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Full Length Research Paper

Comparative study of *Aspergillus niger* and *Penicillium* sp. in the biodegradation of automotive gas oil (AGO) and premium motor spirit (PMS)

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The comparative study of *Aspergillus niger* and *Penicillium* sp. in the biodegradation of automotive gas oil (AGO) and premium motor spirit (PMS) was carried out to ascertain the effectiveness of using these microorganisms in cleaning and restoring the ecosystem when polluted by petroleum products. These fungi were observed to grow at all concentrations of the petroleum products (pollutant) used, but the growth on AGO was considerably reduced compared to the growth on PMS. The mycelia growth of *A. niger* was observed to be faster in PMS than in AGO. The ability of *A. niger* and *Penicillium* sp. to tolerate these pollutants and grow on them, suggest that they can be employed as bioremediation agents and can be used in restoring the ecosystem when contaminated by these pollutants.

Key words: *Aspergillus niger*, *Penicillium* sp., biodegradation, automotive gas oil, premium motor spirit**.**

INTRODUCTION

Worldwide, crude oil contamination of soil and ground water is a severe problem. The negative effects of this pollutant on the environment and on human health are diverse and this depends on the nature of pollution. The search for alternative methods for excavation and incineration to clean polluted site resulted in the application of bioremediation techniques (Atlas and Bartha, 1992).

Mycoremediation as a technique focuses on the degradation of organic compounds by fungi and this is achieved through the production of extra-cellular and intracellular enzymes which catalyses various reactions (Paszezynski and Crowford, 2000). It is practically established that fungi (mostly white rot fungi) are capable of using their mycelia to bioremediate hydrocarbon products due to their high production of organic acids, chelators, oxidative enzymes and extracellular enzymes that enables them to utilize the hydrocarbon product faster (Stamets, 1999). Also, the ability of fungi to lower the pH of its environment appears to be involved in the reduction of some of these compounds.

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This work is a report on the effective biodegradation of automotive gas oil (AGO) and premium motor spirit (PMS) by *A. niger* and *Penicillium* sp.

MATERIALS AND METHODS

Soil samples were collected from Eddy Grace Petrol Station, Km 9, Benin-Lagos Express Road, Ugbowo, Benin City and were transported in a wide mouthed sample bottles to the Microbiology Laboratory, University of Benin, where they were analyzed and stored at room temperature for further analysis.

Media

Growth media used for enumeration and characterization of fungi were formulated according to the cultural requirements of fungi (APHA, 1995). Potato dextrose agar (PDA) was prepared according to manufacturer's instruction and used for the isolation of fungi. PDA-PMS and PDA-AGO media were prepared by adding unused PMS and AGO to four different conical flask of PDA (50 ml) in sequence (2.5, 5.0, 7.5 and 10.0 ml) to obtain a concentration of 5, 10, 15 and 20% mixture. A control was prepared (PDA of 50 ml without PMS and AGO). All media were steam sterilized at 121°C for 15 min. The medium for the primary isolation of fungi was formulated with streptomycin-penicillin antibiotics added at a final concentration of 0.6 ml/Petri dish of PDA. Addition was made by dissolving 5.0 g each of streptomycin and penicillin in 100 ml of sterile distilled water. To the 100 ml of penicillin solution, 20 ml of

Table 1. Growth of *Penicillium* sp. mycelia on PMS in 8 days.

Time (days)	Concentration/growth (mm)			
	5%	10%	15%	20%
0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
1	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
2	10.6 ± 0.07	10.1 ± 0.07	9.7 ± 0.3	9.05 ± 0.07
3	11.0 ± 0.3	10.3 ± 0.3	9.9 ± 0.07	9.1 ± 0.14
4	26.0 ± 0.0	29.0 ± 0.0	24.5 ± 0.7	23.5 ± 0.7
5	32.0 ±0.0	35.0 ± 0.0	34.5 ± 0.7	28.5 ± 0.7
6	35.0 ± 0.0	37.5 ± 2.1	35.5 ± 0.7	29.0 ± 0.0
7	36.0 ± 0.0	37.5 ± 0.7	36.5 ± 0.7	29.5 ± 0.7
8	38.0 ± 0.0	37.0 ± 0.0	37.5 ± 0.7	30.0 ± 0.0

Values are mean ± standard deviation.

Table 2. Growth of *Penicillium* sp. mycelia on AGO in 8 days.

Time (days)	Concentration/growth (mm)			
	5%	10%	15%	20%
0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
1	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
2	9.5 ± 2.1	9.1 ± 1.4	9.0 ± 1.2	9.1 ± 0.1
3	11.5 ± 0.9	10.8 ± 0.8	10.1 ± 0.1	16.6 ± 9.1
4	18.9 ± 10.0	20.3 ± 12.4	17.4 ± 9.3	26.0 ± 2.8
5	26.6 ± 7.6	27.8 ± 10.3	27.5 ± 9.2	26.0 ± 2.8
6	35.5 ± 0.7	36.5 ± 0.7	35.5 ± 0.7	28.8 ± 1.1
7	37.0 ± 1.4	37.3 ± 0.07	36.4 ± 0.2	29.6 ± 0.6
8	39.0 ± 1.4	38.1 ± 0.6	37.0 ± 0.3	30.1 ± 0.2

Values are mean ± standard deviation.

streptomycin was added to constitute the final antibiotic mixture.

