

Isolation and identification of hydrocarbon degrading bacteria and fungi from waste engine oil impacted soil, their distribution frequency and hydrocarbon degradation capacity

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Abstract

The isolation and identification of hydrocarbon degrading bacteria and fungi from waste engine oil contaminated soil obtained from Mechanical Village, Uyo, Akwa Ibom State, their distribution frequency and hydrocarbon degradation capacity were investigated using standard method. The results revealed that *Bacillus myocoides*, *Staphylococcus aureus*, *Listeria murrayi*, *Actinomyces viscosus*, *Clostridium sporogenes*, *Bacillus licheniformis*, *Corynebacterium ulcerans* and *Clostridium histolyticum* were bacteria identified. Fungi identified were *Fusarium oxysporum*, *Cryptococcus terreus*, *Penicillium notatum*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Rhizopus oryzae* and *Rhodotorula mulcilaginosa*. Their distribution frequency were *B. myocoides* (27.9 %), *S. aureus* (8.1 %), *L. murrayi* (9.9 %), *A. viscosus* (11.7 %), *C. sporogenes* (16.2 %), *B. licheniformis* (7.2%), *C. ulcerans* (12.6 %) and *C. histolyticum* (6.3 %) for bacteria. For the fungi, *F. oxysporum* (15.3 %), *C. terreus* (4.1%), *P. notatum* (20.4 %), *A. fumigatus* (11.2 %), *R. stolonifer* (10.2 %), *R. oryzae* (24.5%) and *R. mulcilaginosa* (14.3%). The efficiency of the microbial isolates to degrade waste engine oil from petrol and diesel driven vehicles as their major source of energy were found to vary among the microorganisms. *C. sporogenes* degraded waste engine oil from petrol driven vehicle with the highest efficiency (38.09%), whereas *S. aureus* demonstrated the least efficiency (19.28 %). *C. histolyticum* utilized waste oil from diesel vehicle with the highest efficiency (35.00%), while *C. ulcerans* was the least (17.31%). Among the fungi isolates, *R. mulcilaginosa* showed most degradation potential for spent oil from petrol driven vehicle (34.85 %) whereas *C. terreus* was the least (20.00 %). *A. fumigatus* exhibited highest degradation capacity for spent oil from diesel driven vehicle (29.73 %) while *R. oryzae* demonstrated the least potential (20.76 %). These results implied that microbial consortium could best be used for remediation of hydrocarbon contaminated soil.

Keywords: Isolation; Identification; Bacteria; Fungi Waste Engine Oil; Hydrocarbon Degradation

1 Introduction

Mechanic workshop may be taken as an integral part of the service industry with significant impact of the environment, resulting in seepage of waste engine oil leading to environmental contamination (Owolabi et al., 2013). With increasing demand for automobiles especially in developing countries like Nigeria, automobile workshops proliferate in major cities and town, with waste generated and dumped indiscriminately on every available space, thus contaminating the soil and water ecosystems causing alteration in the microbial populations and distribution, and perturbation of the aquatic and terrestrial environments thereby affecting every living creature in those ecosystems. Hydrocarbon contamination of land and water environment has been a problem since the discovery of oil fuel service particularly in the developing countries (Itah and Essien, 2005; Ikpe et al., 2016; Ikpe et al., 2018). Various forms of petroleum products, viz; petrol, engine oil, diesel, lubricant oils and others are used in mechanical workshops (Kayode et al., 2009;

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Tang et al., 2010). These products tend to harden or change the texture of the soil which have effects on both microbiological and physicochemical properties of the contaminated soil.

