Scientific Research and Essay Vol.4 (8), pp. 728-732, August 2009
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2009 Academic Journals

Full Length Research paper

Comparison of the efficiency of sodium nitrate and sodium nitrite as nutrients in the bioremediation of petroleum hydrocarbon polluted water

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Accepted 6 July, 2009

The role of *Aspergillus niger* (fungus) and the use of 0.2 M sodium nitrate and 0.2 M sodium nitrite as nutrients on the bioremediation of petroleum hydrocarbon polluted water were investigated in this study. 2 samples of petroleum hydrocarbon polluted water each had 0.2 M $NaNO_3$ (aq) and 0.2 M $NaNO_2$ (aq) and *A. niger* added and a control was monitored over a period of 49 days for physicochemical parameters such as Biological Oxygen Demand (BOD), Total Hydrocarbon Content (THC) and pH as indicators of the degree of bioremediation. The results obtained showed the sample amended with 0.2 M $NaNO_3$ (aq) gave a reduction in BOD of 98.4% (1760 - 28 mg/l) compared with that amended with 0.2 M $NaNO_2$ (aq) which gave a BOD reduction of 97.4% (1760 - 46 mg/l). The sample remediated with 0.2 M sodium nitrate showed the greatest reduction in THC of 97.5% (282 - 7.0 mg/l) compared to 94% (282 - 17 mg/l) and 79.5% (282 - 58 mg/l) for the sample with 0.2 M sodium nitrite and control respectively. The pH of all samples fell within acceptable limit of 6 - 9 as stipulated by regulatory agencies such as FEPA and DPR. Over a period of 49 days of study only sample amended with 0.2 M sodium nitrate gave BOD and THC values that fell within stipulated values of 30 and 10 mg/l respectively by FEPA and DPR.

Key words: Bioremediation, efficiency of nutrients, *Aspergillus niger* and environment.

INTRODUCTION

The dominance of petroleum products in world economy creates the conditions for distributing large amount of these toxins into populated areas and ecosystems around the globe (Ojumu, 2004). The transport of petroleum across the world is frequent and the amounts of petroleum stocks in developed countries are enormous. Consequently, the potential for oil spills is significant. The volume of spills usually exceeds the inherent remediation capacity for any given environment, resulting in a significant ecological impact (Yehuda, 2002). Accidental and deliberate crude oil spills have been and still continue to be a significant source of environmental pollution and poses a serious environmental problem due to the possibility of air, water and soil contamination. (Trindade et al., 2005).

The most rational way of decontamination of the environment loaded with petroleum derivatives is an application of methods based mainly on metabolic activity of micro-organisms (Leahy and Colwell, 1990). Microbial degradation is the major mechanisms for the elimination of spilled oil from the environment (Walker et al., 1974; Ibe and Ibe, 1984; Atlas, 1995). The ability to actively decompose specified fractions of petroleum hydrocarbon is expressed by many micro-organisms (Bartha and Atlas, 1997). These microbes are called hydrogen degrading micro-organism. They include *Pseudomonas, Escherichia coli, clostridium, Candida, Aspergillus niger, Yeasts, Penicillium* and a host of others (Okoh et al., 2001; Barth, 2003; Lliros et al., 2003; Chaillana et al., 2004; Adekunle and Adebambo, 2007; Obahiagbon and Owabor, 2008).

The objective of bioremediation is to stimulate the growth of indigenous or introduced microorganisms in regions of subsurface contamination and thus, provide direct contact between the dissolved and sorbed contaminants for biotransformation. Enzymes during normal

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Abbreviations: THC, Total hydrocarbon content; **BOD,** biological oxygen demand; **FEPA,** federal environmental protection agency; **DPR,** department of petroleum resources.

metabolic function of microorganism facilitates biotransformation of organic chemicals (Madhu, 1989).

Microorganisms gain energy by catalyzing energy-producing chemical reactions that involve breaking chemical bonds and transferring electrons away from the contaminant (Atlas, 1981). The type of chemical reaction is called an oxidation-reduction reaction; the organic contaminant is oxidized, the technical term for losing electrons; correspondingly, the chemical that gains the electrons is reduced. The contaminant is called the electron donor, while the electron recipient is called the electron acceptor. The energy gained from these electron transfers is then "invested", along with some electrons and carbon from the contaminant, to produce more cells. These 2 materials — the electron donor and acceptor — are essential for cell growth and are couply called the primary substrates (Atlas, 1995).

