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BIODEGRADATION POTENTIAL OF INDIGENOUS FUNGI ISOLATES FROM WASTE ENGINE OIL CONTAMINATED SOIL

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ABSTRACT

The anthropogenic release of waste engine oil in the environment causes environmental and public health risks. This study aimed at determining the biodegradation potentials of indigenous fungi isolates on waste engine oil. Waste engine oil tainted soils were taken from five different automobile workshops in Benin City. They were analysed using standard laboratory methods. Five fungi isolates; *Penicillium*, *Fusarium*, *Aspergillus*, *Trichoderma* and *Geotrichum* species were isolated and screened for their potential to use engine oil as carbon source. *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sp. had the highest and were used for the bioremediation studies. The pH, electrical conductivity, total nitrogen, total hydrocarbon content and optical density were recorded for 25 days at 5 days interval with different oil concentrations (10%, 20%, 30%, and 40%) to monitor the process. The results obtained showed that pH and total hydrocarbon content values were decreasing with increasing incubation time. *Penicillium* sp. gave the highest electrical conductivity value of 501 us/cm at 30% contamination level at day 25 while *Fusarium* sp. had the lowest, 239 us/cm at 40% contamination level at day 5. The electrical conductivity and optical density values were increasing with increased incubation days. *Penicillium* sp. gave the highest increase in the nitrogen content of 0.91mg/l at 30% contamination level. The study showed that *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sp. were able to biodegrade waste engine oil and therefore recommends that they be used to clean up waste engine oil.

Keywords: *Aspergillius* sp., Public health risk, Sustainable environment, Total hydrocarbon content

INTRODUCTION

The current global technological developments involving the use of products with hydrocarbon origins have continuously increased the amount of environmental pollution linked to hydrocarbons (Adeleye et al., 2018). Engine oil is widely used as a lubricant in motor vehicles and other types of machinery such as hydraulic pumps & motors, compressors, and electrical transformers (Enerijiofi et al., 2020). According to Shahida et al. (2015), engine oil (lubricant) is essentially a substantial mineral fluid that is poured into an engine or machine to reduce the friction between moving parts. Automotive engine oil is one of many products derived from petroleum's initial refining. It is made up of long-chain highly saturated hydrocarbons and employed for lubrication of every part of automotive engines to ensure their best operation at all times (Ajuzie et al., 2015; Shittu & Ogor, 2018). Although lubricating oil typically contains just around 2% crude oil, it is the petroleum fraction that is used most frequently because of its ability to reduce the viscosity of automobiles and machineries (Obayori et al., 2015). The term "used motor oil, spent engine, used motor oil" signifies the dark brown to black oily substance that is drained from an engine after replacing the oil in an automobile or other machine. As the name implies, used motor oils represent oils whose properties undergo destructive changes when exposed to oxygen, thereby combustion gases, and high temperatures. Additionally, the petroleum product goes through additive depletion, oxidation, and viscosity changes (Mark et al., 2018). However, compared to new oil, the polycyclic aromatic hydrocarbon content in used automobile oil is significantly higher (Bharathi et al., 2017).

There has been a problem with hydrocarbon contamination of land and water, especially in developing countries, since the discovery of petroleum as a source of energy (Enerijiofi et al., 2022; Murphy et al., 2022). The use of motor oil has increased as a result of the availability of different kinds of automobiles and machineries. Motor (engine) oil flow into downspouts, sewage drains, agricultural plots, and vineyards which are common practices especially among car technicians (Adenipekun et al, 2018). This inconsiderate disposal adversely impacts plants, microorganisms, aquatic lives and human health (Enerijiofi et al., 2020; Wang et al., 2016; Ikhajiagbe et al., 2021). Heavy metals found in significant amounts in used motor oil, including vanadium, lead, aluminum, nickel, and iron, can persist in topsoil as exchangeable cations, oxides, hydroxide compounds, calcium carbonate, and/or organic compounds (Ying et al., 2017, Nath et al., 2024).

