Effect of Initial pH on the Bioremediation of Crude Oil Polluted Water Using a Consortium of Microbes.

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ABSTRACT

The effect of initial pH on the bioremediation of crude oil polluted water using a consortium of microbes was investigated in this study. Six samples each with initial pH values of 3, 5, 6, 7, 8, and 10 were inoculated with a consortium of microbes. A seventh sample served as a control. The six samples and control were monitored for a total period of five weeks for bioremediation indicating parameters such as pH, Biochemical Oxygen Demand (BOD), Total Microbial Count (TMC), and Residual Hydrocarbon Content (RHC). The pH of the six samples and the control did not vary much throughout the period of investigation. The results showed that the sample with initial pH of 6 had the greatest reduction in BOD and RHC. Similarly, the sample with an initial pH of 6 had the highest percentage increase in TMC. Biodegradation was highly inhibited in the very alkaline (high pH values) and very acidic conditions (low pH values). These results show that initial pH does have an effect on bioremediation of crude oil polluted water.

(Keywords: bioremediation, crude oil, biochemical oxygen demand, bioaugmentation, microorganism)

INTRODUCTION

Petroleum-based products are the major sources of energy for industry and daily life and as the world's dependence on crude oil and its derivatives increases, so does the level of exploration. This has created the conditions for the potential distribution of large amounts of toxins associated with crude oil into the environment (Obahiagbon et al., 2009).

Crude oil is a complex biodegradable substance consisting mainly of petroleum hydrocarbons. It is the major source of pollution in marine environments as a result of its release from activities such as offshore drilling, natural oil seepage, washing of oil tankers as well as well production, transportation, and ruptured pipeline accidents (Hasanuzzaman et al., 2007; Hidayat and Tachibana, 2012). For instance, the negative degradation of the environment experienced by the inhabitants of the oil producing areas in Nigeria has been attributed mainly to oil spillage emanating from the activities of the petroleum industry (Obahiagbon and Akhabue, 2009).

Decontamination of sites polluted with crude oil through biological means has received significant attention. Specifically, bioremediation has been identified as a suitable and sustainable option for the decontamination of crude oil polluted wastewater. It has a relatively low cost of operation, low technology requirement, it is easily implemented and the pollutants are degraded into less toxic forms in a relatively short time (Erdogan and Karaca 2011; Otokunefor and Obiukwu, 2010). It involves the use of microorganisms with the capacity to degrade these hydrocarbons and mineralize them into simpler and less toxic forms such as CO_2 and H_2O (Amenaghawon et al., 2013).

Many microorganisms such as *Pseudomonas, Escherichia coli, Clostridium, Candida, Aspergillus niger, Yeasts, Penicillium,* etc. have been isolated and possess the metabolic capacity to degrade petroleum hydrocarbons by using them as a source of carbon for metabolic activities (Olu-Arotiowa et al., 2007; Mukred et al., 2008; Obahiagbon and Owabor, 2009; Otokunefor and Obiukwu, 2010).

Bioremediation as a clean up method is typically implemented through either of three strategies namely: natural attenuation, biostimulation, or bioaugmentation (Yu et al., 2005). Natural attenuation refers to the combination of natural processes that occur, without human involvement, to decrease or "attenuate" contaminant concentrations and toxicity in wastewater, and thereby reduce the hazards posed by the contaminants. The remediation of wastewater through natural means is still limited by several factors which might inhibit microbial growth and activity. Some contaminated wastewater might contain complex synthetic and recalcitrant pollutants which are not readily amenable to biodegradation. Also, there might be deficiency of electron acceptors or donors and low availability of nutrient sources such as nitrogen and phosphorus (Nyyssönen et al., 2009; Qin et al., 2013). The process of externally stimulating microbial growth and activity for the remediation of contaminants is referred to as biostimulation.

The low population of indigenous microorganisms in wastewater is one of the major limitations of bioremediation through natural attenuation. In some instances, it is possible that the indigenous microorganisms might not even possess the metabolic activity necessary to degrade the hydrocarbon pollutants hence the need for specialized consortium of microorganisms which is added to the remediation medium exogenously (El Fantroussi and Agathos, 2005). This is typically encountered for cases of recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAHs), aromatic and aliphatic halogenated hydrocarbons, pesticides, and nitrated compounds like 2,4,6-trinitrotoluene (TNT). The process of exogenously adding microorganisms to the remediation medium is referred to as bioaugmentation (Obahiagbon and Akhabue, 2009; Yu et al., 2005).

