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Full Length Research Paper

Drilling fluid base oil biodegradation potential of a soil *Staphylococcus* species

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***Staphylococcus* sp. isolated from oil-contaminated soil was grown in 1% drilling fluid base oil, HDF-2000, as a sole source of carbon and energy. The organism has strong affinity for the substrate, growing at the rate of 0.16 h^{-1}. It uses adherence and emulsification as mechanisms for oil uptake. In a nutrient-rich marine broth, base oil (up to 2.0% v/v) and glucose (up to 1.6% w/v) have no significant effect on the growth rates. This showed that the *Staphylococcus* sp. is a strong primary utilizer of the base oil and has potential for application in bioremediation processes involving oil-based drilling fluids.**

Key words: Drilling fluid base oil, *Staphylococcus* sp., biodegradation.

INTRODUCTION

Diesel oils used in oil based drilling mud formulations were found to be toxic to a wide range of microorganisms due to high concentration of potentially toxic materials (20-30% of 2-, 3- and 4-ring aromatics). Consequently, highly refined low toxicity base oil that have low concentration of aromatic compounds (1–10%) become the alternative (Gillam and Gibson, 1986; Candler et al., 1993). Nevertheless, used drilling muds, additives and oil-laden drill cuttings still pose a serious threat to the biota of aquatic and terrestrial ecosystems. As a result, several government programs regulate the use of oilfield chemicals. In Nigeria, Federal Ministry of Environment and Department of Petroleum Resources (DPR) guidelines prohibit the discharge of waste into swamps and the use of permanent waste pits in drilling locations. Biodegradability tests of drilling fluids, therefore, are conducted routinely as part of a package of ecotoxicological tests that are required as part of the use or discharge consent procedure that gives approval for a drilling fluid to be used or discharged into the environment. Information on the biodegradation of drilling fluids is scarce. Limited investigations of biodegradation of cuttings piles have been conducted by Cripps et al. (1999). Other works have described the microbial population associated with drill cuttings. In these investigations, marine *Staphylococcus* species were reported to degrade drilling fluid base oils and oil waste attached to drill cuttings (Benka-Coker and Olumagin, 1995; Nnubia and Okpokwasili 1993; Nnubia and Okpokwasili, 1999).

This work reported the drilling fluid base oil biodegradation potential of a soil *Staphylococcus* species.

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MATERIALS AND METHODS

The drilling fluid base oil, HDF–2000 used in this work was obtained from Baker Hughes Nigeria Limited, Port Harcourt. It is a low aromatic base oil formulated by Total- Fina of France for offshore and onshore drilling operations.

The bacterial isolate was obtained from an oil-polluted soil and purified on nutrient agar plates. The isolate was screened for utilization of drilling fluid base oil, HDF-2000, using mineral salts medium of Okpokwasili and Okorie (1988). Agar and broth mineral salts medium containing 1% base oil was used. Screen test was also done using vapour phase transfer technique of Thijsse and van der Linden (1961). Incubations were at room temperature for 7 days. Colony development and turbidity were indicative of base oil utilization.

Gram reaction, spore staining, motility, oxidative/fermentative (O/F) utilization of sugars, catalase, oxidase, indole production, hydrogen sulphide production, starch hydrolysis, urease, methyl red-Voges Proskauer, citrate and coagulase tests were carried out according to the methods described by Collins and Lyne (1984) and Cruickshank et al. (1980). Identification to the generic level followed the schemes of Holt (1982) and Macfaddin (1979).

Adherence and emulsification assays were done using cell suspensions and cell-free supernatant. The suspensions of the base oil-adapted cells in potassium-urea-magnesium (PUM) buffer were dispensed in 10 ml volumes into duplicate test tubes. The initial absorbances of the suspensions were taken at 420 nm, and 0.1ml of the base oil was added into the tubes. The tubes were shaken vigorously and allowed to stand for 15 min. The lower portions of the tubes were carefully separated into separate cuvettes and the absorbance measured. Adherence is expressed as percentage decrease in absorbance of the cell suspension. The emulsification activity of the cell-free supernatant was determined using the procedure of Marin et al. (1996).