Waste engine oil biodegradation employ naturally existing microbes' biodegradative activities. This frequently makes use of the enzymatic abilities of local populations of microbes that degrade hydrocarbons as well as changing environmental conditions (Femi-Ola et al., 2019). For some hydrocarbons, including polyethylene, fungi are more effective degraders than bacteria (Muhonja et al., 2018; Dhar et al., 2014). They have a better tolerance due to their physiology and environmental adaptation to fluctuations in hydrocarbon toxicities (Barnes et al., 2018). Extracellular enzymes secreted by fungi enable them to rapidly ramify, absorb complex polysaccharides, and subsequently digest the substrate. Under harsh environmental conditions mass production is possible (Abu Bakar et al., 2020). Furthermore, the fungi's filamentous shape makes it simple to filter them out (Pawłowska et al., 2019). As stated above and in corroboration with Maamar et al. (2020), fungi have the capability to neutralize a wide range of ecological contaminants, including hydrocarbons that are polycyclics, aromatics, phenols, halogen-based phenolic substances, polychlorinated biphenyls, and oil-based hydrocarbons

According to Ajuzie et al. (2015), a gathering of microorganisms that are broadly known as hydrocarbonoclastics can utilize hydrocarbons as their sole hope of carbon and metabolic advancement. However, the potential of microorganisms to synthesize hydrocarbons depend on several parameters, including the chemical composition of the constituent molecules in the crude oil mixture and the environmental conditions (Wang et al., 2013; Enerijiofi & Ikhajiagbe, 2021, Ikhajiagbe et al., 2021). In addition, Yeast from the genera, *Pichia*, *Pseudozyma, Rhodotorula*, *Candida* and *Yarrowia* as well as species of *Fusarium*, *Aspergillus*, *Trichoderma*, *Geotrichum*, *Graphium*, *Paecilomyces*, *Penicillium* and *Sporobolomyces* can degrade hydrocarbons (Enerijiofi et al., 2020; Al-Dhabaan, 2021). The contamination of waste engine oil in the environment calls for monitoring and evaluation which necessitates the need for this research. The study is aimed at identifying the fungi species from waste engine oil contaminated soils and determine their bioremediation abilities on waste engine oil.

MATERIALS AND METHODS

Study Area

Soil Samples polluted with waste engine oil were obtained from five (5) distinct automobile workshops in Benin City, Edo State, Nigeria; Ramat Park, Ikpoba Hill (6.37586° N, 5.65998° E), First East Circular, (6.34096° N, 5.63501° E), New Lagos Road (6.34970° N, 5.63105° E), Lucky Igbinedion Way, Ikpoba-Okha (6.37558° N, 566020° E) and Benin- Lagos Expressway opposite University of Benin (6.40376° N, 5.60803° E).

Collection of Sample

The used engine oil-polluted soils were collected from the five (5) automobile repair shops. The Soil samples were acquired at a depth of 0 - 15cm after properly scraping away the outermost soil layer from the automobile shops in labelled sterile polythene bags and transported to the laboratory for fungi analysis and biodegradation studies. In biodegradation experiments, the waste engine oil served as the pollutant.

Fungal Analysis

Enumeration and Isolation of Indigenous Fungi from Soil Samples

Each sample was thoroughly integrated, stones and other undesirable soil particles were carefully eliminated through sorting. The total heterotrophic fungal count were carried out using serial dilution technique and agar plating method. Potato Dextrose Agar (PDA) was used to culture the fungal species. A 10-fold serial dilution was made using 1g of each soil sample. The pour plate method was used to culture 1.0 ml of each sample's 10^{-3}, 10^{-6} and 10^{-9} dilutions on PDA plates (Enerijiofi et al., 2019). However, 0.1% of tetracycline was added to the petri-dish to prevent bacterial growth during incubation at 37^0C for 24 hours. The distinct fungal colonies were isolated through repeated passages (Dhar et al., 2014).

Phenotypic Characterization and Identification of Fungal Isolates

The macroscopic features of the purified isolates were recorded while the microscopic properties of the filamentous fungal and yeast isolates were examined using lactophenol cotton blue stain on with the identification scheme (Dhar et al., 2014)

Determination of Bioremediation Activities of Indigenous Fungal Isolates

The bioremediation studies were carried out using waste engine oil as the only carbon source for the fungal isolates (*Aspergillus*, *Fusarium* and *Penicillium* species) in mineral salt medium (MSM) broth. Sterile conical flasks enclosing 500 ml of MSM broth and 5mls of different concentrations of sterile waste engine oil (10, 20, 30 and 40 % contamination levels) were aseptically prepared. Thereafter, each flask was concealed with cotton wool and enclosed in aluminum foil. This mixture was autoclaved at 121ºC for 20 minutes to allow for growth. Thereafter, a sterile wire loop was used to transfer an inoculum from the pure culture of each fungi isolate to well-labeled flasks containing the mixture. This mixture was incubated using an incubator at 30ºC over a period of 25 days. Each flask was agitated twice daily using a rotary shaker to improve homogenization and ventilation all through the biodegradation studies. At interval of 5 days, during the period of incubation, 10 mls from each culture was collected to determine degradation using four parameters pH, electrical conductivity (EC), nitrogen (N) and total hydrocarbon content (THC). A set-up without the inoculation of fungi isolates were used control (Enerijiofi et al., 2020).

