Influence of Microbial-Induced Calcite Precipitation on Compaction Characteristics of Bioremediated Crude Oil Contaminated Soil using Composting Technique

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ORIGINAL RESEARCH ARTICLE

Abstract- Compaction as a soil improvement method of improving the engineering performance of soil was carried out on remediated contaminated soil with the aim of assessing the influence of microbial induced calcite precipitation (MICP) on compaction characteristics. Autochthonous bacteria were identified as *Bacillus licheniformis* and isolated from oil contaminated soil, collected from Kaduna Refinery at Area W where the crude oil was stored. The bacteria were enhanced with organic liquid nutrient extracted from chicken droppings (CD) in stepped concentration of 10, 20, 30, and 40% by dry weight of soil sample and used to hydrate the contaminated soil. The mixtures were kept in four microcosms for 3 months. The initial total hydrocarbon content was 98g/kg. The bioremediated soil was treated with bacteria solution through MICP technique. The results revealed that sample treated with 30% CD yielded the highest removal efficiency of 89.8% of oil removal. Maximum Dry Density increased with addition of *B. licheniformis* solution to a peak value of 1.93Mg/cm^3 for sample treated with 1.5×10^8 cell/ml. Optimum moisture content (OMC) increased with increased in *B. licheniformis* suspension from 14.4 to 15.8% when treated with 1.5×10^8 and 2.4 ×10^9 cell/ml respectively. Compaction characteristics of soil treated with 30% CD were greatly improved at 1.5×10^8 cell/ml bacteria solution using MICP technology and could be used as liner material in waste management.

Keywords- Bioremediation, compaction, contaminated soil, calcite, liner

1 INTRODUCTION

Soil compaction is an important aspect of civil engineering construction aims at stabilizing or improving soil. It is a soil improvement method that is carried out for the purpose of improving the engineering performance of soil by densification of the soil in order to expel air from the pores through the application of mechanical efforts (Murthy, 2002. Compaction is applied in infrastructures development such as waste containment facility for solid waste disposal, airport runway, earth dam, highway embankment, railway embankment and road pavement.

Maximum dry density (MDD) and optimum moisture content (OMC) are basic indicators of compaction characteristics that describe both laboratory and field compaction operations (Salahudeen *et al.,* 2018). Relationship exists between moisture content and dry density because moisture content dictates behaviour of soil. When moisture content is low, soil becomes stiff and very hard to compress resulting to low unit weight and high air content. However, with increase in moisture content, water serves as a softener to soil making it easy to slip over one another. Sani *et al.,* (2018) and Etim *et al*. (2019) investigated improvement of compaction characteristics of lateritic soil using calcium chloride (at 4, 6, 8%) and periwinkle shell ash, respectively.

Similarly, Yohanna *et al.,* (2021) examined the influence of iron ore tailings on compaction characteristics of black cotton soil and lateritic soil. The Niger Delta region of Nigeria and other parts of the world that produce oil are facing serious challenges due to oil spillage. It has been established that crude oil contamination has negative effects on the geotechnical properties of soil such as compressibility, permeability, bearing capacity, MDD and OMC (Rahman *et al*, 2010; Huang, and Lu, 2014. The physical, mechanical and chemical methods of remediation of oil such as containment, confinement, air stripping, chemical leaching and thermal desorption exist, however, these methods are very expensive, require high energy, acidizing or changing the structure of soil and environmentally unfriendly (Achal *et al*.; 2012). The disadvantages of these methods resulted to the idea of using biological method known as bioremediation which is a sustainable remediation technique that utilises microbial species in hydrocarbon removal (Azubuike *et al*., 2016). Bioremediation is environmentally friendly, up-scalable and can be achieved through biostimulation or bioaugmentation.