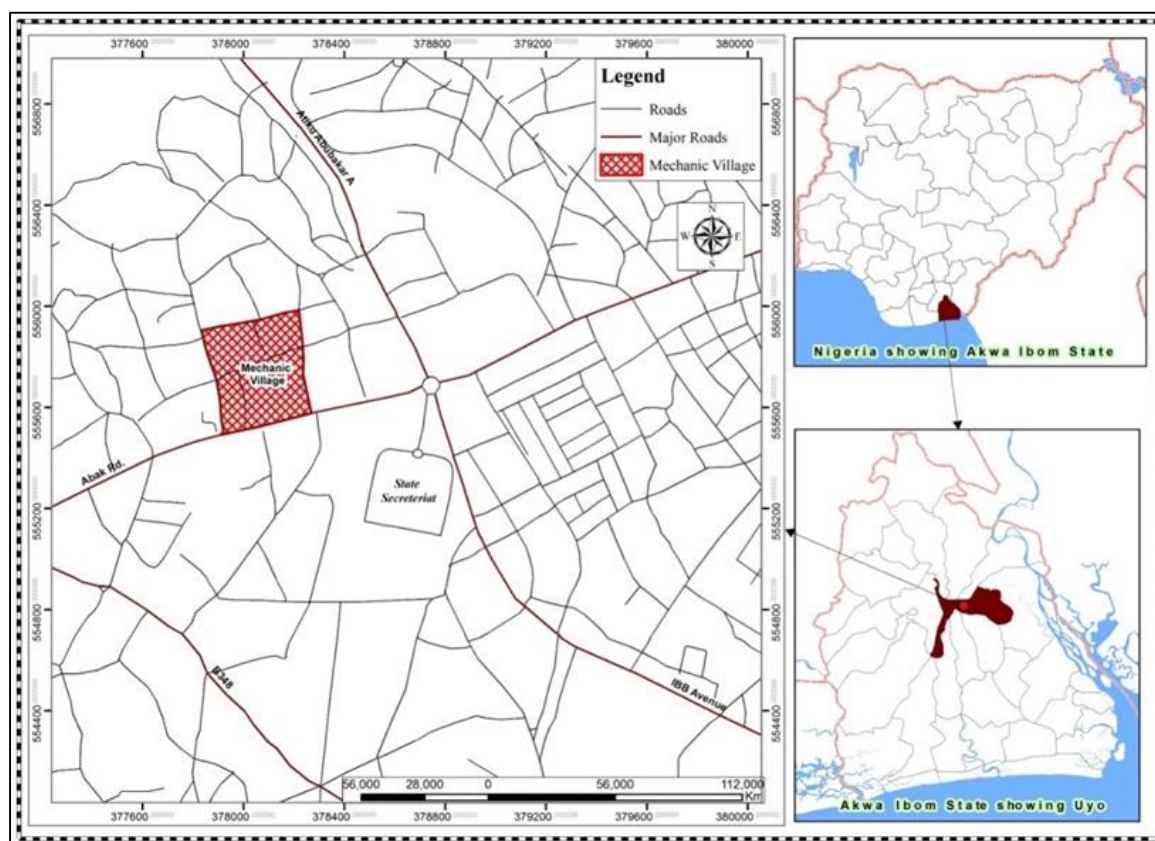
However, microorganisms are reported to be widely distributed on biosphere because of their metabolic capacity which are very impressive and they can easily grow in a wide range of environmental conditions (Velez et al., 2020; Viera et al., 2020). The nutritional versatility of microorganisms can be exploited for decontamination of pollutants. This kind of process is termed as bioremediation (Agarwal and Liu, 2015). There is however, reliable evidence that autochthonous (indigenous) microbes have some advantages over allochthonous microorganisms in the degradation of hydrocarbons as these organisms are able to develop naturally over the years and are well adapted for survival and proliferation in such environment (Adegbola et al., 2014). These microorganisms can convert, modify and utilize toxic pollutants in order to obtain energy and biomass production in the process (Enim, 2013; Su et al., 2023).

Microbial remediation is inexpensive and completely mineralize organic pollutants into carbon(iv)oxide, water, inorganic compounds and cell proteins or convert organic pollutants into other simpler organic compounds (Barua et al., 2011; Das et al., 2012). Meanwhile, for the microorganisms to be employed for bioremediation of organic pollutants research, they must first be isolated from their natural ecosystem, characterized and identified based on their peculiar morphological and biochemical characteristics using standard microbiological techniques (Kufan et al., 2021).

