

# **FUNGI IN BIOREMEDIATION OF OIL POLLUTED ENVIRONMENTS**

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## **Abstract**

This paper highlights how fungi are suited for remediation of oil polluted environments. Many species of fungi are known to occur in oil-polluted environments, and to degrade petroleum hydrocarbons. Further, fungi are amenable to large-scale production, efficiency, genetic engineering or manipulation, cost effectiveness, and ease of transportation. First we examine the sources of crude oil pollution and its effect on the environment and on microorganisms. We also assess the methods currently employed in oil clean-up operations, and their side-effects on ecosystems. Finally, we highlight specific fungi known to possess biodegradation potential for crude oil. Bioremediation in oil clean-up operations is important since environmental pollution problems threaten the well-being of humans and other organisms. In particular, we argue that fungi may have an important role in oil clean-up in the Niger Delta, but further study is needed to implement the approach in that region.

## **Introduction**

Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which possesses a measurable toxicity towards living systems (Nelson-Smith, 1973). The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment (Gutnick and Rosenberg, 1977).

The single largest source of petroleum pollution is routine, low-level discharge such as urban runoff, cleaning operations, and oil treatment of roads for dust control. These sources together account for 90% of total anthropogenic petroleum pollution (Bartha, 1986). Oil pollution also comes from oil-well blowouts, seepage, and deballasting operations, sale and use of petroleum products, pipeline overflow and breakage, and storage tank spills (Plice, 1948; Obire and Wemedo, 1996). Addition of oil to the soil as a deliberate policy of waste disposal also leads to contamination (Flowers *et al.*, 1984). Obire and Amusan (2003) reported deliberate discharge of oilfield wastewater or effluent as a source of environmental contaminant.

Non-anthropogenic sources of hydrocarbons in the environment include natural seepage, and synthesis of hydrocarbons and hydrocarbon-like compounds by fungi (Alexander, 1977).

## **Effect of crude oil pollution on the environment and on microbial populations**

The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration, on environmental factors and on the biological state of the organisms at the time of the contamination. Petroleum distillates up to and including gas oils are more severely toxic on a short time scale than the other components of crude oil. In heavily polluted areas, there are immediate detrimental effects on plant and animal

life, including agriculture (Baker, 1970; Steinhart and Steinhart, 1972; Rowell, 1977; Fagbami *et al.*, 1988; NDWC., 1995). Nevertheless, different species and different life stages of organisms have different susceptibilities to pollution (Nelson-Smith, 1973).

In addition to its effects on visible plants and animals, petroleum contamination impacts microbial populations (Ahearn and Meyers, 1976). The effect of oil on microbial populations depends upon the chemical composition of the oil and on the species of microorganisms present. Populations of some microbes increase; typically, such microbes use the petroleum hydrocarbons as nutrients. The same crude oil can favor different genera at different temperatures (Westlake *et al.*, 1974). However, some crude oils contain volatile bacteriostatic compounds that must degrade before microbial populations can grow (Atlas and Bartha, 1972; Atlas, 1975).

On the other hand, some microbial populations decrease or show a neutral response to petroleum hydrocarbons. The overall effects of petroleum hydrocarbons on total microbial diversity remain unclear.

### **Remediation of crude oil-polluted sites**

Methods for restoring oil-polluted sites vary from complete removal of the affected soil to doing nothing at all and “letting nature take its course” (McGill and Nyborg, 1975). Natural revegetation of an area affected by light crude oil spillage has occurred without any special treatment (Baker, 1970; Stebbings, 1970; Odu, 1978). At low levels of contamination of crude oil, cultivation of soil without nutrient amendment is possible (Toogood, 1974).

Physical methods such as incineration may destroy indigenous organisms, including oil-degrading microbes, and increase the toxicity of the petroleum residue. Sinking the oil with heavy hydrophobic agents such as ground chalk merely removes the oil to anaerobic sediments or deep ocean floor, where long persistence of the oil pollutant is bound to occur. Large quantities of oil accumulating on the bottom foul the ocean floor and also tend to coalesce and rise again as large droplets. Mechanical removal of stranded oil from sand dunes or salt marshes is far more damaging than leaving it alone: Not only is the ecological balance disturbed, but the aesthetic effect may also be irreparable (Nwangwu and Okoye, 1981).

Chemical methods for removing or dispersing spilled oil from the environment were condemned by Nelson-Smith (1973) because of their side-effects on the ecosystem and their toxicity, which is sometimes more pronounced than that of the oil itself. Chemical dispersants may inhibit microbial activity by damaging cell membranes or essential enzymes, or by altering the surface tension of the water in which microbes live. Furthermore, dispersed oil is never recovered from the environment, and its ultimate fate remains unknown.

