Mycoremediation of Petroleum Contaminated Soils: Progress, Prospects and Perspectives

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ABSTRACT

Mycoremediation, an aspect of bioremediation, has been investigated for some decades. However, there seems to be little progress on its commercial application to petroleum-contaminated soils despite some promising outcomes. In this review, mycoremediation is examined to identify development, limitations and perspectives for its optimal utilization on petroleum-contaminated soils. Mycoremediation agents and substrates that have been used for the treatment of petroleum contaminated soils have been identified, application methods discussed, recent advances highlighted and limitations for its applications accentuated. Possible solutions to the challenges in applying mycoremediation to petroleum-contaminated soils have also been discussed. From this review, we conclude that for optimal utilization of mycoremediation of petroleum-contaminated soils, ideal environmental, edaphic and climatic factors of a typical contaminated site must be incorporated into the approach from first principles. Development of application procedures that can easily translate laboratory results to field applications is also required.

KEYWORDS: mycoremediation, fungi, crude oil, soil-contamination

Introduction

Mycoremediation offers an alternative environmentally friendly technique for remediation of contamination in environmental matrices[1-4]. It entails the use of fungi and has been applied to both soil and water[5,6]. The technique has several advantages over other bioremediation, physical and chemical methods. Apart from cost and technical ease, the ubiquitous nature of most fungi species could afford widespread applications in different regions of the world[7,8]. Leonardi[9] and Rahman[10] stated that mycoremediation seems to be the safest means of soil remediation in terms of ecological impact and Human health. This is because most organic contaminants are degraded rather than extracted, which reduces the risk of bioaccumulation and transfer of pollutants into the food chain[11-13]. According to Asamudo[14] and Adenipekun[15] mycoremediation is unique even among other biological techniques such as

bacterial, because there is no requirement for pre-conditioning to a particular pollutant. Asamudo[14] further stated that in mycoremediation, the efficiency is not limited to specific pollutant concentrations. It is, therefore, necessary to examine why the technique of mycoremediation has not been maximized for clean-up of petroleum-contaminated soils and to identify possible solutions to the challenges that may arise.

Although there are several reviews on mycoremediation, none is tailored specifically to that of petroleum-contaminated soils. This review evaluates the development, limitations and perspectives of mycoremediation of petroleum-contaminated soils. It is specifically carried out to elucidate prospects that could further improve the utilization of the technique for the cleanup of petroleum-contaminated soils.

Fungi and Fungal spawns

The kingdom Fungi, distinguished from plants by the possession of chitin in their cell walls, consists of both unicellular and multicellular organisms such as yeast and moulds, as well as macroscopic types such as mushrooms[16,17]. Fungi are classified based on their life cycles, presence or structure of their fruiting body, or the arrangement and type of reproductive cells (spores) they produce[18]. Principally, there are three major groups of fungi. These are multicellular filamentous moulds, macroscopic filamentous fungi that form large fruiting bodies (mushrooms) and single-celled microscopic fungi such as yeasts[19]. Fungi are also broadly classified either as microfungi or macrofungi. The two are distinguished only by the presence of a large (visible), multicellular fruiting body in macrofungi. Macrofungi are generally referred to as mushrooms. Microfungi could be unicellular such as yeast, or multicellular such as moulds. The body of a typical fungus consists of a collection of hyphae which is referred to as the mycelium. A hypha is a long, branching filamentous structure consisting of one or more cells surrounded by a tubular cell wall which extends through the substrate in which the fungi grow[20]. Hyphae have an average diameter of 4–6 μm and serve as the vegetative part of the organism[19]. The hyphae may be divided by internal cross walls, the Septa, into cells. Typically, Septa are perforated by pores large enough for ribosomes, mitochondria and sometimes nuclei to flow between cells[16-19].