Biostimulation involves changing the environment of the microorganism by injection or addition of nutrient, moisture, raising or lowering the pH level, temperature or oxygen, to encourage the population and capacity of microorganisms in bioremediation (Adams *et al*., 2015 and Azubuike, *et al*, 2016). Thus, biostimulation strategy is a kind of natural process of oil removal that could eventually lead to total degradation of the contaminants especially when compatibility of the contaminants, abiotic factors and indigenous microbes are well evaluated. Bioaugmentation is the addition of microorganism to improve the capacity of the indigenous microorganism to degrade oil. In this current study,

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chicken droppings and moisture addition were used to stimulate autochthonous bacteria known as *Bacillus licheniformis* (*B. licheniformis*) Previous studies have shown the potentialities of utilizing animal waste and plants as nutrients in the bioremediation of crude oil contaminated soils (Udeh, Nwaogazie and Momoh, 2013; Ijimdiya and Alhassan, 2017). Researchers have conducted studies on the suitability of different materials as liner (Sabat and Nayak, 2015).

The MICP has been described as a sustainable technique which is useful in the improvement of soil construction materials using microbe (Kadhim and Zheng, 2016). Therefore, MICP is useful in studies that involved strength improvement, permeability reduction, seismic mitigation and erosion control measures (Kim and Youn, 2016). Wei *et al.* (2015) reported that MICP has the potentials of bringing down the cost of construction due to the possibility of producing microbial cement at lower cost with environmental benefits. The chemistry behind MICP is that the bacteria involved releases urease enzymes which catalyses the hydrolysis of urea to yield carbonate ions and Ammonium ion as shown in Equation (1). From Equation (2), the carbonate ions then react with calcium rich solution (i.e., calcium chloride) to form calcium carbonate (calcite) which is useful in increasing strength of soil.

$$CO(NH_4) + 2H_2O \xrightarrow{urease} 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \xrightarrow{yield} CaCO_3(s) \quad (2)$$

Engineered landfill is a modern waste disposal facility designed to contain municipal solid waste deposits in an environmentally safe manner. The components of engineered landfill include liner and cover which prevent the movement of leachate into underground water Sabat and Nayak (2015). However, there is little or no documented report in the literature that mentioned bio-remediated crude oil contaminated soil was treated using microbial induced-calcite precipitation (MICP) with a view to improving the engineering properties of soil such as compaction characteristics.

Therefore, this research aimed at assessing the influence of microbial induced calcite precipitation on compaction characteristics of bioremediated crude oil contaminated soil using composting technique for use as liner material in waste containment management. The specific objectives were to evaluate the compaction, determine the optimum amount of CD and bacteria solution required in bioremediation for MICP, respectively.

2 Materials and Methods

2.1 Collection of Soil Samples

The crude oil contaminated and uncontaminated soil were collected from NNPC Kaduna in accordance with BS 1377 (1990).

2.2 Poultry Manure

Chicken dropping was collected from a Poultry house at Staff Quarters of the Ahmadu Bello University, Zaria-Nigeria. The function of chicken droppings was to supply the autochthonous bacteria in the soil with the necessary nutrients' nitrogen, phosphorus and potassium (N. P. K.) only (Ijimdiya and Alhassan, 2018).

2.3 Extraction of Organic Liquid Nutrient

The CD were air-dried and used in stepped concentrations of 10, 20, 30 and 40% of dry weight of the sample. The CD was ground to powder and the powdered chicken dropping was dissolved in water to form slurry as described by Ford and Fleming (2002). Sieve No 200 was used to filter the bio slurry formed and the liquid nutrient was obtained.

2.4 Composting Technique for Bioremediation

Soil samples were prepared in accordance with BS 1377 (1990). The liquid nutrient extracted was mixed with the contaminated soil containing the indigenous bacteria. The mixture was blended thoroughly in order to obtain a homogenous mix. The blended samples were kept in plastic containers known as microcosms for 90 days. The samples were kept at constant moisture corresponding to the optimum moisture of content of soil (1525, 870, 550, 255 ml for 10, 20, 30 and 40% CD, respectively) for bioremediation by moisture addition at 7days interval (Ijimdiya and Alhassan 2018). The moisture of the microcosms was monitored at 2days interval using VG-200 soil moisture meter and maintained corresponding to the optimum moisture of the soil. This was done to ensure the survival of the microbes and maintain the optimum moisture content of 12-25% recommended by Adams *et al.* (2015).

