International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 10 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.10.009

Effects of Biostimulation and Bioaugmentation strategies on Soil urease activities in crude oil polluted soil microcosm

***Akunna Priya Emmanuel., Christopher E. Nwanyanwu, and Campbell O. Akujobi**
Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.
* Author for correspondence: email: *akunnaemmanuel@gmail.com*

Abstract

Petroleum hydrocarbons can affect soil ecosystems resulting in significant losses in soil health. The aim of the study is to determine the effects of petroleum hydrocarbon on soil urease in crude oil polluted soil. Cow dung application to the contaminated soil was done through Response Surface Methodology (RSM), with a statistical approach, and the Box–Behnken design of the experiment was adopted using design expert 6.0.8 software. Microbial isolates from the polluted site were identified using 16S rRNA coding sequences. The predominant isolate, *Bacillus* (BAB-888) with gene bank accession number KC250233, was used as single strain bioaugmentation. Results from the bioremediation studies showed that the polluted soil contained an array of hydrocarbon utilizing bacteria species. The bacteria population count escalated from $4.0 \times 10^6 - 2.36 \times 10^8$ after biostimulation with cow dung to $3.0 \times 10^5 - 2.99 \times 10^8$. Thus, revealing that the seeded microorganism adapted quickly to the petroleum hydrocarbon polluted soil and was able to make use of the crude oil as the sole carbon source. RSM used with four factors showed that urease decreases with an increase in Stimulant and Time. Pollutant decreases slightly and an exponential sharp increase with time. However, in biostimulated/inoculated soil, urease increases with an increase in Time, Inoculum and Pollutant from level 20, 20 and 50 respectively and progressed to levels 40, 30 and 75 respectively before decreasing. This recommends the potential of a single *Bacillus cereus* strain in hydrocarbon degradation.

Keywords: Biodegradation, Bioremediation, Crude oil, Response Surface Methodology (RSM), Box–Behnken design (BBD), Urease, Biostimulation, Bioaugmentation

Introduction

Soil is a non-renewable resource and its conditions influence food production, enhance water and air quality, environmental potency, world balance, and support human health and habitation (Dick, 1997; Doran and Zeiss, 2000; Lindbo *et al*., 2012).

Petroleum hydrocarbons are toxic compounds that have been classified as priority pollutants (Costa *et al*., 2012). They are resistant to biodegradation, capable of accumulating in plants, as well as in human (Waheed *et al*., 2018; Hunt *et al*., 2019). It also exhibits carcinogenic and neurotoxic properties (Das and Chandran, 2011; Al-Hawash *et al*., 2018; Hunt *et al*., 2019).

The discharge of these pollutants into the ecosystem whether through anthropogenic activities or via accidental discharge, leads to soil and water pollution; thus affecting all forms of life directly or indirectly (Sajna *et al*., 2015) with attendant negative consequence on the economic life of the ecosystem (Paul, 2015; Elum *et al*., 2016).

Bioremediation is the biological approach employed in environmental cleanup of polluted soil. The autochthonous (indigenous) soil microorganisms play great roles in sustainability by keeping essential functions of soil health, involving carbon and nutrient cycling. Biostimulation is another approach of bioremediation that involves the addition of the appropriate nutrients to stimulate the degradation capacity of the native soil microorganisms (Siles and García-Sánchez, 2018). The combined bioremediation technologies of bioaugmentation and biostimulation not only introduce active bacteria, but can also stimulate the indigenous microorganisms and ameliorate the character of the soil. Bacteria species such as *Bacillus, Pseudomonas, Staphylococcus, Flavobacterium* and yeasts have been involved in hydrocarbon degradation (Tremblay *et al*. 2017; Bento *et al*. 2005; Moscoso *et al*. 2012; Nie *et al*., 2014; Fatima *et al*., 2015; Silva *et al*., 2015; Sarkar *et al*,. 2017; Varjani, 2017).

Enzymatic activity is one of the most significant indicators of the characteristic soil health (Singh *et al*. 2017; Utubo and Tewari 2015; Bayarmaa and Purev 2017; Acosta-Martinez *et al*. 2018). Soil enzymes come from plants, soil animals, but mostly from microorganism (Sharma *et al*., 2017). Since remediation technologies a ect not just the oil concentration, but the enzymatic response (Margesin and Schinner, 1999; Gong, 2012), soil enzymes activities is a sensitive indicator and gives a quicker response to both natural and anthropogenic disturbances and also reflect biological changes induced by pollution and contamination (Panettieri *et al*., 2013; Baćmaga *et al*., 2014). Soil enzymes are necessary catalysts for soil functions that include, environmental quality, degradation of xenobiotic compounds (Tejada *et al*. 2011), and nutrient cycling in particular as they are frequently being accumulated, synthesized and decomposed in the soil (Balota and Chaves, 2010).

Urease is an extracellular enzyme whose activities depend on organic and inorganic matter content in soils. It originates from plants, animals, bacteria, fungi, invertebrates, and yeasts (Konieczna *et al*., 2013; Li *et al*., 2014; Dotaniya *et al*., 2019). The urease enzyme catalyzes the hydrolysis of urea fertilizers applied into carbon dioxide (CO_2) and ammonia ($NH_{3)}$ with a concomitant rise in soil pH and N loss to the atmosphere through NH_3 volatilization. Urease plays a major role in global nitrogen cycle (Srinivasa - Rao *et al*., 2017; Kuscu, 2019; Noor - Affendi *et al*., 2020).