2 Materials and Methods

2.1 Sample Collection

The waste engine oil contaminated soil sample used in this study was obtained aseptically at 0-20cm deep with a sterile soil auger from the waste oil dump site in the Mechanical Village, Uyo, Akwa Ibom State, Nigeria into sterile polythene bag for microbiological analysis.



Source: GIS Unit, Geography and Regional Planning Department, University of Uyo, Akwa Ibom State, Nigeria.

Figure 1 Scaled map of Nigeria, and Akwa Ibom State showing Uyo, the study location

2.2 Microbiological analysis

2.2.1 Isolation and Identification of Hydrocarbon Degrading Microbial Isolates

Hydrocarbon degrading bacteria (HDB) and fungi (HDF) were isolated from the soil sample by pour plate method. Culturable bacteria were obtained in a nutrient agar medium plate supplemented with 0.5/L mg Nystatine to inhibit fungi contaminants. The hydrocarbon degrading bacteria were cultured and subcultured onto nutrient agar and incubated at 28°C for 24 hours. Distinct colonies of bacteria were further subcultured onto slant nutrient agar in Bijou bottles and incubated at 28°C for 24 hours. The slant cultures were stored in a refrigerator at 4°C and served as pure stock culture for subsequent characterization and identification of the isolates. For the hydrocarbon degrading fungi, culturable isolates were obtained in a potato dextrose agar medium plate supplemented with 0.5mg streptomycin per litre of the medium to inhibit bacteria growth. The hydrocarbon utilizing fungi were subcultured onto potato dextrose agar and incubated at room temperature for 5 days. Pure colonies were each picked onto potato dextrose agar medium in McCartney's bottles and incubated at room temperature for identification of the isolates.

2.3 Enumeration of Microorganisms

Total viable counts of bacteria and fungi were enumerated after incubation of the microbes by direct plate count method using a colony counter machine. Colonies on each plate were counted and mean number of viable colonies on triplicated plates were obtained before purification of the microorganisms for characterization and identification.

2.4 Characterization and identification of Microbial Isolates

Bacterial isolates were characterized by their morphological and biochemical characteristics. The biochemical tests conducted on each bacterial isolate were gram staining, spore staining oxidation fermentation test, motility test, catalase, oxidase, indole, methyl red (MR), Voges Proskauer (VP), citrate, sugar hydrolysis, urease, coagulase, nitrate reductase and sugar fermentation tests. Sugars fermented were xylose, lactose, manitol sucrose, arabinose, manose, dextrose, fructose and galactose. Identification was done using the identification schemes of Holt et al. (1994), Barrow and Feltham (2003). Fungi isolates were characterized by morphological and cultural characterization and microscopic examination (Cheesbrough, 2006). Identification was done using schemes of Barnett and Hunter (1987) and Sampson et al. (1984).

2.5 Determination of Hydrocarbon degrading Capacity of Isolates

The potential of microbial isolates to utilize waste engine oil from petrol and diesel driven vehicles respectively were evaluated using the method of Okpokwasili and Okorie (1998). The technique was previously adopted by Ijah and Ukpe (1992) and Essien et al. (2003) using mineral salt medium of Zajic and Supplison (1996), three millilitres (3ml) of filtered (0.5µm pore size filter, Millipore Corporation England) sterilized waste engine oil from petrol and diesel driven vehicles respectively were transferred into 15 sterile test tubes based on the number of microorganisms (bacteria and fungi) identified for use in the work. The same was applicable for their respective control test tubes. The test tubes contain 0.5ml each of the sterile mineral salt broth. However, the waste engine oil supplemented mineral salt medium was then inoculated with 0.1ml volume of 24 hours, nutrient broth culture of each bacterial isolates and 5 days potato dextrose broth culture for individual fungi isolates and fifteen uninoculated test tubes containing only mineral salt and spent engine oil from petrol and diesel driven vehicles respectively were included as control. Monitoring of hydrocarbon degradation by each isolate was conducted 48hourly and the amount of oil left in each test tubes was determined by gravimetric method (Itah and Essien, 2002; Nwakanma and Obih, 2015). Percentage weight loss of the oil was determined as follows;

