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Short Communication

Laboratory based degradation of light crude oil by aquatic phycomycetes

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Water samples were collected from Dundaye river (an offshoot of sokoto river Rima) using sterile conical flasks. The samples were immediately transported to the Microbiology Laboratory and introduced into petri dishes containing hemp seeds (*Crotalaria juncea*) and allowed to stand on the bench. The hemp seeds served as baits, after five days a whitish cottony growth surrounding the seeds was observed. The growth was subcultured in potato dextrose broth (PDB) and incubated at 37°C for 48 h. In the degradation experiment, a minimal medium containing 0.2% of the crude oil as sole source of carbon was prepared in replicates. The results obtained indicate that phycomycetes have a potential use in bioremediation.

Key words: Aquatic phycomycetes, crude oil.

INTRODUTION

Environmental protection is gradually gaining serious grounds even particularly in developing countries. In Nigeria, operations of multi national oil companies coupled with poverty-induced vandalization of oil pipelines lead to serious release of crude oil into the environment (Nnadozie, 1995). This spilled oil produces destructive effect on the environment. For example, when the spillage occurs on land, the land loses its agricultural value. Furthermore, when it occurs in water bodies, the aquatic resources such as fish are killed. Many of the standard treatment processes used to decontaminate land and water bodies are not without shortcomings. Apart from additional pollution effect chemical sprays are not only costly but may only be partially effective (Nicholas, 1987; Steven, 1991). There is the need to develop remediation and waste reduction technologies that are efficient, economical and rapidly deployable in a wide range of physical settings (Cattalo and Portier, 1992).

Traditional methods have relied upon removal or containment (Neely, et al, 1981; Brown et al, 1986). Bioremediation (the use of microorganisms to clear oil spills) is now becoming the most efficient and common oil spill clean up processes (Okpokwasili, 2002). A good member of bacteria is now reported to have a chemical "appetite" for oil. These include *Pseudomonas* spp., *Staphylococcus* spp. and *Acinetobacter* spp. (Miklosovicova and Trzilova, 1991; Okoh, 2003; Okerentugba and Ezeronye, 2003; Nweke and Okpokwasili, 2003). According to Atlas et al. (1978), several fungi exhibit more efficient degradation of the petroleum hydrocarbon than bacteria. That is why in this study aquatic phycomycetes were tested for their ability to degrade light crude oil.

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MATERIALS AND METHODS

Water samples were collected from six different points of Dundaye River. The water samples were distributed in equal amounts (20 ml) into petri dishes. Six prepared halved hemp seeds (*Crotalaria juncea*), were placed in each petri dish. Three drops of Streptomycin Sulphate (5 g per liter) were added to each plate to inhibit bacterial growth (Agina and Kpu, 1988). The plates were allowed to stand on the bench at room temperature and examined daily for fungal growth. A whitish cottony growth was obtained surrounding the hemp seeds. The growth was subcultured on potato dextrose agar (PDA) using wire loop.

Lactophenol cotton blue staining technique was used to aid identification of the fungus. A drop of the stain (lactophenol cotton blue) was placed on a clean glass slide using an inoculating needle held with both hands. A small piece of the mycelium was teased out; it was then covered with cover slip and viewed under the microscope. Presence of coenocytic hyphae, granular protoplasm and oil globules were observed.

In the degradation experiment, a minimal medium containing 0.2% of light crude oil (in which small amount of urea was added)