Successful application of bioremediation technology to contaminated systems requires knowledge of the characteristics of the site and the parameters that affect the microbial biodegradation of pollutants (Okoh, 2006).

However, a number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons. Biodegradation is inherently influenced by the composition of the oil pollutant. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen, phosphorus and in some cases iron (Okoh, 2006). Depending on the nature of the impacted environment, some of these nutrients could become limiting thus affecting the biodegradation processes. When a major oil spill occurs in fresh water and marine environments, the supply of carbon is dramatically increased and the availability of nitrogen and phosphorus generally becomes the limiting factor for oil degradation (Atlas et al., 1991). Hence the additions of nutrients are necessary to enhance biodegradation of oil pollutants (Okoh, 2006). Sodium nitrate and sodium nitrite are inorganic salts and act as strong oxidizing agents. They also act as a source of nitrogen required by *A. niger*.

MATERIALS AND METHODS

Sample collection

The crude oil (Escravoes light) used for this study was obtained from an Oil Producing Company located in the Niger Delta region of Nigeria.

Sample preparation

The crude oil polluted water was made by adding 100 ml of Escravoes light (crude oil) to 1000 ml of water. This crude oil polluted water of ratio 1: 10 was then stored in black plastic containers until required. Before the experiment was started the crude oil polluted water was allowed to stand for 1 week allowing the indigenous microbes to grow and accustom to the medium. The 0.2 M sodium nitrate containing sample was made by dissolving 17g of sodium nitrate in 1 litre of petroleum hydrocarbon polluted water while the 0.2 M sodium nitrite containing sample was made by dissolving 13.8 g of sodium nitrite in 1 litre of petroleum hydrocarbon polluted water. To 1 litre each of the hydrocarbon polluted water containing 0.2 M $NaNO_3$(aq) and 0.2 M $NaNO_2$(aq), a fresh innoculum of *A. niger* was added.

Biochemical oxygen demand (BOD)

Reagents used

Winkler's solution A, Winkler's solution B, starch solution;

a) Two 250 ml reagent bottles were filled up completely with the sample and stoppered tightly. To 1 of the bottles, 1.5 ml each of Winkler's solution A and B were added and precipitant was formed. The precipitant was dissolved with 2 ml of concentrated hydrochloric or sulphuric acid to form a golden brown solution. 100 ml of the resulting solution was poured into 250 ml flask and 3 drops of starch indicator were added and titrated against 0.1 N sodium thiosulphate ($Na_2S_2O_4$) initial blue black coloration and the volume of 0.1 N ($Na_2S_2O_4$) solution used was recorded.

b) The second bottle was covered with black cellophane bag or aluminum foil to prevent the penetration of light and then incubated at 20°C for 5 days. At the end of 5 days, step (a) was repeated and the volume of 0.1 N $Na_2S_2O_4$ used was recorded.

The BOD of the sample was calculated as follows:

$$BOD_5 = \frac{(DO_0 - DO_5)}{p} \quad (1)$$

where DO_O = Dissolved oxygen concentration at zero time.
DO_5 = Dissolved oxygen concentration after 5 days incubation period.
P = dilution factor.

Total hydrocarbon content (THC)

Procedure

The oil content of the water was determined by shaking 5 g of a representative waste sample with 10 ml of toluene or carbon tetrachloride and the oil extracted was determined by the absorbance of the extract at 450 nm in a spectronic 70 spectrophotometer.

The wastewater extract ratio was varied where necessary depending on the concentration of oil in the water. In some cases where oil content was very high, it was found more accurate to dilute the extract before reading from the spectrophotometer than reducing the weight of water sample extractant.

A standard curve of the absorbance of different known concentrations of oil in extractant was first drawn; after taking readings from the spectrophotometer, oil concentrations in the water sample were then calculated after reading the concentration of the oil in the extract from the standard curve. With reference to the standard curve and multiplication by the dilution factor, the oil concentration was calculated.

pH measurement

Apparatus

(a) An electronic pH meter (Fisher Accruement pH meter) mode and reference electrode.
(b) Buffer solutions.
(c) Sample.

