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Optimization of Bioremediation Enhancement Factors in an Aged Crude Oil Polluted Soil.

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Abstract

Bioremediation as an environmentally friendly method of restoration of crude oil polluted soil is influenced by several conditions. This study was designed to optimize some bioremediation enhancement factors including soil moisture content, agitation or mixing and nutrient ratio. Baseline properties of the soil samples were determined using standard analytical procedures. The crude oil polluted soil studied was seeded with mixed microbial consortium and differentially supplemented with inorganic nitrogen and phosphorus using carbon, nitrogen and phosphorus ratios 100:10:1 and 100:2:0.2. The initial sample moisture content was adjusted to 80% of its water holding capacity. Subsequently, moisture content adjustment and mixing were done at different intervals while the experiment lasted. Residual total petroleum hydrocarbon was measured every 6 days. Mixing the set-up every three days and moisture content adjustment every six days resulted in more efficient crude oil attenuation in the contaminated soil while carbon, nitrogen and phosphorus ratio 100:2:0.2 yielded statistically significant (p<0.05) higher crude oil degradation (90.99 ± 0.02%) over 100:10:1 ratio (78.15 ± 0.03%) after 36 days of remediation. The results obtained suggest that use of optimized site-specific conditions would enhance the microbial driven process of soil attenuation.

Keywords: Bioremediation, crude oil, moisture content, nutrient ratio, optimization, polluted soil.

1. Introduction

Oil exploration and other related activities remain a global concern because of the attendant environmental degradation and negative effect on the ecosystem (Ugochukwu and Ertel, 2008; Sam *et al*., 2017; Ite *et al*., 2018). Several approaches including physical, chemical and biological techniques are in place to manage this associated pollution (Siles and Margesin, 2018). However, biological remediation or bioremediation is preferred as it is reliable, cheap, efficient and eco - compatible (Azubuike *et al*., 2016; Speight and El-Gendy, 2018). Indigenous microbes with potential to transform pollutants play an important role in this natural process of soil restoration (Azubuike *et al*., 2016; Chauhan *et al*., 2017).

A number of factors influence the optimum functioning of these microbes with effect on the rate of the bioremediation process. Some of these conditions include the nature of the pollutants, pH, temperature, nutrients, aeration, moisture, the impacted soil type and appropriate density of oleophilic microbes (Macaulay, 2015; Azubuike *et al*., 2016; Varjani, 2017; Speight and El-Gendy, 2018). Microbes require nutrients like carbon, nitrogen and phosphorus to support their metabolic activities (Bamforth and Singleton, 2005; Ghaly *et al*., 2013). Crude oil pollution leads to depletion of available nitrogen and phosphorus in impacted soil (Ghaly *et al*., 2013). The introduction of the depleted nutrients stimulates the activity of soil microbes during bioremediation (Walworth *et al*., 2007; Varjani, 2017). These limiting nutrients must be introduced to the soil at optimum levels to enhance biodegradation; nitrogen supplied at high concentration can be inhibitory to microbial activity (Huesemann, 1994; Walworth *et al*., 2007, Onwosi *et al*., 2018).

Since the biotransformation of crude oil in polluted soil occurs mainly by aerobic process with molecular oxygen playing important role, oxygen deficiency reduces the rate of bioremediation (Jain *et al*., 2011). Periodic tilling of the impacted soil helps to increase microbial activity due to enhanced aeration, uniform distribution of nutrients and also the pollutants (Azubuike *et al*., 2016). Water in soil promotes microbial metabolism, diffusion of oxygen, nutrients and degradation products (Tibbett *et al*., 2011). Very high moisture content in soils with low permeability is limiting to bioremediation as it reduces availability of oxygen (Tibbett *et al*., 2011). Soil pH influences the ability of microbes to degrade crude oil (Varjani, 2017). Extremes of pH inhibit microbial activity with negative impact on the rate of bioremediation (Leahy and Colwell, 1990). A soil pH range of 6-8 is reported to be optimum for microbial crude oil degradation (Macaulay, 2015). Fungi are reported to tolerate acidic conditions better than bacteria (Leahy and Colwell, 1990). Soil permeability also affects the process of bioremediation; this is determined by the size of soil particles (Atlas, 1995; Macaulay, 2015). Soil with low permeability such as clay retains the crude oil at the surface resulting in low rate of biodegradation

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while highly permeable soil such as sand is more susceptible to leaching of the pollutant to low oxygen region of the soil (Macaulay, 2015).

