Comparative analysis of microbial loads of molecular potential chain disintegrator (MPCD) and potassium permanganate (PP) in remediation of hydrocarbon contaminated soil/groundwater in three dimensional (3D) laboratory sand tank model

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Abstract. This paper evaluates the comparative analysis of bacterial loads of molecular potential chain disintegrator (MPCD) and potassium permanganate (PP) in remediation of artificial hydrocarbon contaminated soil and groundwater in 3D laboratory sand tank. Alluvial sand was packed in the sand tank model, and 20 cm chamber was packed with prepared artificial diesel contaminated soil. Free flow of water was allowed through the soil medium from the inlet chamber which was being fed continuously from the storage elevated tank with the discharge port opened at flow rate of 58 ml/min. The remediating agents (MPCD and PP) were prepared differently and introduced through the injection wells (IW) to the sand medium at different experimental design stages. Microbial analysis was carried using standard techniques. The microbial loads of unpolluted soil samples collected from the sand tank model was $3.5 \times 10^4 \pm 1.5$ Cfu/g while the oil-degraders from the diesel contaminated soil was $9.5 \times 10^4 \pm 2$ Cfu/g. It could be observed that the population of oil-degraders in the MPCD amended soils and water decreased gradually during the incubation period of 15 days from $36 \times 10^4 \pm 4$ Cfu/g on day 0 to $12.5 \times 10^4 \pm 3.5$ Cfu/g for soil samples and from $58.5 \times 10^2 \pm 1.35$ Cfu/mL to $17.5 \times 10^2 \pm 0.5$ Cfu/mL for water samples. The decrease in the population of oil-degraders could be attributed to the reduction in the concentration of diesel in the soil and water samples undergoing bioremediation. The composition of the two nutrients (MPCD and PP) can be responsible for the ability of the nutrients to enhance the degradation process.

Keywords: Bacterial loads, Degradation, Groundwater, Remediating Agents, 3D Sand Tank.

1. Introduction

In recent years, oil pollution in the environment has been a major concern for most of the developing countries, and this has led to a global interest in the biodegradation of pollutants using biological agents, and other means of decontamination of pollutants. Introduction of chemical dispersants, skimming of the surface oils, inoculating the spilled areas with pertinent microbes are end results of intensive research, using chemical, physical and biological techniques respectively to remove the consequence of oil spillage on the environment and living organisms. The use of microorganisms has been the most encouraging of many researches carried out dealing with large-scale oil spills to provide an effective alternative [1]. This approach is referred to as bioremediation. [2] defined bioremediation as any techniques that utilize...
microorganisms or their enzymes to return the environment altered by contaminants to its original state of uncontaminated. Bioremediation is considered an environmentally and economically attractive alternative to physicochemical environmental decontamination methods, such as land filling, containment and incineration. This is because the microorganisms that are indigenous to the polluted sites have the ability to completely degrade and remove the contaminants from the polluted sites and transform the contaminants into less toxic end products. In addition, the bioremediation helps to restore the biological value of the environment, thereby, preserving the agricultural land. Bioremediation has been found to be a promising, cost-effective, worthwhile and innovative technology for use in the remediation of petroleum products. Introduction of nutrients to soil such as nitrogen fertilizers have showed to enhance biodegradation of PAHs [3]. The potential of animal wastes as hydrocarbon degrader in the remediation of hydrocarbon contaminants has been assessed in the research of [4]. In the earlier research of [5], it was established that animal wastes (Cow dung) has the potential of remediating hydrocarbon contaminated soil. Molecular Potential Chain Disintegrator (MPCD) is a non-flammable, colloid type, water based, pink aqueous solution with a density slightly heavier than water, the ingredients contained in the MPCD are Potassium Hydroxide, Non-ionic Surfactant, Ethanolamine, Dipotassium EDTA, Water. The potential of MPCD was evaluated in a 3D laboratory sand tank [6]. The physical characteristics of MPCD are stated in Table 1.

| Table 1. Physical characteristics of Molecular Potential Chain Disintegrator (MPCD) |
|---------------------------------|------------------|
| Boiling Point                  | 100°C            |
| pH                             | 14±0.5          |
| Specific Gravity               | 1.15±0.005      |
| Odor                           | No distinct odor |

2.0 Methodology
The laboratory sand tank model has a provision of a 20cm chamber in the middle of the cluster multilevel wells (Observation well B, C, and D) and the last multilevel well (Observation well E). (Figure. 1) Alluvial sand was packed in the middle chamber of the sand tank model and the diesel contaminated soils were packed in the 20cm chamber created in the downstream of the middle chamber, water was enabled to flow freely via the sand medium from the inlet chamber, which was being fed uninterruptedly from the storage elevated tank. The preferred discharge port was opened (1st Port) with the flow rate of 58 ml/min, while the other three (3) ports were closely locked.

