BIOREMEDIATION PROSPECTS OF FUNGI ISOLATED FROM WATER SOLUBLE FRACTION OF CRUDE OIL SAMPLES

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ABSTRACT

The fungi associated with water soluble fraction (WSF) of crude oil from two different locations were investigated. The samples were collected from Ezibin oil well (Sample A), Okwagbe village in Ughelli South Local Government Area of Delta State and from NPDC laboratory (Sample B) in Benin City, Oredo Local Government Area of Edo State. The WSF of Samples A and B were used at full strength (100%). Both samples were cultured on sabouraud dextrose agar (SDA). The salinity level, ionic contents, heavy metals and physical characteristics were determined. The fungi isolated and identified were *Trichoderma harizianum* (found in both samples), *Aspergillus accileatus* from Sample A only and *Trichoderma reesei* from Sample B only. The values of sum of ions, physical characteristics (Sum of EC + TDS) and the analyzed values were higher in Sample A than Sample B. Sample A was more acidic (pH5.6) than Sample B (pH6.5). Sample A showed significant difference at ($P<0.005$) in the level of ions, while Sample B showed no significant difference ($P>0.05$), but had significant difference at $P<0.05$ in the level of pH, EC and TDS. The ability of the fungi to adapt to these conditions indicates their potential as a tool for bioremediation of crude oil polluted water.

Keywords: Bioremediation, Crude Oil, Fungi, Polluted Water, Potential.

INTRODUCTION

Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds. Several studies on spills on the environment in the Niger-Delta area and other tropical areas throughout the world consistently showed that the areas which are directly exposed to large or repeated oil spills exhibit long term environmental problems. Oil pollution is one of the environmental consequences of crude oil exploration and exploitation activities producing aqua-toxicological effects which are deleterious to aquatic life (Agbogidi *et al.,* 2005; Edema *et al.* 2007). Water soluble fraction (WSF) is a single phase solution comprised of individual dissolved molecules. Organisms exposed to water soluble fraction of crude oil take up the dissolved hydrocarbon and react to their effects (Overton *et al.,* 1994), and these effects on the growth and development of different organisms have been studied (Edema *et al.*, 2008).

Fungi have evolved a high degree of metabolic versatility that allows them to use a diverse range of organic substrates for growth, including simple compounds such as nitrate, ammonia, acetate or ethanol. The growth of fungi as hyphae and single cells in aquatic environment is adapted for the efficient extraction of nutrients, because these growth forms have high surface area to volume ratio. Fungi are diverse in their ecological adaptation; some of these have been said to help in bioremediation (Kacprzak *et al.*, 2005). They have potential for cleaning up the environment in processes such as detoxifying noxious chemicals and removing oil spills. Fungi are known to degrade aromatic hydrocarbons. They adapt more readily to diverse conditions for low moisture and low pH as compared to most bacteria. They are thus useful in predicting the impact of a particular stress on the environment by their ability to respond to these adverse conditions through a change in their number (Alexopoulos *et al.,* 1996).

Uzoamaka *et al.* (2009) reported that some fungal isolates that were recovered from oil contaminated soils showed potentials for biodegradation. Andrea *et al.* (2001) reported that fungal species such as *Trichoderma* and *Phanerochaete* have been implicated in hydrocarbon degradation of crude oil contaminated soils. Among these isolates, *Aspergillus versicolor* and *Aspergillus niger* were shown to display the fastest and highest extent of biodegradation and the implication of these two organisms in hydrocarbon degradation has been reported by April *et al.* (2006).

Salt level or salinity is the saltiness or dissolved salt

content of a body of water. It is a general term used to describe the levels of different salts such as sodium chloride, magnesium, calcium, sulphates and bicarbonates. Salinity is not expressed as percent, but as part per thousand ($^{o}/_{oo}$) Mantyla (1987), which is approximately amount of salt (in grams) per kilogram of solution. Salt is a natural element of soils and water. The ions responsible for salinization are Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Cl^{-}. Salinity is the sum weight of many different elements within a given volume of water (Mantyla, 1987). The salt found in crude oil is dissolved in water of the crude oil and not in crude oil itself. The salts that are most frequently present in crude oil are calcium, sodium and magnesium chloride (Pinet, 1996).

