Full Length Research Paper

Impact of Nutrients, Aeration and Agitation on the Bioremediation of Crude Oil Polluted Water Using Mixed Microbial Culture

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Abstract. Crude oil polluted water was treated using a bioremediation strategy encompassing natural attenuation and biostimulation using a mixed microbial culture of *Aspergillus niger* and *Pseudomonas aeruginosa*. Four (4) samples of petroleum hydrocarbon polluted water; Control (no nutrient), A (nutrient from NPK 15:15:15), B (nutrient plus aeration), and C (nutrient, aeration and agitation) were monitored for 8 weeks for bioremediation indicating parameters such as Biological Oxygen Demand (BOD), Dissolved Oxygen (DO), Total Hydrocarbon Content (THC) and pH. The results obtained showed an increase in DO levels of 86.4, 87.0 and 87.5 % for samples A, B, and C respectively. The BOD of the samples was observed to decrease in the course of bioremediation with reduction levels of 98.6, 98.7 and 98.8% recorded for samples A, B, and C respectively. Reductions in THC corresponded to 92.3, 93.6, and 94.4% respectively for samples A, B and C. The pH of all samples fell within the acceptable limit of 6-9 as stipulated by regulatory agencies such as the Federal Environmental Protection Agency (FEPA) and the department of petroleum resources (DPR). The final values of BOD and THC fell within the values of 30 and 10 mg/l respectively stipulated by FEPA and DPR with the exception of sample A which had a final THC value of 14 mg/l.

Key words: Bioremediation, Crude oil polluted water, Biochemical oxygen demand, Dissolved Oxygen

1. INTRODUCTION

Crude oil made of petroleum hydrocarbons is the major pollutant in marine environments as a result of their release from activities such as offshore drilling, natural oil seepage, washing of oil tankers as well as well, transportation and ruptured pipeline accidents (Elshafie et al., 2007; Hasanuzzaman et al., 2007; Hidayat and Tachibana, 2012). Crude oil is a complex biodegradable substance containing a large variety of hydrocarbons (Hidayat and Tachibana, 2012; Obahiagbon et al., 2009).

Bioremediation has been identified as a suitable and sustainable choice in the decontamination of water polluted with hydrocarbon derivatives. It involves treating the petroleum pollutants with hydrocarbon degrading microorganisms possessing the kind of enzymes required for such a process. Many microorganisms (bacteria such as: *Escherichia coli, Pseudomonas* sp., *Clostridium,* yeasts *Candida,* and molds *Aspergillus niger, Penicillium* etc.) are known to grow and utilize petroleum and its derivatives for metabolic activities (Adekunle and Adebambo, 2007; Obahiagbon and Owabor, 2008). Bioremediation has been shown to have advantages

including viability, relatively low cost of operation, low technology requirement, widespread use and hydrocarbons are broken down in a relatively short time (Erdogan and Karaca, 2011; Otokunefor and Obiukwu, 2010). The pollutants are broken down into simpler substances such as carbon dioxide and water through biostimulation and bioaugmentation (Agbor et al., 2012). Biostimulation involves the use of nutrients to stimulate the growth of indigenous microorganisms in regions of subsurface contamination. Previous studies on the bioremediation of contaminated wastewater focused on natural attenuation, biostimulation and bioaugmentation with varying degrees of success recorded. Obahiagbon and Aluyor (2009) studied the potential use of inorganic nutrient (sodium nitrate and sodium nitrite) for the biostimulation of *Aspergilllus niger* for the bioremediation of petroleum hydrocarbon polluted water. Kim et al. (2005) reported enhanced bioremediation rates of crude oil contaminated sand as a result of addition of inorganic nutrients. Agbor et al. (2012) applied cocoa pod husk and plantain peels for the biostimulation of microbes during the biodegradation of crude oil polluted soil. Chikere et al. (2009) and Ebere et al. (2011) reported on the

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effectiveness of poultry droppings in enhancing the degradation of crude oil polluted soil in Southeastern Nigeria. One common feature of these studies is that the addition of nutrients is necessary to enhance biodegradation of contaminated wastewater (Okoh, 2006).

The aim of this study was to examine the potential applicability of inorganic fertilizers NPK 15:15:15 for the biostimulation of indigenous microorganisms for the purpose of treating crude oil contaminated wastewater under different treatment conditions. The study is focused on the role of nutrient supplementation, aeration and agitation on the bioremediation process.

