Bioremediation of Crude Petroleum Polluted Stagnant Water with Fermented Cassava Steep

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Abstract: Bioremediation of crude oil polluted stagnant waters was investigated using isolates from cassava steep fermented for a period of seven (7) days to build enough microbial culture. After inoculation of fermented cassava steep into the crude oil polluted stagnant waters, samples of experimental and control waters (not inoculated) were obtained at regular intervals of three days for physico-chemical and microbiological analysis. Results showed the lowest bacterial population in cassava steep (unfermented) was 1.44 x 10^3 cfu/ml, while the highest population was 7.8 x 10^8 cfu/ml (fermented) as determined by standard plate counts. The microorganisms identified from the cassava steep included *Penicillium* spp, *Aspergillus* spp, *Rhizopus* spp, *Bacillus* spp and *Streptococcus* spp. The concentration of crude petroleum in the experimental set-ups inoculated with the cassava steep decreased from 35% at the beginning of the experiment to 4% during the 36-day study period, whereas there were no significant changes in the concentrations of the oil in the control set-ups during the period. It can be concluded that microorganisms isolated from fermented cassava steep can be used for bioremediation of oil polluted stagnant waters. This method can be applied to bioremediate fish ponds along Escravos Creeks in the Niger Delta region of Nigeria upon pollution with crude oil.
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Introduction

In recent years, marine pollution has been of increasing concern both nationally and internationally. Many materials have been demonstrated to have a global distribution in the marine environment. These include petroleum hydrocarbons, chlorinated hydrocarbons such as dichlorodiphenyltrichloroethane (DDT), its derivatives, polychlorinated biphenyls (PCBs); Polybrominated diphenyl ethers (PBDEs) and a number of heavy metal pollutants (de Wit, 2002; de Wit *et al*., 2006). Some of these substances of which great quantities are applied to crops and soil are very recalcitrant and are degraded only very slowly or not at all. Nigeria derives substantial revenue from oil. As oil becomes increasingly important in our society, the production and movement of larger volumes of oil means that more oil is available as potential pollutants. Occasional blowouts from both exploited and unexploited oil deposits spill oil into Nigerian environments thereby, causing pollution (Odiete, 1999). The problem of crude oil pollution is made worse by sabotage and deliberate vandalization of pipelines in the Niger Delta region of Nigeria. Oil degrading bacteria occur naturally in many aquatic and soil ecosystems which help to explain why there is no permanent residual build up of oil in the lands and oceans after oil spills have occurred (Nwachukwu and Ugoji, 1995). The effects of marine oil pollution can be seen in oil coated sands and tar balls on the beaches around the world and on the coastline around Furupa in the Rivers State of Nigeria. The techniques employed to remove oil pollutants from the environment include physical, chemical and biological methods (Atlas, 1981; Wang *et al*., 1995; Solano-Serena *et al*., 2000; Obayori *et al*., 2008). The physical and chemical methods hardly achieve complete elimination of oil from the environment. The use of chemical method involves application of expensive chemical dispersants, thereby introducing even more pollutants in the environment (Jain *et al*., 1992). Bioremediation therefore, is indispensable as the most natural method to eliminate the bulk of oil contaminants from the environment. Ironically, cassava, *Manihot esculenta* Crantz which is cheap and easily obtainable can be used as a bioremediating agent. Cassava is a tropical root crop that serves as a food security and income generation crop for many millions of people in the developing world (Scott *et al*., 2002; Burrell, 2003).). It is grown widely in Nigeria and in many regions of the tropics, where it serves as one of the basic food source for about 200 – 300 million people (FAO, 1991). In 1999, Nigeria produced 33 million tonnes making it the world's largest cassava producer. In this study, degradation of hydrocarbon is enhanced by the application of microorganisms from fermented cassava steep.

Materials and methods

Collection of Samples:

Water samples were aseptically collected from the University of Lagos Lagoon while the crude oil was obtained from the Department of Chemical Engineering, University of Lagos, Nigeria.

Fermentation of Cassava and Development of inoculum:

Fresh cassava tubers were fermented in a bowl containing tap water for a period of seven (7) days. The microbial population density in the cassava steep was determined at the beginning and at the end of the fermentation by standard plate count technique on nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for moulds and yeasts (Nwachukwu and Akpata, 2003). The optical density of the steep was also determined at 600nm to monitor the increase in turbidity of the cassava steep as a function of increase in microbial population.