Within the limits of our literature surveyed, there are no reports of specified optimal conditions for bioremediation in the Niger Delta region of Nigeria; consequently, some factors including aeration (mixing), moisture content and nutrients were investigated in this study to establish site-specific conditions that would enhance the potentials of indigenous microbes in the attenuation of aged crude oil polluted soil in this area.

2. Materials and Methods

2.1. Soil Sample Collection and Preparation

Soil samples were collected from Koko in Warri North Local Government Area of Delta State. The community is potentially exposed to environmental pollution arising from the industrial activities of a number of companies operating in the oil sector. Crude oil polluted soil sample was collected at a depth of 0-50 cm from a crude oil waste handling area while uncontaminated soil sample was collected from a fallow area without any history of crude oil pollution. The composite soil samples were aggregated, taken in sterile polythene bags, kept in ice packs and taken to the laboratory for use within twenty-four hours. The contaminated and uncontaminated soil samples were air – dried for five days and sieved using a 2 mm sieve.

2.2. Baseline Characterization of Soil Samples

Standard analytical procedures were used to determine the following physicochemical properties of the soil samples. Soil temperature was measured on – site and in the laboratory using a digital probe thermometer: H – 9283 Multi – Thermometer (Almaw *et al*., 2017), gravimetric method of Reynolds (1970) was used for moisture content (MC), particle size analysis by hydrometer method (Bouyoucos, 1962), pH by method of McLean (1982), electrical conductivity by method of Richards (1954), porosity and bulk density by weighing bottle method (FAO, 1980), water holding capacity (WHC) by gravimetric method (FAO, 1980), total organic carbon by wet oxidation method (Walkley and Black, 1934), total nitrogen by modified Kjedahl method (FAO, 1980) and available phosphorus by modified sodium bicarbonate extraction (Wantanabe and Olsen, 1965; Olsen and Sommers, 1982).

2.3. Determination of Total Petroleum Hydrocarbon in Contaminated Soil

Spectrophotometric method with n-hexane as the extraction solvent was used (USEPA, 2000; Urum *et al*., 2005, Akpe *et al*., 2015). Five grams contaminated soil sample was weighed into a Nalgene bottle with 5 g sodium sulphate and shaken vigorously to mix. Thereafter, 10 ml n-hexane was added; the bottle was covered and shaken vigorously for 5 min. The soil extract was carefully decanted into a conical flask and covered with foil paper. The extraction process was repeated three times with addition of 10 ml n-hexane to the Nalgene bottle containing the contaminated soil and shaken vigorously for 5 minutes. All extracts were pooled together and transferred to a 50 ml volumetric flask. The volume of the extract in the volumetric flask was adjusted to 50 ml with n-hexane. An aliquot of 10 ml soil extract was centrifuged at 3000 rpm for 10 minutes. Absorbance of supernatant was estimated at 400 nm using a spectrophotometer. The concentration of crude oil in the extract was estimated from n-hexane/crude oil standard curve using the absorbance obtained according to Equation 1.

$$\text{TPH (mg/kg)} = \frac{C \times V \times DF}{W} \quad (1)$$

Where: C = concentration of crude oil in the extract estimated from the standard curve. V = Total volume of the n-hexane/crude oil extract. DF = Dilution factor. W = Mass of soil used

2.4. Daily Moisture Content Monitoring

Three (3) kilograms each of contaminated soil was weighed into 7 plastic containers. The MC of the soil was adjusted to 60 – 80% of WHC. The containers were kept in the laboratory under ambient condition and subjected to agitation daily. A container was used to estimate MC daily by gravimetric method (Reynolds, 1970) till MC was ≤ 60% of sample WHC.