### **Bioremediation**

Owing to the problems associated with physical, mechanical and chemical methods, there is a need for a safer and less expensive approach to remediation of polluted

environments. Bioremediation is a means of cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or by conversion into microbial biomass (Baggott, 1993; Mentzer and Ebere, 1996).

Microbial degradation has emerged as the most significant natural mechanism for the removal of non volatile hydrocarbon pollutants from the environment. Although biodegradation occurs at a distressingly slow rate, it can be enhanced by inoculation with microbial species that will degrade the oil waste more efficiently, and/or by introducing air and nutrients into the environment (Obire, 1988).

Oil-degrading microorganisms are abundant and are not limited to oil producing areas, but are present in any conceivable environment (Odu, 1978). Filamentous fungi, yeasts, actinomycetes and bacteria all have the ability to utilize hydrocarbon substrates—though their ability to do so varies among individual strains and, in some cases, depends on hydrocarbon chain length (Rowell, 1977; Walker *et al.*, 1973). For instance, bacteria and yeasts showed decreasing abilities to degrade alkanes with increasing chain length. Filamentous fungi did not exhibit preferential degradation for particular chain lengths (Walker *et al.* 1973).

Specific fungal genera and species with potential for bioremediation are detailed in a later section of this paper.

### **The parameters of bioremediation processes**

The factors that must be optimized for successful bioremediation are: oxygen and inorganic nutrients, pH, temperature, water availability, and adsorption effects.

- **Adequate supply of oxygen and inorganic nutrients**

Most fungi and bacteria that degrade petroleum hydrocarbons require free or dissolved oxygen (Odu, 1981). In the presence of adequate oxygen, oil degradation also requires mineral elements such as C, Ca, Mg, K, S, Fe, N, P and various trace elements (Odu, 1978).

- **pH**

The optimum pH for biodegradation of hydrocarbons is around pH 6 – 8 (Mentzer and Ebere, 1996). Biodegradation of crude petroleum in an acid soil (pH 4.5) could be doubled by liming to pH 7.4.

- **Temperature**

Temperature as a limiting factor does not seem to be a problem in tropical and temperate zones. Disappearance of hydrocarbon contaminant from agricultural land can be correlated with monthly temperature averages (Dibble and Bartha, 1979); generally, hydrocarbon biodegradation increases with temperature and peaks around 30– 40°C (Mentzer and Ebere, 1996).

- **Water availability**

Soil that is hydrated with 50% to 80% of the maximum water-holding capacity has the greatest microbial activity (Mentzer and Ebere, 1996). Below that level, osmotic and

matrix forces limit the availability of water to microbes; above that level, the reduction of air space and oxygen decrease microbial activity.

- **Adsorption Effects**

Hydrocarbons that are adsorbed onto organic matter are less susceptible to microbial attack. Indeed, the rate-limiting process in biodegradation may be the desorption of contaminants (Mentzer and Ebere, 1996).

### **Alternative Bioremediation Technologies**

The bioremediation technologies for responding to oil spills may be divided into three categories:

#### **Seeding with Naturally-Occurring Microorganisms**

Microbial seeding of petroleum-polluted sites has been proposed by a number of investigators as an alternative to mechanical removal (Obire, 1988, 1990). An active remediation programme using adapted microbes will yield significantly greater, commercially viable rates of oil removal than the experimental rates already reported (Obire, 1988).

#### **Seeding with Genetically-Engineered Microorganisms**

Fungi and bacteria can be genetically engineered to detoxify man-made pollutants (Ogden and Adams, 1989). The oil service industry already utilizes genetically manipulated microbes as producers of surfactants and polysaccharide polymers (Chakrabarty, 1985; 1986).

The use of mutant organisms to get rid of spilled oil has merits and demerits. The induction of a wild type strain by a mutagen such as acridine orange may lead to enhanced ability to degrade oil but it may be difficult to get rid of the mutant population after the desired effect (Obire, 1990). However, disinfectants that selectively kill mutant strains, rather than wild-types, could be used. The disadvantage is that irrelevant genes can also be affected by direct mutation processes. The actual genetic engineering of the relevant genes is what is necessary.

#### **Nutrition (Nutrient Enrichment)**

Nutrient imbalance can hinder biodegradation. Inadequate provision of nitrogen, phosphorus, potassium and sulphur (which is probably the most important and the most easily modified of all the factors) could limit the rate of hydrocarbon degradation in the terrestrial environment (McGill and Nyborg, 1975).