Response Surface Methodology (RSM) is a variety of statistical and mathematical procedures for building of models, designing experiments, analyzing effects of factors, and studying for the optimum situations including bioprocesses (Hsieh *et al*., 2007; Dahyia, *et al*., 2009; Rajkumar and Muthukumar, 2017). Its usage has been mostly in industrial research (Montgomery 1996; Myers and Montgomery 2002). In recent times, numerous studies have described RSM in several fields such as in biochemistry (Martendal *et al*., 2007), biotechnology and life sciences (Anderson-Cook *et al*., 2009; Kockal and Ozturan, 2011; Yolmeh and Jafari, 2017).

This study reports on the effects of biostimulation and bioaugumentation strategies on soil urease activities in crude oil polluted microcosm.

Materials and Methods

Experimental soil

Composite soil sample was collected from an abandoned petroleum hydrocarbon polluted site (HCPS) from Gokana Local Government Area of Rivers State, Nigeria. Sample was collected from topsoil surface layer at depths of 0–30 cm using a soil augur.

Bonny light crude oil (BLCO) used as a pollutant was obtained from Port Harcourt Refinery Company, Eleme, Rivers State, Nigeria. Other chemicals used were analytical grade obtained from United Kingdom.

Experimental Design

Table 1: Experimental range in the Full-factorial Box-Behnken Design and the Level of Variables.

Std Order	Run Order	Pt Type	Blocks	Stimulant	Time	Inoculum	Pollutant Conc
1	1	2	1	20	20	30	75
2	2	2	1	40	20	30	75
3	3	2	1	20	60	30	75
4	4	2	1	40	60	30	75
5	5	2	1	30	40	20	50
6	6	2	1	30	40	40	50
7	7	2	1	30	40	20	100
8	8	2	1	30	40	40	100
9	9	2	1	20	40	30	50
10	10	2	1	40	40	30	50
11	11	2	1	20	40	30	100
12	12	2	1	40	40	30	100
13	13	2	1	30	20	20	75
14	14	2	1	30	60	20	75
15	15	2	1	30	20	40	75
16	16	2	1	30	60	40	75
17	17	2	1	20	40	20	75
18	18	2	1	40	40	20	75
19	19	2	1	20	40	40	75
20	20	2	1	40	40	40	75
21	21	2	1	30	20	30	50
22	22	2	1	30	60	30	50
23	23	2	1	30	20	30	100
24	24	2	1	30	60	30	100
25	25	2	1	30	40	30	75
26	26	2	1	30	40	30	75
27	27	2	1	30	40	30	75

The table above provides the design for four factors (stimulant, time, inoculum, and pollutant concentration) and four levels which includes; concentration of crude oil (50-100 ml), concentration of stimulant (20-40 grams), time (20-60 days) and concentration of inoculum (20-40 ml). These gave 27 runs with specific parameters generated by Response Surface methodology (RSM).

Sample preparation

Two kilograms each of soil sample was weighed into 36 different, slightly perforated, and labeled polyethylene bags (microcosms). The perforations were made for proper aeration of the soil. Nine (9) of the 36 soil samples were used for control. The soil samples were then spiked with crude oil to simulate the soil samples. It was done with different concentrations of the pollutant (crude oil) according to experimental design. Each bag was thoroughly mixed to achieve an even distribution of the crude oil and a maximum percentage of artificial contamination.

Media

Bushnell Haas Agar (**BHA)** was used as the enrichment culture medium for studying hydrocarbon deterioration by microorganisms (Bushnell and Haas, 1941). BHA was prepared following standard method.

Isolation and enumeration of microbes from soil samples

One gram (1 g) from each of the amended soil samples (1-27) was weighed into sterilized universal bottles that contained 9.0 mls of sterile water and was serially diluted to 10^{-5}. Thereafter, 0.1 ml of the suspension was aseptically dispensed to the center of the Nutrient agar (NA) for bacteria, while 0.1ml of the 4th dilution (10^{-4}) was aseptically transferred to the center of the appropriately labelled BHA plates for hydrocarbon degrading bacteria. Whatman filter paper 42 saturated with crude oil was aseptically placed on the lid of the inverted BHA petri plates for vapour phase transfer technique (Amanchukwu *et al*., 1989; Chikere and Azubuike, 2013. The culture plates were then incubated at 28±2°C for 24 hours and 7 days for NA and BHA plates respectively (Odokuma and Ibor, 2002).

Identification of Bacterial Strains

During isolation, colonies that appeared were selected and streaked on nutrient agar plate to obtain a pure culture. The morphologically distinct bacterial colonies that developed on Bushnell Haas Agar supplemented with crude oil were purified on nutrient agar and identified based on morphological, microscopy and biochemical characteristics according to Bergey and Holt (2000), Cappuccino and Sherman (2011) and Mulet *et al*. (2018).

Molecular Identification of Bacterial Strains

The isolates were phylogenetically amplified, identified, and differentiated by 16S rRNA sequencing using the pair of universal primers 27F: 5'- AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'CGGTTACCTTGTTACGACTT-3' (White *et al*. 1990) on an ABI 9700 Applied Biosystems thermal cycler, at the final volume of 40 microliters for 35 cycles. The sequencing conditions were as follows 32 cycles of 96^{o}C for 10s, 55^{o}C for 5s and 60^{o}C for 4 minutes. Resulting sequences were edited using the bioinformatics algorithm Trace edit. Also, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method. Consequently, the 16s rRNA of the isolate showed a percentage similarity to other species at 100% and revealed a closely relatedness to *Bacillus cereus* strain BAB 888 (KC250233).

Soil Urease Enzyme Assay

Urease was determined using spectrophotometry. 5 g of soil sample and 9 ml of distilled water were added into 25 × 150 mm capacity screw-capped tubes. The contents were gently mixed, followed by the addition of 1 ml of 0.2 M urea which were swirled and incubated at 37 ± 0.5^{0}C for 2 hours in a water bath. 15 ml of KCl-Ag_2SO_4 solution was later added and kept for 1 hour. After which 1 ml of supernatant was collected from it and transferred to a 25 ml volumetric flask. Finally, 1 ml of ethylenediaminetetraacetic acid (EDTA) was added, followed by the addition of 2 ml of phenol-nitroprusside and 8 ml of buffered hypochlorite reagent.

