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Effect of Nutrient Source on Indigenous Biodegradation of Diesel Fuel Contaminated Soil

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Abstract

Batch experiment was conducted to investigate the effect of carbon source on biodegradation ability of a diesel fuel contaminated soil. The contaminated soil was collected from near a surface oil tank located at ALBREGA Company sites in Libya. The initial diesel fuel concentration in the soil samples was determined to be 21,419 mg/kg of dry soil. The contaminated soil mixed either with poultry manure or urea fertilizer was applied into 50 ml microcosm jars and incubated at 37 °C for 12 weeks. After 12 weeks of treatment, the maximum TPH removal observed was 79.8% regardless of treatments applied. Soil treated with poultry manure showed 72.2% TPH removal, while soil treated with urea showed 79.8 % TPH removal. The lowest TPH removal (52.4%) was observed in the control treatment. Addition of either urea or poultry to contaminate soil would considerably enhance the biodegradation rate compared to the control treatments.

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1. Introduction

Bioremediation is based on the ability of microorganisms to destroy or degrade organic compounds or minimize the concentration of these materials by the detoxification or mineralization of the organic pollutants to carbon dioxide and water. Bioremediation technologies are an effective technique to mitigate many organic
and inorganic contaminants [3] such as hydrocarbons, halogenated organic compounds, halogenated organic solvent, none chlorinated pesticides and herbicides, nitrogen compounds, radionuclide, heavy metals (lead, mercury, chromium … etc). Consideration of the applicability of bioremediation to oil spills is not new. Bioremediation of petroleum hydrocarbons depends on the specific microbial population present. The composition of the microbial population is affected by the environmental conditions and the composition of the hydrocarbons [1]. Many companies are developing and/or marketing hydrocarbon-degrading seed cultures. Most microorganisms considered for seeding are obtained by enrichment cultures from previously contaminated sites. However, because hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats, adding seed cultures has proven less promising for treating oil spills than adding fertilizers and ensuring adequate aeration. Fertilization and tilling increased total aerobic heterotrophic and hydrocarbon degrading population in contaminated soil by one and three order of magnitudes, respectively [8]. The addition of fertilizer during the bioremediation has been proved very effective and essential to stimulate microbial growth as well as contaminates removal. However, inhibitory effects have also been observed when concentration of nutrients was excessive, mostly due to their toxicity to microbial species [7,9]. Other researchers [12] studied the effects of different concentrations of N-P-K fertilizer on the bioremediation of hydrocarbon in sludge wastes, and found that 240/80/80 mg/kg (N/P/K) addition was the optimal for CO₂ production in sludge samples with TPH concentration ranging from 60,000 to 200,000 mg/kg, while the microbial activities were impeded by almost 50 % when N/P/K concentration reached 6,000 - 2,000-2,000 mg/kg. Others reported that the addition of 15 % fertilizer increase the assimilation of crude oil in soil, especially the linear and branched alkenes [2]. Nutrients amendments had relatively large positive effect on bioremediation of diesel fuel contaminated soil, but showed an inhibitory effect on the biodegradation process through excessive addition [5]. If too much fertilizer is added that may increase soil pH and nitrogen concentration, and the osmotic potential of soil-water to the point where it will be that toxic to soil microbes [9,10].

Although there are many studies were conducted to evaluate the impact of addition of nutrients on the degradation rate of diesel fuel contaminated soil, yet there are some lack of information ‘to my knowledge’ on the importance of addition of either chemical fertilizer or organic fertilizer as an additive to stimulate the degradation rate of hydrocarbon contaminated soil. So this study aimed to compared the degradation rate of diesel fuel by using different types of fertilizer namely urea and poultry manure.

2. Material and Methods

2.1. Samples collection and characterization.

Contaminated soil samples with diesel fuel were collected from location nearby storage tank belongs to ALBREGA Company. Two different types of soil samples were collected: surface contaminated soil and surface uncontaminated soils. Surface contaminated soil samples were collected after marking a 0-20 cm area, digging a depth of 20 cm and transferring mass of 10 kg into plastic bags. A sample of uncontaminated soil, the same amount was collected at the same depth and at a distance of about 300 m from the contaminated area. The soil was packed in plastic bags, transferred to the laboratory and stored at 4 °C until used.

