicts the degradation of TPH in reactor R1(20g) with increase in time. The line equation of y = -1086.5x+26091 was obtained with R^2 value of 0.983 revealing the reliability value of 98.3%. Increase time influence the TPH value with decrease in concentration.

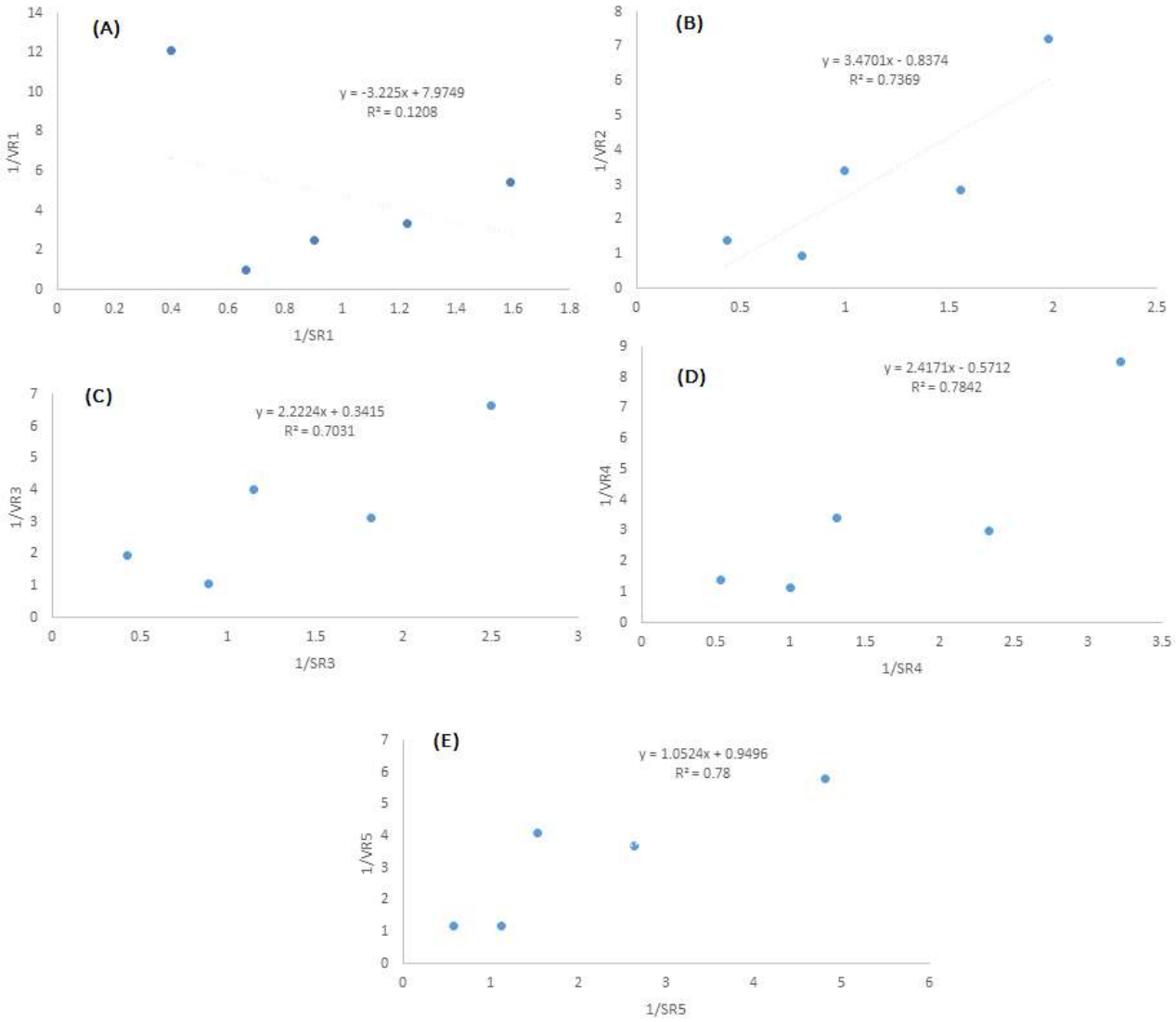

Fig. 8: (A) Lineweaver Burk plot for R1(10^{-4}), (B) Lineweaver Burk plot for R2(10^{-4}), (C) Line Weaver Burk plot for R3(10^{-4}), (D) Lineweaver Burk plot for R4(10^{-4}) and (E) Lineweaver Burk plot for R5(10^{-4})

Figure 2 predicts the TPH degradation in reactor R2(40g) with increase in time. Decrease in TPH concentration was observed with increase in time and the equation of the line obtained reveals y = -1117.2x + 24977 with R^2 value of 0.9249 revealing the reliability of 92.49%.