2. MATERIALS AND METHODS

2.1. Microorganisms

The microorganisms used in this study (bacteria; *Pseudomonas aerugenosa* and molds; *Aspergillus niger*) were obtained from the biotechnology division of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. *Aspergillus niger* was maintained on Potato Dextrose Agar (PDA) slants and stored in a refrigerator at $4^{o}C$ until it was needed. *Pseudomonas aerugenosa* was grown in flasks of 500ml with aeration by mechanical mixing. The separation of bacterial suspension from the liquid medium was achieved by centrifuging. The concentrations of bacterial consortium (numbers of cells in 1 ml of a suspension) were checked using the Thom's chamber (Zawierucha and Malina, 2006).

2.2. Sample collection and preparation

The crude oil (Escravos light) used for this study was obtained from an Oil Producing Company located in the Niger Delta region of Nigeria. The properties of the crude oil sample are as follows: gravity API^{o}; 35.3, gravity SG; 0.85, sulphur (wt%); 0.15, viscosity (cSt at $40^{o}C$); 3.28. Crude oil polluted water was simulated in four vessels. The first vessel which served as the control contained water and crude oil in the ratio 10:1 and was exposed to the atmosphere. The second vessel (A) contained water and crude oil in the ratio 10:1, 2.4 kg of nutrient supplements in the form of NPK 15:15:15 fertilizer and was exposed to the atmosphere. The third vessel (B) contained water and crude oil in the ratio 10:1, 2.4 kg of nutrient supplements in the form of NPK 15:15:15 fertilizer and it was aerated with a fresh oxygen supply using a pump. The fourth vessel (C) contained water and crude oil in the ratio 10:1, 2.4 kg of nutrient supplements in the form of NPK 15:15:15 fertilizer, it was aerated with fresh oxygen and agitated to increase the level of mixing. Samples A, B and C were inoculated with a fresh inoculum of the mixed microbial culture to initiate bioremediation.

2.3. Analyses

The physicochemical parameters of the polluted water were monitored in the course of the remediation process. The following parameters; biochemical oxygen demand (BOD), dissolved oxygen (DO), total hydrocarbon content (THC) and pH were monitored as indicators of the degree of bioremediation. Sampling was done on day zero (before biostimulation) and subsequently at intervals of seven days (one week) for a total of 56 days (eight weeks). The pH of the samples was measured using an electronic pH meter (Fisher Accruement pH meter). The winkler method was used in the estimation of the BOD of the wastewater samples (Woodring and Clifford, 1988). In situ determination of dissolved oxygen of the wastewater sample was done using a dissolved oxygen meter which was calibrated prior to measurement with the appropriate traceable calibration solution (5%HCl) in accordance with the manufacturer's instruction. The total hydrocarbon content of the water was determined by shaking 5g of a representative waste sample with 10 mL of carbon tetrachloride and the oil extracted was determined by the absorbance of the extract at 450 nm using a spectronic 70 spectrophotometer.

3. RESULTS AND DISCUSSIONS

The profile of the BOD of the contaminated water at various remediation conditions is shown in Figure 1. Generally, the BOD value of all samples was observed to reduce in the course of bioremediation. The control (no nutrient added) showed a decrease in BOD of 50.7% indicating that there was an observable level of bioremediation albeit not very significant. The reduction in BOD could be attributed to the activities of the indigenous microbes present in the wastewater which converts the crude oil into less toxic substances such as CO_2, H_2O and many intermediates like organic acids, lipids, esters, complex alcohols and microbial proteins in form of enzymes (Otokunefor and Obiukwu, 2010). Sample A which contained inoculated contaminated water supplemented with nutrients from NPK 15:15:15 fertilizer showed a reduction in BOD of 98.6%. This indicates that nutrient supplementation enhanced the bioremediating capacity of the indigenous microorganisms. The better performance observed can be explained by noting that biodegrading microorganisms need oxygen, carbon and hydrogen to function optimally and these are provided by the added nutrients (Alwan et al., 2013).

Samples B and C showed BOD reductions of 98.7 and 98.8% respectively. These results highlights the positive influence of supplying air to the remediation medium as well as agitation of the remediation medium respectively. Biochemical oxygen demand is a measure of the amount of oxygen consumed by microorganisms in decomposing organic matter in water bodies. It also measures the chemical oxidation of inorganic matter (i.e., the extraction of oxygen from water via chemical reaction).