2.5 Total Hydrocarbon Content (THC) Test

Samples were collected from the microcosms at an interval of 2 weeks for THC test. The THC was determined according to US EPA 418 (1993) by gravimetric method as described by Vinothini *et al.*, (2015). Equation (3) was used in calculating THC:

$$\text{THC} = \frac{(initial\ weight\ of\ soil - weight\ of\ soil\ after\ soil\ extraction)\ g}{initial\ weight\ of\ soil\ kg} \quad (3)$$

2.6 Determination of pH of Soil

The sample's pH determination was performed according to BS 1377-3: 1990 using electrometric method with pH meter (PHS-25; Techmel & Techmel, USA) in ratio of 1: 2.5 (w/v) soil/water mixture.

2.7 Temperature Measurement

The temperature measurement was carried out using temperature meter (PHS-25 by Techmel & Techmel, USA). The probe was inserted into the microcosms at three different positions and allowed for 5 minutes. Temperature values were obtained from the digital display of the temperature meter.

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2.8 Isolation and Identification of Bacteria

The bacteria resident in the soil samples were isolated using serial dilution and agar plating method in which the diluted sample from the last vial bottle was spread on the plate and inoculated at 37°C for 24 hours. The inoculants were then cultured in a liquid media. The liquid media were sterilized by autoclaving for 20 minutes at 121°C. The isolated bacteria were identified by using bacillus test kit. After the inoculation period, plates were observed for growth. The growths on nutrient agar (colonies of bacteria) were enumerated and results were expressed as colony forming unit per gram (Cfu/g) using the following Equation (4):

$$cfu/g = \frac{total\ number \times dilution\ factor}{Volume\ of\ inoculation} \quad (4)$$

2.9 Bacteria Cell Solution for MICP Technology

A cultured *B. licheniformi* bacteria solution was used in stepped concentrations of *B. licheniformis* concentration of 1.5×10^8, 6.0×10^8, 1.2×10^9, 1.8×10^9 and 2.4×10^9 cell/ml.

2.10 Cementation Reagent for MICP Technology

Cementation solution serves as the raw materials for calcite formation in the MICP process and its constituent include 20 g of urea ($CO(NH_2)_2$), 2.8 g of calcium chloride ($CaCl_2$), 3 g nutrient broth, 10 g ammonium chloride (NH_4Cl), and 2.12 g sodium bicarbonate ($NaHCO_3$) per litre of distilled water as described by (Shahraki *et al.*, 2014).

2.11 Sample Preparation for MICP Treatment

After bioremediation, the soil sample was air- dried for 3days, pulverized and sieved in accordance with BS 1377 (1990). The soil samples were treated with bacteria solution. The quantity of the bacteria (QB) and cementation reagent (QCR) concentration were obtained using the following expression:

$$QB = \frac{(50\%\ \text{of the natural OMC for energy level})}{100} \times 3000g \quad (5)$$

$$QCR = \frac{(50\%\ \text{of the natural OMC for energy level})}{100} \times 3000g \quad (6)$$

2.12 MICP Treatment

The soil samples were saturated with *Bacillus licheniformis* in stepped concentrations of 1.5×10^8 /ml, 6.0×10^8 /ml, 1.2×10^8 /ml, 1.8×10^8 /ml and 2.4×10^8 /ml and properly kept in an air-tight material (polythene) for 12hours in the laboratory at room temperature 25 ± 2 °C for the bacteria to fix themselves onto particles of soil. The soil samples were compacted into the 1000cm^3 compaction moulds at optimum moisture contents compacted using three compactive efforts namely BSL, WAS and BSH in accordance to BS 1377 (1990). After fixing the bacteria, the soil samples were mixed on a tray with the quantity of cementation reagent that formed the remaining 50% of the OMC.

2.1.13 Compaction

After mixing both bacteria and cementation reagent with the soil samples, compaction test was conducted on the bio-remediated soil in accordance with BS 1377- 4: 1990 using British standard light, British Standard heavy and West African Standard. were used and compacted using three compactive efforts, namely British Standard light (BSL), West African Standard (WAS) and British Standard heavy (BSH). The 1000 cm^3 mould was used in compacting the sample in three layers of approximately equal part with each layer receiving 27 blows from a 2.5 kg rammer falling through 300 mm for BSL compactive effort; samples compacted using WAS compaction energy were compacted in five layers each receiving 10 blows from a 4.5 kg rammer while samples compacted for BSH compactive effort were compacted in five layers receiving 27 blows from a 4.5 kg rammer. The O.M.C corresponds to the MDD from the graph of dry density against moisture content.

