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Full Length Research Paper

Bioremediation of oil-polluted soil by *Lentinus subnudus*, a Nigerian white-rot fungus

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Inspite of the realization and studies on the use of microorganisms in degrading hydrocarbons there has been very little work on indigenous white-rot fungi in Nigeria, a leading oil – producing country. The ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil was tested. Organic matter and carbon were higher than the control at all concentrations of crude oil contamination in soils inoculated with *L. subnudus* for 3 months. Nutrient contents were generally higher after 6 months of incubations except potassium levels which were not significantly different from the control. As for the total petroleum hydrocarbon (TPH) in crude – oil contaminated soils; the highest rate of biodegradation was at 20% concentration after 3 months and 40% after 6 months of incubation.

Key words: bioremediation, crude oil, Total Petroleum Hydrocarbon oil-polluted soil, *Lentinus subnudus*.

INTRODUCTION

There has been increasing interest by researchers in the application of organisms and nutrients to contaminated soils for effective biodegradation of oil. Various strains of white-rot fungi capable of degrading aromatic compounds were reported by Barr and Aust (1994). In his own contribution, Reddy (1995) observed that lignin-degrading white-rot fungi have the unique ability to degrade or mineralize a broad spectrum of structurally diverse toxic environmental pollutants. Similarly, Lang et al. (1995) reported that lignin-decomposing white-rot fungi show extraordinary abilities to transform recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAH). They added that this unique capability may be used for decontamination of oil polluted soils though a lignocellulose substrate must be supplied for the survival of the fungal species in the soil.

The analysis of Aislabie et al. (1998) indicates that the low availability of nutrients (nitrogen and phosphorus), low water content and alkaline pH of some of the soils were factors likely limiting oil biodegraded rates during summer. Environmental parameters that affect biodegradation of oil in cold climates have also been extensively studied by Atlas (1981) in the Arctic where he found that rates of biodegradation of oil were sensitive to temperature extremes.

Although several studies have been conducted on mineralization or degradation of hydrocarbon by microorganisms (Ojumu et al. 2005) and exotic mushrooms, very little work has been done on indigenous white-rot fungi. In view of this, the present work is aimed at studying the ability of *L. subnudus* to mineralize soil contaminated with various concentrations of crude oil.

MATERIALS AND METHODS

Fungal cultivation and incubation

The culture conditions were according to the method of Baldrian et al. (2000) and modified as follows. 100 g of sterilized soil moistened with 75% distilled water (w/v) were weighed into 9 x 9 x

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Table 1. Nutrient contents of soils contaminated with crude oil and incubated with *L. subnudus* for 3 months.

Treatment (Conc. Of crude oil)	Organic Matter (%)	Carbon (%)	Nitrogen (%)	Phosphorus (ppmig)	Available Potassium (Meq/100g)	pH
Control	3.33^{c}	1.93^{d}	0.14^{a}	19.92^{bc}	0.52^{ab}	6.70^{a}
1%	4.68^{c}	2.71^{c}	0.14^{a}	27.72^{a}	0.50^{a}	6.70^{a}
2.5%	4.68^{c}	2.85^{c}	0.14^{a}	24.19^{ab}	0.49^{b}	6.50^{a}
5%	4.19^{c}	3.00^{c}	0.14^{a}	20.74^{bc}	0.47^{b}	6.50^{a}
10%	5.17^{c}	3.00^{c}	0.14^{a}	16.78^{c}	0.39^{b}	6.80^{a}
20%	8.62^{b}	5.00^{a}	0.14^{a}	9.79^{d}	0.39^{b}	6.53^{a}
40%	10.07^{c}	5.84^{a}	0.14^{a}	3.75^{c}	0.26^{C}	6.70^{a}

Each value is the mean of 3 replicates values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test (0.05).

Table 2. Nutrient contents of soil contaminated with crude oil and incubated with *L.subnudus* for 6 months.

Treatment (conc. of crude oil)	Organic Matter (%)	Carbon (%)	Nitrogen (%)	Phosphorus (PPMLG)	Available potassium (Meq/100g)	pH
Control	1.90^{d}	1.10^{c}	0.14^{a}	0.90^{c}	1.09^{ab}	6.41^{a}
1%	1.90^{d}	1.10^{d}	0.21^{a}	0.90^{c}	1.21^{ab}	6.52^{a}
2.5%	2.44^{cd}	1.41^{cd}	0.22^{a}	1.94^{b}	1.23^{ab}	6.55^{a}
5%	3.17^{bc}	1.84$^{b\,c}$	0.27^{a}	3.58^{bc}	1.27^{a}	6.39^{a}
10%	3.31^{b}	1.92^{b}	0.35^{a}	4.03^{ab}	0.96^{ab}	6.11^{b}
20%	3.53^{b}	2.05^{b}	0.50^{a}	5.23^{a}	0.95^{ab}	5.95^{b}
40%	16.85^{a}	4.03^{a}	0.51^{a}	5.52^{a}	0.75^{b}	5.92^{b}

Each value is the mean of 3 replicates: values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

4 cm (350 cm^3) jam bottles and then mixed thoroughly with crude oil concentrations (1, 2.5, 5, 10, 20 and 40%). 20 g of clean rice straw were laid on the contaminated soil in each bottle, covered with aluminium foil and autoclaved at 15 lbs pressure for 15 min. each bottle was then inoculated with two agar plugs of a vigorously – growing mycelium of *L. subnudus* using a 7 mm sterile cork borer. the bottles were incubated at room temperature for 3 – 6 months.