The volume was then brought up to the mark by the addition of 7 ml of distilled water and placed in the water bath for 40 minutes. The flask was removed from the water bath and brought to room temperature and the absorbance of the blue coloured complex was read at 636 nm.

Statistical Analysis

Differences in the rate of hydrocarbon degradation and microbial analysis across the different experimental conditions were analyzed using graphing data. Frequency distribution table and bar graphs regarding microbiological analysis of crude oil polluted soil bio-stimulated with cow dung and bacterial inoculum were performed using the "two descriptive statistics". Data obtained in the enzyme assay were analyzed using the Second Order Surface Design. Differences were considered significant at $p<0.05$

Results

The bacterial strains isolated from crude oil polluted soil sample were identified based on their colony morphology and microscopic morphology (Table 1), biochemical tests (Table 2), and molecular characterization (Figure 1).

Table 1: Colony Morphology of Bacterial isolates

Characteristic features	X_1	X_2	X_3	X_4
Pigmentation	Off-white or slightly yellow	Greenish - blue	Deep golden yellow or orange	Cream to white or gray
Colony Surface	Shiny, smooth and flat	Smooth, large, and flat	Smooth, raised and shiny	Flat, and rough
Colony Margin	Jagged edges or entire	Undulate	Clustered appearance	Waxy edges or entire
Opacity of Colony	Opaque	Opaque colonies	Shiny opaque	Opaque
Shape	Rod	Rod	Cocci	Rod
Gram-stain	+ve	-ve	-ve	+ve
Motility	Motile	Motile	Non-motile	Motile

Table 2: Biochemical Test Results of Bacterial isolates

Biochemical Tests	X_1	X_2	X_3	X_4
Catalase test	-	+	+	+
Citrate utilization	-	+	-	+
Indole production	-	-	-	-
Methyl-red reaction (M.R)	-	-	+	-
Nitrate reduction	+	+	+	+
Oxidase test	+	+	-	-
Urease activity test	-	-	+	+
Voges-Proskaeur (V P) test	-	-	-	+
Most Probable Identity	*Bacillus subtilis*	*Pseudomonas sp*	*Staphylococcus sp*	*Bacillus cereus*

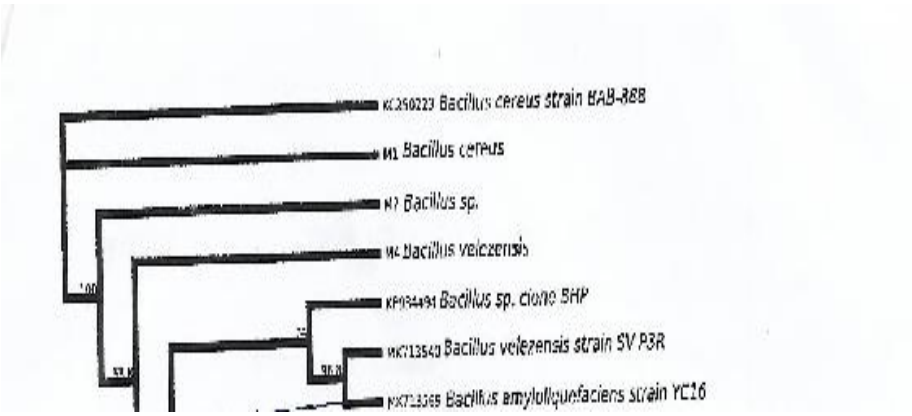

Fig. 1. Phylogenetic tree for bacterial isolates from crude oil polluted soil based on the 16s rRNA gene sequence.

The obtained 16s rRNA sequence from the isolate produced an exact match during the mega blast search for highly similar sequences from the National Center for Biotechnology Information (NCBI) non-redundant nucleotide (nr/nt) database. The 16s rRNA of the isolate M1 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16s rRNA of the isolate M1 within the *Bacillus* sp, and revealed a closely relatedness to *Bacillus cereus* strain BAB 888 (KC250233) than other *Bacillus* sp (Fig. 1) and a phylogenetic tree was made using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6). (Tamura, 2013).

Tables 3 and 4 shows the bacterial counts of heterotrophic bacteria and hydrocarbon degrading bacteria in crude oil polluted soil with biostimulant (cow dung) and microbial analysis of crude oil polluted soil supplemented with biostimulant (cow dung) and inoculum biomass. Total bacterial population was higher on BHA than on NA in Tables 3 and 4.

Table 3: Total count of heterotrophic bacteria and hydrocarbon degrading bacteria in crude oil polluted soil amended with biostimulant (cow dung)

Soil sample code	Bacteria on NA (Cfu/g)	Bacteria on BHA (Cfu/g)
1	3.3×10^7	2.42×10^7
2	5.9×10^7	2.68×10^7
3	1.4×10^7	2.4×10^7
4	3.1×10^7	3.14×10^7
5	9.5×10^7	2.18×10^7
6	2.4×10^7	2.18×10^7
7	2.3×10^7	1.8×10^7
8	2.8×10^7	3.54×10^7
9	4.2×10^7	3.82×10^7
10	2.98×10^7	1.84×10^7
11	3.1×10^7	3.62×10^7
12	4.1×10^7	2.0×10^5
13	3.7×10^7	3.16×10^7
14	7.2×10^7	2.5×10^6
15	1.8×10^7	8.6×10^6
16	$4. \times 10^6$	2.9×10^6
17	3.8×10^7	4.8×10^7
18	4.2×10^7	2.0×10^5
19	3.2×10^7	8.4×10^6
20	4.0×10^7	3.28×10^7
21	3.5×10^7	1.8×10^6
22	7.4×10^7	2.0×10^5
23	2.2×10^7	2.25×10^7
24	2.6×10^7	2.48×10^7
25	2.36×10^8	4.12×10^7
26	9.5×10^7	3.2×10^6
27	1.6×10^7	5.2×10^6

Key: **BHA** = Bushneli Haas Agar, **NA** = Nutrient Agar, **Control 50, 75**, **100** = control containing pollutant (crude oil) only, **Control +1, +2, +3** = control containing biostimulant (cow dung) only, and **Control -** = control without pollutant and amendment.