$$\% \text{ Weight loss} = \frac{\text{Weight of Waste oil (Control)} - \text{Weight of waste oil (degraded)} \times 100}{\text{Weight of Waste oil control}}$$

The degree of weight loss of the medium in the test tubes was used as the index of waste engine oil degradation (utilization) by the microorganisms after 24 days of incubation in the oils.

3 Results and discussion

Bacillus mycoides, *Staphylococcus aureus*, *Listeria murrayi*, *Actinomyces viscosus*, *Clostridium sporogenes*, *Bacillus licheniformis*, *Corynebacterium ulcerans* and *Clostridium histolyticum* were bacteria identified from waste engine oil impacted soil obtained from Mechanic Village Uyo, Akwa Ibom State. (Table 1). Tables 2 and 3 depicted seven fungi (molds and yeasts) identified from the soil sample.

Table 1 Morphological and biochemical characteristics of bacteria isolated and identified in soil contaminated by waste engine oil from the Mechanic Village Uyo, Akwa Ibom State, Nigeria

Study location	S/C	Morphological Characteristics	Gram. Reaction	Spore. Formation	Motility	O-F Test	Catalase	Oxidase	Indole	MR	VP	Citrate	Sugar Hydrolysis	Urease	Coagulase	Nitrate Reductase	Sugar Fermentation										Probable Organism				
																	Manitol	Lactose	Glucose	Maltose	Xylose	Sucrose	Arabinose	Mannose	Dextrose	Galactose		Fructose			
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	A A ₁	Expansive white hairy colonies with characteristic swirls	Rod +	+	-	+	+	+	-	+	+	+	+	+	-	+		AG	AG	AG	AG	OG	AG	AO	AG	OG	AG	AG	AG	<i>Bacillus mycoides</i>	
	B B ₂	Round golden yellow colonies with grapelike clusters	Cocci cluster +	-	-	+	+	-	-	+	+	+	+	+	+	+		AG	AG	AG	AG	OG	AO	AO	AG	AG	AG	AG	AG	<i>Staphylococcus aureus</i>	
	C C ₃	Small translucent, grayish colonies	Short rod +	-	+	+	+	-	-	+	+	+	+	+	-	+		AG	AG	AG	OO	OG	OO	AO	AO	OO	OO	OO	OO	OO	<i>Listeria murrayi</i>
	D D ₄	Gray and white colonies form fungus-like branched	Rod +	-	-	+	+	-	-	+	-	-	-	+	-	+		AG	AG	AG	AO	OG	AG	AO	AO	AO	AO	AG	AG	AG	<i>Actinomyces viscosus</i>

	SS ₄	White to brown	Milky	Cottony	Aseptate and unbranched hyphae	Smaller sporangia are globose with air-dispersed sporangiospores	Reproduced asexually by sporangiospores	Sporangiospores are elliptical	<i>Rhizopus oryzae</i>
	TT ₅	White	Pale violet	Cottony	Septate and branched hyphae	Chlamydo-spores are multicellular, with cells connected by pores in the septae	Reproduced asexually by fragmentation of chlamydo-spores	Fusiform, slightly curved, pointed at the tip with septation	<i>Fusarium oxysporum</i>

Table 3 Morphological and biochemical characteristics of yeasts isolated and identified in soil contaminated by waste engine oil from the Mechanic Village Uyo, Akwa Ibom State, Nigeria