For commercial propagation of mushrooms, mushroom spawns are used[21]. The propagative part of a fungus is the mycelium (a collection of hyphae). Mushroom spawns refer to any material which has been inoculated with mycelium for the propagation of mushrooms[22]. The spawn is used to transfer the fungal mycelium onto any material (substrate) from which the mushroom can grow. Although mushrooms can grow straight from spawn without a substrate, most times this is not ideal[23]. It has been demonstrated that a small quantity of mushroom spawn can inoculate a much greater amount of substrate resulting in better mushroom growth and yield compared to using the spawn alone[24]. Mushroom spawns are named according to the material used for inoculation of the fungal mycelia. Thus, sawdust spawn consists of a sterilized sawdust that has been inoculated with mycelium, while grain spawn is made of a sterilized grain inoculated with spores or a sterile culture of mycelium. Examples of grains that have been used for grain spawns are rye, rice, corn and wheat. Other types of spawns are plug or dowel spawn, woodchip spawn, straw spawn and liquid spawn (water enriched with mushroom spores or mycelia slurry)[25].

Factors affecting mycoremediation

The efficiency of mycoremediation is affected by factors such as temperature, sunlight, oxygen level, nutrients and moisture content[26]. It has been demonstrated that mycoremediation is optimal at temperatures of 25-30^{0}C[27]. Aguilarivera[28] reported that 70% relative humidity is ideal for mycoremediation with *P. ostreatus*. According to Brady[29] a carbon-nitrogen ratio of 10 in soil is optimal. Nutrient requirement is usually maintained using both organic and inorganic manures[27]. Gueren[30] demonstrated that a combination of mycoremediation with compost resulted in up to 50% increase in the remediation efficiency of polycyclic aromatic hydrocarbons (PAHs). The addition of compost also aids temperature optimization during the process[6]. Amjad[31] further listed factors affecting mycoremediation efficiency to include environmental and genetic factors such as pH, ecology, type of substrate, enzyme type and mycelium age. Das[32] reported that nutrients such as nitrogen and phosphorus could become limiting factors. Mushroom biomass content, length of remediation process and type of substrates as well as mobilizing agents are also known to

affect the efficiency of mycoremediation[33]. Other factors include the life cycle of fungal agents, fungal species, soil geochemistry as well as surface active and chelating agents[34,35].

Effects of bioavailability on mycoremediation

Petroleum contaminants, particularly the semi-volatiles, are hydrophobic[36]. Thus, solubility and bioavailability are important factors in bioremediation of petroleum-contaminated soils. Boopathy[37] stated that the rate of contaminant conversion during bioremediation depends on their uptake and metabolism rate and the rate of contact with the cells of the organism. Manning[38] reported that activities which result in the homogenous spread of contaminants in soils can drastically stimulate their biodegradation. Singh[39] demonstrated that bioavailability of organic contaminants decreases with time. In a study on the degradation of DDT in soil, it was found that there was a substantial decrease in the degradation rate with time even in the presence of aged DDT. According to Boopathy[37], several physicochemical processes such as sorption and desorption, diffusion, and dissolution influence contaminants bioavailability. Thus, these factors must be considered in planning for mycoremediation of petroleum contaminated soils. The use of surface-active agents can help overcome the problem of contaminants bioavailability during remediation of petroleum-contaminated soils[37].

Effects of Temperature on mycoremediation

Temperature generally plays a critical role in bioremediation processes[40]. It has been demonstrated that the rate of degradation of organic contaminants is comparably higher at elevated temperatures[41]. Higher rates of degradation of organic contaminants are also reported in tropical soils compared to soils from temperate regions[42,43]. Dimond[44] stated that temperature affects the half-life of organic contaminants which increases with lowering temperatures. Hong[45] demonstrated that a temperature range of 20–40 °C was optimal for degradation of the contaminant fenitrothion. Siddique[40] further demonstrated that the highest degradation of Hexachlorocyclohexane in water and a soil slurry was achieved at an incubation temperature of 30 °C.

Effects of pH on mycoremediation

The degradation of organic contaminants in soil is favoured by high pH because of increased solubility[46]. Owen[42] demonstrated that alkaline soil pH resulted in faster degradation of organic contaminants compared to those carried out in acidic soil. According to Andrea[47], the degradation of organic contaminants is inhibited at low soil pH of 4.5 to 4.8. Nash[48] in a study of effects of pH on stability of DDT, observed that maximal degradation in both moist and dry soils were obtained at pH values above 7. Hong[45] reported a bioremediation of fenitrothion-contaminated soil using *Burkholderia sp*. *FDS-1* with an optimal degradation at a slightly alkaline pH of 7.5. Thus, pH is one of the factors that should be considered in mycoremediation of petroleum contaminated soils.

