

## Investigating the ability of five fungal species to utilize Gasoline as sole carbon source.

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### ABSTRACT

This study investigated the abilities of five fungal isolates indigenously polluted mechanic soils to utilise gasoline. Of all the fungal isolates obtained in this study *Aspergillus* species. were found to be more predominant in the polluted mechanic soils. The growth profiles were determined by monitoring total viable counts, dry weights and pH of the culture utilizing gasoline as carbon and energy source. Total viable counts increased significantly and dry weights of fungi as the days of incubation progressed until the 14th day (P<.001). There was significant difference (P<.002) in the pH values of the fungal isolates. The pH values decreased significantly (P<.001) as fungal cells metabolised after 14 days of incubation. *A. terreus* recorded the lowest pH of 4.9 after 14 days of incubation. *A. ochraceus* had the highest pH value of 5.25 after 14 days of incubation on gasoline. *Trichoderma* sp had the lowest pH of 5.05 on gasoline. Of all the fungal isolates *A. ochraceus* had the highest viable count value of 6.26 on gasoline after 14 days of incubation. *A. niger* had the highest dry weight value of 19 on gasoline while *Trichoderma* sp. had the lowest dry weight value of 13.3 on gasoline. *A. terreus* recorded the highest dry weight value of 16.8 while *A. niger* had the lowest dry weight value of 13.3 on gasoline after the 14th day of incubation. Of all the fungal isolates used in this study *A. ochraceus* have shown the best abilities to utilise gasoline in-vitro. All the organisms used in this study are all indigenous to the environment from which they were isolated.

**Keywords:** Bacteria, fungi, crude oil, gasoline, biodegradation

### INTRODUCTION

All activities of using crude oil led to pollution risks that can be minimised, but not totally eliminated, causing several problems for the environment (Pala *et al.*, 2006). Crude oil consists of four main groups of hydrocarbons including aliphatics, aromatics, resins and asphaltines (Colwell and Walker, 1997). The leakage of crude oil into soil damages the biological systems residing in the soil, including microorganisms and plants (Dariush *et al.*, 2007). Some fractions of crude oil are toxic for living organisms. However various microorganisms are able to use some crude oil fractions as sole carbon source and change these

component to non-toxic materials such as CO<sub>2</sub> and H<sub>2</sub>O (Ewis *et al.*, 1998). The contamination of soil and aquifer systems by gasoline hydrocarbons as a consequence of accidental spillage can cause serious environmental problems. The major gasoline constituents (benzene, toluene and xylene- BTX) are relatively soluble in water and are considered human carcinogens (Claudia and Selma, 2000).

One of the best approaches to restoring contaminated environments is to make use of the physiological potentials of microorganisms able to degrade the pollutants in a bioremediation process. It is an attractive approach to cleaning up hydrocarbons

because it is simple to maintain, applicable over large areas, cost effective and leads to complete destruction of the contaminant (Bento *et al.*, 2005). Numerous microorganisms are known for their ability to degrade hydrocarbons. The biodegradation capabilities of bacteria have been recognised (Elisane *et al.*, 2008) but fungi have been subject recent research (Colombo *et al.*, 1996; Krivobok *et al.*, 1998; Salicis *et al.*, 1999; Garcia *et al.*, 2000; Santos and Linardi, 2004; Potin *et al.*, 2004), due to their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin degradation, which are capable of degrading high molecular weight, complex or more recalcitrant compounds, including aromatic structures.

The aim of this work is to determine the ability of indigenous fungi to utilise gasoline as carbon source and for growth thus degrading both petroleum fractions.

## MATERIALS AND METHODS

### Sample collection

Contaminated soils were collected from a mechanic workshops in triplicates.

### Isolation and characterisation of hydrocarbon utilizing fungi

The fungi species indigenous to the mechanic soils were isolated using Potato Dextrose Agar (PDA) into which streptomycin (50mg/ml) had been added to suppress bacterial growth. Fungal isolates were characterized as described by Barnett and Hunter (1998).

The obtained cultures of fungi were screened for the ability to utilise gasoline as substrate.

### Determination of the ability of fungal isolates to utilise gasoline

A known volume of 150ml of the basal medium (minimal salt medium, composition 10 g NaCl, 29 g KCl, 42 g MgSO<sub>4</sub>, .83 g KH<sub>2</sub>PO<sub>4</sub>, .42 g NaNO<sub>3</sub>, 1.25 g NaHPO<sub>4</sub>, 100 ml distilled water,

pH 7.2) was dispensed into 250ml conical flasks and gasoline were introduced separately into flasks at 1.0% v/v after sterilization (Okpokwasili and Okorie, 1988). Overnight broth cultures (35g/l of malt extract broth for fungi) of each organism was seeded into each flask and incubated in a gyratory shaker incubator (New Brunswick Scientific Incubator Shaker) at 150rpm/min and 30°C. Utilisation of gasoline was monitored at two days interval for 14 days by monitoring fungal growth measured by viable count on nutrient agar. The optical density was determined at 600nm wavelength with PG T70 U.V/VIS spectrophotometer and changes in ionic concentration, pH, was determined with pH meter. Fungi was harvested on the filter paper by filtration and dried in the oven, the weight was determined.