Isolation, characterization and identification

Serial dilutions of soil samples were made in sterile distilled water by adding 1.0 g of soil sample to 9.0 ml of water and were mixed vigorously for uniform distribution to make a stock solution. 1.0 ml of the stock solution was serially diluted to 10^{-10} and 0.5 ml of each of the inoculums was used in a pour plate method incorporated with antibiotic mixture. The plates were incubated at room temperature (28 ± 2°C) for 5 days, where characteristic fungi growths were isolated for use in degradation. The fungi isolated were characterized based on their microscopic appearance on culture medium, morphology and types of asexual spores produced and were identified as illustrated in Genera of Imperfect Fungi (Barnett and Hunter, 1972) and Fungi in Agricultural Soil (Domsch and Gams, 1972).

Biodegradation experiment

The prepared PDA-PMS and PDA-AGO media together with the control (PDA only) were dispensed into Petri dishes in duplicate of each concentration and allowed to solidify. Pure cultures of *A. niger* and *Penicillium* sp. were inoculated into the center of the plates containing the different concentrations of the media. Inoculation of each plate was done with the aid of a sterile 8.0 mm diameter cork borer. The Petri dishes were incubated at room temperature of 28 ± 2°C for 10 days. Growth of *A. niger* and *Penicillium* sp. were taken by measuring the mycelia spread from the center of the plate using a transparent rule.

RESULTS AND DISCUSSION

The mycelia of *A. niger* and *Penicillium* sp. both grew in the AGO and PMS at all concentrations, but the growth on AGO was considerably reduced compared to that of PMS. The mycelia growth of *A. niger* was observed to be faster in PMS than in AGO. However, the radial growth of *A. niger* and *Penicillium* sp. were inhibited at concentration of 20% mostly with the AGO. Generally, there was significant reduction in the radial growth of *A. niger* and *Penicillium* sp. as the concentration of the pollutants increased. All these are shown in Tables 1 - 4.

The tolerance of the mycelia of *A. niger* and *Penicillium* sp. on the pollutants (AGO and PMS) which served as treatment in this study, varied. The outstanding mycelia growth of both organisms on all treatments and at all con-

Table 3. Growth of *A. niger* mycelia on PMS in 8 days.

Time (days)	Concentration/Growth (mm)			
	5%	10%	15%	20%
0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
1	10.5 ± 0.07	8.1 ± 0.07	8.0 ± 0.1	8.1 ± 0.07
2	15.1 ± 0.07	9.6 ± 0.4	9.2 ± 0.07	9.05 ± 0.07
3	27.0 ± 0.0	25.0 ± 0.0	18.0 ± 0.0	14.1 ± 0.07
4	32.0 ± 0.0	30.0 ± 0.0	25.5 ± 0.7	27.0 ± 1.4
5	35.1 ± 0.07	31.7 ± 0.07	28.7 ± 0.07	28.0 ± 0.07
6	40.5 ± 0.0	34.0 ± 1.4	29.0 ± 0.07	29.0 ± 1.4
7	41.4 ± 0.2	36 ± 2.3	35.5 ± 0.3	28.7 ± 0.9
8	45.1 ± 0.07	40.1 ± 0.07	37.3 ± 0.4	29.8 ± 0.4

Values are mean ± standard deviation.

Table 4. Growth of *A. niger* mycelia on AGO in 8 days.

Time (days)	Concentration/Growth (mm)			
	5%	10%	15%	20%
0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
1	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
2	8.1 ± 0.07	8.2 ± 0.07	8.8 ± 0.4	8.8 ± 0.3
3	11.3 ± 1.3	9.0 ± 0.4	9.1 ± 0.1	9.1 ± 0.0
4	15.1 ± 0.1	10.6 ± 0.9	10.8 ±2.2	10.7 ± 1.9
5	26.5 ± 0.7	19.0 ± 1.4	14.6 ± 0.6	13.2 ± 1.3
6	33.5 ± 2.1	22.0 ± 4.2	21.2 ± 1.6	20.4 ± 2.7
7	37.5 ± 3.5	31.9 ± 0.2	28.6 ± 0.1	28.0 ± 0.0
8	39.2 ± 1.4	37.2 ± 1.4	33.1 ± 1.4	35.2 ± 0.3

Values are mean ± standard deviation.

centrations used may be due to the higher production of extra-cellular enzymes and organic acids that enabled them to utilize the hydrocarbon faster. This agree with the findings of Stamets (1999), that mycelia mats are used for bioremediation because they produce extra-cellular enzymes and acids that break and dismantle the long chains of hydrocarbon, the base structure common to oils, petroleum products and many other pollutants.

The growth pattern of both organisms on AGO and PMS may be due to the fact that AGO and PMS are just a single petroleum product and may contain chemical additives responsible for inhibiting growth. Although both organisms grew at all concentrations of pollutant, the growth was reduced in AGO compared to PMS. Odjegba and Sadiq (2002) reported that engine oil usually contains chemical additives (amines, phenol, benzene, calcium, zinc, barium, magnesium, phosphorus, sulphur and lead) and that metals present in AGO are not the same as those present in PMS. This may be the reason why the growth of *A. niger* remained constant for some days in AGO. It could also be that *A. niger* may have synthesized its extra-cellular enzyme in large quantity so as to overcome the inhibition posed on it by the metals in the pollutants.

The reduction in mycelia growth as concentration increased could be due to the toxicity of pollutants as PDA in the mixture was significantly reduced and growth was on PDA alone. The control did well as it covered almost the entire plate.

The ability of *A. niger* and *Penicillium* sp. to tolerate these pollutants and grow on them, suggest that they can be employed as bioremediation agent and can be used in restoring the ecosystem when contaminated by these pollutants.

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