Effects of Relative humidity on mycoremediation

For mycoremediation of contaminated soils, relative humidity is generally maintained at above 60%[49,50]. A study carried out by Xiao[49] utilised a relative humidity of 70%, that by Migliore[50] was 60% while the relative humility in Singh[51] was between 60-70%. Higher relative humidity values of up to 85-95% have also been reported[52]. Seidu[53] reported that mushroom growth and fruiting is favoured by a relative humidity of 70-80%.

Fungal types used for mycoremediation of petroleum contaminated soils

Different species of fungi have been used for remediation of petroleum-contaminated soils (Table 1). These include microfungi such as *Arbuscular mycorrhiza* and yeast [54-56] as well as *Penicillium* and *Aspergillus species*[57,58]. Mycoremediation with macrofungi (mushrooms) is also known[15,59]. Abioye[56] reported degradation of crude oil by the yeast *Saccharomyces cerevisiae* isolated from a fermented local juice (Zobo). The yeast was used to inoculate a sterile mineral salt media containing 1 g of crude oil maintained at 30 ^{0}C for 28 days. Obire[60] established that microfungal communities were actively involved in remediation potentials of cow and poultry manure on petroleum contaminated soils. In the study, isolated yeasts and moulds from cow manure used for remediation of the petroleum contaminated soils include *Alternaria sp., Aspergillus sp., Cephalosporium sp., Cladosporium sp., Geotrichum sp., Monilia sp., Mucor sp., Penicillium sp., Rhizopus sp., Sporotrichum sp., Thamnidum sp., Candida sp.,*

Rhodotorula sp. and Torulopsis sp. Yeast such as *Saccharomyces spp* has also been demonstrated to transform PAHs to simpler and non-toxic forms[61].

One significant class of fungi with demonstrated mycoremediation of petroleum contaminated soils are the ligninolytic mushrooms such as white rot fungi [62-64]. Lebo[65] and Fetzer[66] stated that the ability of white rot fungi to degrade recalcitrant organic pollutants stems from the fact that these mushrooms naturally feed on and degrade lignin, a substance with similar monomeric unit to organic contaminants. Stamet[2] demonstrated up to 99% degradation in a large-scale study of naturally diesel-contaminated soils with initial concentrations of 20,000 parts per million of PAHs after 8 weeks using the white rot mushroom *P. ostreatus.* Kristanti[67] established that up to 93% degradation of crude oil in the soil can be obtained using the white rot mushroom *Polyporus sp. S133* pre-grown on wood meal.

Mycoremediation by mushrooms other than white rot have also been reported. These include brown rot fungi such as *Ganoderma species,* and edible (button) mushrooms such as *Agaricus species* (which grows naturally on soils)[35,68]. It has been demonstrated that the litter-decomposing mushroom *Stropharia coronilla,* can metabolise PAHs compounds such as benzo[a]pyrene (BaP) at an initial concentration of 200 µM and this activity can be increased up to 12 times in presence of supplementary Mn^{2+}. Mohammadi-Sichani[69] established that the litter decomposing mushroom *A. bisporus* can yield a higher ability to degrade total petroleum hydrocarbons in soils than white rot mushroom such as *Pleurotus ostreatus* and *Ganoderma lucidum.*