Figure 3 predicts the effect of remediant on TPH degradation in R3(60) with increase in time. The linear equation is given as y = -1123 + 23093 and R^2 value 0.9241 showing the reliability value of 92.41%. Increase in period of exposure influence the TPH degradation in reactor R3 of 60g of the remediant.

Figure 4 reveals the effect of remediant on the TPH in R4 of 80 g of remediant with increase in time. Decrease in TPH was observed with increase in time and the linear equation obtained is y = -1137x + 22975 with R^2 value of 0.9133, revealing 91.33% reliability.

Figure 5 reveals the relationship between TPH of R5 of 100g remediant with increase in time. Decrease in TPH value was experienced with increase in time' The equation of the line is given as y = -1158x + 22356 with R^2 value of 0.9007, which shows 90.07 reliability.

Figure 6 reveals the TPH behaviour with increase in time for control sample. The equation was given as y = -1026.1x + 27423 with R^2 value 0.9654 which is 96.54% reliability.

Comparison of TPH degradation in various reactor with control.

The TPH concentration was monitored with variation in five, for each reactor set-up and the obtained result was compared with the control and the obtained result reveals variation as shown in figure 7.

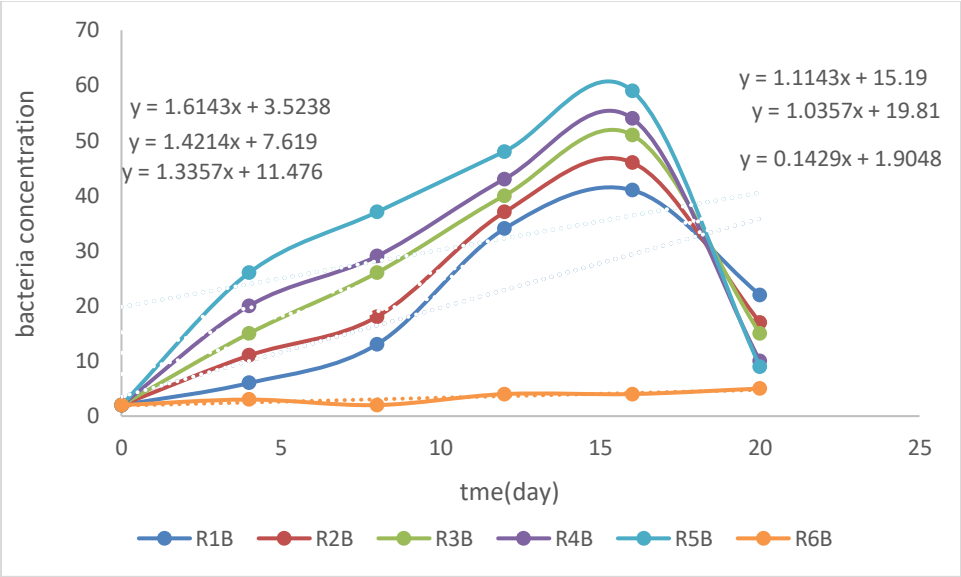

Fig. 9: Bacteria concentration versus time for all reactors

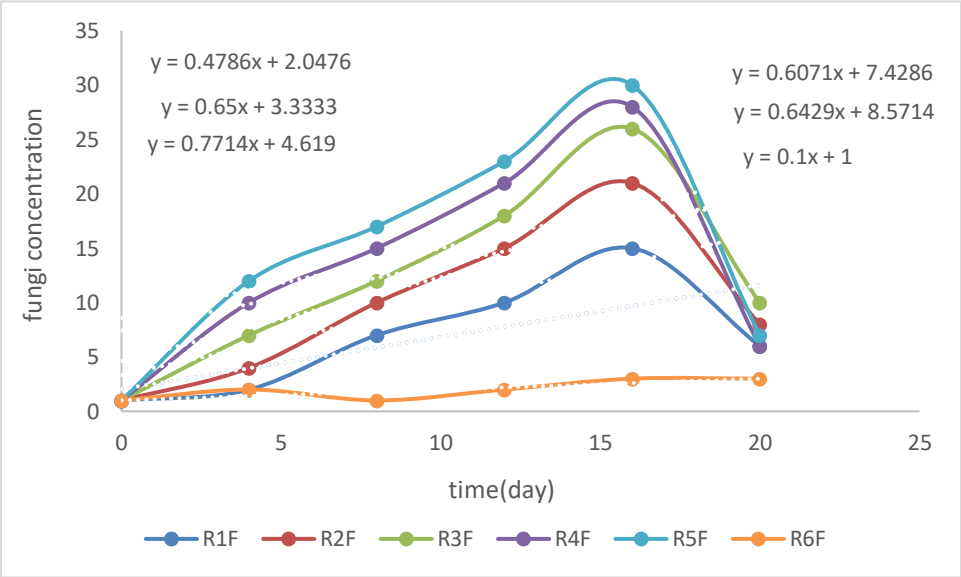

Fig. 10: Fungi concentration versus time for all reactors

Figure 7 demonstrate the comparison of the TPH values of R1 (20), R2(40), R3(60), R4(80), R5(100), R6(control) with increase in time. Decrease in TPH R1, R2, R3, R4, R5, and control was experienced with increase in time. The linear equation values are obtained, that is yR1 = -1086.5x + 26091, yR2 = -1117.2x + 24977, yR3 = -1123.9x + 23903, yR4 = -1137.7x + 22975, yR5 = -11158.2x + 22356, yR6 (control) = -256.53x + 26397

Result of Line Weaver Burk plot and evaluation of functional parameters