2.5. Evaluation of Effect of Agitation and Moisture Content on Crude Oil Degradation in the Contaminated Soil

Three (3) kilograms each of polluted soil was weighed into 4 plastic containers per group (5 groups) and control. Each container was amended with microbial consortium, composed of all microbial isolates with >50% crude oil degradation potential from our previous work (Edemhanria et al., 2020), and nutrient at carbon, nitrogen, phosphorus ratio 100:2:0.2. Urea was used as source of nitrogen while potassium dihydrogen phosphate supplied phosphorus. Sample MC was adjusted to 80% of WHC. Each group was subjected to the following treatment at the specified interval indicated in Table 1. Crude oil degradation was measured as residual total petroleum hydrocarbon at 6 days interval for 24 days following the procedure described earlier.

Table 1. Treatment groups and intervals investigated

	Treatment Interval (days)	
Group	Agitation	Moisture Adjustment
A1	Daily	3
A2	3	3
A3	Daily	6
A4	6	6
A5	3	6
Control	None	None

2.6. Effect of Carbon, Nitrogen and Phosphorus Ratio on Crude Oil Degradation in Soil

Three (3) kilograms each of crude oil polluted soil was weighed into 7 plastic containers per group (2 groups) and subjected to the following treatment: Group one was treated with microbial consortium and supplemented with nutrient at carbon, nitrogen and phosphorus ratio 100:10:1 while Group two had microbial consortium with nutrient supplementation at ratio 100:2:0.2. The MC of the contaminated soil sample was adjusted to 60 – 80% field WHC for both groups. Each container was agitated every 3 days and moisture adjustment done every 6 days in both groups. The experiment lasted for 36 days with residual TPH evaluated every 6 days.

2.7. Data Analysis

All experiments were performed in triplicates, and data were analyzed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics 23 software for Windows. The data were presented as mean ± standard error (SE). One-way Analysis of Variance (ANOVA) was used in comparing the means followed by Duncan's Multiple Range (DMRT) Post Hoc test. Student's t test was used to compare means for the nutrient ratios studied. $P<0.05$ was taken as statistically significant.

3. Results and Discussion

The soil is a key natural resource and part of the terrestrial ecosystem; it serves as habitat to an enormous diversity of organisms such as microorganisms, insects, earthworms and other invertebrates while also supporting plant growth and other agricultural practices (Dominati *et al*., 2010; Blum, 2013). However, the sustainable use of the soil as a key natural resource and its ability to function can be affected by a number of activities including pollution (Polyak *et al*., 2018). This is particularly true of Nigeria's Niger Delta where soil pollution from crude oil exploration and utilization is a major issue (Sam *et al*., 2017; Ite *et al*., 2018). Baseline site characterization is useful in the identification of pollutants and establishing their effect on the properties of the impacted soil (Azubuike *et al*., 2016). In this study, the baseline data presented in Table 2 confirmed crude oil pollution with TPH level higher than the regulatory intervention limit of 5000 mg/kg in Nigeria (DPR, 2002). Some soil properties are affected by crude oil in an impacted-soil (Bosma *et al*., 1997; Michel and Fingas, 2016). This possibly explains the higher values for electrical conductivity, bulk density and WHC in the polluted soil compared to the uncontaminated soil (Barua *et al*., 2011). Other properties like pH, porosity, total nitrogen and available phosphorus were higher in the uncontaminated soil compared to the contaminated sample. The sandy nature, porosity and the pH of the contaminated soil supports leaching and possible contamination of the ground water (Blum, 2013; Michel and Fingas, 2016). However, the low silt and clay content of the soil is suitable for microbial activity that drives bioremediation (Vidali, 2001). The acidic nature of the soil in Koko area due to petroleum hydrocarbon contamination corroborates report by Imasuen *et al*. (2014). The leaching of the basic cations due to heavy annual rainfall in the area (Imasuen *et al*., 2014) and production of organic acid intermediates from microbial action on the oil contributed to the acidic pH (Nwachukwu and Ugoji, 1995; Barua *et al*., 2011).