In the first set of control treatments, crude oil was not added to the soils while in the second set, different levels of oil were added to all soils but not inoculated with the fungus. At 3 and 6 months after incubation, the mycelial-ramified waste was separated from the soils which were analyzed for physico-hemical parameters after air-drying.

Soil pH, organic carbon, nitrogen and phosphorus

The soil pH was determined according to the procedure of Bates (1954). Organic matter and percentage nitrogen were determined following the method of the Association of Official Agricultural Chemists (AOAC, 1980). Determination of percentage nitrogen was done first by titrating distilled digested sample and 0.02 N NaOH and %n calculated using the formula:

$\%N = (A_1 \times 0.08 \times 100) / \text{wt of soil titre}$

Where A_1 = titre value

Phosphorus and potassium constituents were determined as outlined in aoac (1980). Total petroleum hydrocarbon content of the soil was determined using a Fourier transform infrared spectrometry at the Global Environmental Consultants Laboratory Warri, Nigeria. a completely randomized design experiment was used for biodegradation studies and each replicated three times.

RESULTS

In soils contaminated with crude oil and inoculated with *L. subnudus* for 3 months, organic matter and carbon were higher than the control at all concentrations of contamination (Table 1) while nitrogen remained constant. Phosphorus constituents fluctuated and the potassium levels were not significantly different from the control except at 40% concentration. As shown in Table 2, nutrient contents were generally higher than the control after six months of incubation except potassium levels which were not significantly different from the control.

The Total Petroleum Hydrocarbon (TPH) of crude oil contaminated soils incubated with *L. subnudus* for 3 and 6 month are shown in Table 3 The rate of biodegradation increased at 20% concentrations of crude

Table 3. TPH of crude-oil contaminated soil incubated with *Lentinus subnudus*.

TPH (Mg / Kg)			
Treatment (concen. of crude oil)	**0 Months**	**3 Months**	**6 Months**
1%	9423 ± 0.05	6310 ±0.03	3712 ± 0.01
2.5%	34664 ± 0.10	15019 ± 0.20	7538 ± 0.10
5%	62241 ± 0.20	52996 ± 0.18	8937 ± 0.30
10%	140305 ± 0.15	104849 ± 0.25	14677 ± 0.20
20%	256980 ± 0.25	198980 ± 0.25	12535 ± 0.13
40%	336930 ±0.40	285532 ± 0.30	16447 ± 0.25

Each value is a mean of 3 readings ± standard error

after 3 months while values decreased after 6 months of incubation.

DISCUSSION

Lentinus subnudus inoculated on crude oil contaminated soil showed higher nutrient distribution of organic matter, carbon and phosphorus compared to the control with increase in crude oil concentration after 3 and 6 months. This indicates that biodegradation has taken place. A lower value of phosphorus and potassium was observed with increase in crude oil concentration. Lehtomake and Niemela (1973) reported a low value of nitrogen, potassium, phosphorus reserves in petroleum hydrocarbon contamination. From this study, a reduction in nutrient contents of contaminated soils after introduction of the white-rot fungi was observed at higher levels of 10–20% crude oil concentration compared to lower levels of contaminated oil. This is similar to the findings of Calder and Lader (1976) who found that toluene and phenol may stimulate growth at low concentrations, but show bacteriocidal action at high concentration and solubility of the crude oil components. Leahy and Colwell (1990) also reported that very high concentrations of hydrocarbon will inhibit biodegradation by nutrient or oxygen limitation.

Leahy and Colwell (1990) stated that pH is a predominant factor in determining biodegradation in soil. in this work the pH of contaminated soils reduced after the introduction of the white-rot fungi from 6.90 to 6.62 and finally to 6.25 after 3 and 6 months of incubation, respectively. Dibble and Bartha (1979) found that the pH range of 5.0 to 7.8 favoured the degradation of oily sludge in the soil.

The present study revealed that the total petroleum hydrocarbon values were higher on contaminated soils compared to the control suggesting the presence of more petroleum hydrocarbon. Odu (1978) reported similar effect in soil in mangrove and rain forest soils polluted with crude oil. the best rate of biodegradation of crude oil by *L. subnudus* was after 3 months is similar to the findings of Odu (1972) who reported that incubation for biodegradation takes several months. A decrease in TPH was recorded from 3 to 6 months and this agrees with the observation of Sorkoh et al. (1997).

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