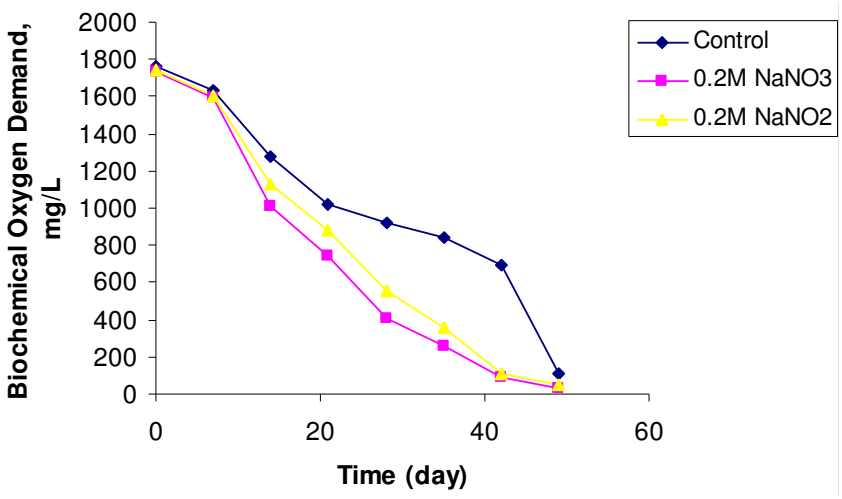

Figure 1. Variation of biochemical oxygen demand with time for samples amended with sodium nitrate and sodium nitrite.

(d) Stirrers.
(e) Thermometer.

Procedure

The temperatures of the buffer solutions and sample were taken using a thermometer and the temperature is manually compensated for in the meter. The electrode system of the pH was then calibrated. After calibration, the sample was thoroughly mixed together using a stirrer and its pH was taken.

The pH value obtained was then recorded. Based on the efficiency of the electrodes, the corrected value was then recorded.

RESULTS AND DISCUSSIONS

The role of *A. niger* (fungus), 0.2 M sodium nitrate and 0.2 M sodium nitrite on the bioremediation of petroleum hydrocarbons was investigated in this study. The 2 samples of 0.2 M sodium nitrate and sodium nitrite respectively and the control (Petroleum hydrocarbon polluted water only) was monitored for physicochemical parameters such as biological oxygen demand (BOD), total hydrocarbon content (THC) and pH as indicators of the degree of bioremediation. Different samples of the hydrocarbon polluted water was innoculated with *A. niger* (fungus), 0.2 M sodium nitrate and 0.2 M sodium nitrite under appropriate condition of pH, temperature and oxygen supply (Trindade et al., 2005). The samples were monitored for 49 days.

The BOD values of all samples were observed to reduce as time progresses for the period investigated. Figure 1 shows the trend of BOD reduction for the various samples. The sample amended with 0.2 M $NaNO_3$ (aq) showed a reduction in BOD of 98.4% (1760 - 28 mg/l) compared with that amended with 0.2 M $NaNO_2$ (aq) which shows a BOD reduction of 97.4% (1760 - 46 mg/l). The control sample had a BOD reduction of 93.8% (1760 - 110 mg/l). Of the 3 samples, only the sample remediated with 0.2 M $NaNO_3$ (aq) fell below maximum values of 30 mg/l stipulated by regulatory agencies like FEPA and DPR during the experimentation period of 49 days. The reduction in BOD for samples can be attributed to activities of the *A. niger* and other indigenous microbes present in the samples which converts the hydrocarbons 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 and Aluyor, 2002).

The total hydrocarbon content for all samples was observed to reduce with time within the period investigated. The control sample showed the least reduction in THC (282 - 58 mg/l) in 49 days signifying the activity of indigenous micro-organism (Obahiagbon and Ezeokeke, 2000). The sample remediated with 0.2 M sodium nitrate as shown in Figure 2 showed the greatest reduction in THC of 97.5% (282 - 7.0 mg/l) compared to the sample with 0.2 M sodium nitrite which showed a THC reduction of 94% (282 - 17 mg/l). The increased efficiency of nitrate (NO_3^-) over the nitrite (NO_2^-) could be attributable to the extra oxygen which makes it more readily assimilable by the microorganisms. The reduction in the THC values signifies a reduction in hydrocarbon contents as a result of the release of enzymes by the microorganisms to mineralize the organics to less toxic substances such as CO_2 and H_2O (Da Cunha, 1996; Obahiagbon and Aluyor, 2001). At the end of the remediation period (49 days) the sample remediated with 0.2 M sodium nitrate fell within the acceptable limit of 10 mg/l recommended by regulatory agencies such as Federal Environmental Protection Agency (FEPA, 1997) and DPR.