This paper focuses on the effect of initial pH on bioremediation of crude oil polluted water bioaugmented with a consortium of microbes. Bioremediation indicating parameters such as pH, Biochemical Oxygen Demand (BOD), Total Microbial Count (TMC), and Residual Hydrocarbon Content (RHC) were monitored for a treatment duration of five weeks to determine the extent of bioremediation.

MATERIALS AND METHODS

Microorganisms

A microbial consortium made up of *Aspergillus niger*, *Clostridium*, *Proteus,* and *Pseudomonas aeruginosa* was used in this study. The microorganisms 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. The bacteria (*Pseudomonas aeruginosa, Clostridium* and *Proteus*) were grown in flasks of 500 mL 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).

Sample Collection and Preparation

The crude oil (Escravoes light) used for this study was obtained from an oil producing company located in the Niger Delta region of southern Nigeria. Crude oil polluted water was simulated in seven vessels. To each vessel were added crude oil and water in the ratio 1:10. The vessels were allowed to stand for one week to allow indigenous microbes adapt to their new environment. The initial pH of the first six vessels was adjusted to 3, 5, 6, 7, 8, and 10, respectively. These six vessels were subsequently inoculated with a fresh inoculum of the mixed microbial culture to initiate bioremediation. The seventh vessel which acted as a control was not inoculated and its pH was not adjusted. Bioremediation indicating parameters of the polluted water such as pH, BOD, TMC, and RHC were monitored in the course of the remediation process. Sampling was done on day zero (before bioaugmentation) and subsequently at intervals of seven days (one week) for a total of 35 days (five weeks).

Analytical Methods

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). The total microbial count (concentration of microbial consortium) expressed as the numbers of cells in 1 mL of a suspension was determined using the Thom's chamber (Zawierucha and Malina, 2006).The residual 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.

RESULTS AND DISCUSSION

Figure 1 shows the variation of the pH of the samples in the vessels with time in the course of bioremediation. It was observed that the pH of the six samples, as well as the control, did not vary significantly throughout the five weeks treatment period. This may be an indication of low rate of biodegradation and may not be unconnected with the use of a consortium of microbes in the investigation, as the use of a microbial consortium sometimes leads to competition among the microorganisms with detrimental effect on the biodegradation process (Gonzalez, 2011; Thomassin-Lacroix et al., 2002).

Notwithstanding, the samples with initial pH of 5 and 6 gave the highest increase in pH and the pH change was 0.32 for pH-5 sample and 0.24 for pH-6 sample. This was closely followed by pH-7 sample and the control with changes of 0.23 and 0.20, respectively. The pH change for pH-3, pH-8, and pH-10 samples were respectively 0.15, 0.10, and 0.02; an indication of very low biodegradation and which, by extension, implies unfavourable environment for the microbes at those pH values.

Figure 2 shows the variation of BOD of the samples with remediation time. It was observed that the BOD of all the samples investigated reduced in the course of bioremediation. The BOD of the control also showed a decrease indicating some level of bioremediation resulting from the activity of the indigenous microorganisms. BOD is a measure of the amount of oxygen consumed by microorganisms in degrading organic matter in aqueous systems. In this regard, a reduction in BOD is indicative of a reduction in the organic matter present in the wastewater which in this case is crude oil (Amenaghawon et al., 2013).

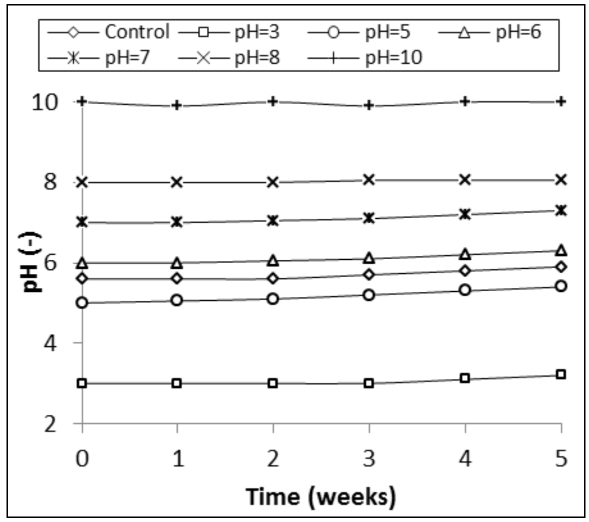

Figure 1: Variation of pH with Remediation Time.