Bioremediation protocols

Lagoon water (1000ml) and Nigerian crude oil (5%) were mixed in six separate bowls. Three of the bowls were inoculated with 100ml of fermented cassava steep as experimental set-ups. The other three bowls served as controls and were not inoculated with the fermented steep. Samples from the experimental and control set-ups were taken aseptically at intervals of 3 days for 36 days and analyzed for physico-chemical parameters which included optical density, pH, viscosity and specific gravity.

Residual oil

The residual crude oil was extracted from both the experimental and control samples using n-hexane: dichloromethane solvent systems (1:1) and quantified gravimetrically (Yveline *et al*., 1997). The oil extract was placed at ambient temperature for 3 days to evaporate the solvent system after which the residual oil was obtained by difference in mass.

Microbial Enumeration

Estimation of total heterotrophic microorganisms (THM)

During the study period, THM was estimated thus: NA and PDA plates were prepared and used for isolation of bacteria and fungi respectively. Aliquots (1.0ml) of appropriate diluted fermented cassava steep were plated on the above media in three replicates, spread aseptically and incubated aerobically with NA plates at 37^0C for 1-3 days and PDA plates at 30^0C for 3-5 days, At the end of the incubation period, developed colonies were counted. The relative abundance i.e. the population density estimate of the organisms was obtained by multiplying the plate count per ml for each organism by the dilution factor used (Nwachukwu and Ugoji, 1995).

Identification of isolates

Isolated organisms were identified based on their biochemical properties according to previous descriptions by Cowan and Steel (Barrow and Feltham, 1995; Smith, 1969).

Determination of physico-chemical parameters

Optical density:

The optical density of the experimental and control set-ups was determined using the photoelectric colorimeter (Model: AE- 11C Tokyo Erma Optical works, Ltd Japan) at 600nm (Rieck *et al*., 1993).

Determination of pH:

The pH of the experimental and control samples were determined with the use of a digital pH meter (Model: Jenway M50/Rev model CE 350 EU) in 1:1 sample solution in distilled water. The pH meter was calibrated using phthalate buffer (pH, 4.0) and phosphate buffer solutions (pH, 7.0).

Determination of viscosity:

The viscosity of the samples was measured according to the procedure of Rammohan and Yassen (2003) using a glass capillary viscometer (Model: Capirograph Toyoseikl Seisaku-Sho Ltd.). The sample was allowed to flow through an outlet tube (measuring tube which is narrowed into a capillary tube above the outlet). Two annular reference marks on the measuring tube were used. The time it took for the sample meniscus to drop from the upper to the lower reference mark was measured manually with a stop watch (seconds). The centistokes (CST) of the experimental and control samples were calculated by multiplying the measured time by the viscometer calibration factor at room temperature (30 ± 2^0C). The pH and CST measurements were done at three days intervals for thirty-six days.

Specific gravity:

The specific gravity determinations of the experimental and control set-ups were carried out by pycnometry as described by Ohwoavworhua and Adelakun (2005). The pycnometer was first weighed when empty, it was then washed thoroughly before introducing samples of the experimental and control set-ups into it, after which it was weighed again. Specific gravity was determined by dividing the weight of the sample by $50cm^3$ which is a constant for the pycnometer.