The growth profile of the bacteria in 1% drilling fluid–mineral salts broth were followed by monitoring the optical density at 420 nm, total viable counts and pH of the pure cultures at 48-h intervals for 14 days. The effects of drilling fluid and glucose on bacterial isolates were assessed by monitoring their growth on 0.5, 1.0 and 2.0% (v/v) drilling fluid as well as on 0.4, 0.8 and 1.6% (w/v) glucose in marine broth of Okpokwasili and Nnubia (1995). Using the total viable count data, specific growth rate and the doubling time of the isolate were calculated.

RESULTS AND DISCUSSION

The *Staphylococcus* sp. has natural ability to biodegrade the base oil. It produced heavy growth in the mineral salt-base oil broth and agar media. It also grew well with the vapour phase transfer technique and appeared to prefer gaseous base oil or its gaseous components.

The growth profile determined by monitoring the optical density, total viable counts and pH of the culture utilizing HDF-2000 drilling fluid base oil as sole carbon and energy source is shown in Figure 1. The total viable counts increased with the optical density. There was a decrease in pH from 7.75 to 6.23 as the bacterial cells metabolize the drilling fluid. The changes in pH could be ascribed to the production of acidic metabolites. Aerobic biodegradation of aliphatic and aromatic hydrocarbons leads to production of organic acids (Madigan et al., 1997; Gottschalk, 1986; Cerniglia, 1992; Cerniglia and Heitkamp, 1989). Growth was fast in the base oil-mineral salts medium with lag phase of less than 24 h. The exponential phase growth rate was 0.16 h^{-1}.

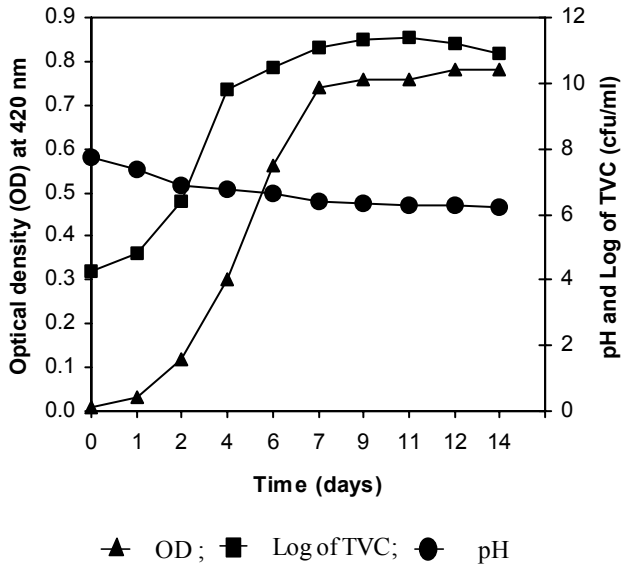

Figure 1. Growth profile of *Staphylococcus* sp. in mineral salts-HDF 2000 broth as sole source of carbon and energy.

The adherence and emulsification activities were 27.73 and 24.53%, respectively. The cells, therefore, have strong affinity for the base oil and was able to produce extracellullar biosurfactant capable of emulsifying the base oil. These are the mechanisms used by microorgnisms to take up substrates with low water solubility. *Pseudomonas*, *Alcaligenes*, *Bacillus*, *Klebsiella*, *Actinomyces*, *Aeromonas*, *Nocardia*, *Xanthomonas* and *Streptomyces* are among the well known microbial genera that have been associated with hydrocarbon degradation. Drilling wastes have been reported to be degraded by bacterial isolates identified as species of *Staphylococcus*, *Acinetobacter*, *Alcaligenes*, *Serratia*, *Clostridium*, *Enterobacter*, *Nocardia*, *Bacillus*, *Actinomyces*, *Micrococcus* and *Pseudomonas* (Benka-Coker and Olumagin, 1995). *Bacillus* and *Staphylococcus* have been identified by Nnubia and Okpokwasili (1993) as major primary users of diesel oil and drilling fluid base oils.

