THE ROLE OF BACTERIA IN THE MINERALIZATION OF DIESEL-BASE ENGINE OIL

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ABSTRACT

The mineralization of diesel-based engine oils produced by African Petroleum (AP) was investigated for a period of twenty-eight (28) days. Two different samples of AP engine oils, Heavy Duty Visco SAE40 (H40) and Diesel Motor Oil SAE 40 (D40) were investigated. Soil from a mechanic workshop at Irediawa Junction, along Ekenwan Road, Benin City, Edo State, Nigeria served as seed for the mineralization. The study was carried out at room temperature (27-29^{0}C). The pH of the test systems ranged from 6.5 to 7.3. The counts on the first day were 2.52x10^4 and 2.71 x10^4 for H40 and D40 respectively. Whereas the corresponding counts in 28th day were 2.04 x10^5 and 2.03 x10^5 in colony forming unit per milliliters (cfu/ml). The hydrocarbon utilizing bacteria (HUB) isolated include Alcaligene, Bacillus, Citrobacter, Micrococcus, Proteus, Pseudomonas, and Vibrio. The physicochemical analysis showed that nitrate, phosphate, sulphate, biochemical oxygen demand, (BOD), oil and grease content and total organic carbon (TOC) decreased with time. The rate mineralization between the two engine oils was in accordance with the normal growth rate for a close system. There was no significant difference between the rates of mineralization of the two engine oils.

Key Word(s): *Mineralization, Hydrocarbon Utilizing Bacteria (HUB), Diesel-Base Engine Oils, Biochemical Oxygen Demand (BOD) and Total Organic Carbon (TOC).*

INTRODUCTION

As the usage of petroleum hydrocarbon products increase, soil contamination with engine oils is becoming one of the major environmental problems. Mineralization is the complete conversion organic compounds to the more mobile energetic state or a minerals form [1]. An oil polluted soil can be remedied by mineralising the engine oil pollutants which is carried out through biodegradation and biotransformation of the organic constituent of the engine oil. Microbial degradation of complex compounds does not always result in mineralization. Incomplete degradation, also called transformation of

the compound may occur as a result of microbial activity [2]. Most mechanical methods used to reduce hydrocarbon pollution is expensive, time consuming and depends mainly on excavating of these soil, treating in separate area or better treatment facilities. These treatments include incineration and/or burial in secure landfills. These are effective treatments but after burning, this soil loses most of its nutritional value and structure. These methods do not remove the contamination but only relocate the problem [3]. Bioremediation processes have been shown to be effective methods that stimulate the biodegradation of contaminated soil [4] and may restore contaminated soils through the broad biodegradation capabilities evolved by microorganisms towards undesirable organic compounds [5].

The present study was therefore undertaken with a view to elucidate the ability of microbial isolates on the mineralization of used engine oil and oil contaminated soil by bacterial consortium. Bacteria and fungi are the key agents of degradation, with bacteria assuming the dominant role in marine ecosystems and fungi becoming more important in freshwater and terrestrial environments. While hydrocarbon-degrading microorganisms are ubiquitous, hydrocarbon-degraders normally constitute less than 1% of the total microbial community. When oil pollutants are present these hydrocarbon-degrading populations increase, typically to 10% of the community [6]. Biodegradation of oil contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as one of the efficient, economic, versatile and environmentally sound treatment [7]. Biodegradation of petroleum hydrocarbon pollutants and petrochemicals by bacteria have been extensively investigated by [8];[9];[10];[11];[12].

MATERIALS AND METHODS

The soil samples were collected at Irediawa junction along Ekenwa road, Benin City, Edo State, Nigeria. Soil samples for biological analysis were obtained from different points (about ten meters apart) and pooled together to form composite samples [13]. Two types of used engine oils produced by African Petroleum (AP) were obtained from the filling station located along Akpakpava Road, Benin City, Edo State, Nigeria. Heavy Duty Visco SAE40 (D40) and Diesel Motor Oil SAE 40 (D40) were aseptically taken to the laboratory for analysis. The soil sample was weighed and 10g was dissolved in 100ml of distilled water to obtain a ten-fold (10 fold) dilution of the soil sample as used by [14]. Toxicity test was conducted to obtain non-toxic concentration. The test was conducted by measuring equal volumes (10ml) from the 10-fold dilution of the soil sample into four conical flasks. Different volumes of 0.1ml, 1.0ml and 10.0ml of the engine oils were transfer into the flasks. One of the flasks served as control which was free from engine oil. The volumes were made up to 100ml with sterile distilled water. This resulted to concentrations of 1mg/l, 0.1mg/l and 0.01mg/l of the engine oils. The culture was incubated for 24 hours at 37^0C. The non-toxic concentrations of the two engine oil were determined by plating out in a nutrient media by spread plate method and were incubated for 48 hours at 37^0C. The sample inoculated with engine oil that produced similar counts with control after two days of incubation was taken as the non-toxic concentration of the oil to the organisms.