The soil was sandy type with more than 88.76% sand, 2 % silt and 9.24% clay. Using the hydrometer test, the water holding capacity (WHC) and the initial moisture content of the contaminated soil were 9% and 4.8%, respectively. The total microbial population was determined to be $2.12 \times 10^5$ cfu/g of soil on nutrient agar plates incubated at 37 °C. The microbial populations that were found to grow on diesel fuel - mineral salts medium was $9.9 \times 10^4$ cfu /g of soil at 37 °C. All types were defined to be Gram +ve and Gram -ve Bacillus spp. These isolates have successfully survived repeated subcultures on diesel fuel - mineral salts medium.
However, they grew more slowly on mineral salts medium compared to nutrient agar medium.

The contaminated soil had total extractable petroleum hydrocarbon (TPH) of 2.1% or (2,149 mg/kg) of dry soil; whereas, the total extracted hydrocarbon of the uncontaminated soil was 0.0105% (105.51 mg/kg) of dry soil. GC analysis of the contaminated soil prior to any treatment revealed that the soil was contaminated with weathered petroleum hydrocarbons in the range of C12 – C23. The absence of hydrocarbons shorter than C11 reflected the influence of weathering (evaporation). Furthermore, compounds in the range of C5 – C10 have been known to be inhibitory to the majority of hydrocarbon degraders. As solvents, these compounds tend to disrupt lipid membrane structure of microorganisms [6]. The absence of such volatile and toxic components should make biodegradation easier and more useful.

2.2. Treatments and Experimental Set-up

To investigate the effects of different types of nutrient on the rate of diesel fuel degradation, three different treatments in microcosm experiments were carried out in duplicate. The first treatment consisted of contaminated soil plus poultry manure (CS-org), the second consisted of contaminated soil plus urea (CS-U), and the third consisted of contaminated soil only (CS) as control.

All treatments were set-up in sets of screw cap glass jars as microcosms containing 50g of soil. Each treatment consisted of 12 jars. A total of 72 jars sacrificially sampled in duplicate sets every two weeks over a period of 12 weeks. The evaporation of water during incubation period was compensated by placing Derham tube filled with distilled water inside each jar. Also weekly tilling of all treatments was performed by inserting a stainless steel wire loop into the soil and mixing it in order to supply oxygen to microorganisms. Extraction of crude oil was carried out using soxhlet extraction method.

3. Results and Discussion

In this experiment, all values were expressed as the mean of two replicates. Fig. 1 to 3 represents the percentage of TPH removal in each treatment. For contaminated soil without any additive (CS), Fig. 1 shows that after the first two weeks, the (TPH) removal was 27% of the initial concentration. It is hypothesized that the reduction of TPH may be due to the improvement of some conditions such as humidity and ventilation. At the end of experiment, the total TPH removal was 52.4%. This suggests that there has been improvement in soil properties and conditions comparing with the conditions in the beginning of the experiment.

While previous studies by other researchers [4, 11] were not in support of the hypothesis that nitrogen and phosphorous based additives improve the biodegradation process, the result of this study found that TPH removal in the first two weeks for the contaminated soil received poultry manure (CS+ORG) remarkably increased to 58.8% comparing to that obtained with CS sample during the same period (Fig.1). After two weeks, a gradual increase in the removal of TPH (2.3%) was observed with CS-Org until the end of treatment period where the removal rate reached its maximum of 72%. Comparing CS-Org sample to the CS, the rate difference was 19.8% which is confirming the fact that the natural organic fertilizers would improve the rates of biodegradation.
For the treatment received urea additive (CS-U), it found that a TPH in the first two weeks was 63.4% (Fig. 2). After the second week as much as 20.6% removal was achieved which make up a total removal of 79.4% by the end of the experimental period. Comparing the effect of different type of fertilizer on the biodegradation of diesel fuel (Fig. 3), it found that the highest reduction rate was with the CS-U treatment. The results also demonstrate that regardless of type of fertilizer used, whether organic or chemical, the
addition of fertilizer had a positive impact on the rate of biodegradation of soil contaminated with diesel fuel compared to that observed with the CS treatment. This reduction was clearly observed during the first two weeks of the experimental run where the TPH reduction was about 64%, 59% and 27% for CS-U, CS-org, and CS, respectively. In other words, the addition of nutrients has enhanced biodegradation of petroleum hydrocarbons by almost 30% compared to the control treatment.

4. Conclusions

Both urea and poultry manure enhanced the removal of diesel fuel when compared to the control. However, this study demonstrated that urea as inorganic nutrients would stimulate more the rate and the extent of biodegradation of diesel fuel in soil. This may be attributed to nutrients bioavailability (nitrogen and/or phosphorus) is an enhancing factor in the removal of TPH of diesel fuel contaminated soils.

References


