Journal of Engineering, Science, and Technology
ISSN: 2714-2396, Volume 5, Issue 1.

ASSESSMENT OF CHANGES IN FACTORS AFFECTING BIOREMEDIATION

R. Babalola[1*], Y. Atiku[2], U. Isaac[3], E. Udoetuk[1], and U. Aniediong[1]

[1] Department of Chemical / Petrochemical Engineering, Akwa Ibom State University Ikot Akpaden, Akwa Ibom State-Nigeria
[2] 2Department of Chemical Engineering, Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University Bauchi, PMB 0248 Bauchi, Bauchi State-Nigeria.
[3]Federal Polytechnic Oko, College of Engineering, Department of Chemical Engineering, Oko, Anambra State, Nigeria
E-mail: babalolarasheed72@gmail.com* ; ukemeisaac2018@gmail.com, ekeminiudoetuk@aksu.edu.ng, umoaniediong@gmail.com

Received Date 1st March 2021 Accepted Date 3rd April 2021 Published Date 30th April 2021

Abstract
The assessment of changes in factors affecting Bioremediation, biostimulation, and bio-augmentation of the petroleum-contaminated soil by adding nutrient (urea) and long cultured bacteria to the soil was examined in this research work. The materials employed during the bioremediation process include petroleum, contaminated soil from Ogoni, pretreated soil, isolated bacteria, mineral salt medium, nutrient agar, nutrients, solution of concentrated pH, and distilled water. Bioremediation took about 21 days. The microbial variation results are; pseudomonas aeruginosa degrades about 20% of crude oil compared to bacillus cereus, which degrades about 15.6%. In contrast, the mixed culture degrades 28.04% compared with the microbial control, which degrades 1%. Treatment options using bioreactors other than those discussed here revealed different percentage degradation (bio-degradation), which may be attributed to the variations in their compositions. Assessing pH variation, pH 7 degrades 28.04% of crude oil compared to pH 6, which degrades about 20.92% o and pH 8 degrade about 0.44% percent. It was observed that most of the bioreactors used for the treatment process with pH values outside the acceptable range of 6.5-8.5 for effective Bioremediation affected the extent of biodegradation of the crude oil hydrocarbon contaminant. From the results of nutrient variations, 2.0 g of urea degrades about 28.04% of crude oil compared to 1.5 g of urea which degrades about 21.38%. In comparison, 2.5 g of urea degrades 17%. The control for the nutrient degrades about 14.36% of the crude oil, and it was observed that treatment options with more quantity of nutrients become inhibitory to the microorganisms; hence, the more the nutrient, the less the rate of biodegradation. In conclusion, it could be said that all the environmental factors considered in this study influence the effective bioremediation process.
Keywords: Bio-augmentation, variation, microbes, Bioremediation, and bio-stimulation

1. Introduction
Interaction of man with the environment has made man research, develop, and improve on the surrounding. In these several activities, man has also caused harm to the ecosystem; in the long run, these activities have altered the normal processes of the ecosystem. This as a whole has been one of the major problems of man to date. The effect of these several factors can be seen in the following; mutation of the gene, depletion of the ozone layer, biodiversity loss, extinction of several species of plant and animals (mass extinction), ecological crisis, ecological collapse, global warming, climate changes, natural hazards, production of greenhouse gases among others. All

these activities and effect occurs in the biosphere (where man's activity takes place or life exists) [1].

Remediation is the process of removal of pollutants from the media of contamination, such as the environment. Remediation can be divided into chemical, physical, and biological remediation. Physical and chemical remediation consists of steps used to separate contaminants from the media of contamination by extracting the polluted substance as a concentrated liquid or substance from the soil. Examples of these processes are soil vapour extraction (SVE), bio sparging, bioventing, and bio slurping. In contrast, biological remediation consists of both phytoremediation and Bioremediation. Bioremediation is sub-classified under two broad aspects: in situ and ex situ bioremediation processes [2]. Also, the in-situ process is concerned with natural attenuation, bio slurping, bio venting, bio sparging, and phytoremediation. At the same time, the ex-situ method deals with land farming, windrow, bioreactors, and biopile.

The key players in Bioremediation are bacteria, microscopic organisms that live virtually everywhere. Microorganisms are ideally suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminants as food and because they are so small that they can contact contaminants quickly [3]. In situ bioremediation can be regarded as an extension of the purpose that microorganisms have served in nature for billions of years: the breakdown of complex human, animal, and plant wastes so that life can continue from one generation to the next. The extent to which microorganisms successfully destroy man-made contaminants in the subsurface depends on three factors: the type of organisms, the type of contaminant, and the geological and chemical conditions at the contaminated site [4, 5]. Bioremediation aims to stimulate microorganisms with nutrients and other chemicals that will enable them to destroy the contaminants [6]. The bioremediation systems in operation today rely on microorganisms native to the contaminated sites, encouraging them to work by supplying them with the optimum levels of nutrients and other chemicals essential for their metabolism. Thus, today's bioremediation systems are limited by the capabilities of the native microbes [3]. However, researchers are currently investigating ways to augment contaminated sites with non-native microbes, including genetically engineered microorganisms, which are suited to degrading the contaminants of concern at particular sites [7, 8].