The biodegradation conditions of the experimental set up were monitored by withdrawing the samples before initiation of the experiment at the first day and subsequently at day 7, 14, 21 and 28 for microbiological and physical chemical analysis. Total heterotrophic bacterial (THB) counts of the withdrawn samples were performed. Serial dilution using normal saline (0.85%) as diluents was done and spread plate technique was adopted using Nutrient agar and incubated for 48h at 37^0C and room temperature for 5days respectively [15]. Vapour phase transfer method was adopted in estimating the population of hydrocarbon utilizing microorganisms. Mineral salt agar with a sterile filter paper (whatmann No. 1) saturated with the oil which supplied the hydrocarbon by vapour phase transfer to the inocula were incubated (room temperature for 5 days). Physicochemical analyses were carried out such as pH, total organic carbon, biochemical oxygen demand (BOD), oil and grease analysis, alkalinity analysis, sulphate content, nitrate content and phosphate content to determine the rate of degradability of the engine oils.

RESULT

The growth of bacteria population in the medium is made possible by the availability of nutrient. Therefore, limitation of nitrogen and phosphate content in the medium limits the bacteria mineralization of hydrocarbon in the medium. The pH ranged between 6.5 and 7.3 and such that favours heterotrophic bacteria (fig. 3). This was as a result of the production of metabolic products released into the test system; thereby contributing to the pH fluctuation around neutrality. The nutrient content (nitrate. fig 5, phosphate. fig 8, and sulphate, fig 7) decrease during the investigation even when they were also produced as metabolic products. The reduction showed that the rate of bacteria utilization of nutrient was faster than the rate which product were released, hence the nutrient reduction. For biomass building, there was a correspondence in nutrient utilization indicating the relative importance in cell metabolism as state by Odum's combine law. Total organic carbon (TOC), was used as a quality control in the mineralization process (fig 1). The percentage of change during the process apparently showed that the H40 (16.24%) was most mineralised than D40 (30.03%), but T-test conducted indicated that there was no significance difference between the mineralization of both test samples. The biochemical oxygen demand of the test sample decreased continuously indicating a gradual re- aeration of the test system. (fig 4).

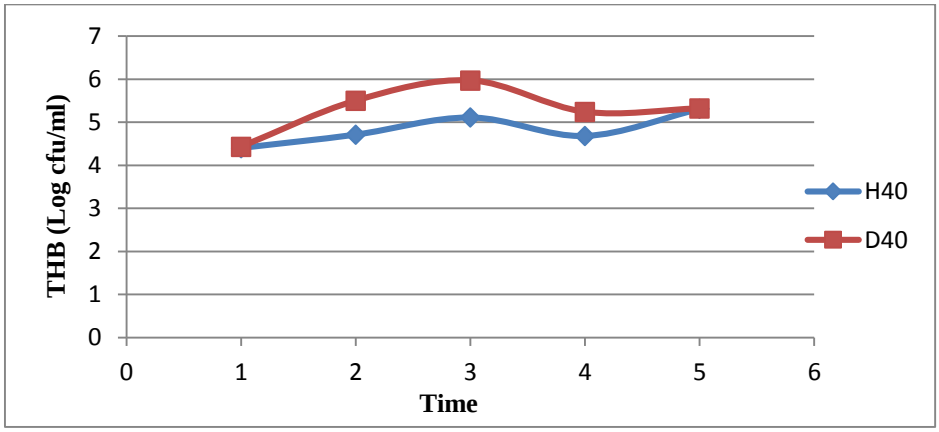

Fig 1. Change in total heterotrophic bacterial count (Log cfu/ml) of the test system