The pH values was observed to reduce sharply initially as can be seen from Figure 3 for the samples remediated

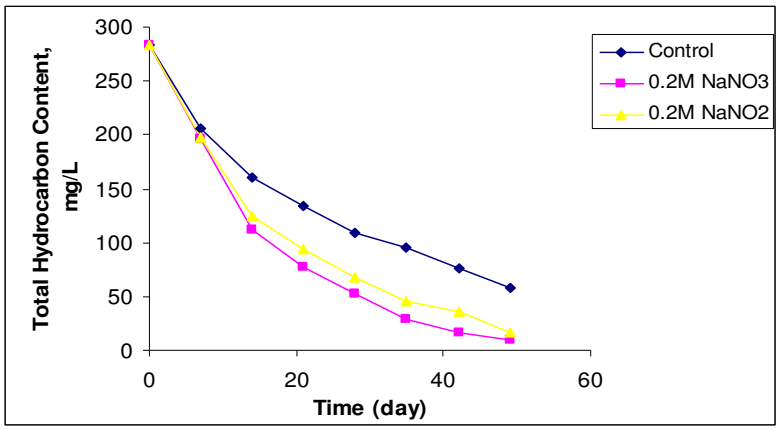

Figure 2. Variation of total hydrocarbon content with time for samples amended with sodium nitrate and sodium nitrite.

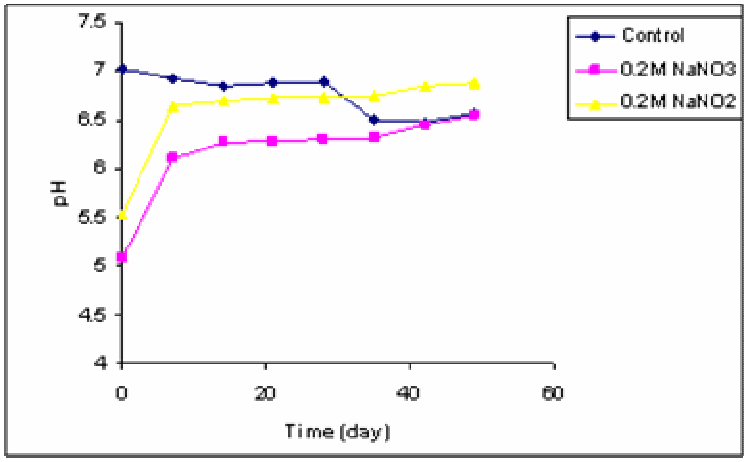

Figure 3. Variation of pH with time for samples amended with sodium nitrate and sodium nitrite.

with 0.2 M $NaNO_3$ (aq) and 0.2 M $NaNO_2$ (aq) due to the acidic nature of the salt. The control sample showed a gradual decline signifying the activities of indigenous microbes converting the hydrocarbons into acidic products such as alkanoic acids. By and large all samples showed a steady rise in pH with time for the period investigated, showing the conversion of hydrocarbons into less toxic acidic products. The pH of all samples fell within acceptable limit of 6 - 9 as stipulated by regulatory agencies such as Federal Environmental Protection Agency (FEPA, 1997) and DPR.

Conclusion

Bioremediation as a strategy for clean up of petroleum hydrocarbon polluted water has been shown to be sufficient considering the level of reduction in THC and BOD over the period of study.

a) *A. niger* offer an efficient and interesting possibility of degrading petroleum hydrocarbon polluted water.

b) 0.2 M $NaNO_3$ (aq) is more efficient in stimulating the microorganisms as seen from the reduction of 97.5 and 98.4% in THC and BOD compared with that of 94 and 97.4% reduction in THC and BOD for 0.2 M $NaNO_2$ (aq) amended sample.

c) Only the sample amended with 0.2 M $NaNO_3$ (aq) fell within the stipulated levels of 10 and 30 mg/l for THC and BOD by regulatory agencies such as FEPA and DPR over the experimentation period of 49 days.

d) Scale up of this bioremediation strategy is very promising and should be encouraged.

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