Table 2: Physicochemical properties of soil samples

Parameter	Contaminated	Uncontaminated
Particle size distribution		
Clay (%)	4.23 ± 0.14	4.26 ± 0.15
Silt (%)	1.78 ± 0.19	1.64 ± 0.26
Sand (%)	94.00 ± 0.30	94.47 ± 0.34
pH	5.55 ± 0.05	6.68 ± 0.31
Temperature (ºC)	28.70 ± 0.12	28.93 ± 0.30
Electrical Conductivity (µS/cm)	133.00 ± 3.61	74.53 ± 2.85
Bulk Density (g/cm^3)	1.31 ± 0.02	1.24 ± 0.02
Moisture Content (%)	9.44 ± 0.09	7.15 ± 0.37
Porosity (%)	50.72 ± 0.76	53.21 ± 0.65
Water Holding Capacity (%)	24.26 ± 0.63	9.47 ± 0.44
Total Organic Carbon (%)	2.87 ± 0.49	0.61 ± 0.16
Total Nitrogen (mg/kg)	265.87 ± 3.41	289.18 ± 1.73
Available Phosphorus (mg/kg)	40.47 ± 2.38	48.07 ± 2.09
Total Petroleum Hydrocarbon (mg/kg)	9906.40 ± 1.48	ND

Values are mean ± SE. ND is not determined.

3.1. Effect of Agitation and Moisture Content on Crude Oil Degradation in Polluted Soil

The various combinations of mixing or agitation and moisture content replenishment studied yielded different results for residual total petroleum hydrocarbon performed every 6 days. The results are presented in Figure 1. All treatment groups evaluated were significantly different from the control ($p<0.05$) with higher crude oil degradation. However, A5 with 79.34 ± 0.03% crude oil degradation following sample agitation every 3 days and moisture adjustment every 6 days for 24 days was more efficient, and so it was used in further experiments in the study. The daily monitoring of the MC of the contaminated soil sample after adjustment to about 80% of field WHC revealed that this reduced to 57.34 ± 0.72% on day 5 at room temperature (Table 3). The minimum benchmark WHC for optimum bioremediation used in this study was 60% (Bahmani *et al*., 2018).

3.2. Effect of CNP Ratio on Crude Oil Degradation in Polluted Soil

The CNP ratio 100:10:1 that is widely reported in literature to be the optimum nutrient ratio for microbial transformation of crude oil in soil was compared with CNP ratio 100:2:0.2. The later resulted in 90.99 ± 0.02% against 78.15 ± 0.03% of the former (Figure 2) after 36 days. This difference was significant ($p<0.05$).

Table 3. Daily moisture content monitoring in contaminated soil sample after moisture adjustment

Day	Moisture Content (%)	Water Holding Capacity (%)
	9.44 ± 0.09*	38.91 ± 0.38
0	19.49 ± 0.10	80.33 ± 0.42
1	19.01 ± 0.19	78.34 ± 0.78
2	18.46 ± 0.10	76.09 ± 0.42
3	16.96 ± 0.30	69.92 ± 1.26
4	15.05 ± 0.10	62.03 ± 0.41
5	13.91 ± 0.17	57.34 ± 0.72
6	12.48 ± 0.21	51.42 ± 0.85

Values are mean ± SE of triplicate determinations. *Moisture content of sample before adjustment to 80% of water holding cpacity. Baseline water holding capacity = 24.26 ± 0.63%.

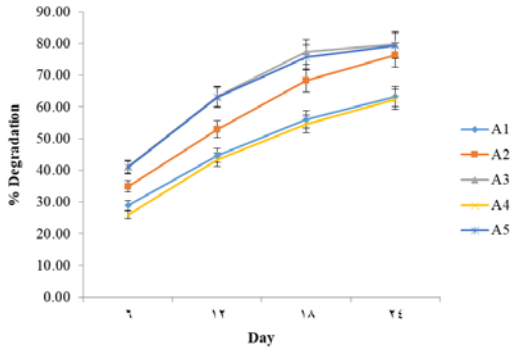

Figure 1. Effect of agitation and moisture adjustment on crude oil degradation. A1 (daily agitation with moisture adjustment every 3 days), A2 (agitation and moisture adjustment every 3 days), A3 (daily agitation and moisture adjustment every 6 days), A4 (agitation and moisture adjustment every 6 days), A5 (agitation every 3 days and moisture adjustment every 6 days).