Study location	S/C	Surface colour	Reverse colour	Appearance	Nature of Hyphae	Vegetative reproductive structure	Sugar Fermentation						Sugar Assimilation						Probable organism
							Glucose	Lactose	Mannose	Sucrose	Galactose	Arabinose	Glucose	Lactose	Mannose	Sucrose	Galactose	Arrabinose	
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	PP ₁	Milky	Milky	Oval, mucoid appearance	No true hyphae	Reproduced asexually by budding	00	00	00	00	00	00	+	+	+	-	+	+	<i>Cryptococcus terreus</i>
	QQ ₂	Orange to pink	Orange	Soft, smooth, moist and spherical appearance	Absent of hyphae	Reproduced asexually by multilateral budding	00	00	00	00	00	00	+	-	-	+	+	+	<i>Rhodotorula multilateralis</i>

KEY: 00 - Non-fermentative; + - Positive; - - Negative

They include; *Penicillium notatum*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Rhizopus oryzae*, *Fusarium oxysporum*, *Cryptococcus terreus* and *Rhodotorula mulcilaginos*a. The variability of the microorganisms in the oil contaminated soil agrees with studies of Jobson et al. (2002) that hydrocarbon degrading microbes are usually present in large numbers in many soils impacted by hydrocarbons when compared with uncontaminated environment. It is also in accordance with Ekundayo et al. (2012) that the present of microorganisms in sites contaminated with petroleum products suggest the capacity to degrade and use the hydrocarbons as their only source of carbon for growth.

Table 4 depicted the distribution frequency of bacteria isolated and identified from the engine oil impacted soil. *B. mycoides* demonstrated the highest percentage occurrence of 27.9% while *C. histolyticum* was the lowest with 6.3% percentage occurrence. Among the fungi isolated, *P. notatum* showed the highest percentage occurrence of 20.4%, whereas *C. terreus* was the lowest with 4.1% percentage occurrence. Furthermore, it was noticed in this study the relatively low bacterial and fungal population dynamics which might be so due to the toxic impacts of the waste engine oil in the soil. This agreed with report of Jensen (1995), who suggested a toxic or inimical impact of the spent oil on the heterotrophic microbial counts in hydrocarbon contaminated environment.

Table 4 Distribution frequency of bacteria isolated and identified in soil contaminated by waste

Probable orgasms	Distribution frequency (n=111)	Percentage occurrence (%)
<i>Bacillus mycoides</i>	31	27.9
<i>Staphylococcus aureus</i>	9	8.1
<i>Listeria murrayi</i>	11	9.9
<i>Actinomycete viscosus</i>	13	11.7
<i>Clostridium sporogenes</i>	18	16.2
<i>Bacillus licheniformis</i>	8	7.2
<i>Corynebacterium ulcerans</i>	14	12.6
<i>Clostridium histolyticum</i>	7	6.3

Table 5 Distribution frequency of fungi isolated and identified in soil contaminated by waste engine oil obtained from the Mechanic Village Uyo, Akwa Ibom State, Nigeria

Probable orgasms	Distribution frequency (n=98)	Percentage occurrence (%)
<i>Penicillium notatum</i>	20	20.4
<i>Aspergillus fumigatus</i>	11	11.2
<i>Rhizopus stolonifer</i>	10	10.2
<i>Rhizopus oryzae</i>	24	24.5
<i>Fusarium oxysporum</i>	15	15.3
<i>Cryptococcus terreus</i>	4	4.1
<i>Rhodotorula mulcilaginos</i> a	14	14.3