## RESULTS

The characterisation of fungi isolated from contaminated sites have revealed five fungal species which include: *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. ochraceus*, and *Trichoderma* sp that could utilize gasoline as carbon source.

No significant difference was observed in the changes in pH values obtained on gasoline by fungal isolates respectively (P=.226). There was significant difference (P<.002) in the pH values of the fungal isolates. The pH values dropped significantly (P<.001) during utilisation of gasoline by all fungal isolates from 0h to the 14th day of incubation. *A. terreus* had the lowest pH of 4.9 after 14 days of incubation. *A. ochraceus* had the highest pH value of 5.25 after 14 days of incubation (Table 1). *Trichoderma* sp produced the lowest pH of 5.05 on gasoline while *A. flavus* recorded the highest pH of 5.55 on gasoline (Table 1).

Table 1: Changes in pH produced by fungal isolates during utilization of gasoline

Time (days)	Microorganisms				
	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Aspergillus terreus</i>	<i>Aspergillus ochraceus</i>	<i>Trichoderma viride</i>
0	7.00	7.00	7.00	7.00	7.00
2	6.95	6.85	6.95	6.80	6.90
4	6.80	6.70	6.80	6.50	6.70
6	6.50	6.80	6.50	6.30	6.50
8	6.40	6.30	6.40	6.00	6.30
10	5.90	6.00	5.90	5.80	5.90
12	5.70	5.50	5.70	5.60	5.60
14	5.50	5.30	5.50	5.60	5.40

The viable counts of all fungal isolates increased significantly ( $P < .001$ ) from 0h to the 14th day of incubation during the utilisation of gasoline. There was no significant difference observed for viable counts on gasoline for fungal isolates respectively.

*A. ochraceus* had the highest viable count value of 6.26 on gasoline after 14 days of incubation on crude oil while *A. fumigatus* and *A. niger* both recorded the lowest viable count value of 8.11 (Fig. 1) after the 14th day of incubation.

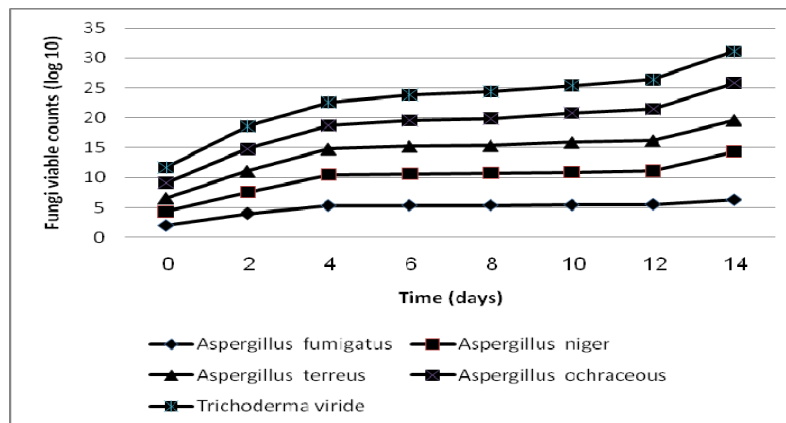


Fig. 1: Changes in viable counts produced by fungal isolates during utilization of gasoline

Dry weight values increased significantly ( $P < .001$ ) from 0h to 14th day of incubation during the utilisation of gasoline by fungal isolates. Meanwhile dry weight values on gasoline showed no significant difference ( $P = .212$ ). There was significant

difference ( $P < .036$ ) in dry weights of fungal isolates. *A. niger* had the highest dry weight value of 19 on gasoline while *Trichoderma* sp. had the lowest dry weight value of 13.3 on gasoline (Fig 2).

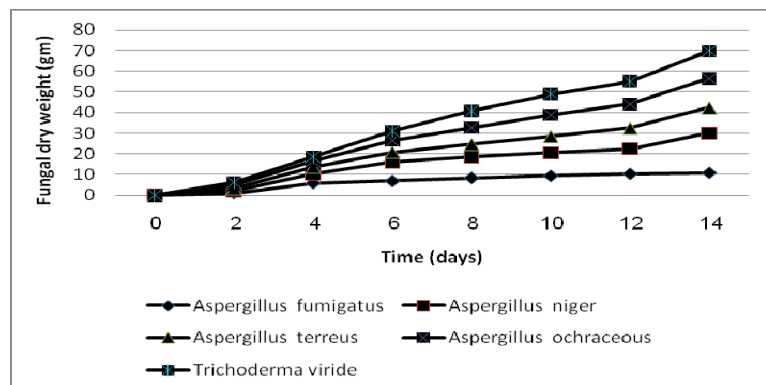


Fig. 2: Changes in dry weights produced by fungal isolates during utilization of gasoline.

In Fig. 2, *A. terreus* recorded the highest dry weight value of 16.8 while *A. niger* had the lowest dry weight value of 13.3 on gasoline after the 14th day of incubation.