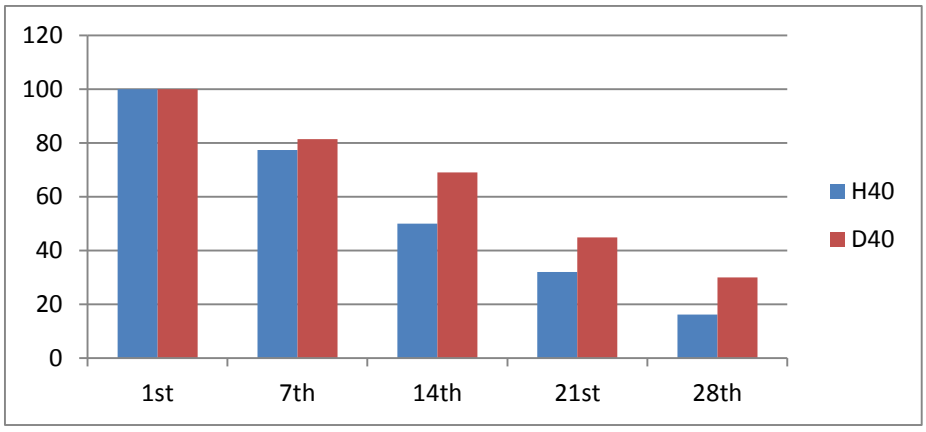

Fig 2. Change in percentage (%) of total organic carbon of the test system with days.

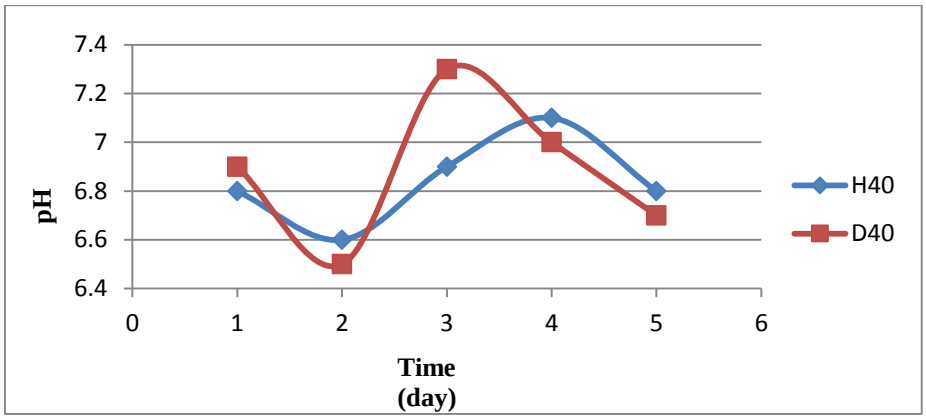

Fig. 3. Change in pH of the medium for the test systems.

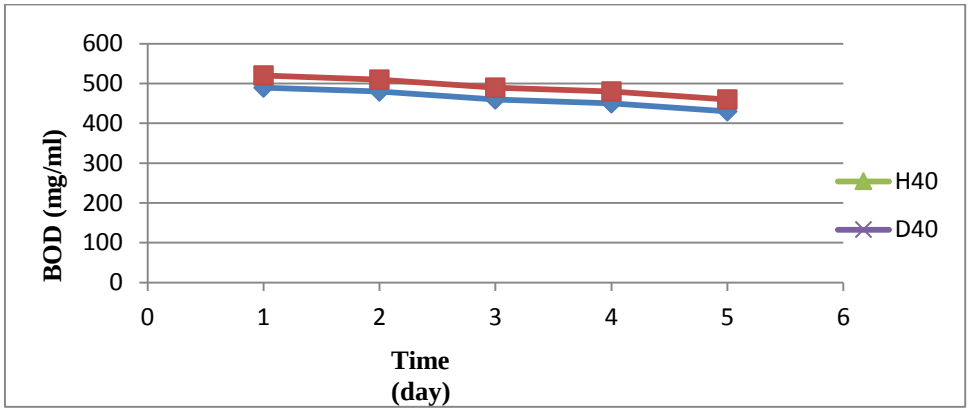

Fig. 4. Change in BOD of the test systems.