Results and discussion

The bacterial population in the cassava steep (unfermented) was 1.44 x 10^3 cfu/ml, while the highest population was 7.8 x 10^8 cfu/ml (fermented) as determined by standard plate counts. The microorganisms identified from the cassava steep included *Penicillium* spp, *Aspergillus* spp, *Rhizopus* spp, *Bacillus* spp and *Streptococcus* spp. Rapid degradation occurred between days 0 and 18 after which the remediation becomes notably slower for experimental set-ups. This might be because the nutrient was becoming limiting. In addition, a good number of the organisms might have died after day 18, due to competition for available space and decreasing level of nutrients. The concentration of crude petroleum in the experimental set-ups inoculated with the cassava steep decreased from 35% at the beginning of the experiment to 4% during the 36-day study period, whereas there were no significant changes in the concentrations of the oil in the control set-ups during the period. The reduction in oil from the experimental set-ups is an indication that bioremediation really took place. The control set-ups almost maintained the oil concentration throughout the duration of the experiment. The mean pH of the experimental set-up was slightly acidic, that of the control set-up was near neutrality. This was probably because the microorganisms were degrading the oil much rapidly in the experimental set-ups than in the control set-ups. Fig. 1 shows that the optical density of the experimental set-ups reached its peak (0.56) on the 18th day. It is noteworthy to observe that the viscosity of the experimental set-ups was at its lowest point (0.76 cp) on the same day. The optical density decreased from 0.39 on day 0 to 0.20 on day 9. It then increased again to 0.56 on day 18 before it started decreasing remarkably to 0.16 on day 36. From Figs 1-3, days 0-9 can be regarded as lag phase when the microorganisms were becoming adapted to their environment and the nutrients provided. Between days 9 and 18, the sharp rise in optical density indicates that the growth had been initiated, quite rapidly and that bacterial cells began to reproduce by binary fission. The maximum growth could be said to occur on day 18. The period between days 20 and 27 could be regarded as stationary phase, since there was no more rapid growth observed. According to Venosa et al. (1991), most biodegradation, when it occurs starts after 3-5 days lag period and reaches significant levels after 20 days. The period between days 27 and 36 was endogenous phase where respiration and death predominated. In contrast, there was variation of average values of optical density with time in the control set-ups. Here, it was observed that the average maximum optical density was 0.09. This indicates that if at all, growth or reproduction was taking place, it would be at a very slow rate. This probably is because the microorganisms in the control set-ups were destitute of nutrients, which could aid in growing and multiplying before biodegrading and bioremediation of the oil polluted water. The microorganisms identified from the experimental samples were *Penicillium* spp, *Aspergillus* spp, *Rhizopus* spp, *Bacillus* spp and *Streptococcus* spp. They were responsible for remediation of oil under favourable conditions. It was also observed that the average pH values of the experimental set-ups were higher than the control set-ups. The mean change in pH of experimental set-ups decreased from day 0 to day12 as well as from day 15 to 24. The maximum pH was 6.65 on days 27, 30 and 36. The mean changes pH of the control set-ups gradually decreased from day 0 to end of the study. Crude oil fragments penetrate a lot into the electrode during pH determination, despite the continual calibration. This prevented us from obtaining reliable pH values. Meanwhile, while the mean pH of the experimental set-up was slightly acidic, that of the control set-up was near neutrality. The mean viscosity values were a little bit generally higher in the control set-ups during the study period. This was because the microorganisms were degrading the oil much rapidly in the experimental set-ups than in the control set-ups. In contrast to the control samples, an obvious decline in the average specific gravity was observed in the experimental samples from days 15 to 36.