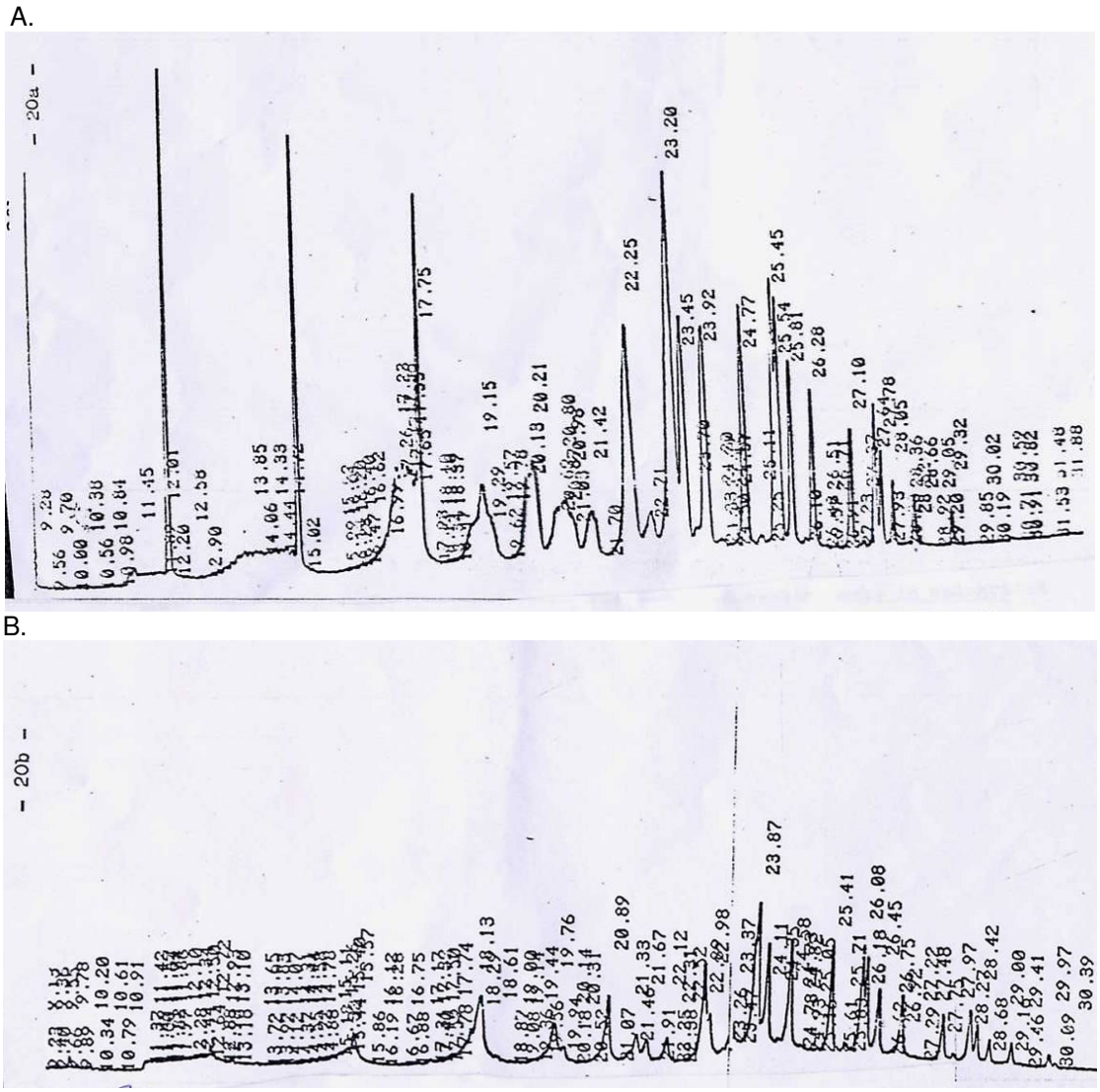

Figure 1. A. Chromatogram showing the carbon peaks of crude oil. B. Chromatogram showing the carbon peaks after degradation of crude oil by aquatic phycomycetes.

was prepared and dispensed in small conical flasks. The medium was inoculated with young cultures of the isolates. The experiment was allowed to stand for two weeks. After two weeks the fungal growth was separated from the oil using gas chromatography. The hydrocarbon that dissolved in carbon tetrachloride was drained through a funnel in which a filter paper containing sodium sulphate was used. The Carbon tetrachloride was evaporated leaving only the hydrocarbon. The separated hydrocarbon was again dissolved in dichloromethane (5.5 mg/ml of undegraded crude oil and 5.2 mg/ml of degraded crude oil).

RESULTS AND DISCUSSION

The fungal colonies were identified as *Saprolegnia* spp.

The chromatographic machine used could not exceed the temperature of 280°C. Therefore, all fractions of crude oil above 280°C were not eluted. The figure below shows results for only gasoline (40-170), Kerosene (170-230) and some gas oil (230-300).

The appearance of dark stains within the hyphal fragments even after the separation of hydrocarbon and the culture suggest that it is the degraded component of the oil that becomes adsorbed to hyphal cell wall. It is also clear from the carbon peaks that aquatic phycomycetes can degrade kerosene component of the oil to a large extent. Gasoline and gas oil were also degraded (Figure 1).

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