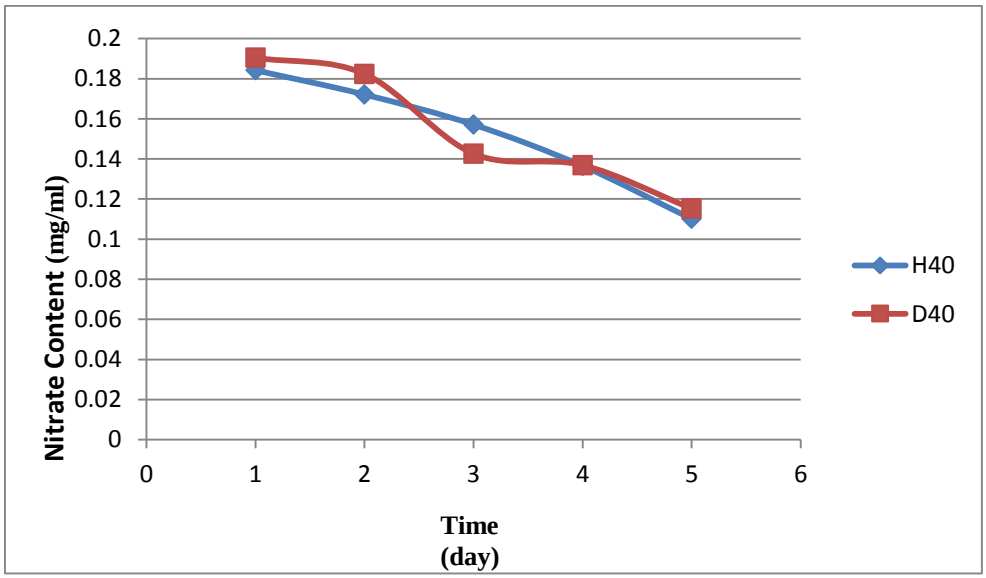

Fig. 5. Change in concentration of nitrate content for the test systems.

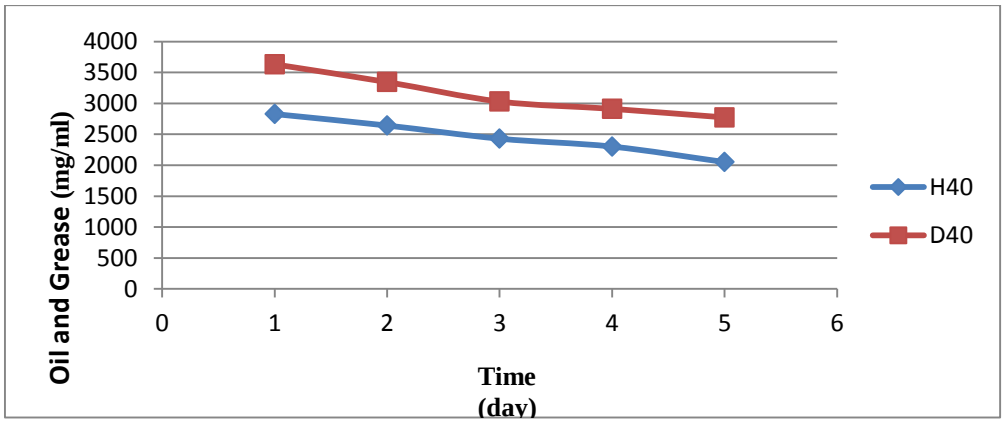

Fig. 6. Change in oil and grease of the test systems.