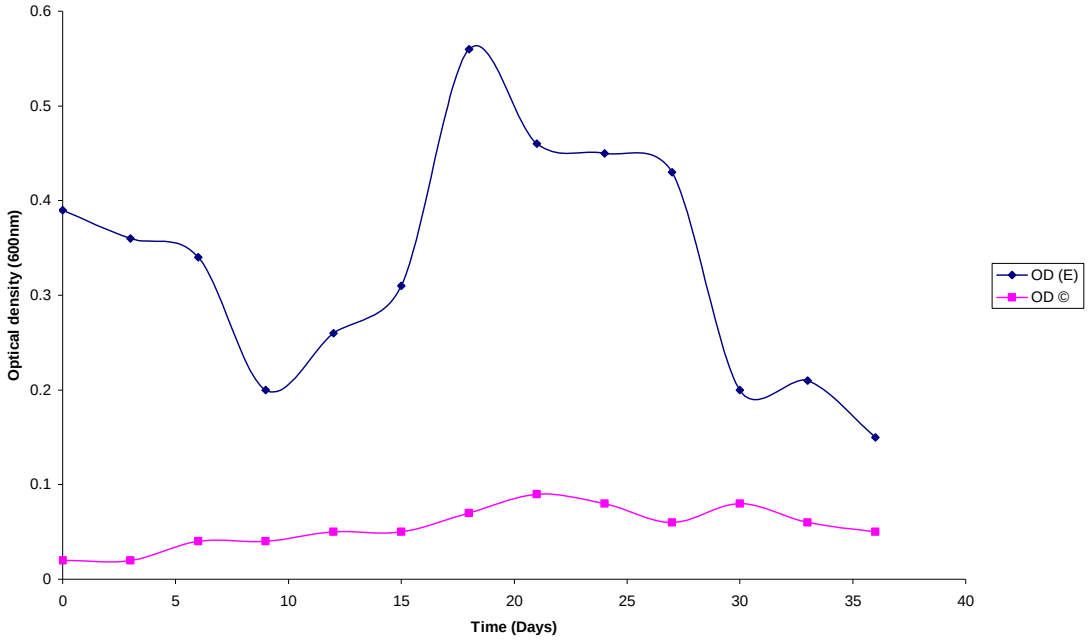

Fig.1 Optical density of experimental and control samples OD (E), Optical density of experimental samples; OD©, Optical density of control samples

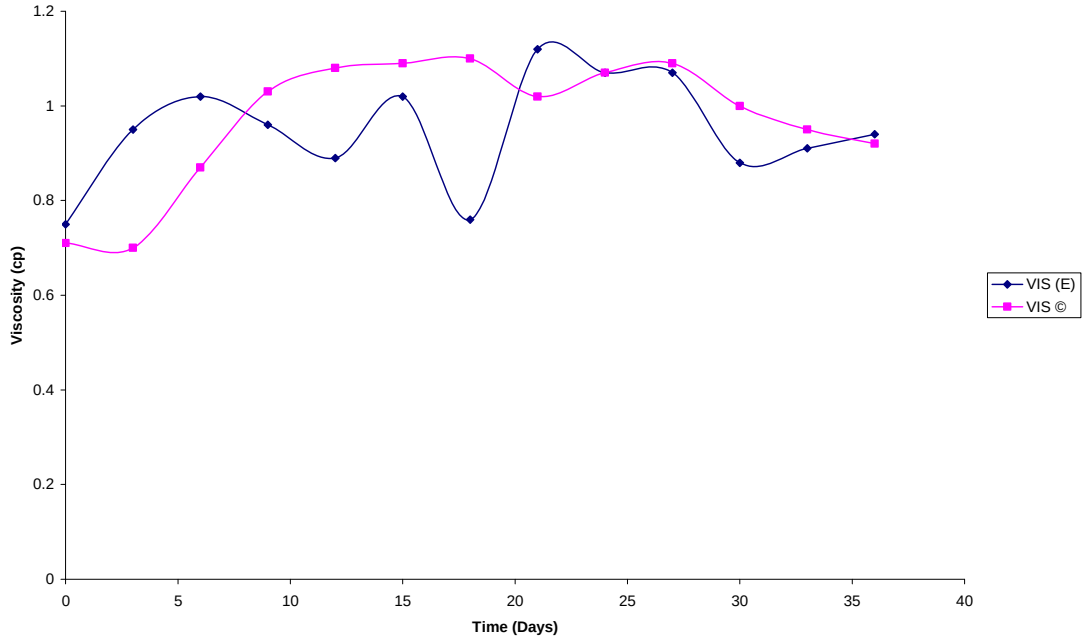

Fig. 2 Viscosity of experimental and control samples Vis (e), viscosity of experimental samples; Vis ©, viscosity of control samples