Microbial transformation of organic contaminants occurs typically because they can use the contaminants for their growth and reproduction. Organic contaminants serve two purposes for the organisms: they provide a source of carbon, which is one of the basic building blocks of new cell constituents. They provide electrons that the organisms can extract to obtain energy. Microbes degrade contaminants because, in the process, they gain energy that allows them to grow and reproduce. Microbes get energy from the contaminants by breaking chemical bonds and transferring electrons from the contaminants to an electron acceptor, such as oxygen. They "invest" the energy and some electrons and carbon from the contaminant to produce more cells. The type of chemical reaction is called an oxidation-reduction reaction: the organic contaminant is oxidized, the technical term for losing electrons; correspondingly, the chemical that gains the electrons is reduced. The contaminant is called the electron donor, while the electron recipient is called the electron acceptor. These two materials, the electron donor and acceptor, are essential for cell growth and are commonly called the primary substrates. Regardless of the mechanism microbes use to degrade contaminants, the microbe uses to degrade contaminants; the microbes' cellular components have relatively fixed elemental compositions. A typical bacterial cell is 50 percent carbon; 14 percent nitrogen; 3 percent phosphorus; 2 percent potassium; 1 percent sulfur; 0.2 percent iron; and 0.5 percent each of calcium, magnesium, and chloride. If any of these or other

elements essentials to cell building is in short supply relative to the carbon present as organic contaminants, competition for nutrients within the microbial communities may limit overall microbial growth and slow contaminant removal. Thus, the bioremediation system must be designed to supply the proper concentration and ratios of these nutrients if the natural habitat does not provide such nutrients [7].

According to [9,10,3], most of the microorganisms that are frequently identified as active members of bioremediation microbial consortia belong to the genera Acinetobacter, Actinobacter, Alcaligenes, Arthrobacter, Bacillus, Berjerinckia, Flavobacterium, Methylosinus, Mycobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctonia, Serratia, Trametes, and Xanthobacter. The in situ bioremediation techniques involve treating polluted substances at the site of pollution. It does not require any excavation; therefore, it is accompanied by little or no disturbance to soil structure. Ideally, these techniques ought to be less expensive than ex-situ bioremediation techniques due to no extra cost required for excavation processes; nonetheless, the cost of design and on-site installation of some sophisticated equipment to improve microbial activities during Bioremediation is of major concern [11,3]. Some in situ bioremediation techniques might be enhanced (bioventing, biosparging, and phytoremediation), while others might proceed without any form of enhancement (intrinsic Bioremediation or natural attenuation). Besides, in situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals, and hydrocarbons polluted sites [11-14]. The ex-situ bioremediation techniques involve excavating pollutants from polluted sites and transporting them to another site for treatment. Ex-situ bioremediation techniques are usually considered based on the cost of treatment, depth of pollution, type of pollutant, degree of pollution, geographical location, and geology of the polluted site. Performance criteria, which also determine the choice of ex situ bioremediation techniques, have been described [15, 2].

2. Material and Methods

2.1 Materials

Soil samples A, B, C, were collected from contaminated sites in Ogoni, mainland and riverside, River State. The non-oil polluted soil was collected from the Ogoni mainland. The soil samples were collected using a soil auger. These samples were stored in pre-sterilized sample containers to avoid physical and chemical properties alteration at a depth of 5-15 cm, packed in ice-block coolers, and transported to the laboratory. The samples were duly labeled then taken to the laboratory for analysis. The non-contaminated soil is pretreated for the bioremediation process; it is watched and autoclaved and dried for about 2 hrs and sieved using sieve no. 4, about 1 mm [16]. Sample (A) is contaminated soil from the main-land, sample (B) is contaminated soil from the riverside, sample (C) is a non-contaminated soil.

Five millimeters (5 mL) of desalted crude oil is used to contaminate the pretreated soil. Urea granules (99 %, Lab Tech Chemicals) were used as nutrients. Nutrients, especially nitrogen and phosphorus, are highly needed and added to grow the microbial population to consume the total petroleum hydrocarbon (TPH) at a reasonable rate. Agar obtained from the Department of Microbiology Akwa Ibom State University was used for bacteria growth. The solution of the following mineral salts were used experiment; K_2HPO_4 (99 %, Sigma), KH_2PO_4 (99 %, Sigma), $MgSO_4.7H_2O$ e (99.9 %, Aldrich), NaCl (98 %, Aldrich),, $NaNO_3$ (97.5 %, BDH), $(NH_4)_2SO_4$ (99.9 %, Aldrich),, $FeSO_4.7H_2O$ (99 %, Sigma), and $CaCl_2$ (97 %, Aldrich).

2.2 Experimental Methods

2.2.1 Enumeration of total heterotrophic bacteria in the oil-polluted and non-oil polluted soil sample

Ten grams (10 g) of each soil sample were measured into the conical flask, and 90 mL of sterile water was mixed with the sample. The samples were shaken to dislodge and homogenize the solution [17].