Correlation analysis results of the fungal isolates showed positive (correlation coefficient value=0.820) correlation between viable counts and dry weights. Hence dry weights increased with a concomitant increase in viable counts. High negative correlation (correlation coefficient value= -0.844) occurred between viable counts and pH. Similar trend as mentioned above for bacterial isolates was observed. There was also high negative correlation (correlation coefficient value= -0.940) between dry weights and pH, hence as dry weights increased pH decreased concomitantly.

### DISCUSSION

The fungi isolated from contaminated soils indicated the prevalence of *A. ochraceus* have shown the best abilities to utilise and degrade gasoline. Oboh *et al.* (2006) have reported the abilities of fungal species which included; *Aspergillus* sp., *Penicillium*, *Rhizopus* and *Rhodotorula* species to grow on gasoline as the sole carbon and energy source when screened for hydrocarbon utilisation. The major genera of fungi active in polluted soils were *Aspergillus*, *Penicillium* and *Mucor* (Nkwelang *et al.*, 2008). Uzoamaka *et al.* (2009) reported that the eight isolates showed potentials for hydrocarbon biodegradation are *Aspergillus versicolor*, *Aspergillus niger*, *Aspergillus flavus*, *Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila*, *Rhizopus arrhizus* and *Mucor* spp. Santos

*et al.* (2008) reported the ability of *Aspergillus* sp. to biodegrade gasoline.

Atlas and Bartha (1972) observed that both water in oil and oil in water emulsions are formed following oil spillage. The two phase liquid medium where the bulk of the carbon and energy source are found is water insoluble and all other minerals nutrients are dissolved in the water phase, microbial growth typically occurs at the interface of the two liquids. The ability of the microorganisms to lower the interfacial tension will increase the interface and thus accessibility of the hydrocarbon substrate. Similar observations were made in this study as the role being played by agitation during hydrocarbon degradation was visibly observed. While flasks placed on shaker resulted in crude oil and gasoline complete disappearance, those put in the incubator without shaking showed little or no degradative effect. Agitation breaks the hydrocarbon into droplets, thereby providing increased surface area to accelerate biodegradation. The oil in this state is not only made readily available but its movement through water column makes oxygen and other nutrient more readily available to the organism. The physical state of the petroleum hydrocarbons is known to have a marked effect on its biodegradation; the more soluble it is in water the more liable it is to microbial attack. Hydrocarbon degrading organisms act mainly at the oil and water interface. This investigation however shows that gasoline was utilised to a greater extent than crude oil.

The reduction in pH of the culture fluids in flasks within the 14 day incubation period confirmed chemical changes of the hydrocarbon substrates

which must have been precipitated by microbial enzymes (Atlas and Bartha, 1972). The growth profiles of the fungal isolates on gasoline revealed a sharp drop in pH. Hydrogen ion concentration is a major variable governing the activity and composition of fungi. Many species can metabolise over a wide pH range from the highly acidic to alkaline extremes. Thus, the insensitivity of the fungi to high hydrogen ion concentration and narrow pH range of most bacteria account for the sharp drop in pH. Microbial degradation of hydrocarbons often leads to production of organic acids and other metabolic products (Nwachukwu and Ugoji, 1995; Okpokwasili and James, 1995). Thus organic acids probably produced account for the reduction in pH levels (Oboh *et al.*, 2006).

The growth profiles showed that none of the fungal isolates exhibited lag phases. This observation has been reported previously (Oboh *et al.*, 2006) and can be attributed to genetic make-up due to the constitutive expression of hydrocarbon catalysing enzymes or physiological owing to previous exposure to exogenous hydrocarbons present in the contaminated soils. This may be followed by a concomitant development of the ability of the organisms to emulsify petroleum hydrocarbons and which is a major factor in hydrocarbon uptake and assimilation. Ptaek *et al.* (1987) reported that many of the petroleum degrading bacteria produce extracellular emulsifying agents. Utilisation of gasoline resulted in increase in cell densities with a concomitant reduction in the oil layer complete disappearance of crude oil and gasoline with prolonged incubation. According to Dariush *et al.* (2007) increasing crude oil concentration decreased the reduction of crude oil in vegetated and non vegetated soil samples. In all the contaminated vegetated soils, the reduction of the crude oil was higher than non vegetated soils.

In the higher concentrations (7% and 10%) the difference of crude oil reduction between the vegetated and non vegetated soil samples was not significant, while the reduction was significant between the vegetated and non vegetated samples in concentrations up to 5% (Dariush *et al.*, 2007).

It is interesting to note that all the organisms used in this study are all indigenous to the environment from which they were isolated and that all of those, which were tested for biodegradation, are able to biodegrade organic contaminants actively. The biodegradation of contaminants is the best means to completely remove oil pollutants. Biodegradation should be accelerated in order to develop a faster means of cleaning up pollutants. The ultimate success of bioremediation is dependent upon microorganisms staying in close physical contact with the substance to be degraded. The key to increasing the rate of biodegradation of contaminant is to optimise the growth rate of indigenous soil degrading micro flora. Finally increasing biodegradation rates of indigenous microorganisms is the best option to maximise contaminant clean up when all avenues have been exhausted.

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