Figure 8 demonstrates the relationship between the 1/v and 1/s of each bioreactor set-up for the determination of the functional parameters of the maximum specific rate of substrate degradation and dissociation or equilibrium constant rate of TPH.

Figure 8A predicts the Lineweaver Burk Plot line of 1/VR1 with 1/SR1. The equation is of the line given as y = -3.225x + 7.9749 revealing the slope of -3.225 and of 7.97. Therefore, the Michael`s Menten equation of $V = \frac{V_{max}S}{K_s+S} = \frac{0.125S}{2.48+S}$, thus $1/V_{max}$ = intercept and slope = K_s/V_{max}, where, V_{max} =0.125 $(ppm/day)^{-1}$, K_s = 2.48 $(ppm)^{-1}$

Figure 8B predicts the Lineweaver Burk Plot line of 1/VR2 with 1/SR2. The equation is of the line given as y = 3.4701x - 0.8374 revealing the slope of 3.4701 and intercept of 0.8374. Therefore, the Michael`s Menten equation of $V = \frac{V_{max}S}{K_s+S} = \frac{0.125S}{2.48+S}$, thus $1/V_{max}$ = intercept and slope = K_s/V_{max}, where, V_{max} =1.19 $(ppm/day)^{-1}$, K_s = 4.145 $(ppm)^{-1}$

Figure 8C represents the Lineweaver Burk Plot line of 1/VR3 with 1/SR3. The equation was obtained as y = 2.2224x + 0.3415 revealing the slope of 2.2224 and intercept of 0.3415.Therefore the Michael`s Menten equation of $V = \underline{V_{max}S}/K_s+S = 0.703S/6.5+S$, thus $1/V_{max}$ = intercept and slope = K_s/V_{max}, where V_{max} =2.39 (ppm/day)$^{-1}$, K_s = 6.5 (ppm)$^{-1}$

Figure 8D shows the Lineweaver Burk Plot line of 1/VR4 with 1/SR4. The equation was obtained as y = 2.4171x - 0.5712 revealing the slope of 2.417 and intercept of 0.5712. Therefore, the Michael`s Menten equation of $V = \underline{V_{max}S}/K_S+S = 0.78S/4.2+S$, thus $1/V_{max}$ = intercept and slope = K_S/V_{max}, where V_{max} =1.75(ppm/day)$^{-1}$, K_S = 4.2 (ppm)$^{-1}$

Figure 8E predicts the Lineweaver Burk Plot line of 1/VR5 with 1/SR5. The equation was obtained as y = 1.0524x + 0.9496 revealing the slope of 1.0524 and intercept of 0.9496.Therefore the Michael`s Menten equation of $V = \underline{V_{max}S}/K_s+S = 0.78S/1.1+S$, thus $1/V_{max}$ = intercept and slope = K_S/V_{max}, where V_{max} =1.053 (ppm/day)$^{-1}$, K_S = 1.1 (ppm)$^{-1}$.