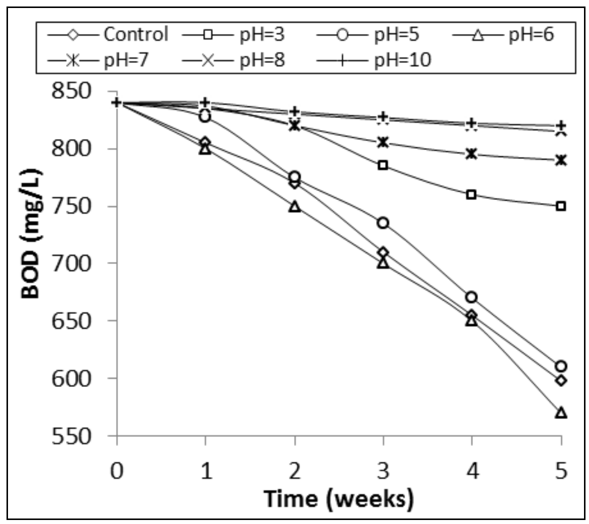

Figure 2: Variation of Biochemical Oxygen Demand (BOD) with Remediation Time.

The decrease in the value of BOD of the samples may be as a result of the metabolic activities of the microorganisms either those indigenous to the wastewater samples or those exogenously added. These organisms have the capacity to convert 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 (Obahiagbon et al., 2009; Otokunefor and Obiukwu, 2010).

Similar observations were reported by Obahiagbon and Aluyor, (2009) for the bioremediation of crude oil contaminated water supplemented with nitrates. Satyawali and Balakrishnan, (2008) also reported reductions in BOD for the treatment of wastewater from molasses-based alcohol distilleries.

Amenaghawon et al. (2013) investigated the treatment of domestic wastewater supplemented with inorganic fertilizers. They reported reductions in the BOD of the wastewater and attributed this observation to the activity of the stimulated indigenous microorganisms. The most significant reductions in BOD were obtained for the samples with initial pH value of 6 followed by that with an initial pH value of 5 both of which are slightly acidic. On the other hand, very slight BOD reduction was observed for samples with initial pH of 8 and 10, both of which are alkaline. The same trend was observed for the samples with initial pH values of 3 which is an acidic condition. These results show that biodegradation was highly inhibited in the very alkaline and acidic conditions. This is as a result of the harsh environment imposed under those conditions, with possible death of microbial cells in which case bioremediation is slowed and possibly halted (Sharma, 2012). It is evident from the results that the optimum initial pH for biodegradation was 6.

The variation of the total microbial count with time in the course of bioremediation is shown in Figure 3. The total microbial count expressed as the numbers of cells in 1 mL of a suspension is indicative of the concentration of microbial consortium present in the crude oil sample. It was observed that there was an increase in TMC with remediation time for samples with initial pH of 3, 5, and 6, as well as the control. This trend could be attributed to the growth of microorganism which is reflected in the increase in the population of the microorganism. The final TMC of the sample with an initial pH of 7 was exactly the same as the start value at the end of the six weeks period of investigation, with slight variations observed within the period. On the other hand, TMC decreased for the samples with initial pH of 8 and 10. This is an indication of microbial death resulting in a decrease in the microbial population with a concomitant negative impact on biodegradation, as a result of the alkaline conditions. This observation corroborates that reported for the case of BOD for samples with initial pH of 8 and 10. It was observed once again that the optimum initial pH for biodegradation was 6 as this resulted in the greatest increase in TMC.