Table 4: Microbial analysis of the crude oil polluted soil containing Biostimulant (cow dung) and inoculum biomass

Soil sample code	Bacteria on BHA (cfu/g)	Bacteria on NA (cfu/g)
1	2.98×10^7	1.1×10^7
2	2.96×10^7	2.7×10^7
3	2.95×10^7	1.6×10^7
4	2.86×10^7	1.3×10^7
5	7.8×10^6	5.2×10^7
6	2.99×10^8	1.14×10^8
7	3.0×10^5	7.0×10^6
8	2.97×10^7	1.1×10^7
9	2.86×10^7	3.1×10^6
10	2.69×10^7	1.76×10^8
11	2.56×10^7	6.2×10^7
12	2.81×10^7	6.4×10^7
13	2.97×10^7	9.8×10^7
14	2.56×10^6	2.0×10^7
15	2.89×10^6	1.2×10^7
16	2.86×10^6	2.3×10^7
17	2.92×10^7	1.4×10^7
18	2.8×10^7	4.5×10^7
19	2.86×10^7	1.76×10^8
20	2.99×10^7	3.1×10^7
21	2.97×10^7	3.0×10^6
22	1.38×10^7	6.4×10^7
23	2.89×10^7	7.2×10^7
24	1.4×10^6	2.1×10^7
25	2.98×10^7	5.0×10^6
26	2.99×10^7	3.1×10^7
27	2.58×10^7	4.9×10^7

Key: **BHA** = Bushneii Haas Agar, **NA** = Nutrient Agar, **Control 50, 75, 100** = control containing concentration of pollutant (crude oil) only, **Control +1, +2, +3** = control containing biostimulant (cow dung) only, and **Control -** = control without pollutant and amendment.

Figure 2 shows Residual plots for soil urease activity in biostimulation/inoculum in crude oil polluted soil. Plot indicating interaction effects of urease vs pollutant, time in biostimulated/augmented crude oil contaminated soil is shown in Figure 3. Contour Plot indicating interaction effects of urease vs inoculum, time in soil crude oil biostimulation/inoculum biomass and Plot indicating interaction effects of urease vs pollutant, inoculum in crude oil biostimulated and bioaugmented soil are shown in Figures 4 and 5 respectively.

Figure 6 shows the main effect plot and the mean of Urease for the different levels of the factors (Stimulant, Time, Inoculum and Pollutant). Interaction Plot for Catalase showing the mean urease for the interaction between Inoculum and pollutant in biostimulated and inoculated crude and Optimization Plot showing the value of each factors (values in red color) which produces the maximum value for Urease (blue color) in amended and bioaugmented crude oil polluted soil are shown in Figures 7 and 8 respectively.

Optimization for Effects of Factors on Urease Activity versus Stimulant, Time, Inoculum, and Pollutant

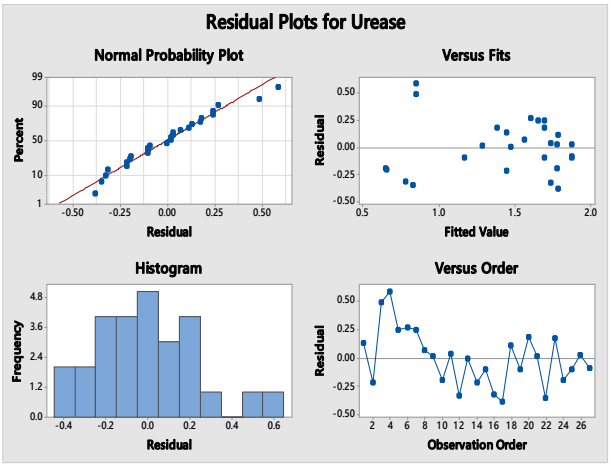

Figure 2: ***Residual plots for soil urease activity in biostimulation/inoculum in crude oil polluted soil***

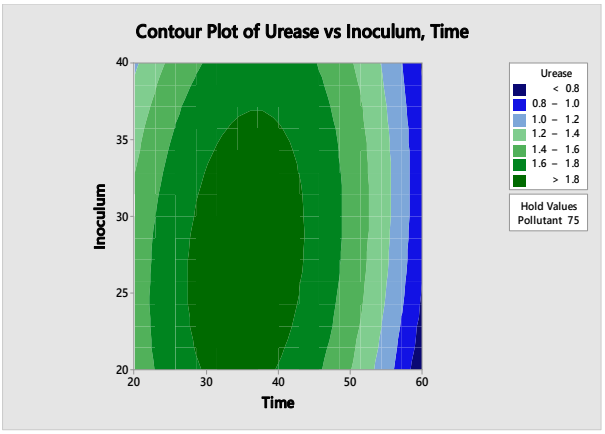

Figure 4: ***Contour Plot indicating interaction effects of urease vs inoculum, time in soil crude oil biostimulation/inoculum biomass***