2.2.2 Serial dilution of the samples

One millimeter (1 mL) of the stock sample was aseptically transferred into sterile test tubes containing 9 mL of diluent to give a dilution of 10^{-1}. This was repeated until the seventh dilution factor was attained [18, 19].

2.2.3 Inoculation of culture medium

One millimeter (1 mL) from 10^{-4} dilution from each soil sample was transferred into sterile Petri dishes in duplicates. About 20 mL of sterile nutrient agar was aseptically poured into the seeded plates (pour plate technique). 0.1 mL from 10^{-4} dilution was transferred into sabouraud dextrose agar plates in duplicates. A hockey stick was used to spread the inoculums evenly on the surface of the plates (spread plate technique) [20]. All nutrient agar plates were incubated at 28ºC for 24-48 hrs, sabouraud dextrose agar plates were incubated for 5-7 days, and mineral salt medium plates for 11-21 days at 28ºC. Colonies that emerged after incubation were enumerated. The nutrient agar for bacteria growth utilized was sabouraud dextrose agar for fungi growth and mineral salt medium for hydrocarbon-degrading bacteria growth [17].

2.2.4 Enumeration of hydrocarbon degraders

The estimation of densities of hydrocarbon-degrading bacteria and fungi were determined using the vapour phase transfer method with sterile crude oil as a carbon and energy source supplied from the lid of the plates. 1ml from dilutions 10^{-4} from each soil sample were used to seed the sterile Petri dishes in duplicates. Sterile molten mineral salt medium (MSM) is supplemented with chlorophenicol (0.5 mL) to suppress bacterial growth and 0.5ml of nystatin to suppress fungal growth on different preparations. These media were poured into the seeded plates and swirled to mix with the inocula. All plates were inoculated for 14 days at 28ºC. Plates that developed colonies were counted and recorded as colony-forming unit/g (cfu/g) (microbial density) [17].

2.2.5 Purification and maintenance of microbial isolates

Discrete colonies from culture plates were picked for characterization. Bacteria colonies were repeatedly sub-cultured into freshly prepared nutrient agar by the streaking method. They incubated for growth before transferring to agar slant. Pure isolates of bacteria were maintained on agar slant as stock and preserved in the refrigerator [21].

2.2.6 Characterization and identification of bacteria isolates

Bacteria isolates were characterized and identified presumptively based on their morphological, cultural, and physiological characterization. Confirmatory identification was based on biochemical reactions. The following biochemical tests were carried out; Gram staining, motility, catalase, spore staining, oxidase, urease, citrate, starch hydrolysis, methyl red - Voges Proskauer (MR-VP) test, hydrogen sulphide production test, and sugar fermentation. The tests for various isolates were collected, and the identification was carried out by comparing it with the standard procedures. Fungal isolates were characterized and identified using standard procedures for fungal identification and bacterial isolates via the Biochemical test [17].

2.2.7 Solution pH preparation

The pH of each solution was certified by using about 0.1M of HCl and 0.1M NaOH, varying these, the pH of the various solution in the reactor is stabilized at 6, 7, and 8. Some quantity of distilled water is placed into a beaker, using a pH meter after calibration. The pH of the distilled water is measured at about 6.44. Also, using a syringe, some quantity of 0.1M of HCl, that is for a lower pH (6), is added into the solution in less than drops, the solution is stirred, and the pH is measured, both acid and base are added interchangeably until the setpoint is reached, the same is repeated for both the neutral pH, i.e., 7, also for pH of 8, using the acid and base respectively [22].

2.2.8 Extraction of petroleum hydrocarbon

After Bioremediation, about 3 weeks, the petroleum hydrocarbon was extracted to evaluate the amount of hydrocarbon remediated and the residual oil. The contaminated soil was washed with dichloromethane until the colour of the solution and soil in the reactor becomes colourless (clean). The solution from washing the soil is transferred into a separating funnel to separate the oil dissolve in the organic solvent (dichloromethane) from the water. The extract from the funnel is put into a soxhlet extractor or is distillation set-up to recover the organic solvent and leave the oil as residue. The solution was heated at a range of 20-39.7ºC. The residual oil is weighed in the round bottom flask, weighing the empty round bottom flask to know the amount (mass) of residual oil present [23].