According to the United States Office of Technology Assessment (1990), the addition of limiting nutrients to the spill site is necessary. There are enough hydrocarbon-utilizing organisms in the soil environment to perform biodegradation once nutrient limitation is alleviated (Stone *et al.*, 1942). Soybean lecithin and ethyl allophanate, which are natural phospholipids, are the best available phosphorus and nitrogen sources, respectively, for microorganisms that degrade oil (Olivieri *et al.*, 1978).

### **Interaction between Microorganisms and Hydrocarbons**

Petroleum contains a wide range of organic compounds that are nutrients for microorganisms. Petroleum degradation is primarily an oxidation process, although there is some evidence for anaerobic hydrocarbon degradation (Gutnick and Rosenberg, 1977). Microbes capable of degrading petroleum hydrocarbons share the following characteristics:

- Efficient hydrocarbon uptake via special receptor sites for binding hydrocarbons and/or unique compounds that assist in the emulsification and transport of hydrocarbons into the cell.
- Enzymes that introduce molecular oxygen into the hydrocarbon and generate intermediates that subsequently enter common energy-yielding catabolic pathways.
- Inducer specificity: Exposure to petroleum and its constituents activate the two systems above.

### **Fungi as agents of bioremediation**

Fungi can possess all three traits, are found in oil-contaminated environments, and are known to degrade hydrocarbons (Llanos and Kjoller, 1976). Further, the ease of transportation, genetic engineering, and scaling-up makes fungi the organisms of choice in bioremediation. In a taxonomic study of fungi, Nyns *et al.* (1969) found that hydrocarbon assimilation is most common in the orders Mucorales and Moniliales, as well as in the genera *Aspergillus* and *Penicillium* (order Eurotiales). Furthermore, in comparison with eight other genera, *Aspergillus* and *Penicillium* species were the most efficient metabolizers of hydrocarbons (Obire *et al.*, 2008). Hydrocarbon assimilation was, however, relatively rare, and was a property of individual strains, not of species or higher taxa (Nyns *et al.* 1968). Nevertheless, diverse fungi have been isolated from oil-contaminated environments, and/or shown to degrade hydrocarbons in the lab. As one striking example, yeast populations in a fresh water stream increased by several orders of magnitude in the five days after an oil spill (Turner and Ahearn, 1970, cited by Jones, 1976).

In addition to degrading hydrocarbons directly, fungal mycelia can penetrate oil, thereby increasing the surface area available for biodegradation and bacterial attack. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited (Davis and Westlake, 1979). It was also reported that although bacteria initiated the degradation of a synthetic petroleum mixture, twice as much was degraded when both bacteria and fungi were present.

Species in many fungal genera are known to metabolize hydrocarbons/ and or thrive in oil-contaminated sites. They include:

*Acremonium* (Llanos and Kjoller, 1976)

*Aspergillus* (Bartha and Atlas, 1977; Obire *et al.*, 2008)

*Aureobasidium* (Bartha and Atlas, 1977)  
*Candida* (Bartha and Atlas, 1977; Obire *et al.*, 2008)  
*Cephalosporium* (Bartha and Atlas, 1977; Obire *et al.*, 2008)  
*Cladosporium* (Walker *et al.*, 1973; Bartha and Atlas, 1977; Obire *et al.*, 2008)  
*Cunninghamella* (Bartha and Atlas, 1977)  
*Fusarium* (Llanos and Kjoller, 1976; Obire *et al.*, 2008)  
*Geotrichum* (Obire *et al.*, 2008)  
*Giiocladium* (Llanos and Kjoller, 1976)  
*Graphium* (Llanos and Kjoller, 1976)  
*Hansenula* (Bartha and Atlas, 1977)  
*Mortierella* (Llanos and Kjoller, 1976)  
*Mucor* (Obire *et al.*, 2008)  
*Paecilomyces* (Llanos and Kjoller, 1976)  
*Penicillium* (Llanos and Kjoller, 1976; Bartha and Atlas, 1977; Obire *et al.*, 2008)  
*Rhodospiridium* (Ahearn and Meyers, 1976; Bartha and Atlas, 1977)  
*Rhodotorula* (Bartha and Atlas, 1977; Obire *et al.*, 2008)  
*Saccharomyces* (Bartha and Atlas, 1977)  
*Sphaeropsidales* (Llanos and Kjoller, 1976)  
*Sporobolomyces* (Bartha and Atlas, 1977)  
*Torulopsis* (Bartha and Atlas, 1977)  
*Trichoderma* (Llanos and Kjoller, 1976; Obire *et al.*, 2008)  
*Trichosporon* (Ahearn and Meyers, 1976; Bartha and Atlas, 1977)

### **Fungi for bioremediation in the Niger Delta**

Although there have been reports of commercial production of fungal and bacteria inocula for treatment of oil spills in developed countries (Bartha and Atlas, 1977), species of microorganisms are habitat specific (Obire, 1988). One region that might benefit from fungal bioremediation is the Niger Delta. Nigeria is one of the world's largest producers of crude oil. In recent times, the Niger Delta region has become a hotbed of violent conflicts that portend several dangerous outcomes for national political and socio-economic stability. These conflicts, exacerbated by environmental factors, have also affected the oil industry, as well as international economies and security. Table 1 shows the oil spillage data in the Eastern Operations in Nigeria.