The environment contains a wide range of different filamentous fungi that degrade hydrocarbon (Bijofp, 2003). Microbiota populations show a rapid increase in response to moderate level of oil, much of the population increase is due to increase in oil degrading microflora. Most of the restoration of polycyclic aromatic hydrocarbon (PAH) contaminated sites depends on the use of plants for phytoremediation or the use of fungi for mycoremediation.

The aim of this study was to determine the salinity, ionic content and physical properties of water soluble fraction of crude oil as well as to isolate and identify the fungi associated with it, in order to ascertain the possible use of the fungi in bioremediation.

MATERIALS AND METHODS

Collection of crude oil samples

The crude oil samples were collected from Ezibin oil well, Okwagbe Village, Ughelli South Local Government Area, Delta State (Sample A) and Oredo flow-station, National Petroleum Development Corporation (NPDC) laboratory in Benin City, Edo State. The experiments were conducted in Botany, Chemistry and Microbiology laboratories of the Delta State University, Abraka.

Preparation of WSF

The WSF was prepared according to the method by Anderson *et al.* (1974). A sample of crude oil (500 ml) was slowly mixed in 500 ml de-ionized water in a 2 litre conical flask. Using an orbital magnetic stirrer with a magnetic rod, stirring was done for 24 hours at room temperature (28±2°C) for both samples (A and B) of crude oil. After 24 hours of stirring, the oil-water mixture was allowed to stand for another 24 hours in a separating funnel; the lower phase of the oil-water mixture was collected and used as 100% strength for both samples.

Preparation of WSF culture

The WSF samples were cultured on potato dextrose agar (PDA) (Oxoid, England) using the pour plate method. A volume of 1ml of each WSF concentration was transferred into a Petri dish. Then 20 ml of PDA, prepared according to manufacturer's instructions and containing 50µg/ml chloramphenicol to inhibit bacteria growth, was poured into the plate. The plate was swirled round for even mixing and allowed to solidify. Control plates contained 1ml de-ionized water instead of WSF. All culture plates were incubated at room temperature (28±2°C) for 7 days. Culture samples were examined under low (x40) and high (x100) powers of an optical microscope for fungal growth. Fungal identification was done by CABI, U.K.

Determination of properties of WSF

The physical characteristics, ionic contents and heavy metals determinations were done according to the methods described by Ademoroti (1996) and Trivedi and Raji (1997).

Statistical analysis

The data were subjected to statistical analysis of variance (ANOVA) using mean ± standard error, comparison test (Turkey Multiple Comparison Test). The significance was tested at 5% probability level.

RESULTS

Three fungal species were isolated (Table 1) and identified as *Trichoderma harziarium, Trichoderma reesei* and *Aspergillus acculeatus. Trichoderma harziarium* and *Aspergillus aculeatus* were associated with Sample A, while *Trichoderma harzianum* and *T. reesei* were associated with Sample B. The study showed that the WSF contained some amount of salt (Table 2). The total salinity in Sample B was higher than Sample A. Table 3 shows that the pH values for Samples A and B were 5.60 and 6.50, respectively. Sample A was more acidic than

Sample B. Samples A and B showed a significant difference (P<0.05) in the level of EC and TDS (Table 4). The sum of physical characteristics had the least values.

Heavy metals namely, Fe, Cu, Cr, Pb, Va, Zn and Mn were found to be present (Table 5). Zinc had the highest value (2.46) in Sample B followed by Mn (2.45) in Sample A. Vanadium had the least value (0.01) in Sample A while Cr was not detected in Sample B.

Table 1: Fungi Isolated from WSF Samples

Fungi Isolated	Sample A	Sample B
Trichoderma harzianum	+	+
Trichoderma reesei	—	+
Aspergillus aculeatus	+	—

Fungi identification was done by CABI, UK.