Table 1: Fungal types used for mycoremediation of petroleum contaminated soils

S/N	STUDIES	FUNGI SPECIES	EXPERIMENT DESIGN	CLIMATIC REGION	OUTCOME
1.	Adenipekun[70]	1. *Pleurotus pulmonarius* 2. *Pleurotus ostreatus* (All macroscopic)	1. Laboratory /glasshouse Scale: 2 months 2. Artificially contaminated soils (composition: 0, 10, 20 or 30%) with spent cutting fluid-SCF and fresh cutting fluids-FCF 3. Target contaminants: 16 priority PAHs 4. Solvent for extraction: Hexane, Dichloromethane (3:1). 5. Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ Overall range of PAHs degradation by *P. pulmonarius* inoculated on FCF contaminated soil was 17.3 to 27.3%, while for *P. ostreatus* inoculated soil was 69.0 to 99.07% at different contamination levels. ➢ Overall PAHs degradation for *P. pulmonarius* and *P. ostreatus* inoculated on SCF ranged from 27.4 to 57.4% and from 37.8 to 45.2%, respectively.
2.	Nicholas[71]	1. *Heterobasidion annosum* 2. *Resinicium bicolor* (All macroscopic)	1. Laboratory /glasshouse Scale: 36 days 2. Artificially contaminated soils with diesel (3.5 and 7.0%) 3. Target contaminants: TPHs 4. Solvent for extraction: Dichloromethane 5. Method of application: prepared mushroom spawn (rice grain colonised with fungi- substrates) were transferred to and mixed with soil	Temperate Climate	➢ ANOVA showed significant decrease in TPHs over time.
3.	Young[72]	1. *Irpex lacteus* 2. *Trichaptum biforme* 3. *Phlebia radiata* 4. *Trametes versicolor* 5. *Pleurotus ostreatus* (All macroscopic)	1. Laboratory /glasshouse Scale: 180 days 2. Artificially contaminated soil samples were used 3. Principal substrate: white pine (*Pinus strobus*) sawdust was used for *Irpex lacteus* while others were grown on wheat bran and sawdust, which was properly moistened. 4. 20 g of Bunker C oil was added to each substrate, which were then maintained at 27^0C for 180 days 5. Target contaminants: TPHs and PAHs 6. Solvent for extraction: Methylene Chloride	Temperate Climate	➢ Average degradation efficiency between C10 and C14 alkanes was observed to be 98.1% and 48.6%, respectively after 180 days. ➢ Highest efficiency was obtained for P. ostreatus against Phenanthrene (94.9%) after 180 days
4.	El Hanafy[58]	1. *Aspergillus niger* 2. *Penicillium commune* (all microscopic)	1. Laboratory /glasshouse Scale: 2 weeks 2. Crude oil samples were used 3. Germinating fungi pre-cultured for one week were transferred to 100ml of Bushnell Haas media then to 500ml conical flask containing 1% crude oil, 0.1% V/V Tween 80 and 0.016 mg/ml of redox oxidation. The flask was incubated for 2 weeks before assessment.	Temperate climate	➢ *Aspergillus niger* degraded 54% of crude oil, while ➢ *Penicillium commune* degraded 48%
5.	Flayyih[73]	1. *Aspergillus niger,* 2. *Aspergillus fumigatus,* 3. *Fusarium solani* 4. *Penicllium funiculosum* (all microscopic)	1. Laboratory /glasshouse Scale: 28 days 2. Artificially contaminated soil samples with crude oil (2% w/w) were used 3. Target contaminant: TPHs 4. Extracting solvent for TPHs: Dichloromethane	Temperate climate	➢ Highlighted that time is factor on remediation efficiency ➢ Highest remediation efficiency was 95% with A. *niger* after 28 days of treatment. ➢ Highest remediation efficiency by mixed cultures of fungi were 90% with *A. niger* and *A. fumigatus*.
6.	Fana[74]	Yeast- *Candida tropicalis SK2* (all microscopic)	1. Laboratory /glasshouse Scale:180days 2. Typical petroleum contaminated-soil samples with crude oil 3. The Candida tropicalis SK21 was inoculated into the soil to reach a density of 1.0×106 CFU/g. 4. Target contaminant: TPHs and PAHs Extracting solvent for TPHs: Dichloromethane	Temperate climate	➢ Microbial enumeration showed that the yeast SK21 could grow well in the contaminated soil ➢ Yeast removed 83% of TPH in 180 days ➢ 81.5% of PAHs were removed by the fungi during the period of 180days