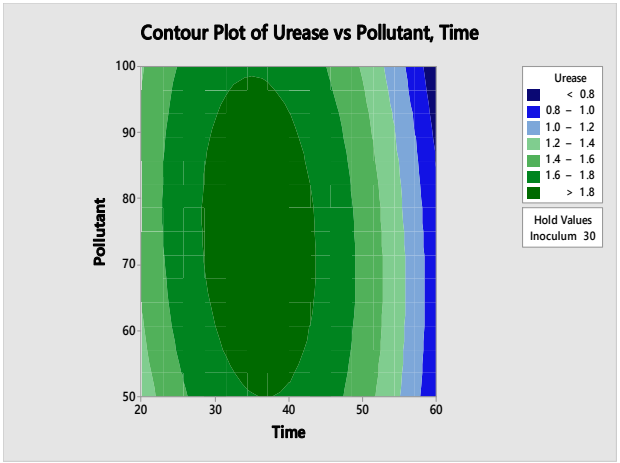

Figure 3: ***Plot indicating interaction effects of urease vs pollutant, time in biostimulatied/augmented crude oil contaminated soil***

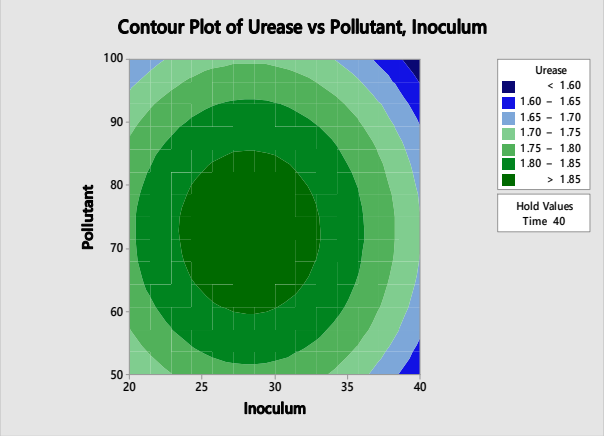

Figure 5: ***Plot indicating interaction effects of urease vs pollutant, inoculum in crude oil biostimulated and bioaugmented soil***

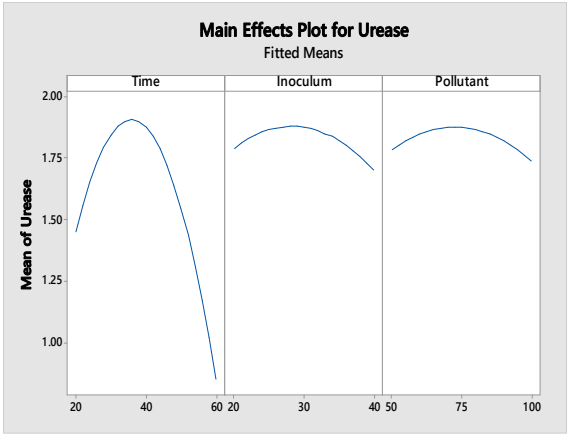

Figure 6: The main effect plot shows the mean of Urease for the different levels of the factors (Stimulant, Time, Inoculum and Pollutant).

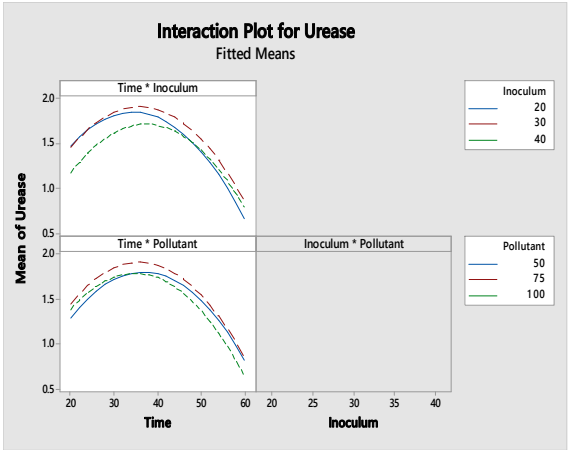

Figure 7: ***Interaction Plot for Catalase displaying the mean urease for the interaction between Inoculum and pollutant in biostimulated and inoculated crude oil polluted soil***

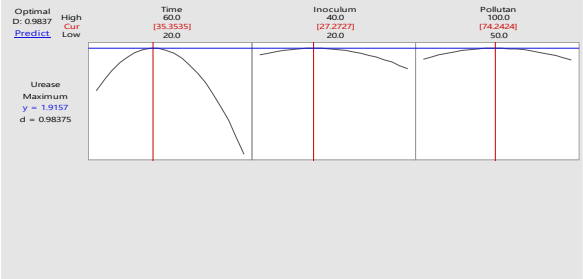

Figure 8: ***Optimization Plot showing the value of each factors (values in red color) which produces the maximum value for Urease (blue color) in amended and bioaugmented crude oil polluted soil***

Discussion

The colony cell morphology and the biochemical tests of petroleum hydrocarbon degrading organisms from the soils were identified by the methods of Udgire *et al*. (2015).

Results from the bioremediation studies showed that the polluted soil contained an array of hydrocarbon utilizing bacteria belonging to the genus *Bacillus*, *Pseudomonas*, and *Staphylococcus* genera. Some of these bacteria genera have also been reported in hydrocarbon degradation by Wokem *et al*. (2017). Al-Dhabaan (2019) and Olawep**o** *et al*. (2018) recorded the presence of *Staphylococcus aureus* and *Bacillus subtilis* in polluted soil amended with cow dung. Tavassoli *et al*. (2012) and Jahromi *et al*. (2014), reported that species of *Pseudomonas Staphylococcus* and *Bacillus* prefer resins and asphaltenes, petroleum hydrocarbon component as substrates.

Morphological, microscopic, physiological, and biochemical characteristics in addition to the 16s rRNA gene sequences suggested close relationship with 98-100% identity to *Bacillus cereus* strain BAB 888 (KC250233) as the major degrading bacteria isolated from the polluted soil.