2.2.9 Bioremediation test for soil contaminated with petroleum hydrocarbons

Fifty grams (50 g) of the prepared soil sample were weighed into plastic cups as reactors, nine (9) altogether. About 10 mL of distilled water is added to every vessel of pH 7. These bioremediation tests were designed to investigate the effects of pH value, nutrients, and microorganisms on the rate of crude oil degradation. Three reactors 1, 2, 3 were used to consider variation in nutrient (urea), about 1.5 g, 2.0 g, and 2.5 g for each vessel. The control reactor for variation in nutrients contains distilled water and no nutrient solution. Three reactors, 4, 5, and 6 also used to monitor variation in pH 6, pH 7, and pH 8. Furthermore, to evaluate the control, the reactor with pH 7 was used as control. Another set of reactors 7, 8, 9 were used to study variation in microbial constituents. Two microbes were considered pseudomonas aeruginosa and bacillus cereus. The degradation rate for both single microbes and mixed microbes was compared with that without microbe (control). The first reactor contained pseudomonas aeruginosa, the second Bacillus cereus, and the third contained both inoculums. Reactors for nutrient and pH variation contain both microbes. Each of the control vessels was used to monitor the different factors which influence the rate of Bioremediation. This remediation process was carried out for 3 weeks, i.e., 21 days. At some regular intervals, the soil was stirred, thus enabling for aeration of the soil samples, which assist in contaminant degradation [24].

3. Results and Discussion

3.1 Biochemical Characteristics of the Bacterial Isolates

Results from the isolation, subculturing, characterization, and determination of the indigenous microbes show the presence of the following bacteria; Pseudomonas Protista, Bacillus cereus, Bacillus subtilis, Micrococcus sp, Pseudomonas aeruginosa, Staphylococcus aereus, and Chromatium sp. (Table 3 and 6) of which Pseudomonas aeruginosa and Bacillus cereus were chosen to carry out the remediation process (M1 and M2). This confirmation on these organisms' application as hydrocarbon degraders is in line with other similar findings, as reported by [25, 26; 27]. The densities of the microbes as obtained and presented in Tables 1 and 4 for both MSM media and NA were enough to be used for the bioremediation experiment. The finding is in agreement with other similar findings as reported by [5, 28]. Also, Table 2 and 5 show the different

morphological characteristics of the microorganisms for both media, and Table 3 and 6 show the microorganisms' biochemical characteristics for both media. All the results obtained from the morphological and biochemical characteristics on the microorganisms' identities are in line with the literature, as reported by [10, 29; 30].

Table 1: Microbial density using MSM

Sample	Medium	HUB
A	MSA_1	2.0×10^5
B	MSA_2	1.1×10^5
	MSA_1	1.3×10^5
	MSA_2	1.5×10^5
C	MSA_1	1.9×10^5
	MSA_2	1.7×10^5

Table 2: Morphological Characteristics

Shape	Pigment	Elevation	Colony Surf.	Consistency	Odour	Optical char.
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Milky	Raised	Shiny	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Irregular	Green	Flat	Shiny	Viscose	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Purple	Raised	Shiny	Butyrous	Present	Opaque
Regular	Purple	Raised	Shiny	Butyrous	Present	Opaque
Regular	Milky	Raised	Shiny	Butyrous	Present	Opaque

Table 3: Biochemical characteristics

Gram reaction	Shape	Catalase	Coagulase	Motility	Starch hydrolysis	Citrate	Urease	Methyl red	Voge Proskauer	Spore formation	H_2S	Oxidase	Glucose	Maltose	Lactose	Fructose	Sucrose	Manitol	Gal. &Dex.	Probable organism
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	$A/_A$	*B. cereus*
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	AG	A	-	A	-	-	$A/_-$	*B. substilis*
+ve	Rod	+	-	+	-	+	-	-	+	-	-	+	A	A	-	AG	AG	-	$AG/_{AG}$	*Pseudomonas aeruginosa*
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	AG	A	-	A	-	-	$A/_-$	*B. substilis*
+ve	Cocci	+	-	-	+	+	+	+	-	-	+	+	A	-	A	-	A	A	$A/_A$	*Micrococcus sp*
+ve	Rod	-	-	+	+	+	-	+	-	+	+	-	A	A	-	-	A	A	$AG/_A$	*Chromatiumsp*
+ve	Rod	-	-	+	+	+	-	+	-	+	+	-	A	A	-	-	A	A	$AG/_A$	*Chromatiumsp*
+ve	Cocci	+	-	-	+	+	+	+	-	-	+	-	A	-	-	-	A	A	$A/_A$	*Micrococcus sp*
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	AG	A	A	A	-	-	$A/_-$	*B. substilis*
+ve	Cocci	+	-	-	+	+	+	+	-	-	+	+	A	-	-	-	A	A	$A/_A$	*Micrococcus sp*

Table 4: Microbial density of the indigenous microbes using NA

Samples	Medium	THBC
A	NA_1	1.3 X 10^6
	NA_2	1.5 X 10^6
B	NA_1	1.9 X 10^6
	NA_2	1.7 X 10^6
C	NA_1	1.4 X 10^6
	NA_2	1.2 X 10^6

Table 5: Morphological characteristics of the indigenous microbes

Morphological characteristics						
Shape	**Pigment**	**Elevation**	**Colony Surf.**	**Consistency**	**Odour**	**Optical characteristics**
Irregular	Light yellow	Flat	Shiny	Viscose	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dry	Butyrous	Present	Opaque
Regular	Milky	Raised	Shiny	Butyrous	Present	Opaque
Irregular	Green	Flat	Shiny	Viscose	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Yellow	Raised	Shiny	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Irregular	Green	Flat	Shiny	Viscose	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Purple	Raised	Shiny	Butyrous	Present	Opaque
Regular	Milky	Raised	Shiny	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque
Regular	Purple	Raised	Shiny	Butyrous	Present	Opaque
Irregular	Milky	Raised	Dull	Butyrous	Present	Opaque