Obahiagbon and Aluyor (2009) reported enhanced bioremediation levels when crude oil contaminated water were supplemented with nitrates. They reported BOD reductions of up to 98%. Similar results were reported by Satyawali and Balakrishnan (2008) for the treatment of wastewater from molasses-based alcohol distilleries.The final BOD values of Samples A, B and C fell below maximum values of 30 mg/L stipulated by regulatory agencies like the federal environmental protection agency (FEPA) and the department of petroleum resources (DPR) during the study period of 56 days (FEPA, 1997).
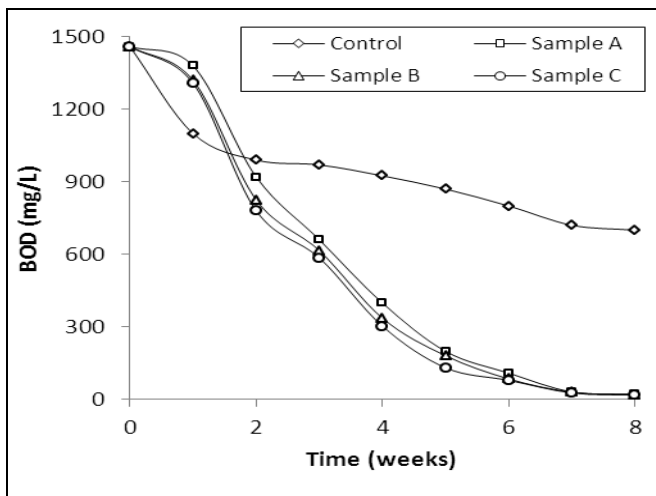

Fig. 1: Variation of BOD with time for wastewater remediated with NPK fertilizer
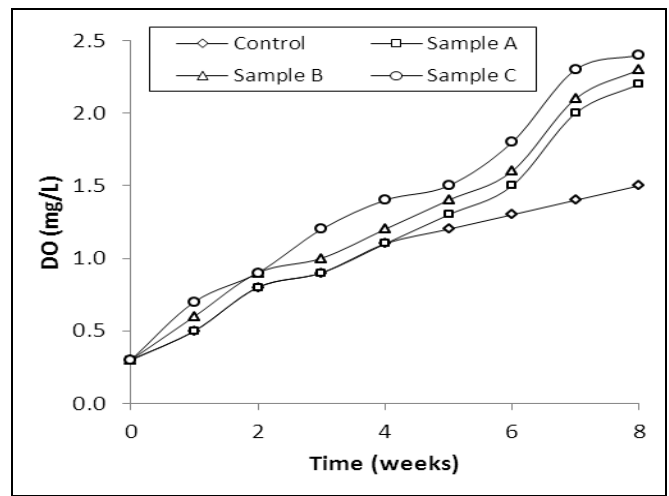

Fig. 2: Variation of DO with time for wastewater remediated with NPK fertilizer

Figure 2 shows the variation of the dissolved oxygen (DO) content of the crude oil contaminated water with time. The trend evident from the Figure shows that there was a steady and progressive increase in the DO of the samples in the course of the bioremediation process. This is an indication of the degradation of the crude oil in the contaminated water which makes it possible for more air to permeate into the water (Droste, 1997). The increase in the DO corresponds to the decrease in BOD of the contaminated water as indicated in Figure 1. The BOD directly affects the amount of dissolved oxygen in water bodies. The higher the BOD, the more rapidly oxygen is depleted in the water body (Amenaghawon et al., 2013). This means that less oxygen is available to aquatic life. The effects of high BOD are the same as those of low dissolved oxygen: aquatic organisms become stressed, suffocate, and die. BOD is affected by the same factors that affect dissolved oxygen. Aeration and agitation of stream water by rapids and waterfalls, for example will accelerate the decomposition of organic and inorganic material. This explains the higher levels of DO and higher levels of BOD reduction recorded for samples B and C. Although the initial DO of the wastewater (0.3mg/l) was far below the limit of 2.0 mg/L set by FEPA, the indigenous microbes upon stimulation by the fertilizer were able to improve DO level to 2.2, 2.3 and 2.4 mg/l respectively for samples A, B and C.

The total hydrocarbon content (THC) of all samples was observed to reduce with time within the period investigated as shown in Figure 3. The control sample showed the least reduction in THC corresponding to 44.1% reduction in THC suggesting the activity of indigenous micro-organism. Enhanced reduction levels of 92.3, 93.6 and 94.8% were recorded for samples A, B and C respectively highlighting the importance of nutrient supplementation, aeration and agitation respectively. The reduction in the THC values is indicative of a reduction in hydrocarbon content which results from the mineralisation of the hydrocarbons by the microorganisms to less toxic substances such as CO_2 and H_2O (Alwan et al., 2013; Okoh, 2006; Otokunefor and Obiukwu, 2010). These results are similar to those obtained by Alwan et al. (2013) who investigated the bioremediation of the water contaminated by waste of hydrocarbon using Ceratophyllaceae and Potamogetonaceae plants. At the end of the remediation period only samples B and C had THC value that fell within the acceptable limit of 10 mg/l recommended by FEPA and DPR (FEPA, 1997). The