Result of transient growth of bacteria and fungi

The microbial growth rate behavior of the bacteria and fungi isolated and identified from each bioreactor set-up are presented in figure 9 and 10. Figure 9 predicts the bacteria growth characteristics in each reactor with increase in time. No log phase was experienced but acceleration, progressive, stationery and decline phases are experienced with increase in time and a slight increase was in control reactor. However, increase in microbial growth resulted toincrease in substrate utilization with increase in time. The equation of each curve is expressed as Y(R1)= 1.6143x + 3.5238, Y(R2)= 1.4214x + 7.619, Y(R3)= 1.3357x + 11.476, Y(R4)= 1.1143x + 15.19, Y(R5)=1.0357x + 19.81 and Y(R6)= 0.1429x + 1.9048. The variation on bacteria concentration was caused by variation in time as well as the substrate utilization and environmental factors.

Figure 10 predicts the characteristics of fungi in substrate utilization (Total Petroleum Hydrocarbon) in different bioreactors with increase in time. The result obtained revealed no log phase but acceleration, progressive, stationery and decline phase were observed as the substrate declined in each reactor. However, the equation of the behaviour of the curve is given as y(R1) = 0.4786x + 2.0476, y(R2) = 0.65x + 3.3333, y(R3) = 0.7714x + 4.619, y(R4) = 0.6071x + 7.4286, y(R5) = 0.6429x + 8.5714 and y(R6) = 0.1x + 1. The variation in in the fungi concentration was caused by variation in time as well as state of substrate utilization.

Samples were analyzed for several physicochemical parameters at 4-day intervals during the study period. The baseline data showed that the pH of the crude oil and all reactors were slightly acidic. However, the pH of R1-R5 increased slightly while there was a slight decrease in the pH of the control during study period.

The ambient temperature was 29.1±4.0^{o}C throughout the study period. It was observed that the conductivity of the reactors containing the remediant is to a large extent greater than that of the control. This is attributed to the fermented Guava leaf (Psidium guajava) extract. Similarly, the concentrations of total nitrogen, available phosphorus, and available potassium also recorded a noticeable decrease.

The application of gas chromatography allowed detection of carbon fractions (C8–C33) in the crude oil sample. The chromatogram obtained at day 20 of the study shows that more than half of the carbon fractions of the crude oil in R1-R5 samples were attenuated. However, for the control (R6), very little changes were observed in the magnitude of the peaks obtained in the gas chromatograms during the study period. Generally, the decrease in TPH concentration in the samples by the end of the study are: R_1 = 75.65% R_2 = 80.47% R_3 = 84.51% R_4 = 87.98% R_5 = 91.95% and control=18.44%. This result shows that the TPH concentration of soil in the reactor containing more volume of the remediant came lowest.

CONCLUSIONS

This study demonstrated that Guava leaf (*Psidium guajava*) extract is a good organic substrate containing nitrogen, phosphorus and potassium, which have great potentials for enhanced bioremediation of crude oil polluted soil. Guava leaf (*Psidium guajava*) extract when applied in appropriate concentration to crude oil polluted soil would enhance the bioremediation of such sites by increasing microbial activities. Interestingly, the decrease in TPH and peak attenuation obtained were just in 20 days of monitoring, which suggests that the use of Guava leaf (*Psidium guajava*) extract could be a cost-efficient and process-efficient bioremediation option for hydrocarbon-contaminated soils. This research was able to address the following contribution to the field of Engineering and Science as demonstrated below:

i. The remediant (*Psidium guajava*) used possess the capacity of degrading petroleum hydrocarbon in soil environment
ii. The available nutrient in the remediant (*Psidium guajava*) was able to catalyze the biodegradation of the petroleum hydrocarbon present in the bioreactors.
iii. The research demonstrates that the Guava leaf (*Psidium guajava*) is major substance identified to possess high quality characteristics to facilitate bioremediation process, if the material contains the relevance of the nutrient.

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