Table 6: Biochemical characteristics of the indigenous microbes

Gram reaction	Shape	Catalase	Coagulase	Motility	Starch hydrolysis	Citrate	Urease	Methyl red	Opaque	Spore form	Hydrogen sulphide	Oxidase	Glucose	Maltose	Lactose	Fructose	Sucrose	Mannitol	Gal	Dex	Probable organism
-ve	Rod	+	-	+	-	+	-	-	+	-	-	+	A	A	-	AG	AG	-	AG	AG	*Pseudomonas Protista*
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*Bacillus cereus*
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	AG	A	-	A	-	-	A	-	*Bacillus subtilis*
+ve	Cocci inpains	+	-	-	+	+	+	+	-	-	-	+	-	A	-	A	-	A	A	A	*Micrococcus sp*
-ve	Rod	+	-	+	-	+	-	-	+	-	-	+	A	A	-	AG	AG	-	AG	AG	*Pseudomonas aeruginosa*
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*Bacillus cereus*
+ve	Cocci	-	-	-	+	+	-	-	+	-	+	-	AG	A	-	A	A	AG	AG	A	*Staphylococcus aereus*
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*Bacillus cereus*
-ve	Rod	+	-	+	-	+	-	-	+	-	-	+	A	A	-	AG	AG	-	AG	AG	*Pseudomonas aeruginosa*
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	AG	A	-	A	-	-	A	-	*Bacillus subtilis*
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*Bacillus cereus*
+ve	Rod	-	-	+	+	+	-	+	-	+	+	-	A	A	-	-	A	A	AG	A	*Chromatiumsp*
+ve	Cocci	+	-	-	+	+	+	+	-	-	-	+	-	A	-	A	-	A	A	A	*Micrococcus sp*
+ve	Rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*B. subtilis*
+ve	rod	-	-	+	+	+	-	+	-	+	+	-	A	A	-	-	A	A	AG	A	*Chromatium sp*
+ve	rod	+	-	+	+	-	-	-	+	+	-	-	AG	A	-	A	A	-	A	A	*B. cereus*

3.2 Bioremediation Experiment and Variations in Factors Affecting Bioremediation

It was observed that nine (9) reactors treatment options were used for the bioremediation experiment. According to the results from microbial variation, *pseudomonas aeroginosa* degrades

about 20% of crude oil used during the contamination process compared to *bacillus cereus,* which degrades about 15.6% of the crude oil. In comparison, the mixed culture degrades 28.04%. That is, both cultures mixed to give a better hydrocarbon-degrading tendency as revealed. The microbial control reactor degrades 1% of the crude oil detail of that is as shown in figure1, other treatment options other than the ones discussed here revealed different percentage degradation, and this may be attributed to the variations in the composition of other respective reactors used for the bioremediation experiment particularly their nutrient content, this finding is in line with the literature as reported by [29, 4]. Details on the variables used for the bioreactor experiment are presented in Table 7. Figure 1 is the percentage of oil degraded versus microbial reactors (reactors with varying microorganism (s).

However, evaluating pH variation, pH 7 degrades 28.04% of crude oil compared to pH 6, which degrades about 20.92% of the oil, and pH 8 degrades about 0.44% percent. In addition, it was observed that among the bioreactors used for the treatment process, most of the bioreactors with pH values outside the acceptable range of 6.5-8.5 for effective Bioremediation affected the extent of biodegradation of the crude oil hydrocarbon contaminated soil. This finding complies with the similar work conducted, as reported by [3, 5]. The detail on the pH variation is as presented in Tables 7 and 8 respectively, in this work, pH 7 is used as the control for the pH variation. Figure 2 is the percentage of oil degraded versus pH reactors.

Furthermore, from the results of nutrient variations, 2.0 g of urea degrades about 28.04% of crude oil compared to 1.5 g of urea, which degrades about 21.38% of oil. In comparison, 2.5 g of urea degrades 17%. The control for the nutrient degrades about 14.36% of the crude oil. Besides, it was observed that among the treatment options reported, those with more quantity of nutrient becomes inhibitory, thereby lowering the percentage degradation among the treatment options. This finding agrees with the literature work as revealed by similar work conducted and reported by [3,31,5]. It was observed that the more the nutrient, the less the rate of degradation, details on the formulation used and the results obtained is also as presented in Table 7 and 8 respectively. Figure 3 shows the percentage of oil degraded versus nutrient reactors.