In petroleum-producing regions of Nigeria, Obire (1988) found several species oil-degrading aquatic fungi in the genera *Candida*, *Rhodotorula*, *Saccharomyces* and *Sporobolomyces* (yeasts) and, among filamentous fungi, *Aspergillus niger*, *Aspergillus terreus*, *Blastomyces* sp., *Botryodiplodia theobromae*, *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum*, *Penicillium glabrum*, *Pleurofragmium* sp., and *Trichoderma harzianum*.

In Nigeria, no information is yet available regarding the commercial production of fungi or microbial inocula for use in bioremediation of oil polluted environments. Efforts should therefore be focused on developing indigenous fungi for use in large scale operations in the Niger Delta area of Nigeria. The data in Table 1 show that between 66% and 100% of crude oil is lost after recovery efforts. This quantity of oil is lost because of

the geographical terrain of the Niger Delta, which is mostly a mangrove swamp and marsh. The soft flowing mud of the swamps and prop roots of the mangrove trees are usually the natural obstacles during oil recovery efforts in this region; through which heavy machinery cannot be moved for oil recovery efforts. Bioremediation is therefore the only answer to the removal of oil spilled in these areas and the best means of remediation in such ecosystems.

Scientific investigations have shown that crude oil degrading fungi (microorganisms) abound in the Niger Delta region. The specific steps that need to be taken to start the implementation of fungi for bioremediation in Nigeria would be the isolation and screening for the best crude oil degrading fungi. The potentials and abilities of these fungi would further be enhanced or improved upon, probably through genetic engineering and the provision of the appropriate nutrients during the bioremediation programmes.

**Table 1: Oil Spillage in the Eastern Operations in Nigeria (1989 – Feb. 2000)**

Year	Total No. of Incidents	Approx. No. of Barrels Spilled	Approx. No. of Barrels Recovered	Approx. No. of Barrels Lost
1989	92	6,147.51	1,467.25	4,680.34
1990	119	15,264.50	5,172.50	10,091.61
1991	117	155,031.33	1,402.25	153,629.08
1992	184	27,161.54	721.00	26,440.54
1993	251	7,310.34	1,973.50	5,336.64
1994	270	32,259.70	1,692.25	30,567.45
1995	245	67,561.41	8,846.39	58,715.02
1996	264	43,841.35	0.92	43,840.43
1997	266	74,749.52	1,243.50	73,506.02
1998	133	69,338.68	383.50	68,955.18
1999	260	28,013.72	100.80	27,912.92
Jan – Feb.2000	51	10,179.75	NIL	10,179.75
<b>Total</b>	<b>2252</b>	<b>536,858.84</b>	<b>23,003.86</b>	<b>513,854.98</b>

Source: Department of Petroleum Resources, cited by Azaiki, 2009.

### Summary and Conclusion

Biodegradation is increasingly being considered as a less expensive alternative to physical, mechanical and chemical means of disposing hydrocarbon pollutants. The ubiquitous distribution of fungi and their ready isolation from oil-contaminated environments indicate that they play an important role in the degradation of oil spilled in the environment.

Fungi have evolved the ability to degrade petroleum hydrocarbons, and, unlike other groups of microorganisms, filamentous fungi do not exhibit preferential degradation for particular chain lengths of alkanes. Fungal mycelia penetrate oil and increase the surface area available for degradation by other microbes. Fungi are notably aerobic and can also grow under environmentally stressed conditions such as low pH and poor nutrient status,

where bacterial growth might be limited. Finally, fungi are easy to transport, genetically engineer, and produce in large quantities.

Although developed countries have commercially produced fungal cultures for oil cleanup, this is less common in developing countries. One region subject to frequent detrimental oil spills, and lacking access to commercially produced cleanup strains, is the Niger Delta. This region may benefit from development of indigenous fungi for bioremediation. It is recommended that the crude oil which cannot be recovered after a spill should therefore be subjected to bioremediation using fungi and fungal products such as their enzymes.

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