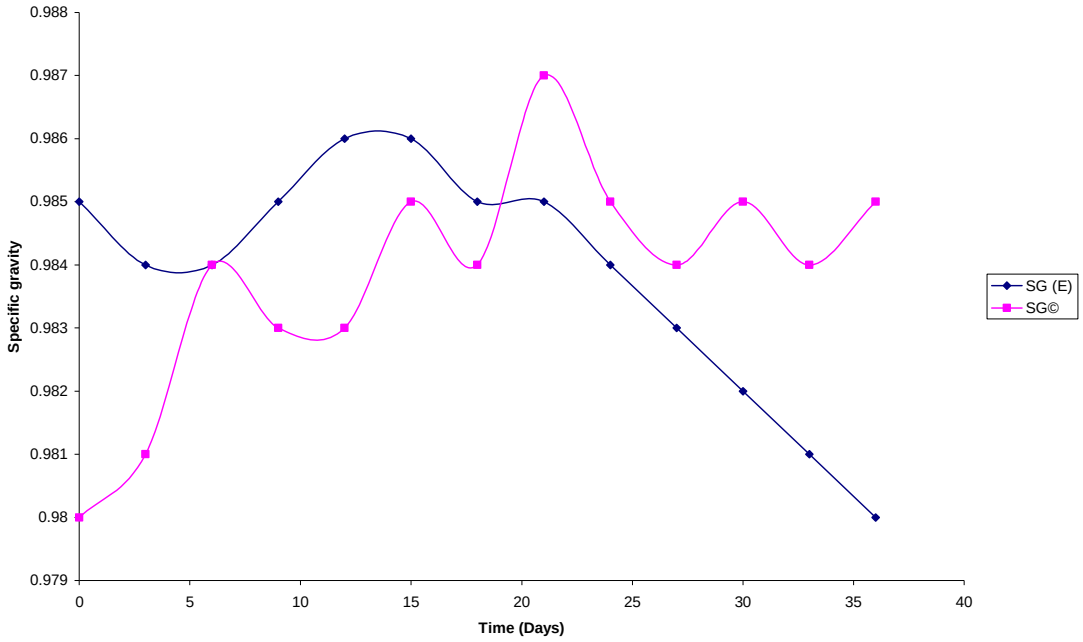

Fig. 3 Specific gravity of experimental and control samples SG (E), specific gravity of experimental samples; SG ©, specific gravity of control samples

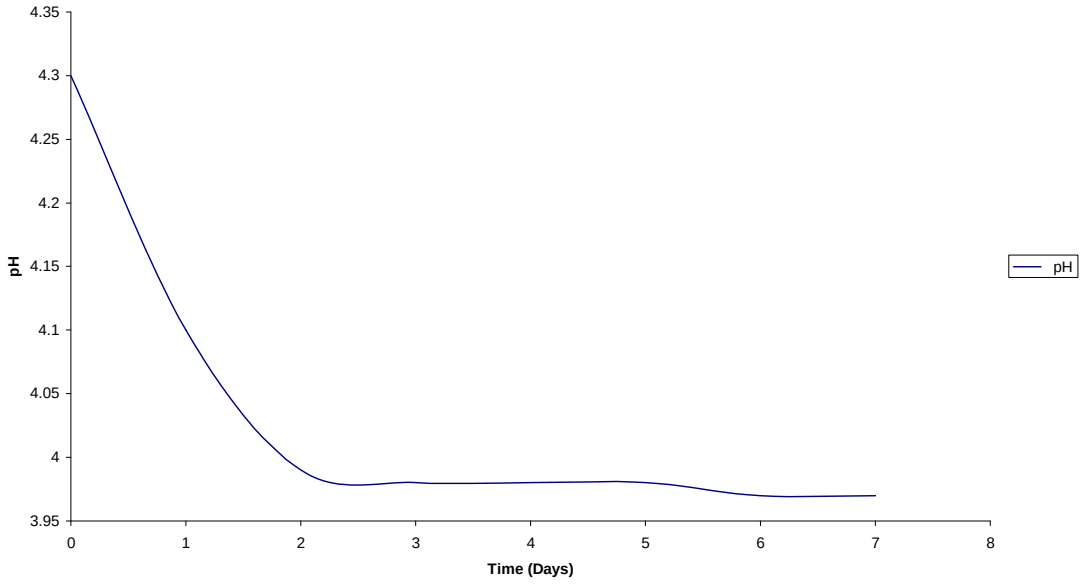

Fig. 4 pH of cassava steep during fermentation

Conclusion

This preliminary study focused on the possibility of bioremediating crude oil polluted stagnant water which results from accidental discharges from refinery effluents, vessels carrying crude oil, pipeline vandalization, loading and off loading operations and oil pipe leakages. Our data from results of the experiments carried out over a period of 36 days, and observations made, emphasise the interesting potential of microorganisms isolated from fermented cassava steep in the bioremediation of oil polluted stagnant water and perhaps, larger water bodies.

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