Table 7: Variables used for the reactor experiment

Reactor	10 mL solution	Soil added (50 g)	Bacterial inoculated	Nutrient (Urea) g	pH	Contaminant (Oil) mL
1	Yes	Yes	M_1	2.0	7	5
2	Yes	Yes	M_2	2.0	7	5
3	Yes	Yes	M_1, M_2	2.0	7	5
4	Yes	Yes	No	2.0	7 (control)	5
5	Yes	Yes	M_1, M_2	1.5	7	5
6	Yes	Yes	M_1, M_2	2.5	7	5
7	Yes	Yes	M_1, M_2	No	7 (control)	5
8	Yes	Yes	M_1, M_2	2.0	6	5
9	Yes	Yes	M_1, M_2	2.0	8	5

*Note: M_1 represent Pseudomonas aeruginosa and M_2 represent Bacillus cereus.

Table 8: Results from bioremediation process

Reactors	Composition	W_1(g)	W_2(g)	W_3(g)	V_1 (mL)	V_2 (mL)	P (%)
1	$7M_1N_{2.0}McC$	229.115	232.662	3.547	4.000	1.000	20%

2	$7M_2N_{2.0}McC$	120.760	124.502	3.742	4.220	0.780	15.6%
3	$7M_1M_2N_{2.0}McC$	107.746	110.936	3.190	3.598	1.402	28.04%
4	$7N_{2.0}McC$(Control)	107.291	112.766	5.475	4.950	0.050	1%
5	$6M_1M_2N_{2.0}McC$	120.746	124.252	3.506	3.954	1.046	20.92%
6	$8M_1M_2N_{2.0}McC$	229.071	233.485	4.414	4.978	0.022	0.44%
7	$7M_1M_2N_{1.5}McC$	120.766	124.252	3.486	3.931	1.069	21.38%
8	$7M_1M_2N_{2.5}McC$	229.129	232.809	3.680	4.150	0.850	17%
9	$7M_1M_2McC$(Control)	120.771	124.568	3.797	4.282	0.718	14.36%

Reactor 3($7M1M_2N_{2.0}McC$) also serves as a control for the pH evaluation. The relationship used in relating mass to volume is the density formula, as shown in equation (1)

$$Density = \frac{Mass}{Volume} \quad (1)$$

Density of crude oil used = $0.8867gcm^{-3}$.