7.	Rahman[10]	Oyster mushroom (macroscopic)	1. Laboratory /glasshouse Scale: 31 days 2. Artificially contaminated soil samples with crude oil were used 3. Layer of oyster mushrooms substrate were equally distributed on top of the soil and gently compacted	Temperate climate	➢ Fruit bodies of mushroom was found 7 days after inoculation ➢ 35% of TPH was removed
8.	Abioye[56]	Yeast-*Saccharomyces cerevisiae* (microscopic)	1. Laboratory /glasshouse Scale: 28 days 2. crude oil samples (3.5 and 7.0%) 3. Yeast was isolated from Zobo drink and developed on Sabauroud dextrose agar by spread plate method incubated at 25^0C for 48 hours. 4. Cultured yeasts were then inoculated on a 50ml Mineral salt medium containing 1g of crude oil and maintained at 30^0C for 28 days 5. Target contaminants: TPHs 6. Solvent for extraction: Dimethyl ether	Tropical climate	➢ Degradation activities increased with days ➢ 49.29% of crude oil degradation was achieved after 28 days.
9.	Al-Nasrawi[57]	1. *Aspergillus niger* 2. *Penicillium documbens,* 3. *Cochliobolus lutanus* 4. *Fusarium solani.* (all microscopic)	1. Laboratory /glasshouse Scale: 21 days 2. Typical petroleum-contaminated soils taken from 0-15cm of contaminated sites were used 3. Prepared fungi on nutrient medium were used to inoculates soils and maintained at 4. Extracting solvent: acetone and dichloromethane (DCM)-1:1 5. Target contaminants: PAHs Method of application: Layering growing substrates on top of soil	Temperate climate	➢ Highest degradation was recorded for *Pennicillin documbens* at 21 days.
10.	Edema[75]	*Basidiomycetes* (macroscopic)	1. Laboratory /glasshouse Scale: 4 weeks 2. Artificially contaminated soils (soils thoroughly mixed with crude oil 1l/5.0 Kg). 3. Extracting solvent: acetone and dichloromethane (DCM)-1:1 4. Target contaminants: PAHs Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ 98.93% PAHs reduction was achieved
11.	Kristanti[67]	*Polyporus sp* (macroscopic)	1. Laboratory /glasshouse Scale: 60 days 2. Artificially contaminated soils with crude oil(3000mg) 3. Extracting solvent: hexane, dichloromethane (DCM) and chloroform successively 4. Target contaminants: TPHs Method of application: wood meal pre-grown fungi were applied to contaminated soil surface, then mixed thoroughly	Temperate climate	➢ highest degradation rate of crude oil was 93% in the soil after 60 days
12.	Adenipekun[15]	*Pleurotus pulmonarius* (macroscopic)	1. Laboratory /glasshouse Scale: 2 months 2. Artificially contaminated soils with crude oil and Palm kernel oil (0- 40%). 3. Target contaminants : Total Petroleum Hydrocarbons (TPHs) Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ trace metal contents decreased during treatment ➢ There was a 40.80% degradation of TPHs at 1% crude oil concentration and 9.28% at 40% after 2 months.
13.	Adenipekun[76]	*Lentinus subnudus* (macroscopic)	4. Laboratory /glasshouse Scale: 3-6 months 5. Artificially contaminated soils (soils thoroughly mixed with crude oil concentrations (0, 1, 2.5, 5, 10, 20 and 40%). 6. Target contaminants : Total Petroleum Hydrocarbons (TPHs) 7. Method of application: Layering growing substrates and active spawn on top of soil	Tropical climate	➢ Total Petroleum Hydrocarbon decrease were 33.04%, 56.67%, 14.85%, 25.27%, 22.57% and 15.25% respectively for each concentration after 3months, and ➢ 60.60%, 78.25%, 85.64%, 89.54%, 95.12% and 95.12% respectively after 6months

14.	Stamets[2]	*Pleurotus ostreatus* (macroscopic)	1. Large Scale: 8 weeks 2. Typical petroleum- contaminated soils of diesel and oils approximately 20,000 parts per million of Total Aromatic Hydrocarbons 3. 4 piles of contaminated soils where place on a large sheet of 6mm black polythene tarps. Each pile measured about 4 X 20 X 8 feet in width 4. A corresponding 30 % of sawdust spawns were mixed to contaminated soils. 5. Spawn where placed in layers between contaminated soils in a parallel sheet spawning 6.	Temperate Climate	➢ About 99% of TPHs were degraded after 8 weeks
15.	Isikhuemhen[62]	*Pleurotus tuberregium* (macroscopic)	1. Laboratory /glasshouse Scale: 30 days 2. Artificially contaminated soils (1, 3, 5, 10 and 15%, w/w) of crude oil 3. Target contaminants: TPHs 8. Solvent for extraction: Xylene Method of application: mixing contaminated soils with substrates, then inoculation active spawn (25% w/w)	Tropical climate	➢ There was 85% reduction in TPHs after 30days

(Many other studies exist on mycoremediation of organic/ inorganic contaminants. In preparing this table emphasis was on soil contaminants directly relating to crude oil/petroleum products. Where similar studies-involving similar fungi and identical scenarios/outcomes existed, only one of such was taken as a representative case based on a 'first published' criteria).