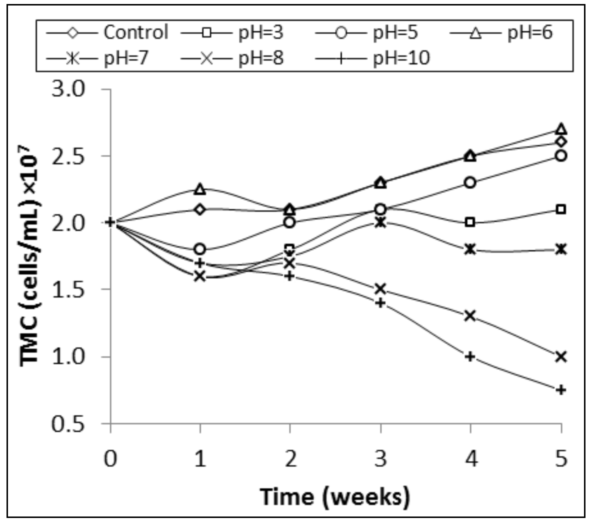

Figure 3: Variation of Total Microbial Count (TMC) with Remediation Time.

Figure 4 shows the variation of residual hydrocarbon content of the wastewater samples in the course of bioremediation. It was observed that the trend of RHC was similar to that of BOD as indicated in Figure 2. The RHC of all the samples investigated decreased with remediation time including the control. The reduction in the RHC values is indicative of a reduction in the crude oil content of the wastewater samples as a result of the mineralisation of the hydrocarbons by the microorganisms to less toxic substances such as CO_2 and H_2O (Okoh, 2006; Otokunefor and Obiukwu, 2010;). The trend observed is similar to that reported by Alwan et al. (2013) who investigated the bioremediation of water contaminated by waste of hydrocarbons using *Ceratophyllaceae* and *Potamogetonaceae* plants. Mukred et al. (2008) examined the growth of microbial populations and their effectiveness in the bioremediation of crude oil polluted water. They reported similar reduction in total hydrocarbon content with a bacteria consortium of *Acinetobacter faecalis, Staphylococcus* sp. and *Neisseria elongate.*

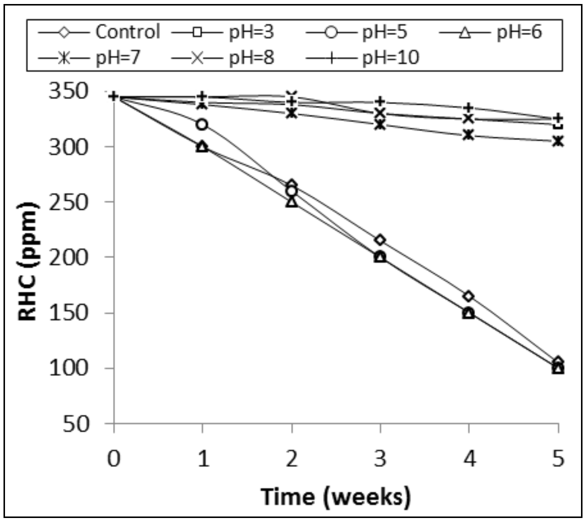

Figure 4: Variations of Residual Hydrocarbon Content (RHC) with Remediation Time.

The most significant reductions in RHC were observed for samples with initial pH of 6 followed by that with 5. The control also recorded a significant decrease in the RHC. On the other hand, small reductions in RHC were recorded for samples with initial pH of 8 and 10 both of which are alkaline. The percentage RHC reduction for the pH-3 sample (very acidic) was also small. Again, the results show that biodegradation was inhibited in the alkaline samples as well as the very acidic samples. This is not different from the observations recorded for the case of BOD. From the RHC results, the optimum initial pH was 6; which agrees with those of BOD and TMC earlier discussed.

CONCLUSION

Crude oil contaminated wastewater was treated through bioremediation using a consortium of microorganisms. Bioremediation of crude oil polluted water using a consortium of microbes is a function of the initial pH of the polluted water. The optimal remediation occurred in the sample with an initial pH of 6 which was closely followed by the sample with an initial pH of 5 and the control in that order. Microbial death occurred in the alkaline samples having initial pH of 8 and 10, with negative impact on remediation efficiency. Also, the high acidity in the sample with initial pH of 3 inhibited microbial activities with detrimental effect on remediation efficiency.

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