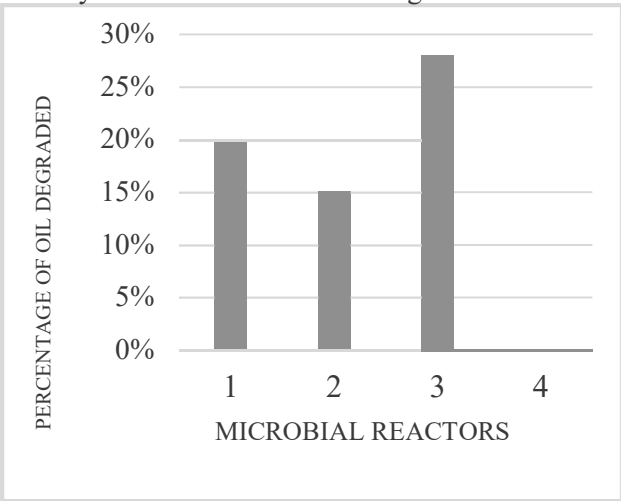

Fig. 1: Percentage of oil degraded versus Microbial Reactors

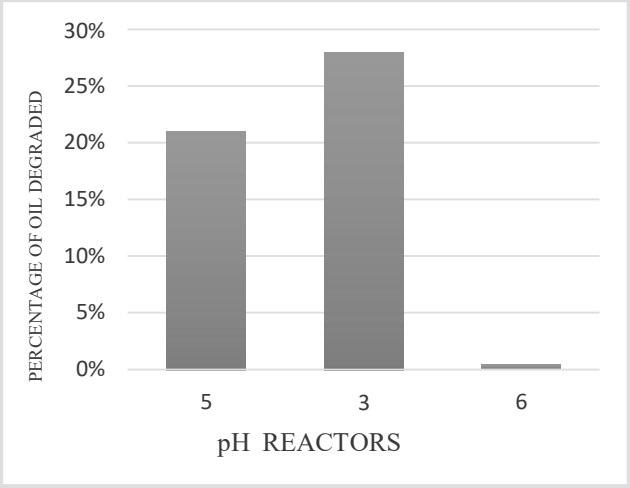

Fig. 2: Percentage of oil degraded against pH reactors

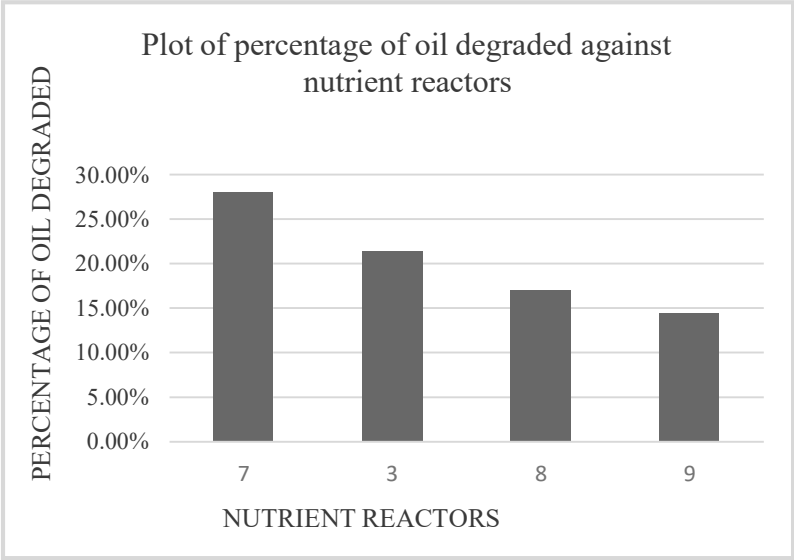

Fig. 3: Percentage of oil degraded against nutrient reactors

4. Conclusions

The research examined the evaluation of variations in factors affecting Bioremediation, biostimulation, and bioaugmentation of the crude oil contaminated soil from Ogoni. The bioremediation experiment lasted for 21 days. The results from microbial variation, pH, and nutrient variation evaluated as factors revealed that the extent of degradation (biodegradation) is affected due to those mentioned factors. It was observed the highest biodegradation of 28.04% at the pH of 7 and treatment option number three (3) out of the nine (9) bioreactors used as the treatment option during the 21 days study period. However, more time and optimization of the factors considered in this study are required to achieve accelerated percentage biodegradation. In a nutshell, it could be said that all the environmental factors considered in this study influence effective Bioremediation, as revealed by the results obtained concerning the extent of biodegradation of the crude oil hydrocarbon contaminant.

Nomenclature

HUB	Hydrocarbon Utilizing Bacteria
Colony surf.	Colony surface
H_2S	Hydrogen sulphide
+ve	Positive
-ve	Negative
AG	Acid & Gas
A	Acid
W_1	Weight of empty bottle (g)
W_2	Weight of empty bottle and residual oil (g)
W_3	Weight of residual oil (g)
V_2	Volume of oil degraded (mL)
V_1	Volume of residual oil (mL)
P	Percentage of oil degraded
6, 7 and 8	pH 6, 7 and 8
M_1	Pseudomonas aeruginosa
M_2	Bacillus cereus
$N_{1.5}$	Nutrient (urea) 1.5 g
$N_{2.0}$	Nutrient (urea) 2.0 g
$N_{2.5}$	Nutrient (urea) 2.5 g
Mc	Moisture content
C	Crude oil

References

[1] Bioremediation, n.d. Wikipedia, viewed 21 June 2019, https://en.m.wikipedia.org/wiki/Bioremediation

[2] Abdulsalam, S., 2012. Mathematical Modeling of Bioremediation of Soil Contaminated with Spent Motor Oil. *Journal of Emerging Trends Engineering and Applied Science, 3(4)*, pp. 654-659.

[3] Vidali, M., 2001. Bioremediation an overview, *Journal of Pure and Applied Chemistry*, *73(7)*, pp. 1163– 1172.

[4] Samuel, E.A., 2013. Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in In-situ Soil Bioremediation of Diesel Oil: Experimental Analysis and

Kinetic Modeling. *Journal of Environment and Earth Science, 3 (7),* pp. 51-61.

[5] Atiku, Y. M., Abdulsalam, S. and Muhammad, I.M., 2018. Biostimulation of Soil Contaminated with Spent Motor Oil Using Cow Dung Poultry Litter in Land Farming Microcosm. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 12(3)*, pp. 01-10.

[6] Abdulsalam, S., Muhammad, I.M. and Atiku, Y.M. 2018. Exact Approach to Bioremediation of Soil Contaminated with Spent Motor Oil Using Cow Dung and Poultry Litter in Land Farming Microcosm, *Journal of the Nigerian Society of ChemicalEngineers*, *33(2),* ISSN: 0794-6754.

[7] National Research Council, 2003. Principles of Bioremediation. In Bioremediation: when does it work?. Washington D. C. *The National Academy Press*.

[8] Abdulsalam, S., Adefilia, S.S., Bugaje, I.M. and Ibrahim, S., 2011. Comparison of Biostimulation and Bioaugmentation for Remediation of Soil Contaminated with Spent Motor Oil, *International Journal of Environmental Science and Technology, 8(1),* pp. 187– 194, ISSN: 1735:1472.

[9] Oyeleke, S.B. and Manga, S.B., 2008. Essential of Laboratory Practical, *Best Publisher, Minna,* pp. 36-63, First edition.

[10] Abioye, O.P., Agamuthu, P. and Abdul Aziz, A.R. 2012. Biodegradation of Used Lubricating Oil by Microbes Isolated from pristine Soil Environment, *Malaysian Journal of Science,* pp. 31.

[11] Folch, A., Vilaplana, M., Amado, L.,Vicent, R. and Caminal, G., 2013. Fungal permeable reactive barrier to remediate groundwater in an artificial aquifer. *J Hazard Mater. 2013, 262*, pp. 554–560. doi:10.1016/ j.jhazmat.2013.09.004.