Mechanism of mycoremediation

The mechanism of fungal degradation of organic contaminants in soils is presently thought to follow a similar mechanism for degradation of lignin[32,77,78]. Several mechanisms have been proposed including both direct and indirect oxidation of the organic molecule by the fungal enzymes namely Lignin-peroxidase (LiP), Manganese Peroxidase (MnP) (Figure 1), Versatile peroxidase (VP) and Lacasses[52,79,80]. Hatakka[81] suggested a possible combination of two or more enzyme mechanisms in the degradation process. Hofrichter[82] proposed a radical-mediated reaction initiated by manganese peroxidase (MnP). This involves indirect oxidation of aromatic (phenolic) rings (ether peroxide formation), spontaneous ring opening to produce muconic acid derivatives and decarboxylation of the formed carboxyl groups to carbon dioxide[83]. Several intermediate fragments are produced in the processes (Figure 1).

Application procedures for mycoremediation of petroleum-contaminated soils

The general procedure for application of mushrooms to contaminated soils is by layering[84-86]. This has been carried out by layering actively growing substrates on topsoil or by way of vertical and horizontal sandwiching of active mushroom substrates between contaminated soils[2,70]. The actively growing mushrooms substrates may be pre-developed to a level where mycelia are actively sprouting before inoculation of soils, or spawns may be inoculated directly on substrates layered on soils[76,87].

Adenipekun[70] described a procedure whereby 400 g of soils was artificially contaminated with 0-30% of spent cutting fluid-SCF and fresh cutting fluids-FCF and placed in sterile 350 ml bottles. 80 g of moistened rice straw were then laid on these soils, and after sterilization and cooling, 10 g of the actively growing mushrooms spawns were inoculated on the samples. In Bhatt[87], 250 g of contaminated soil was placed between two layers of rice straw colonized with fungal mycelium (i.e. 50 g of the fungal mycelium on top and 50 g at bottom of the soil).

For microscopic fungi, these are often prepared first on their respective growth media before inoculation on soils[57,73,74].