Statistical analyses of Data

The Data obtained and recorded for pH, EC, N and THC were statistically analyzed by two-way analysis of variance with replication. The results were compared to determine if there were statistical significant differences based on the days of interval monitored during the bioremediation studies at 95% probability level (Ogbeibu, 2005).

RESULTS AND DISCUSSION

Results

Soils from location B had the highest count, 8.0±0.13 x 10^3 cfu/g while locations C and D had the least count, 1.1±0.10 x10^3cfu/g 1.1±0.10 x10^3cfu/g respectively (Table 1). The distribution pattern of fungal isolates is recorded in Table 2. *Penicillium* and *Fusarium* species were present in all polluted soil samples. However, *Aspergillus* sp. was present in all contaminated soil sample except Sample A while *Trichoderma* and *Geotrichum* species were present in only contaminated soil sample B. The result of pH values as recorded showed that *Aspergillius* sp. with 30% and 40% waste engine oil contamination level had the highest of 5.8 at day 5 while the least at 25 day period was 5.0 (Table 3). In all the fungal isolates studied, *Penicillium* sp. gave the highest electrical conductivity value of 508 us/cm at 30% contamination level at day 25 while *Fusarium* sp. had the lowest of 239 us/cm at 40% contamination level at day 5 (Table 4). *Penicillium* sp. gave the highest increase in the nitrogen content of 0.96mg/l at 30% contamination level with while *Aspergillus* and *Fusarium* species gave the least reduction of 0.11mg/l at 10% contamination level (Table 5). The Total Hydrocarbon Content (THC) values at varying concentrations of waste engine oil with fungal inoculum in a mineral salt medium showed that at 10%, 20%, 30% and 40% waste engine oil contamination level, the three isolates gave reductions with the highest value at 0.9 (Table 6). The optical density (OD) values at varying concentrations of waste engine oil had *Aspergillus* sp. recording the highest growth of 0.460 at 10% contamination level while *Fusarium* sp. at 40% had the least of 0.320. The fungal isolates show no significant difference in the values at the different waste engine oil contamination level (Table 7).

Table 1: Total Heterotrophic Fungi Count

Samples	THFC($X10^{-3}$)
A	1.3±0.02
B	8.0±0.13
C	1.1±0.10
D	1.1±0.21
E	1.6±0.01

Legend: A: Ramat Park, B: First East Circular, C: New Lagos Road, D: Lucky Igbinedion Way, E: Benin- Lagos Expressway

Table 2: Distribution of Isolated Fungi

Samples	*Penicillium sp.*	*Fusarium sp.*	*Aspergillus sp.*	*Trichoderma sp.*	*Geotrichum sp.*
A	+	+	_	_	__
B	+	+	+	+	+
C	+	+	+	_	_
D	+	+	+	_	_
E	+	+	+	_	_

Legend: A: Ramat Park, B: First East Circular, C: New Lagos Road, D: Lucky Igbinedion Way, E: Benin- Lagos Expressway

Table 3: pH values of MSM amended with varying concentrations of waste engine oil inoculated with selected fungal isolates

Fungal Isolates	Conc. WEO (%)	Incubation period (Days)					
		Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
***Aspergillius* sp.**	10	6.5	5.6	5.4	5.2	5.1	5.0
	20	6.8	5.7	5.6	5.5	5.5	5.5
	30	7.3	5.8	5.8	5.8	5.8	5.7
	40	7.5	5.8	5.8	5.8	5.7	5.6
	Control	7.1	6.3	6.3	6.3	6.3	6.4
	10	6.7	5.8	5.7	5.6	5.6	5.6
***Fusarium* sp.**	20	6.8	6.5	6.5	6.4	6.3	6.2
	30	6.9	6.8	6.8	5.8	5.9	5.8
	40	7.2	6.8	6.8	6.6	5.8	5.5
	Control	7.1	6.6	6.6	6.6	6.7	6.6
	10	6.5	5.6	5.4	5.3	5.2	5.1
***Penicillium* sp.**	20	6.6	5.8	5.8	5.8	5.7	5.7
	30	7.1	5.8	5.8	5.8	5.6	5.7
	40	7.6	5.8	5.7	5.6	5.6	5.5
	Control	7.1	6.6	6.6	6.6	6.6	6.4

Table 4: EC (us/cm) values of MSM amended with varying concentrations of waste engine oil inoculated with selected fungal isolates