2.1 Sample Preparation
2.1.1 Preparation of Soil Sample
Artificial soil Contamination was prepared as follows:
Mass of uncontaminated alluvial soil  20kg
Mass of diesel  2.1kg
Percentage by mass of diesel used

\[
\frac{2.1}{20} \times 100 = 10.5\% \\
= 1.05 \times 10^5 \, \text{mg of diesel/kg of the porous media} \quad [7]
\]

Plate 1a and 1b displayed the uncontaminated and contaminated sample [7].
Figure 1. View of 3D Laboratory Sand Tank Model (Isometric and Sectional) [8a and 8b]

Plate 1a. Alluvial Sand without Diesel Contaminant.

Plate 1b. Alluvial Sand mixed with 10.5% Diesel Contaminant.
2.1.2 Chemical Preparation (MPCD Preparation)
Concentrated MPCD (0.5 l) was diluted with 3l of water, thereafter the solution was introduced into the laboratory Sand tank through the 4 multilevel injection wells. Both water and soil samples were taken at 7th day and 15th day for dissolved oxygen test and microbial analysis.

2.2. Sample Preparation for 2nd Phase of the Experiment.
2.2.1. Soil Sample Preparation
The method used in the preparation of soil samples for the sand tank in the first phase of the experiment (see 2.1.1) was also adopted for the second phase experiment.

2.2.2. Preparation of Oxidizing Agent (PP)
175g of PP was measured in the laboratory and dissolved in 3.5l of distilled water (equivalent to ratio 50 g of PP to 1 litre of distilled water) stirred for about 20 minutes to acquired well dissolved solution of PP. Thereafter, the dissolved PP was injected via the 4 Multilevel wells (precisely, 29cl per injection well), soil and water samples were taken at 7th and 15th day respectively for both dissolved oxygen test and microbial analysis.

2.3. Microbial Analysis
The enrichment medium, Bushnell Hass Agar (BHA) was sterilized at 121°C for 15 min after which it was supplemented with 2% (v/v) filter sterilized diesel to serve as the only source of carbon [12]. The soil (g) and water (ml) samples were serially diluted and 1ml suspension was aseptically transferred from each 10^4 and 10^2 dilution respectively into sterile Petri dishes and seeded with BHA using pour plate technique. The medium was allowed to solidify and incubated at 30°C for 1 - 3 days. A control devoid of the sample was prepared for each set of experiments. All experiments were performed in triplicate. After incubation, the colonies that grew on the agar were counted.

3. Results and Discussion
3.1. Dissolved Oxygen Test. (DO)
The dissolved oxygen in the background water recorded was 5.48mg/l, while the result of the dissolved oxygen test carried out on the water sample artificially contaminated with diesel before the injection of any of the remediating agents gives 0.59mg/l. Table 2 shows the results of both the MPCD and PP remediated water samples, the results show the effect of the remediating agent on contaminant, the DO increases with day although not up to the value of DO obtained at the background.

| Samples | Dissolved Oxygen (mg/l) from Samples Remediated with MPCD Solution | Dissolved Oxygen (mg/l) from Remediated with PP Solution |
|---------|---------------------------------------------------------------|-------------------------------------------------|
| 7th Day | 2.14                                                          | 2.34                                                |
| 15th Day| 3.43                                                          | 3.63                                                |