3 Results and Discussion

3.1 Characterization, Identification and Enumeration of the Bacteria

The bacteria species in the crude oil contaminated and uncontaminated soils were characterized based on their reaction with Gram's staining reagent, morphology and cell arrangement. The result of Gram staining reaction was positive and large motile with chain arrangement and rod–shaped with pair arrangement were observed in contaminated and uncontaminated soil respectively, thus *Bacilli sp* were suspected and this is in agreement with Nabti *et al.* (2013). Results of the 24- and 48-hours reaction yielded the profile number (octal code) of 70772036 and 50672004. Similarly, when the numbers were submitted to the GENMAT software (microbiology software) the bacteria were identified as *Bacillus licheniformis* and *Bacillus megaterium* respectively.

Table 1 shows the population of bacteria in samples treated with dosage of chicken droppings. It was observed that microcosm treated with 30% CD had the highest population of 7.24×10^8 while further increase in the quantity of CD produced 6.67×10^8. This may probably be attributed to the ability of the 30% CD to satisfy the ratio of C: N: P needed for bioremediation. This is in agreement with Burghal *et al*. (2015) who reported that microorganism's stimulation must be in a proper ratio of carbon, nitrogen and phosphorus (C: N: P) ratio of 100:10:1, to facilitate microbial proliferation and activity.

Table 1. Microbial population of *B. licheniformis* of samples collected at 12 weeks

CD content (%)	Microbial Population (*cfu/g*)
0	4.97E+07
10	5.26E+08
20	6.41E+08
30	7.24E+08
40	6.67E+08

3.2 Effects of Chicken Droppings and Bioremediation Time on the Total Hydrocarbon Content Contaminated Soil

Figure 1 shows the variation of total hydrocarbon content with time of bioremediation for various CD contents. The concentration of hydrocarbon in contaminated soil before

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treatment was 98g/kg, when chicken droppings (CD) were added in stepped concentrations of 10, 20, 30, and 40% by dry weight of the sample, it was observed that the sample without chicken droppings (control or 0%CD) had the least hydrocarbon concentration of 8g/kg at the end of 12 weeks of remediation period. The probable reason may be due to lack of nutrient to activate the indigenous bacteria. However, sample treated with 10% CD showed an improvement in oil degradation over the 0% CD wherein 50g/kg of THC was lost. Similarly, for sample treated with 20%CD, a high value of 63g/kg was obtained at 12th week of remediation time. For sample treated with 30% CD, hydrocarbon content increase from 22 to 88g/kg at 2 to12 weeks, while 40% CD yielded 86g//kg crude oil loss in 12 weeks. This indicated that 30% CD resulted to lowest decrease in hydrocarbon content of 10g/kg. This might be due to increase in microbial population from 4.97×10^7 for the untreated to 7.24×10^8 *(cfu/g)* for sample treated with 30%CD which led to higher microbial activity.

The samples treated with 30% CD recorded highest removal efficiency of 89.8%, followed by sample treated with 40%CD with 87.67%. The least removal efficiency of 10.2% at 12 weeks period of remediation was obtained when there was no addition of CD to the sample (0%CD). This indicated the ability of the stimulant (chicken droppings) to activate the bacteria. This agrees with finding made by Checkroud *et al.*, (2011) who reported a reduction of 88-90% of oil when studied the biodegradation of oil in marine medium using consortium of *Pseudomonas sp* and *Rhodococcus sp*. However, the result is in contrast with that of Jabbar *et al.* (2017) who reported a removal efficiency of 75% from the sample treated using biopile system for bioremediation while 38% reduction was reported for the control.