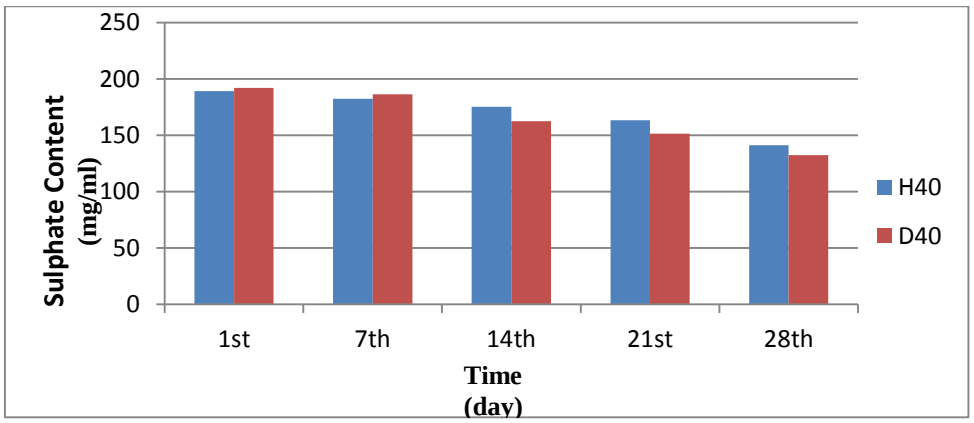

Fig. 7. Change in the concentration of sulphate content of the test systems.

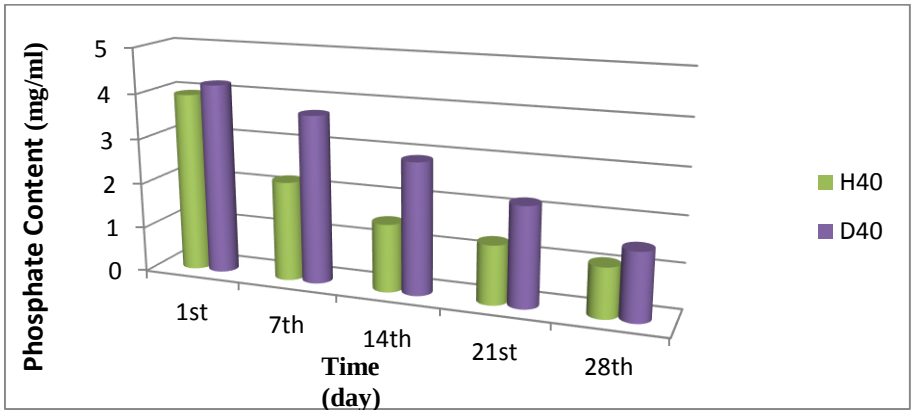

Fig. 8. Change in the concentration of phosphate content of the test systems.

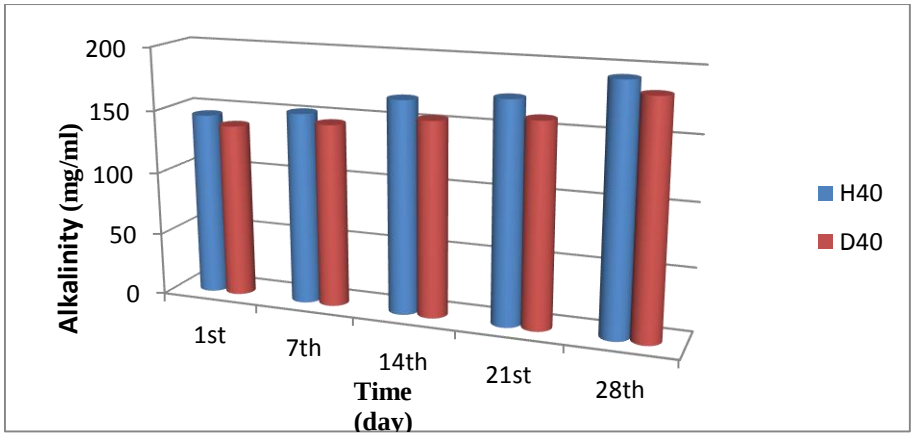

Fig. 9. Change in alkalinity of the test systems.

Most microorganisms isolated from the test sample which is in according with [17] show that they are able to attack engine oil. The bacteria isolates were predominantly Gram negative and have earlier been isolated and identified from various petroleum products. The isolates were identified as *Alcaligene, Bacillus, Citrobacter, Micrococcus, Proteus, Pseudomonas, and Vibrio* [17]. Early researchers reported that *Pseudomonas aerogenosa* is the predonant species in petroleum product biodegradation and this is expected as the genus is always found everywhere oil pollution is analysed [18].

CONCLUSION

The result from this research has shown that the mineralization process of the engine oils were positive and correlates with that of the previous researchers. Evidently, variations of physiochemical analysis of the samples have shown a gradual break down of petroleum products. Therefore, further research on diesel-base engine oil mineralization with some genetically modified microbial species has a future prospect and will reduce the half-life of the engine oil. This will lead to less time and resource consuming bioremediation process.

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