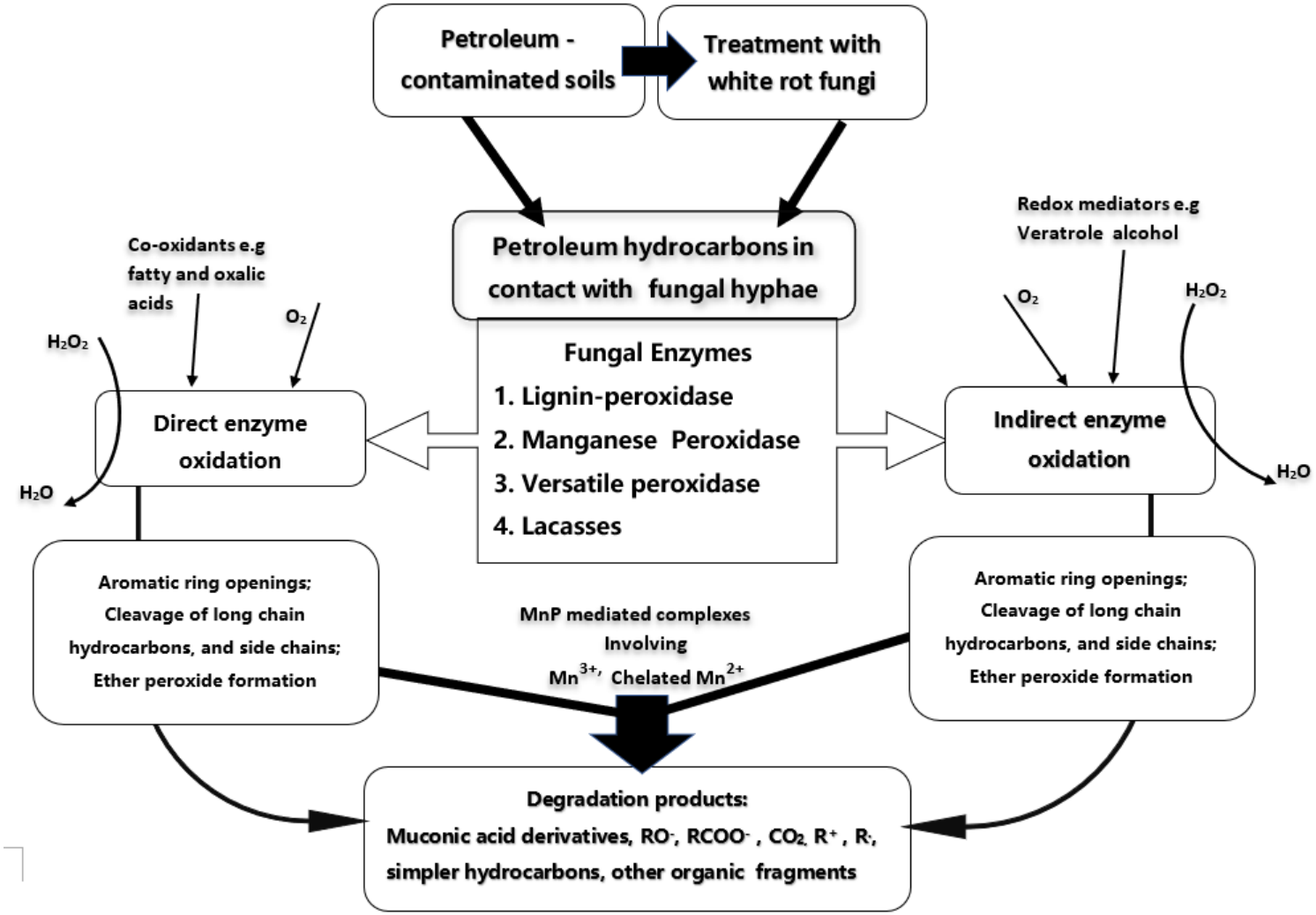

Figure 1: Mechanism of mycoremediation of petroleum-contaminated soil by white rot fungi

Substrates for mycoremediation of contaminated soils

Substrates that have been used for growing white-rot fungi include rice straw, cotton waste, wheat bran, rice bran, shredded straw, corn cobs, soybeans flour, pasteurized/fermented/fresh cereal straw, pine barks, fragmented woods (sawdust), straw bales, sugar beet pulps and coffee pulps[88,89]. These substrates are often enriched with animal manures in different proportions for effective growth[60,90]. Composting has also been used as soil amendment for mycoremediation[91]. Wright[92] demonstrated an overall degradation of PAHs between 80 and 92 % in contaminated soils treated with spent sawdust compost.

Substrates for mycoremediation of petroleum contaminated soils are also usually sterilized before inoculation of the fungal spawns[2,15]. Adenipekun[70] reported that sterilization of fungal substrates reduces competition by other microbial organisms which could inhibit the growth of the mushrooms.

Advances in mycoremediation of petroleum contaminated soils

Studies on mycoremediation have evolved from the direct use of fungi and mushrooms, to that of fungal-derived enzymes as well as spent fungal substrates[84-86,94]. These studies have also been carried out both in situ and ex situ, and there are a some large-scale applications[94,95]. There are also reports on mycoremediation in both temperate and tropical regions (Table 1), as well as in developed and developing countries[2,59,75,96,97]. It has further been demonstrated that a combination of fungi and bacteria can lead to enhanced remediation efficiency [98].

Studies such as Aranda[99], Hirantsuka[100] and Sack[101] have demonstrated that fungal enzyme extracts can degrade organic contaminants in soils. Anasonye[96] however, reported that MnP-enzyme extracts of the fungus *Kuehneromyces mutabilis* could not replicate the activity exhibited by the fungi itself on contaminated soils. These observations support the proposition of Hatakka[81] who stated that a combination of more enzyme systems and other physiological processes are involved in the mycoremediation process.

Winquist[97] demonstrated that laboratory outcomes of mycoremediation can also be applied in the field. PAHs-contaminated soils were mixed with composted green waste (1:1) and treated with fungal inoculum of *Phanerochaete velutina.* The study was performed both at the laboratory and field scales. Results from laboratory study with an initial concentration of 3500 mg/kg revealed up to 96% degradation of 4-ring PAHs and 39% of 5- and 6-ring PAHs after three months. For the field study with an initial PAHs concentration of 1400 mg/kg of soil, 94% of the 16 PAHs were degraded after three months. Okparanma[102], Zitte[103] and Albert[104] have also demonstrated that spent mushroom substrates could be used for mycoremediation of petroleum contaminated soils.