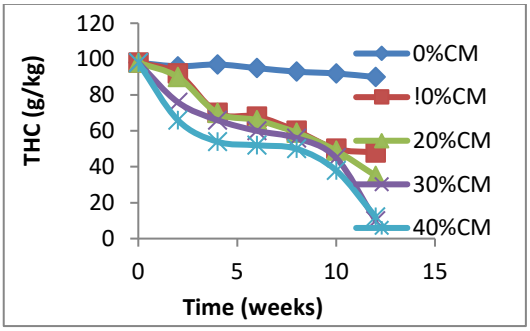

Fig. 1: Variation of THC with time of remediation

3.3 Effects of pH and Temperature on the Bioremediation of Crude Oil Contaminated Soil

The pH and temperature are important parameters that influence the growth and survival of microorganism during bioremediation process (Abatehn *et al.*2017). Table 2 shows the results of the pH and temperature of the microcosms at the 12th week. It was observed that the highest pH value was obtained from sample treated with 30%CD content while sample treated with no nutrient (0%CD) produced the lowest value of pH due to presence of crude oil. Therefore, higher pH might probably be attributed to higher microbial activity in 30% CD treatment which led to higher population of microbes that resulted to greater hydrocarbon degradation. Generally, the pH of the samples ranged from 6.92 to 7.99, which fall within the recommended optimal pH range of 6.9-8.5 for bioremediation (Ujuwundu *et al.*, 2011 and Abatehn *et al.*2017).

The population of microbes in sample treated with 30% CD was higher which led to higher microbial activity thus; a higher temperature value was obtained. Temperature encourages microbe to be metabolically active and speed up the degradation process of the contaminants (Abatehn *et al.*,2017). It was observed from Table 2 that crude oil loss increased with increase in temperature from 26.74, 27.09, 27.84 and 28.39 °C for 50, 63, 86 and 88g/kg, respectively. This may be the reason for proportionality with temperature. This finding is in agreement with that of Ujuwundu *et al.*, (2011).

Table 2. pH and Temperature of samples treated with CD contents at week 12

Parameter	CD (%)				
	0	10	20	30	40
pH	6.92	7.5	7.71	7.99	7.81
Temperature	26.1	26.74	27.09	28.39	27.84
THC (g/kg)	8	50	63	89.8	87.6

3.4 Index Properties

The percentage passing BS No 200 sieve for the crude oil contaminated soil (untreated) and natural soil were 13.01% and 46.5%, respectively, with liquid and plastic limits of 42.2% and 35.44%, respectively, for the crude oil contaminated soil and 45.06% and 37.09% for natural soil respectively. It was observed from Fig. 4.1 that the untreated soil has percentage of fine and coarse sand fraction of 36% and 52%, respectively. This may be due to the presence of crude oil in the soil as contaminants which adhere to silt and clay sized particles and agglomerated them to form coarse sand particles. Similarly, the natural soil has 27% fine sand fraction with 43% coarse sand fraction. Similar findings were reported by Habeb-ur-Rahman *et al*, 2007; Rahman *et al.*,2010

3.5 Compaction Characteristics

3.5.1 Maximum Dry Density

Figure 2 shows the variation of MDD of bioremediated crude oil contaminated with dosages of *B. licheniformis* solution at BSL, WAS and BSH. It was observed that the values of MDD increased with increase in bacteria solution as shown in Figure 2. The MDD of sample treated with 1.5×10^8 cell/ml of *B. licheniformis* solution increased from 1.67, 1.72, and 1.83 Mg/m^3 for the untreated to 1.76, 1.81 and 1.93 Mg/m^3 when compacted using BSL, WAS, and BSH compactive efforts, respectively. for samples treated with 6.0×10^8 cell/ml of *B. licheniformis* solution, MDD values 1.7, 1.81 and 1.86 Mg/m^3 were obtained, compacted at BSL, WAS and BSH energy level respectively. When the bacteria content was increased to 1.2×10^9 cell/ml, MDD values of 1.69, 1.75 and 1.87Mg/m^3 were obtained for BSL, WAS and BSH compactive efforts,

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respectively. Similarly, 1.8×10^9 cell/ml of *B. licheniformis* solution, produced MDD values of 1.68, 1.77 and 1.84Mg/m^3 compacted at BSL, WAS and BSH energy level, while the highest population of microbial flora of 2.4×10^9 cell/ml, yielded the MDD values of 1.69, 1.73 and 1.86 Mg/m^3 at BSL, WAS and BSH compactive efforts.