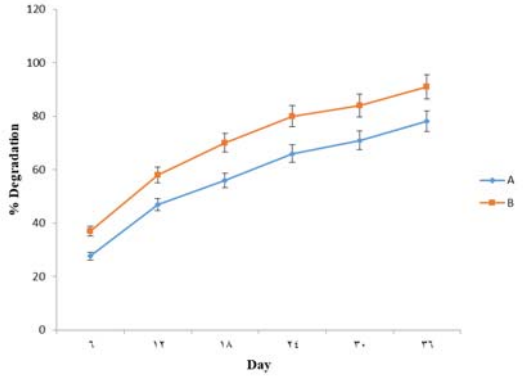

Figure 2: Effect of carbon, nitrogen and phosphorus ratio on crude oil degradation in polluted soil. A (100:10:1) and B (100:2:0.2).

Agitation (mixing) every 3 days and moisture content adjustment every 6 days resulted in more efficient crude oil degradation in this research. These strategies have been reported to enhance oil degradation (Azubuike *et al*., 2016). Periodic tilling or agitation of soil increases microbial activity during bioremediation through improved aeration, increased nutrient availability and also pollutants (Tibbett *et al*., 2011; Azubuike *et al*., 2016). Adequate water is needed for growth and mobility of microbes as well as movement of nutrients, oxygen and waste products (Bahmani *et al*., 2018). The addition of water at regular intervals helps to compensate for moisture loss due to evaporation and maintain optimum level of moisture content in the soil during bioremediation (Bahmani *et al*., 2018).

Microbes in soil require nutrients like carbon, nitrogen and phosphorus to support their metabolic activities (Bamforth and Singleton, 2005; Ghaly *et al*., 2013). The indigenous oleophilic microbes in a crude oil polluted soil mineralize the pollutant as source of carbon to bring about a distortion of the nutrient ratio following depletion of available nitrogen and phosphorus in the impacted soil with time (Ghaly *et al*., 2013). This is responsible for the lower values of total nitrogen and available phosphorus obtained in this study for the polluted soil (Table 2). The addition of the limiting nutrients (nitrogen and phosphorus) to the crude oil polluted soil stimulates microbial metabolic activities during the remediation process (Walworth *et al*., 2007; Jiang *et al*., 2016; Safdari *et al*., 2018).

Several nutrient sources that may be organic or inorganic have been used to enhance bioremediation (Koshlaf *et al*., 2016; Kumari *et al*., 2016). Quantification of required nutrient level in bioremediation studies is easier when inorganic nutrients are used (Suja *et al*., 2014; Shahi *et al*., 2016). Achieving the desired CNP ratio is an important consideration in bioremediation optimization (Huesseman, 1994; Onwosi *et al*., 2018). Urea containing 46.6% of nitrogen and potassium dihydrogen phosphate composed of 22.8% phosphorus were used in this study. Again, the limiting nutrients when supplied at high concentration to the bioremediation system can inhibit microbial activity hence the need for optimum CNP ratio (Huesemann, 1994; Walworth *et al*., 2007). Although the CNP ratio 100:10:1 has been reported to be the optimum ratio for oil bioremediation in soil (Wu *et al*., 2016), in this study, 90.99 ± 0.02% degradation with CNP ratio of 100:2:0.2 after 36 days remediation was significantly different from 78.15 ± 0.03% obtained with CNP ratio 100:10:1.

4. Conclusion

The widespread crude oil pollution with the associated negative impact on the environment in oil producing regions of Nigeria requires an enhanced strategy for bioremediation, which is the preferred cleanup approach. Such a plan will benefit from site-specific characterization to identify its peculiarity that will be factored into the design of an effective restoration approach. In this study, the optimization of parameters including agitation, moisture level and nutrient ratio enhanced the rate of bioremediation of aged crude oil polluted soil.

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