The bacterial inoculum biomass and amendment under soil microcosms experiment also showed positive degradation ability. The highest colony forming units per gram (CFU/g) of the utilizing bacterial populations in the amended and bioaugmented microcosms using a single strain of isolate via inoculum biomass recorded 3.0×10^6 to 1.76×10^8 CFU/g on nutrient agar and 3.0×10^5 to 2.99×10^8 CFU/g^{-1} on BHA. The HUB counts increased remarkably from 10^7 – 10^8 Cfu/g. Thus, suggesting that the seeded microorganism adapted quickly to the petroleum hydrocarbon polluted soil and was able to make use of the crude oil as the sole carbon source. Diaz-Ramirez *et al*. (2013) reported that the number of viable counts increased with the bioaugmentation strains. According to Okerentugba *et al*. (2016), microbial communities exposed to hydrocarbons adapt to this exposure through selective enrichment and genetic changes resulting in an increase in hydrocarbon degradation. The increase in the density of the microbial populations could ensure the rapid degradation of the pollutants.

Residual plot was obtained from Response Surface Regression of urease versus Stimulant, Time, inoculum, and Pollutant analyzed. The data satisfies the assumptions of the second order model and can be used to make decisions. The residuals also have a constant variance since there is no systematic pattern from the graph of residual against the fitted value. Also, there is no trend in the graph of residual against observation order which suggest that the residuals are independent from each other. The main effect plots shows how Urease increases with an increase in Time, Inoculum and Pollutant from level 20, 20 and 50 respectively till it gets to level 40, 30 and 75 respectively after which it starts decreasing.

In this study, the response of the soil enzyme urease to crude oil was coincident with microorganism response to these compounds, that is, the amended and inoculated crude oil polluted soil had an increased counts of microorganisms and also responses of the soil enzymes. This is logical, because microbial populations represent the major source of soil enzymes (Datta *et al*., 2017). It should be noted that organic matter not only activates metabolism of microorganisms but also is favourable for decomposition of organic pollutants (Junter *et al*., 2002).

Conclusion and Recommendations

This study concluded that bioremediation of crude oil-polluted soil enhanced petroleum hydrocarbon degradation. Regardless of some contradictory reports, biostimulation and bioaugmentation although distinct from each other, proved to be an effective strategy for cleaning up oil spill sites and should be used as complementary techniques for the remediation of oil contaminated sites. It can be concluded that that RSM is a reliable and powerful tool for modeling and optimization of enzyme activity and PAH bioremediation processes. In addition, bioremediation of crude oil polluted soil using bioaugmentation technique should be amended with organic nutrient to enhance its efficiency.

References

Acosta-Martinez, V., Cano, A and Jahnson, J. (2018). Simultaneous Determination of Multiple Soil Enzyme Activities for Soil Health-Biogeochemical Indices. *Applied Soil Ecology*. **126**: 121–128.

Al-Dhabaan, F. A (2019). Morphological, Biochemical and Molecular Identification of Petroleum Hydrocarbons Biodegradation Bacteria Isolated from Oil Polluted Soil in Dhahran, Saud Arabia. *Saudi Journal of Biological Sciences*. **26**(6):1247-1252.

Amanchukwu, S. C., Obafemi, A and Okpokwasili, G. C. (1989). Hydrocarbon degradation and utilization by a palm wine yeast isolates. *FEMS Microbiology Letter*. **57**(2): 151—154. https://doi.org/10.1111/j.1574-6968.1989.tb03290.x

Anderson-Cook, C. M., Borror, C. M and Montgomery, D. C. (2009). Response Surface Design Evaluation and Comparison. *Journal of Statistics Planning and Inference*. **139**: 629–641.

Baćmaga, M., Wyszkowska, J., Borowik, A., Tomkiel, M and Kucharski, J. (2014). Response of Fungi, B-Glucosidase and Arylsulfatase to Soil Contamination by Alister Grande 190 OD, Fuego 500 SC and Lumax 357.5 SE Herbicides. *Polish Journal of Environmental Studies*. **23** (1):19–25

Balota, E. L and Chaves, J. C. D. (2010). Enzymatic Activity and Mineralization of Carbon and Nitrogen in Soil Sultivated with Coffee and Green Manures. *Revista Brasileira de Ciência do Solo*. **34**: 1573-1583.

Bayarmaa, J and Purev, D. (2017). Role of enzymes in soil. Mongolian Journal of Agricultural Sciences. **22**(03): 96-102. https://doi.org/10.5564/mjas.v22i03.950

Bento, F. M., Camargo, F. A. O., Okeke, B. C and Frankenberger, W. T (2005). Diversity of Biosurfactant Producing Microorganisms Isolated from Soils Contaminated with Diesel Oil. *Microbiological Research*. **160**: 249–255.

Bergey, D. H. 1 and Holt, J. G. (Eds) (2000). Bergey's manual of determinative bacteriology. 9th ed. Philadelphia: Lippincott Williams & Wilkins.

Bushnell, L. D and Haas, H. F (1941). The Utilization of Certain Hydrocarbons by Microorganisms. *Journal of Bacteriology*. **41**: 653-673.

Cappuccino, J. G and Sherman, N. (2011). *Microbiology: A laboratory Manual* (9th ed.). The Benjamin-Cummings Publishing Co., Inc., Menlo Park, California.

Costa, A. S., Romão, L. P., Araújo, B. R., Lucas, S. C., Maciel, S. T., Wisniewski, A and Alexandre, M. R. (2012). Environmental Strategies to Remove Volatile Aromatic Fractions (BTEX) From Petroleum Industry Wastewater Using Biomass. *Bioresource Technology*. **105:** 31-39.