The effects of varying concentrations of drilling fluid base oil and glucose on growth of the isolate are shown in Figure 2. Generally, drilling fluid prolonged the lag phase of the bacteria. Nevertheless, various concentrations of the base oil and glucose had little or no effect on the lag phases and the maximum bacterial density was reduced by the drilling fluid. This indicates that glucose is a more readily utilized substrate. The exponential phases were stimulated at all concentrations of base oil and glucose. In the marine broth (control), the growth rate and generation time were 0.58 h^{-1} and 1.23 h,

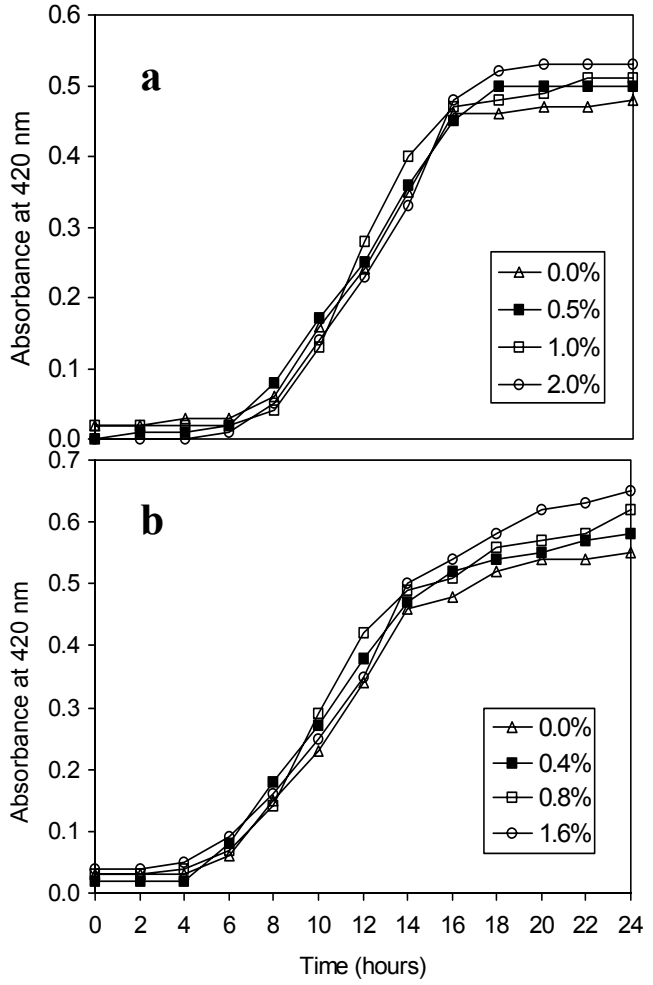

Figure 2. Effects of different concentrations of drilling fluid (a) and glucose (b) on the growth of *Staphylococcus* sp.

respectively. The results showed that there was an increase in growth rates and decrease in generation times at all concentrations of the base oil and glucose. The growth rates varied between 0.60 and 0. 66 h^{-1} for the base oil, and between 0.64 and 0.77 h^{-1} for glucose. On the other hand, the generation times varied between 1.16 and 1.06 h for the base oil and between 1.08 to 0.90 h for the glucose. However, the analysis of variance at 95% confidence limit showed that these variations were insignificant. It therefore could be reasoned that 2% drilling fluid base oil and 1.6% glucose are below toxic or inhibitory concentrations for the *Staphylococcus* sp. Glucose toxicity and impaired glucose transport and utilization have been reported for *Bacteroides ruminicola* (Russel, 1992).

The results of this work showed that this organism has potential application in the bioremediation of sites polluted by oil-based drilling fluid base oil.

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