Fungal Isolate	Conc. WEO (%)	Incubation period (Days)					
		Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
***Aspergillius* sp.**	10	305	326	347	371	395	415
	20	346	352	368	379	410	438
	30	343	364	381	404	429	451
	40	237	269	294	321	350	385
	Control	332	334	335	337	343	346
	10	403	428	441	469	473	491
***Fusarium* sp.**	20	346	361	387	399	417	431
	30	344	351	367	389	396	424
	40	238	239	254	269	281	309
	Control	332	339	344	349	361	373
	10	403	441	468	481	493	501
***Penicillium* sp.**	20	346	357	373	386	391	498
	30	343	362	374	380	392	508
	40	236	257	284	291	315	337
	Control	332	339	342	349	356	371

Table 5: Nitrogen content (mg/l) values of MSM amended with varying concentrations of waste engine oil inoculated with selected fungal isolates

Fungal Isolates	Conc. WEO (%)	Incubation period (Days)					
		Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
***Aspergillius* sp.**	10	0.15	0.11	0.20	0.40	0.58	0.85
	20	0.16	0.12	0.30	0.50	0.69	0.88
	30	0.16	0.12	0.22	0.42	0.49	0.92
	40	0.18	0.13	0.43	0.52	0.10	0.72
	Control	0.16	0.15	0.15	0.15	0.15	0.17
	10	0.15	0.11	0.30	0.40	0.40	0.58
***Fusarium* sp.**	20	0.15	0.11	0.12	0.32	0.42	0.79
	30	0.16	0.12	0.32	0.16	0.62	0.50
	40	0.18	0.12	0.32	0.53	0.73	0.70
	Control	0.16	0.16	0.16	0.16	0.16	0.16
	10	0.15	0.11	0.21	0.41	0.71	0.86
***Penicillium* sp.**	20	0.16	0.12	0.31	0.40	0.41	0.88
	30	0.17	0.12	0.32	0.52	0.62	0.96
	40	0.17	0.12	0.52	0.62	0.81	0.10
	Control	0.16	0.15	0.15	0.15	0.15	0.15

Table 6: THC (mg/l) values of MSM amended with varying concentrations of waste engine oil inoculated with selected fungal isolates

Fungal Isolates	Conc. WEO (%)	Incubation period (Days)					
		Day 0	Day 5	Day 10	Day 15	Day 20	Day 25
***Aspergillius* sp.**	10	2.12	1.14	1.12	1.11	1.10	0.90
	20	3.20	1.18	1.14	1.14	1.12	1.11
	30	3.51	1.29	1.28	1.26	1.24	1.20
	40	3.80	1.34	1.30	1.30	1.26	1.22
	Control	3.10	3.0	3.0	3.0	3.0	3.0
	10	2.12	1.18	1.14	1.12	1.12	1.11
***Fusarium* sp.**	20	3.19	1.26	1.23	1.23	1.20	1.20
	30	3.50	1.30	1.30	1.29	1.28	1.26
	40	3.80	1.34	1.30	1.28	1.28	1.28
	Control	3.10	3.10	3.10	3.10	3.10	3.10
	10	0.12	1.15	1.14	1.12	1.10	0.98
***Penicillium* sp.**	20	3.20	1.16	1.16	1.15	1.14	1.10
	30	3.50	1.26	1.26	1.24	1.24	1.21
	40	3.80	1.28	1.28	1.28	1.26	1.24
	Control	3.10	3.0	3.0	3.0	3.0	3.0

Table 7: The Optical density values of MSM amended with varying concentrations of waste engine oil inoculated with selected fungal isolates

Fungal Isolates	Conc. WEO (%)	Incubation period (Days)				
		Day 2	Day 5	Day 10	Day 15	Day 20
***Aspergillius* sp.**	10	0.419	0.419	0.419	0.430	0.460
	20	0.410	0.410	0.410	0.422	0.452
	30	0.402	0.402	0.402	0.418	0.448
	40	0.390	0.390	0.390	0.412	0.440
	Control	0.774	0.774	0.774	0.774	0.780
	10	0.326	0.326	0.326	0.315	0.340
***Fusarium* sp.**	20	0.324	0.324	0.324	0.312	0.335
	30	0.324	0.324	0.324	0.304	0.330
	40	0.301	0.301	0.301	0.302	0.320
	Control	0.780	0.780	0.780	0.780	0.784
	10	0.396	0.396	0.396	0.412	0.433
***Penicillium* sp.**	20	0.396	0.396	0.396	0.402	0.430
	30	0.370	0.370	0.370	0.398	0.410
	40	0.368	0.368	0.368	0.398	0.390
	Control	0.780	0.780	0.780	0.782	0.784