Dahiyia, S., Singh, N., and Rana, J. S (2009). Optimization of growth parameter of phytase producing funus using RSM. *Journal of Scientific and Industrial Research*. **68**: 955-959.

Das, S. K Varma, A. (2010). Role of enzymes in maintaining soil health. *Soil Enzymology*. 25-42

Datta, R., Anand, S., Moulick, A., Baraniya, D., Imran Pathan, S., Rejsek, K., Vranová, V., Sharma, M., Sharma, D and Kelkar, A. (2017). How Enzymes Are Adsorbed on Soil Solid Phase and Factors Limiting its Activity: A Review. *International Agrophysics*. **31**:287–302.

Dick, R. P. (1997). *Soil Enzyme activities as integrative indicators of Soil Health*. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CABI Publishers, Wellingford. pp 121–156.

Doran, J. W and Zeiss, M. R. (2000). Soil Health and Sustainability: Managing the Biotic Component of Soil Quality. *Applied Soil Ecology*. **15**: 3–11

Dotaniya, M., Aparna, K., Dotaniya, C. K., Singh, M and Regar, K. L. (2019). Role of Soil Enzymes in Sustainable Crop Production. In M. Kuddus (Ed.), Enzymes in Food Biotechnology. Oxford, UK: Academic Press. pp 569-589.

Elum, Z. A., Monini, K and Henri-Ukoha A. (2016). Oil Exploitation and Its Socioeconomic Effects on the Niger Delta region of Nigeria. *Environmental Science and Pollution Research*. **23** (13): 12880–12889

Fatima, K., Afzal, M., Imran, A and Khan, Q. M. (2015) Bacterial Rhizosphere and Endosphere Populations Associated with Grasses and Trees to Be Used for Phytoremediation of Crude Oil Contaminated Soil. *Bulletin of Environmental Contamination and Toxicology*. **94**: 314–320

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution; International Journal of Organic Evolution. **39**(4): 783–791.

Freitas, D. S., Wurr Rodak, B and dos Reis, R. A. (2018). Hidden nickel deficiency. Nickel fertilization via soil improves nitrogen metabolism and grain yield in soybean genotypes. *Frontiers in Plant Science*.

Gong, Xiao-Bao (2012). Remediation of Weathered Petroleum Oil Contaminated Soil Using a Combination of Biostimulation and Modified Fenton Oxidation. *International Biodeterioration ad Biodegradation*. **70**: 89-95.

Hsieh, C., Tsai, M. J., Hsu, T. H., Chang, D. M and Lo, C. T (2007). Medium Optimization for Polysaccharide Production of *Cordyceps sinensis*. *Applied Biochemistry and Biotechnology*. **120**: 145-157.

Hunt, L. J., Duca, D., Dan, T and Knopper, L. D. (2019). Petroleum Hydrocarbon (PHC) Uptake in Plants: A literature review. *Environmental Pollution*. **245**: 472–484.

Jahromi, H., Fazaelipoor, M. H., Ayatollahi, S and Niazi, A. (2014). Asphaltenes biodegradation under shaking and static conditions. *Fuel*. **117:** 230-235.

Junter, G. A., Coquet, L., Vilain, S and Jouenne, T. (2002). Immobilized-Cell Physiology, Current Data and Potentialities of Proteomics. *Enzyme and Microbial Technology*. **31**: 201–212.

Kockal, N and Ozturan, T (2011). Optimization of Properties of Fly Ash Aggregates for High-Strength Lightweight Concrete Production. *Material Design*. **32**:3586–3593

Konieczna, I., Zarnowiec, P and Kwinkowski, M. (2013). Bacterial urease and its role in long-lasting human diseases. *Current Protein and Peptide Science*. **13**:789–806.

Kuscu, I. S. K. (2019). Changing of soil properties and urease-catalase enzyme activity depending on plant type and shading. Environmental Monitoring and Assessment, 191(3): 24-31.

Li, Q., Csetenyi, L and Gadd, G. M (2014). Biomineralization of metal carbonates by *Neurospora crassa*. *Environmental Science and Technology*. **48**: 12-19.

Lindbo, D. L., Kozlowski, D. A and Robinson, C. (2012). (eds).. Know Soil Know Life. Madison, WI: *Soil Science Society of America*. **70**: 1774-1787.

Margesin, R and F Schinner, 1999. Biological decontamination of oil spills in cold environments. *Journal of Chemical Technology and Biotechnology*. **20**: 313-333.

Martendal E, Budziak D and Carasek E. (2007) Application of fractional factorial experimental and Box–Behnken designs for optimization of single drop microextraction of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole from wine samples. *Journal of Chromatography A.* **1148**:131–136

Montgomery, D. C (1996). *Design and Analysis of Experiments*, 4th edn. Wiley, USA

Moscoso, F., Teijiz, I., Deive, F. J and Sanromán, M. A. (2012). Efficient PAHs biodegradation by a bacterial consortium at flask and bioreactor scale. *Bioresource Technology*. **119**: 270–276

Mulet, M., Sanchez, D., Rodriguez, A. C., Nogales, B., Bosch, R., Busquets, A., Gomila, M., Lalucat J and Garcia-Valdes, E. (2018). *Pseudomonas gallaeciensis* sp. nov., isolated from crude-oil-contaminated intertidal sand samples after the Prestige oil spill. *Systematic and Applied Microbiology*. **41** (4): 340–347.

Myers, R. H and Montgomery, D. C (2002). *Response surface methodology: process and product optimization using designed experiments*, 2nd edn. Wiley, New York.

Nie, Y., Liang, J. L., Fang, H., Tang, Y. Q and Wu, X. L. (2014). Characterization of a CYP153 alkane hydroxylase gene in a gram-positive Dietzia sp. DQ12-45-1b and its team role with alkw1 in alkane degradation. *Applied Microbiology and Biotechnology*. **98**: 163–173.