results obtained are also comparable to those reported by Thavasi et al. (2011) who investigated the effect of biosurfactants and nutrient supplementation from fertilizers on the biodegradation of crude oil by marine isolates of *Bacillus megaterium, Corynebacterium kutscheri* and *Pseudomonas aeruginosa*. They observed that *Pseudomonas aeruginosa* and the biosurfactants produced by it resulted in the maximum crude oil degradation of about 89%. They further noted that there was no significant difference between the degree of biodegradation when bacteria cells and biosurfactants were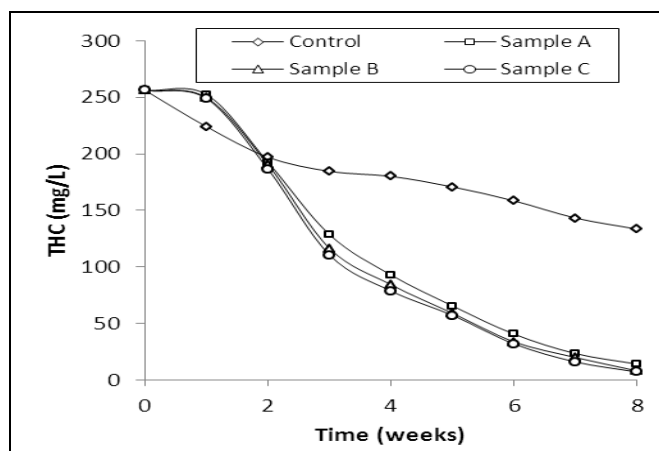 used compared with when fertilizer was used alongside the biosurfactant. This led to the conclusion that biosurfactants alone are capable of enhancing biodegradation to a large extent without the need for nutrient supplementation from fertilizers. Zahed et al. (2012) applied response surface methodology for the optimisation dispersed crude oil bioremediation. Using reduction in total petroleum hydrocarbon as an indication of bioremediation, they reported 22.1% crude oil removal through natural attenuation. When the remediation medium was supplemented with external sources of nitrogen and phosphorus, the optimized degree of bioremediation increased to 69.5%.
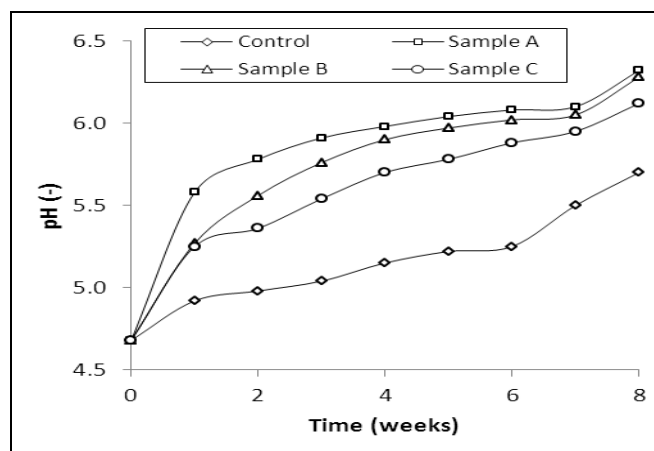

Fig. 3: Variation of THC with time for wastewater remediated with NPK fertilizer

Fig. 4: Variation of pH with time for wastewater remediated with NPK fertilizer

Figure 4 shows the variation of the pH of the polluted water with time in the course of bioremediation. The general trend observed indicates that the pH of all samples tested increased with increase in bioremediation time with the smallest increase observed for the control. The steady rise in pH with time for the period investigated, suggests the conversion of hydrocarbons into less toxic and less acidic products. Similar observations were reported by Amenaghawon et al. (2013) for the application of urea and NPK 15:15:15 fertilizers as biostimulants in the bioremediation of domestic wastewater. The pH of all samples fell within acceptable limit of 6 - 9 as stipulated by regulatory agencies such as FEPA and DPR (FEPA, 1997).

4. CONCLUSION

The use of bioremediation as a strategy for cleanup of petroleum hydrocarbon contaminated water was studied. The study focused on the role of nutrient supplementation, aeration and agitation on the bioremediation process. The following conclusions can be drawn.

(a) The hydrocarbon contaminated water contained some indigenous microbes as seen in the response to key indicators of the degree of bioremediation such as BOD, DO, and THC in the absence of inoculation and nutrient supplementation.

(b) The use of NPK 15:15:15 fertilizer as a nutrient source enhanced the bioremediation capability of the indigenous microbes present in the wastewater. This was evident in the significant reductions in BOD and THC as well as increase in the DO of the polluted water in the course of bioremediation.

(c) Conclusively, nutrient supplementation should be combined with aeration and agitation during bioremediation of crude oil contaminated water as this is more efficient in stimulating the microorganisms as seen in the higher reduction of 98.8 and 94.8% in BOD and THC (sample C) compared with that of samples B (98.7 and 93.6%) and A (98.6 and 92.3%) respectively.

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