Meanwhile, the microbes exhibited variable hydrocarbons degradation efficiency in the waste engine oil from petrol and diesel driven vehicles respectively. For example, *C. sporogenes* showed the highest hydrocarbon degradation potential in wastes engine oil from petrol driven vehicle with 38.09% utilization on the 24th day of incubation in the oil. *S. aureus* exhibited the lowest hydrocarbon degradation efficacy (19.28%). These results were in accordance with the report by Pinholt et al. (1999) who observed that the efficiency of bioremediation of crude oil by bacteria in soil ranges from 0.13% to 50%. However, Cameota and Singh (2008) reported 90% degradation potential of hydrocarbons in six weeks in liquid culture by a consortium of *P. aeruginosa* and *Rhodococcus erythropholis*. In this study, *C. histolyticum* demonstrated the highest hydrocarbon degradation potential in waste engine oil from diesel driven vehicle (35%) after 24 days incubation in the oil (Table 6). The results showed that *Clostridium* sp. exhibited the best

degradation potentials for waste oil. *C. ulcerans* showed the lowest hydrocarbon degradation ability with 17.31% degradation on the 24th day of incubation in the waste engine oil from diesel driven vehicle.

This results agreed with the report by Itah and Essien (2005) that *Corynebacterium* sp. and *Cryptococcus albidus* demonstrated very weak tarball degradation. *Rhodotorula mulcilaginosa* exhibited 35.85% degradation capacity of hydrocarbons in waste engine oil from petrol driven vehicle among the fungi used in this study. This was in accordance with Bhatt et al. (2002), who reported that *Rhodotorula* sp. contributed to effective degradation of low molecular weight PAHs and other hydrocarbon components in crude oil. *C. terreus* showed lowest potential to degrade hydrocarbons in waste engine oil from petrol driven vehicle. Moreso, *A. fumigatus* exhibited highest hydrocarbon utilization capacity in waste oil from diesel driven vehicle with 29.73% degradation whereas, *R. oryzae* was the lowest with 20.76% degradation efficiency. The variability in the capacity of the microbial isolates to degrade hydrocarbons in the waste engine oils agreed with Margesin et al. (2013); Nie et al. (2014) who suggested that microbial species secrete different catalytic enzymes responsible for the degradation of various fractions in the petroleum oil. Therefore, for bioremediation of petroleum contaminated sites to be effective, a consortium of microbial species with variable hydrocarbon degradation capacity should be employed.

Table 6 Waste engine oil degradation by monoculture of bacterial and fungal isolates

	Microorganism	Incubation Period (Days)											
		2	4	6	8	10	12	14	16	18	20	22	24
Petrol Driven Engine	Bacteria Monoculture	%	%	%	%	%	%	%	%	%	%	%	%
	<i>Bacillus mycoides</i>	1.98	3.96	4.95	5.94	8.74	9.90	10.89	12.87	13.86	15.84	17.82	20.79
	<i>Staphylococcus aureus</i>	1.43	4.29	5.00	7.14	9.29	10.71	12.14	14.29	15.71	16.43	17.86	19.28*
	<i>Listeria murrayi</i>	2.94	5.88	7.84	9.80	11.76	10.80	15.69	17.65	20.58	23.53	25.49	27.45
	<i>Actinomycece viscosus</i>	2.56	5.13	7.69	9.40	10.26	11.97	13.68	15.38	17.09	18.80	19.66	21.37
	<i>Clostridium sporogenes</i>	11.43	13.33	14.29	15.24	18.09	21.90	23.81	25.71	27.62	30.48	33.33	38.09*
	<i>Bacillus licheniformis</i>	9.28	11.34	12.37	14.43	15.46	17.53	19.59	23.71	26.80	29.89	32.99	35.05
	<i>Corynebacterium ulcerans</i>	3.03	4.14	6.06	9.50	11.41	12.12	14.20	16.18	18.23	20.18	22.22	24.26
	<i>Clostridium histolyticum</i>	4.08	6.12	7.14	10.20	12.24	14.29	15.31	18.39	19.38	21.43	22.45	24.49
Diesel Driven Engine	<i>Bacillus mycoides</i>	1.94	3.88	5.83	8.74	8.91	12.62	14.56	18.45	20.39	23.30	25.24	27.18
	<i>Staphylococcus aureus</i>	2.02	3.03	4.10	8.18	11.11	12.10	13.17	16.16	19.20	22.12	24.24	27.18
	<i>Listeria murrayi</i>	1.09	1.09	3.26	5.43	6.52	8.69	9.78	12.04	15.22	18.48	21.74	22.83
	<i>Actinomycece viscosus</i>	2.41	2.87	3.61	8.43	9.64	12.05	15.66	18.07	21.69	25.30	27.71	30.12
	<i>Clostridium sporogenes</i>	2.68	5.36	7.14	9.82	12.50	14.29	16.96	18.75	21.43	24.11	26.79	28.57
	<i>Bacillus licheniformis</i>	1.94	4.85	5.83	7.77	9.71	11.65	12.62	14.56	16.50	18.45	20.39	23.30
	<i>Corynebacterium ulcerans</i>	0.96	0.96	2.88	5.77	6.73	7.69	9.62	10.58	13.46	15.38	16.35	17.31*
	<i>Clostridium histolyticum</i>	8.67	17.50	19.17	20.83	22.50	24.17	25.83	27.50	29.17	30.83	32.50	35.00*
Petrol Driven Engine	Fungi Monoculture												
	<i>Penicillium notatum</i>	1.94	4.85	6.79	9.71	10.68	11.65	13.59	15.53	16.50	18.48	20.39	22.33
	<i>Aspergillus fumigatus</i>	1.04	2.08	4.17	6.25	7.29	9.38	11.56	13.54	15.63	18.75	19.79	21.88
	<i>Rhizopus stolonifer</i>	4.76	6.67	9.52	12.38	14.29	16.19	17.14	18.09	20.00	21.90	21.81	26.67
	<i>Rhizopus oryzae</i>	2.04	5.10	7.14	10.20	12.24	14.29	15.31	18.37	20.41	20.98	23.47	25.51
	<i>Fusarium oxysporum</i>	1.89	3.26	5.43	6.52	7.61	9.78	13.04	15.22	17.39	18.48	19.56	21.74
	<i>Cryptococcus terreus</i>	3.16	5.26	7.37	9.47	10.53	11.58	13.68	13.79	14.74	15.79	18.95	20.00*