DISCUSSION

Most studies of mycoremediation of petroleum-contaminated soils are carried out using artificially contaminated soils in glasshouses, under sterile conditions[10,56]. This could lead to several issues in applying laboratory results to the field.

Firstly, the varying concentrations added to soils to create artificial contamination cannot be compared to real crude oil spills with massive quantities of petroleum contaminants in soils. Liu[105] reported up to 50% content of total petroleum hydrocarbons per Kg dry soil in petroleum contaminated sites. Concentrations of up to 420 g/kg of total petroleum hydrocarbons were also reported by Kim[106]. Thus, it would be ideal to use typical petroleum-contaminated soils during mycoremediation studies. The use of typical petroleum-contaminated soils such as those of Kim[106] would allow potential incorporation of measures for possible dilution of highly contaminated soils prior to application of mycoremediation[107].

Secondly, the aspect of substrates sterilization and incubation at room temperatures creates an artificial environment which is different from those of the field during in situ applications. Using unsterilised substrates and conditions identical to field environments would aid replication of laboratory outcomes in the field.

Thirdly, climatic and edaphic factors are not usually incorporated into laboratory or glasshouse studies and this will certainly be encountered in the natural environment. Ideal research in mycoremediation of petroleum-contaminated soils should be tailored towards real-life situations using typical petroleum-contaminated soils and unsterilised conditions.

This would involve investigations into various substrates and conditions which can be easily replicated during in situ applications. Using substrates and fungi species which are ubiquitous or native to sites of contamination would also help mitigate potential adaptation problems.

There seem to be limited reports on application of enhancement agents such as the use of surfactants, combination with other microbial communities, as well as combinations of mycoremediation with phytoremediation for treatment of petroleum contaminated soils. Surfactants can increase the bioavailability of organic contaminants[108,109]. Therefore, integrating surfactants with mycoremediation, and combination with other biological agents could lead to increase efficiency of the process on petroleum contaminated soils.

Biotechnology and engineering solutions are not often incorporated into mycoremediation for the management of petroleum contaminated soils. Bamforth[110] and D'Annibale[35], reported that factors such as life cycle, size of fruity body and mycelium biomass influence the efficiency of mycoremediation. Biotechnology can be integrated to developed mushrooms with better size of the fruity body, mycelium biomass and improved enzyme yield[111,112], which will result in increased mycoremediation efficiency.

Although many studies exist supporting the potential of mycoremediation of soil contaminants (organic and inorganic), only few of such studies relate to soil contamination by crude oil and other petroleum products. Most of the studies so far carried out on mycoremediation of petroleum-contaminated soils are still at laboratory or glasshouse scale. There is a need to initiate research that applies glasshouse findings to field applications.

CONCLUSION

Mycoremediation is also capable of providing reliable options for the treatment of petroleum-contaminated soils. This is because fungi can provide cheaper and safer means for the simultaneous degradation of organic contaminants and the extraction of inorganic species. In addition, most fungi are ubiquitous which ensures their widespread applications. Furthermore, the warm temperatures for fungal growth makes the technique ideal for tropical regions with warm temperatures, locally available fungi and substrates.

There are several innovations in this field, notably, the use of fungal enzymes, and spent mushroom substrates. These provide options in situations where mushrooms cannot be

cultivated. Petroleum-contaminated sites often also contain high levels of trace metals. Most of the mushroom species are hyperaccumulators of trace metals. It is therefore worth not only studying their degradation or extraction efficiencies but possible speciation and transformation of the inorganic (trace metals) species. Mushrooms have short life cycles, which is somewhat an advantage, because a remediation cycle can be achieved within a short time. However, care must be taken such that substances already taken up are not returned to soils via putrefaction. The end use and treatment of the harvested mushrooms should also be integrated into remediation programs.

Finally, the challenge in the development of mycoremediation from laboratory studies to large-scale field applications on petroleum-contaminated soils lies in incorporating ideal environmental, edaphic and climatic factors of a typical contaminated site into the process from first principles.

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