Noor - Affendi N. M., Mansor N and Samiri, S. S (2020). Addition of chemical and natural urease inhibitors in reducing ammonia and nitrous oxide losses. *Journal of Soil Science and Plant Nutrition*. **20**:253–258.

Odokuma, L. O. and Ibor, M. N. (2002). Nitrogen fixing bacteria enhanced bioremediation of crude oil polluted soil. *Global Journal of Pure and Applied Sciences***. 8** (4): 455-468.

Olawepo, G., Ogunkunle, C., Adebisi, O and Fatoba, P. (2018). Enhanced Bioremediation of Brass Crude-oil (Hydrocarbon), Using Cow Dung and Implication on Microbial Population. *Pollution*. **4** (2): 273-280.

Panettieri, M., Lazaro, L., Lopez-Garrido, R., Murillo, J. M., Madejon, E. (2013). Glyphosate effect on soil biochemical properties under conservation tillage. *Soil Tillage Research*. **133**:16–24.

Paul, I. A. (2015). A Historical Perspective of Petroleum on Nigeria's Economic Crisis since Independence: *Global Journal of Human-Social Science and Economics*. **15**(2): 17–24.

Rajkumar, K and Muthukumar, M. (2017). Response surface optimization of electro-oxidation process for the treatment of C.I. Reactive Yellow 186 dye: reaction pathways. *Applied Water Science*. **7**(2): 637–652.

Saitou, N and Nei, M. (1987). The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*. **4**: 406-425.

Sajna, K. V., Sukumaran, R. K., Gottumukkala, L. D and Pandey, A. (2015). Crude Oil Biodegradation Aided by Biosurfactants from *Pseudozyma* sp. NII 08165 or its Culture Broth. Bioresource. *Technology*. **191**: 133-139.

Sarkar, P., Roy, A., Pal, S., Mohapatra, B., Kazy, S and Maiti, M. K. (2017). Enrichment and Characterization of Hydrocarbon-degrading Bacteria from Petroleum Refinery Waste as Potent Bioaugumentation agent for in situ Bioremediation. *Bioresource Technology*. **242**: 15-27

Sharma S., Kumar V and Tripathi R. B. (2017). Isolation of phosphate solubilizing microorganism (PSMs) from soil. *Journal of Microbiology and Biotechnology Research*. **1** (2): 90.

Siles, J.A., and García-Sánchez, M. (2018). Microbial Dynamics during the Bioremediation of Petroleum Hydrocarbon-Contaminated Soils through Biostimulation: An Overview. In: Prasad R., Aranda E. (eds) Approaches in Bioremediation. Nanotechnology in the Life Sciences. Springer, Cham. pp 115–134.

Silva, D. P., Cavalcanti, D. D, de Melo, E. J. V., dos Santos, P. N. R., da Luz, E. L P., de Gusmao N. B and Sousa, M. F (2015). Bio-removal of Diesel Oil through a Microbial Consortium Isolated from a Polluted Environment. *International Biodeterioration and Biodegradation.* **97**:85–89.

Srinivasa Rao, C., Grover, M., Kundu, S and Desai, S. (2017). *Soil Enzymes*. In R. Lal (Ed.), Encyclopedia of Soil Science. Boca Raton: Taylor & Francis Group. pp. 2100-2107.

Tamura, K., Stecher, G., Peterson, D., Filipski, A and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*. **3**0(12): 2725–2729.

Tejada, M., Gomez, I., Garcia-Martinez, A. M., Osta, P, and Parrado, J. (2011). Effects of Prochloraz fungicide on soil enzymatic activities and bacterial communities. *Ecotoxicology and Environmental Safety*. **74**:1708–1714

Tremblay, J., Yergeau, E., Fortin N., Cobanli, S., Elias, M and King, T. L (2017). Chemical Dispersants Enhance the Activity of Oil and Gas Condensate-Degrading Marine Bacteria. *Journal of International Society for Microbial Ecology*. **11**:2793-2808

Udgire, M., Shah, N and Jadhav, M. (2015). Enrichment, Isolation and Identification of Hydrocarbon Degrading Bacteria. *International Journal in Current Microbiology Application and Science*. **4**(6):708-713

Utubo, E. B and Tewari, L (2015). Soil Enzymes as Bioindicators of Soil Ecosystem Status. *Applied Ecology and Environmental Research*. **13**(1): 147–169.

Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology*. **223**: 277–286.

Waheed, S.M., Sangal, P and Shukla, S. (2018). Microbiological analysis for hydrocarbon exploration. *Environmental Risk Assessment and Remediation*. **2**(3): 1–7.

Wokem, V. C., Odokuma, L. O and Ariole, C, N. (2017). Isolation and Characterization of Hydrocarbon Utilizing Bacteria from Petroleum Sludge Samples Obtained from Crude Oil Processing Facility in Nigeria. *Journal of Applied Environmental Management*. **21**(2): 355- 359.

Wyszkowska, J., Borowik, A., and Kucharski J. (2015). Response of Avena sativa, microorganisms and enzymes to contamination of soil with diesel oil. *Plant Soil and Environment*. **61** (11): 483–488.

Yolmeh, M and Jafari, S.M. (2017). Applications of Response Surface Methodology in the Food Industry Processes. *Food Bioprocess Technology*. **10**: 413–433.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Microbiology
DOI:10.22192/ijarbs.2021.08.10.009	

How to cite this article:
Akunna Priya Emmanuel., Christopher E. Nwanyanwu, and Campbell O. Akujobi. (2021). Effects of Biostimulation and Bioaugmentation strategies on Soil urease activities in crude oil polluted soil microcosm . Int. J. Adv. Res. Biol. Sci. 8(10): 71-85.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.10.009