Thus, the peak result of MDD occurred at 1.5×10^8 cell/ml, probably due to better attachment of bacteria cell in the soil that facilitated site of nucleation for calcite formation (Mujah *et al.*2016). The probable reason for the increase of MDD might be attributed to calcite formation within the soil matrix of the compacted specimens. This finding conforms to that of Sani *et al,* (2018) who explained that MDD increased with increased in calcium chloride and energy levels.

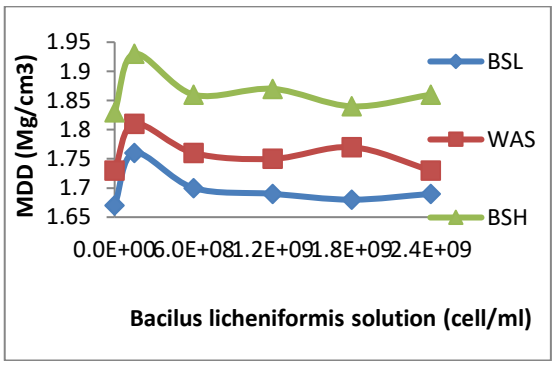

Fig. 2: Variation of MDD with *Bacillus licheniformis* suspension

3.5.2 Optimum Moisture Content

Figure 3 shows the variation of OMC of the remediated crude oil contaminated soil with five levels of *B. licheniformis* solution compacted using BSL, WAS and BSH compactive efforts. It was found that the OMC generally increase with bacteria solution concentration and decrease with increase in compactive efforts. When sample was treated with 1.5×10^8 cell/ml of *B. licheniformis* solution, compacted using BSL, WAS and BSH, the values of OMC obtained were 14.4, 13.1 and 12.5% respectively. For sample treated with 6.0×10^8 cell/ml of *B. licheniformis* solution, the values of OMC were 14.6,13.2 and 12.9 % for BSL, WAS and BSH respectively. Similarly, treatment with 1.2×10^9 cell/ml of *B. licheniformis* solution, produced 14.8,13.6 and 13.1 % compacted using for BSL, WAS and BSH compactive efforts, respectively. Further increase of bacteria concentration to 1.8×10^9 cell/ml yielded 14.9, 13.9 and 13.3 % values of OMC for BSL, WAS and BSH energy levels, respectively. The peak bacteria concentration used was 2.4×10^9 cell/ml which produced the highest values of OMC of 15.4, 14.1 and 13.9% for BSL, WAS and BSH energy level, respectively. The increase in OMC may be attributed to the calcite produced by the microbial flora. This is similar to findings made by Yohanna *et al.* (2021).

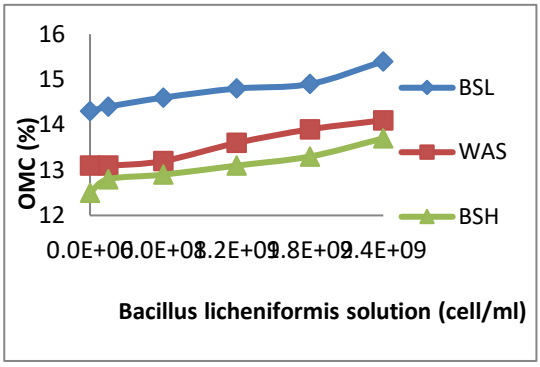

Fig. 3: Variation of OMC with *Bacillus licheniformis* solution

4 Conclusion

This study has shown that biostimulation strategy of bioremediation using composting technique with 30% CD as source of nutrient is achievable in crude oil degradation with *B. licheniformis*. The compaction characteristics as engineering properties of soil can be greatly improved by the MICP technology using culture of *B. licheniformis* at 1.5×10^8 cell/ml. Bioremediation of crude oil contaminated soil and MICP treatment are useful technologies for improvement of liner construction materials can reduce.

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