[12] Kim, S., Krajmalnik-Brown, R., Kim, J-O. and Chung, J. 2014. Remediation of petroleum hydrocarbon-contaminated sites by DNA diagnosis-based bioslurping technology. *Sci Total Environ 2014, 497,* pp. 250–259. doi:10.1016/j.scitotenv.2014.08.002.

[13] Frascari, D., Zanaroli, G. and Danko, A.S., 2014. In situ aerobic co-metabolism of chlorinated solvents: a review. *J Hazard Mater, 2015, 283*, pp. 382–399. doi:10.1016/j.jhazmat.2014.09.041.

[14] Roy, M., Giri, A.K., Dutta, S. and Mukherjee, P. 2015. Integrated phytobial remediation for sustainable management of arsenic in soil and water. *Environment International, 2015,75,* pp. 180–198. doi:10.1016/j.envint.2014. 11.010.

[15] Philp, J.C. and Atlas, R.M. 2005. *Bioremediation of contaminated soils and aquifers. In: Atlas R. M, Philp J.C (eds) Bioremediation: applied microbial solutions for real-world environmental cleanup.* American Society for Microbiology (ASM) Press, Washington: 2005: 139–236

[16] Mwaura, N.A. 2017. Screening, isolation and characterization of hydrocarbonoclastic bacteria from oil contaminated soil. pp. 25-36.

[17] Kingsley, C.A., Bassey, E.E., Iloasusi, C.A., Awah, N.S., Okeke, B.C., Anaukwu, C.G., Ezenwa, C.U., Orji, M.U. and Okafor, A.C. 2015. Isolation and characterization of microorganism from oil polluted soil Kwata, Awka South, Nigeria. *American Journal of Current Microbiology, 3(1),* pp. 3.

[18] Prescott, L.M., Harley, J.P. and Klein, D., 2005. Microbilogy, Sixth edition. *McGraw Hill International edition, New York*.

[19] Cheesbrough, M., 2004. District laboratory practice in tropical countries (2). *Cambridge, University Press, United Kingdom*, pp. 157-179.

[20] Https://www.microbeonline.com/spread-plate-technique-principle-procedure-result assessed January, 2020.

[21] Anupama, M. and Padma, S., 2009. Isolation of hydrocarbon degrading bacteria from soils contaminated with crude oil spills. *Indian Journal of Experimental Biology, 47,* pp.760-765.

[22] Https://www.pharmaguideline.com/2011/08/preparation-and-standardization-of-0.1mL html?m=1.

[23] Zhongyan, M., 1998. Bioremediation of petroleum hydrocarbon contaminated soil using indigenous cultures. pp. 35.

[24] Chijioke, O.A., Chioma, B. C. and Christopher, C., 2016. Bioremediation technique – Classification based on site of application: principles, advantages, limitations and prospects. *World J. Microbiol Biotechnol 2016, 32*, pp180. Doi: 10.1007/s11274-016-2137-x.

[25] Das, N. and Chandran, G., 2011. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, *Biotechnology Research International,* 2011,20(11),Article ID94180, Available online @ http:// dx.doi.org/10.4061/2011/941810.

[26] Subathra, M.K., Immanuel, G. and Suresh, A., 2013. Isolation and Identification of Hydrocarbon Degrading Bacteria from Ennore Creek. *Bioinformation, 9(3):* pp. 150–157, Doi: 10.6026/97320630009150.

[27] Drzewiecka, D., 2016. Significance and Roles of *Proteus* spp. Bacteria in Natural Environments, *Microbial Ecology 72(4),* pp. 741–758.

[28] Abdulyekeen, K.A., Muhammad, I.M., Giwa, S.O. and Abdulsalam, S., 2016. Bioremediation of Used Motor Oil Contaminated Soil Using Elephant and Horse Dung as Stimulants, *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 10(12), ISSN: 2319-2399***,** pp. 73-78, DOI: 10.9790/2402-1012027378.

[29] Adams, G,O., Prekeyi, T.F., Samson, E.O. and Igelenyah, E., 2015. Bioremediation, Biostimulation and Bioaugmentation A Review *International Journal of Bioremediation & Biodegradation*, *2015, 3(1),* pp. 28-39.

[30] Victor, T.O., Celestine, U.A., Emmanuel, O.E. and Oluwatayo, M., 2015. Biostimulation Hydrocarbon Utilizing Bacteria in Soil Contaminated with Spent Engine Oil Using Banana and Plantain Agro-waste, *Journal of Soil Science and Environmental Management, 6(8),* pp. 225-233, Doi 10.5897/JSSEM15.0505.ISSN: 2141-2391. http://www.academic journals. Org. / JSSEM.

[31] Ajao, A. T., Oluwajobi, A.O. and Olatayo, V.S., 2011. Bioremediation of Soil Microcosm from Auto-Mechanic Workshops.*J.APP.SCI.Environ.Management, 15(3),* pp. 473-477, ISSN: 1119-8362.