Table 2: Total Salinity of WSF of Samples A and B using three Measurements

Total Salinity	Sample A $^{o}/_{00}$	Sample B $^{o}/_{00}$ (parts per thousand)
Sum of Ions	8.53	10.18
Sum of physical characteristics		
EC + TDS	0.38	0.45
Analysed values	0.73	1.23

Table 3: Ions in WSF of Samples A and B

Ions	Sample A	Sample B
Mg^{2+}	0.82±0.00	0.83±0.00
Ca^{2+}	0.86±0.00	0.93±0.00
K^{+}	0. 78±0.00	1.06±0.00
Na^{+}	1.25±0.01	1.46±0.01
Cl^{-}	2.04±0.02	2.05±0.01
NO_3^{-}	1.01±0.34	1.48±0.01
SO_4^{2-}	0.88±0.02	0.92±0.01
HCO_3^{-}	0.87±0.01	0.98±0.02
PO_4^{-}	0.02±0.00	0.03±0.00
Sum of Ions	8.53	10.18

*Values in mean ± S.E. (P<0.05)

Table 4: Physical Characteristics of the WSF of Crude Oil of Samples A and B

Physical characteristics	Sample A	Sample B
pH	5.60±0.03	6.50±0.00
EC	0.31±0.00	0.33±0.00
TDS	0.07±0.00	0.12±0.00
Sum (EC + TDS)	0.38±0.00	0.45±0.00

*Values of mean ± S.E. (P<0.05)

Table 5: Heavy Metals in WSF of Crude Oil of Samples A and B

Heavy Metals	Sample A	Sample B
Fe	0.16±0.00	0.14±0.00
Cu	0.16±0.00	0.12±0.00
Cr	0.02±0.00	ND
Pb	0.03±0.00	0.02±0.00
Va	0.01±0.00	0.02±0.00
Zn	2.10±0.01	2.46±0.00
Mn	2.45±0.01	2.12±0.00

*Values in mean ± S.E. ($P<0.05$)
ND: Not Detected

DISCUSSION

Trichoderma species have been implicated in hydrocarbon degradation of crude oil contaminated soil (Andrea *et al.,* 2001). Also, some fungal isolates such as *Aspergillus* species and *Trichoderma* species have been reported by April (2000) to show potentials for hydrocarbon biodegradation. Also, April (2000) showed that *Aspergillus* species display the fastest and highest extent of biodegradation and implication of the organism in hydrocarbon degradation. *Trichoderma* and *Aspergillus* spp among other genera of fungi are known to metabolize hydrocarbon or thrive in oil contaminated sites (Llanos and Kjoller, 1976; Bartha and Atlas, 1977). It has also been reported that *Aspergillus* and *Penicillium* species were more efficient metabolizers of hydrocarbon than other fungal species such as *Candida, Mucor, Fusarium, Geotrichum, Rhodotorula* and *Trichoderma* (Obire *et al.,* 2008). The isolation of *Trichoderma* and *Aspergillus* species from the WSF samples therefore, supports these previous findings.

The salinity result was in agreement with Edema (2006). The salts that are most frequently present in crude oil are calcium, sodium and magnesium chloride (Pinet, 1996) and these were among the detected salts in the current report. The ions responsible for salinization are Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Cl^{-}. These ions invariably contributed to the high salinity of the samples recorded in this work and partly explains the higher salinity of sample B. Salinity is the sum weight of many different elements within a given volume of water (Mantyla, 1987). Salinity is an ecological factor of considerable importance influencing the type of organisms that live in a water body (Lewis, 1982).

Several elements were detected. However, the salinity result may be attributed to the absence of ionized components of other elements such as N, P, Fe and numerous minor elements which are of immense biological importance but are usually minor contributors to total salinity (Wetzel, 2007).

Total dissolved solids and ionic conductivity of water are generally used measurements (Covich, 1993). These properties regulate the pH. The low pH conditions under which the fungi thrived is in agreement with (Alexopoulos *et al.,* 1996) that fungi adapt more readily to diverse conditions of low pH.

Sites contaminated by organic compounds have been shown to be characterized by heavy metals (Bouchez *et al.,* 2000). In such a difficult case, some workers opine that the use of filamentous fungi may give some advantage over bacterial bioaugmentation. Fungi display a high ability to immobilize toxic metals (Baldrian, 2003). Fungal bioremediation has been successful for clean-up of pentachlorophenol (PCP), a wood preservative and polycyclic aromatic hydrocarbon (Andrea *et al.,* 2001).

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