	<i>Rhodotorula malcilaginosa</i>	3.03	4.55	9.09	10.61	13.64	16.67	19.69	22.73	27.27	28.79	31.82	34.85*
Diesel Driven Engine	<i>Penicillium notatum</i>	1.19	2.38	4.76	7.14	9.52	10.71	11.90	13.09	14.28	16.67	19.05	22.62
	<i>Aspergillus fumigatus</i>	2.70	5.41	6.76	9.46	10.81	12.16	14.86	18.92	21.62	24.32	24.68	29.73*
	<i>Rhizopus stolonifer</i>	1.08	2.15	4.30	7.53	9.68	11.83	13.98	16.13	19.35	22.58	24.73	27.96
	<i>Rhizopus oryzae</i>	1.98	3.96	4.95	5.94	6.93	7.92	10.89	12.87	14.85	15.84	17.82	20.76*
	<i>Fusarium oxysporum</i>	1.25	2.50	6.25	7.50	10.00	12.50	13.75	16.25	18.75	21.25	23.75	27.50
	<i>Cryptococcus terreus</i>	1.01	5.26	6.06	8.08	9.19	11.12	13.15	14.12	15.20	17.17	21.50	24.18
	<i>Rhodotorula malcilaginosa</i>	4.04	4.55	7.07	10.10	12.13	14.20	15.15	16.30	19.15	21.10	24.24	25.30

* Lowest and highest percentage hydrocarbon degradation potentials

4 Conclusion

Eight viable bacteria and seven fungi with variable percentage occurrence were isolated and identified in waste engine oil obtained from the Mechanic Village Uyo, Akwa Ibom State. Among the bacterial isolates, *Clostridium* sp. exhibited highest hydrocarbon degradation capacity, while *Rhodotorula malcilaginosa* and *Aspergillus fumigatus* were fungi with highest hydrocarbon utilization potentials. However, these hydrocarbon degraders could be used to decontaminate petroleum polluted sites.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interest exist.

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