3.2 Microbial Analysis
The total bacterial loads of unpolluted soil samples collected from the sand tank model was 3.5 x 10^4 ± 1.5 CFU/g while the total loads of oil-degraders from diesel contaminated soil was 9.5 x 10^4 ± 2 CFU/g. The total loads of oil-degraders contained in MPCD and PP amended soils and water undergoing bioremediation are presented in Figures 2a and 2b respectively. The oil-degraders present in the unpolluted soil samples were lower than the diesel polluted soil samples (Table 3). The diesel was a source of carbon to the microbes and the microbes obtained energy by breaking down the chemical bonds and transferring electrons away from
the diesel. The energy gained from the electron transfer was used along with the carbon and some electrons to produce more cells [9]. The presence of oil-degraders in the diesel contaminated soil and water in the 3D laboratory Sand tank is an indication that the indigenous microbes were carrying out their metabolic activity. The activities of these microorganisms could be responsible for the bioremediation of the environment as suggested by [10]. The MCPD and PP were to serve as sources of nutrients for the indigenous microbes, so as to enhance the biodegradation of diesel polluted soil and water [3]. The comparisons of the biodegradation of MPCD and PP on the soil and water samples undergoing bioremediation are shown in Figures 2a and 2b. It could be observed that the population of oil-degraders in the MPCD amended soils and water decreased gradually during the incubation period of 15 days from $36 \times 10^4 \pm 4 \text{Cfu/g}$ on day 0 to $12.5 \times 10^4 \pm 3.5 \text{Cfu/g}$ on day 15 for soil samples and from $58.5 \times 10^2 \pm 1.35 \text{Cfu/mL}$ to $17.5 \times 10^2 \pm 0.5 \text{Cfu/mL}$ for water samples. The decrease in the population of oil-degraders could be attributed to the reduction in the concentration of diesel in the soil and water samples undergoing bioremediation. This is in agreement with an earlier work carried out on bioremediation where the highest bacterial population was observed on day 7 and decreased throughout the incubation period of 35 days [11]. On the other hand, the population of oil-degraders in the PP amended soil and water increased from $22.5 \times 10^4 \pm 0.5 \text{Cfu/g}$ to $40.5 \times 10^4 \pm 0.5 \text{Cfu/g}$ on day 7 and the decreased to $31.5 \times 10^4 \pm 0.5 \text{Cfu/g}$ on day 15 for soil samples while there was reduction in the loads of oil-degraders from $5 \times 10^2 \pm 0 \text{Cfu/mL}$ to $2.5 \times 10^2 \pm 0.5 \text{Cfu/mL}$ on day 7 and increased to $8 \times 10^2 \pm 1 \text{Cfu/mL}$ on day 15. It could be observed that in PP amended samples there was no gradual decrease in the loads of oil-degraders as was in case of MCPD amended samples. Although bacterial loads can be used to monitor bioremediation process, it cannot be used in isolation. Other parameters such as optical density, pH, CO$_2$ evolution, temperature, GCMS/HPLS, etc are necessary to confirm the degradation of the diesel. The composition of the two nutrients (MPCD and PP) can be responsible for the ability of the nutrients to enhance the degradation process.

**Table 3. Population of Oil-Degraders from Soil and Water Samples used for the Bioremediation**

| Samples | Total Loads of Oil-degraders |
|---------|------------------------------|
| Unpolluted Soil samples collected from Sand Tank Model | $3.5 \times 10^4 \pm 1.5 \text{Cfu/g}$ |
| Diesel polluted soil | $9.5 \times 10^5 \pm 2.0 \text{Cfu/g}$ |
| Water sample used for MCDP amendments | $110 \times 10^2 \pm 1.0 \text{Cfu/mL}$ |
| Water sample used for PP amendments | $17.5 \times 10^2 \pm 0.5 \text{Cfu/mL}$ |
Figure 2a: Total loads of oil-degraders from the soil samples undergoing bioremediation

Legend:
- MPCD: Molecular potential chain disintegration
- PP: Potassium permanganate

Figure 2b: Total loads of oil-degraders from the water samples undergoing bioremediation

Legend:
- MPCD: Molecular potential chain disintegration
- PP: Potassium permanganate
4. Conclusion
From the study, the following conclusions were drawn:

i. the DO was 5.48 mg/l at the background water and reduced significantly to 0.59 mg/l after contaminated with diesel, the DO increases with day although not up to the value of DO obtained at the background.

ii. the MPCD and PP remediated samples DO increased but not up to the background value of the DO.

iii. it could be observed that the population of oil-degraders in the MPCD altered soils and water decreased gradually during the incubation period of 15 days from $36 \times 10^4 \pm 4$ Cfu/g on day 0 to $12.5 \times 10^4 \pm 3.5$ Cfu/g for soil samples and from $58.5 \times 10^2 \pm 1.35$ Cfu/mL to $17.5 \times 10^2 \pm 0.5$ Cfu/mL for water samples

iv. the composition of the two nutrients (MPCD and PP) can be responsible for the ability of the nutrients to enhance the degradation process.

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