Discussion

Fungi are known effective degraders of a wide range of simple and complex substances. The fact that fungi utilize crude oil and its products as a substrate for their survival and growth attests to employing extra cellular enzymes in the breakdown of the substrate within the media containing crude oil. In this study, a higher number of fungal isolates from the soil tainted with waste engine oil were reported compared to the control. This revealed that more nutrients were available in the waste engine oil polluted soils than the control. Also, the report of this study was corroborated by earlier report of Enerjiofi et al. (2020) where they reported the isolation of high bacteria numbers in similar polluted environment. However, *Penicillium*, *Fusarium* and *Aspergillus* species were the most predominant fungi isolates identified in this study as they were isolated from the five locations. *Aspergillus* sp was isolated from four locations except at Ramat Park. In contrast, *Trichoderma* sp. and *Geotrichum* sp. were only isolated in soils from one location, First East Circular. The isolation of these fungal isolates though at varying numbers were not surprising as they are soil borne microorganisms, resident within the studied environment and had earlier been documented to grow and proliferate during the remediation process signifying their capabilities to consume waste engine oil as their only source of carbon and energy (Dhar et al., 2014).

The preponderance of the fungal isolates particularly *Penicillium*, *Fusarium* and *Aspergillus* species in waste engine oil contaminated soil environment confirms their high prowess as hydrocarbon biodegraders. This corroborates the reports of earlier studies which documented their presence is crude oil contaminated soil environment (Singh, 2006; Dhar et al., 2014). The preliminary screening indicated that *Penicillium*, *Fusarium* and *Aspergillus* species were capable of utilizing waste engine oil in mineral salt medium broth for growth better than other fungal isolates. Hence, they were utilized for the bioremediation studies in this work.

Four physiochemical parameters; pH, electrical conductivity (EC), nitrogen and total hydrocarbon content (THC) were used as indicators to monitor the bioremediation potentials of the three selected isolates (*Penicillium*, *Fusarium* and *Aspergillus* species) on waste engine oil. The results obtained revealed that pH and total hydrocarbon content values were decreasing with increasing period of incubation. This showed that bioremediation activity was in progress. The reduced values recorded for the pH were possibly due to the medium becoming acidic resulting from the breakdown of hydrocarbon that was added to the mineral salt medium. This catabolic reaction of the hydrocarbon content could have been responsible for the release of carbon (iv) oxide and water, which combined giving rise to the formation of carbonic acid. The carbonic acid could be responsible for contributing to the increased acidity and the recorded decreasing pH values. The decrease in the total hydrocarbon content indicated that the fungi isolates were able to make use of waste engine oil as substrate for carbon and energy. However, there were no significant difference in the values for both pH and THC ($p > 0.05$). Earlier, Kumar *et al*. (2016) reported that *Penicillium* sp. was identified as the effective strain in the biodegradation of petroleum component which corroborated with the result of this study. However, the values for the control experiment remained practically the same all through the study period. This means that bioremediation did not take place all through the 25 day study period as no fungal isolate was inoculated. In contrast to pH and THC, the electrical conductivity values were reported to increase proportionately with increasing days of incubation, resulting in the release of more ions (cations and anions). These increasing values resulting from the breakdown of waste engine oil by the three selected fungal isolates points to their effectiveness in using waste engine oil as source of carbon and energy. This is as reported earlier (Femi-Ola et al., 2019). However, there was no significant difference in the values recorded for EC concentrations despite the increase ($p > 0.05$).

The concentration of nitrogen reduced throughout the entire biodegradation studies at all concentrations with the exception of *Penicillium* sp. at 10%, 20% and 30% contamination levels. The general understanding could be that nitrogen as limiting within the substrate. However, the optical density values increased with incubation period in this study. This also indicated that fungal growth was on the increase because they were able to use of waste engine oil as a source of carbon and energy as reported earlier by Ajuzie et al. (2015). As the incubation period increases, the growth of the fungus is shown to rise, which suggests that the degradation of petroleum increases.

CONCLUSION

The result obtained from this study revealed that, the studied fungal isolates; *Penicillium*, *Fusarium* and *Aspergillus* species isolated from waste engine oil polluted soils hold promise for the development and discovery of new bioremediation approach for the effective waste engine oil treatment. This study recommends that bioremediation should be highly considered and utilized in the management of waste engine oil tainted soil as this will guarantee a sustainable environment for mankind and the ecosystem.

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