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Characterization of hydrocarbon utilizing bacteria in waste engine oil-impacted sites

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Abstract

Changes in soil physicochemical properties and bacterial species present in soil contaminated with waste engine oil were evaluated at three auto-mechanical workshops in Uyo, Nigeria. This work was aimed at isolating and identifying hydrocarbon degrading bacteria from waste engine oil polluted soil, and assessing their hydrocarbon-utilizing ability. Waste engine oil pollution affected soils significantly with increases in soil physicochemical properties, and heterotrophic bacterial population counts. Eight bacterial species *Corynebacterium kutscheri, Pseudomonas aeruginosa, Flavobacterium aquatile, Serratia odorifera, Micrococcus agilis, Staphylococcus aureus, Micrococcus luteus* and *Bacillus substilis* were isolated by the selective enrichment technique and screened for hydrocarbon utilization capability in mineral salt media with 1% (v/v) waste engine oil as a sole carbon and energy source. The extent of bacterial growth observed was related to the ability of organisms to biodegrade hydrocarbons present in the medium bacterium species, which showed varying hydrocarbon utilization during the 15 days of incubation. Growth in hydrocarbon medium was the most efficient in cultures of *Corynebacterium kutscheri*. All isolates also showed variable emulsification ability, with *Corynebacterium kutscheri*, showing the highest ability. These results demonstrate the presence of indigenous bacteria in hydrocarbon-polluted soils and the potential toward the remediation of hydrocarbons.

Keywords: Hydrocarbon-utilizing bacteria, selective enrichment technique, *Corynebacterium kutscheri*

Introduction

Petroleum utilization as fuel and petroleum products leads to severe environmental pollution [1]. Large-scale accidental spills pose a great threat to the ecosystem [2]. Soil pollution by petroleum hydrocarbons has been shown to produce pronounced changes in the physicochemical and microstructure of the oil-contaminated soil [3]. This affects parameters such as soil porosity, bulk density, and adsorption [4-5]. Fresh spills and/or high levels of pollutants may often result in the reduction of large sectors of soil microbial population, although soils with lower levels or old pollution may show an increase in numbers and diversity of microorganisms [6-7]. The diversity and the number of microorganisms at polluted soil sites may assist in the characterization of such a site, such as the toxicity of petroleum hydrocarbons to the microbiome, age of the spill and concentration of the pollutant [8].

Additionally, microorganisms in soils exposed to hydrocarbon pollution usually exhibit a higher potential for biodegradation of such pollutant compounds than others with no history of such exposure. Percentages of hydrocarbonoclastic microbes are quite low in soil when there is no oil spill, but may increase 1,000 fold after oil spill [9].

Conventional remediation methods do not seem to be able to address this problem, or tends to aggravate the problem [10]. Mechanical methods such as incineration, excavation and/or burial in secure land fill, as well as a host of other chemical decomprelocates osition methods are expensive, time consuming and only the pollution [11]. An efficient way of remediating the oil-contaminated sites could be employment of microorganisms, such as bacteria, microscopic algae, and fungi, isolated from polluted environments or enhanced from the organisms already present in the same environment [12-13]. Waste engine oil-polluted soils also serve as a source of indigenous bacteria capable of hydrocarbon degradation.

The employment of microorganisms in the biodegradation of hydrocarbons over chemical or conventional treatment is preferred for many reasons; end products are comparatively safer and cost-effectiveness [11]. Ogunbayo et al., [18] evaluated the effectiveness of bacteria indigenous to soil in remediating engine oil-polluted, soil and isolated Bacillus, Pseudomonas, Flavobacterium, Micrococcus and Rhodococcus species, with Pseudomonas and Rhodococcus species giving most favorable degradation effectiveness and efficiencies.

This study therefore considered the isolation of indigenous bacterial communities in waste engine oil-polluted soil using selective enrichment technique, and the assessment of hydrocarbon-utilization capability in waste engine oil-augmented mineral salt medium.

Materials and Methods

Sample collection

Waste engine oil –contaminated soil samples used in this study were collected from three auto-mechanic workshops within the mechanic village, Uyo, Akwa Ibom State, Nigeria. Composite soil samples were obtained at each sampling point using a soil auger from 0-10 cm below the soil surface. The soils were labeled "Unpolluted" for the unpolluted sample, "MA" for the mechanic workshop 1 sample, "MB" for the mechanic workshop 2 samples, and "MC" for the mechanic workshop 3 samples. This was followed by bulking and transportation to the laboratory in sterile polythene bags within six hours for isolation of organisms.

Physico-chemical analysis of soil samples

The soil pH was measured using HANNA Instruments Model 209 pH meter [14]. Moisture content was calculated on the basis of the air dry weight as described by AOAC [15]. Total organic carbon was calculated by weighing exactly 0.5 g of the soil sample into a flask, and 10 ml of 1.0 M $K_2Cr_2O_7$ was added and swirled to mix. 20 ml conc. H_2SO_4 was added, gently swirled for a minute and allowed to stand for 20 minutes. The suspension was diluted to about 100 ml of distilled water. Five drops of o-phenanthroline indicator were added to each sample and was titrated with 0.5 M ferrous ammonium sulfate to a light blue end point. The reagent blank was also run and the titre values recorded, and used to calculate the organic carbon content [15].

The total hydrocarbon content (THC) was determined by first extracting hydrocarbons by acidifying 2 g of representative soil samples using H_2SO_4, and extracting upon addition of 20 ml of toluene in a separatory funnel. The contents of the funnel were shaken, and allowed to settle into two layers. The absorbance of the supernatant (extract) was read at 420 nm with UNICAM UV/VIS spectrophotometer (Spectronic 20D). Readings were recorded from the spectrophotometer and using the determined curve to obtain the figure [16].

Phosphorus was determined using the ascorbic acid method as described by AOAC, [15]. 50 ml of the soil dilution was pipetted into 250 ml Erlenmeyer flask, and 1 drop of phenolphthalein indicator was added. Exactly 5 N H_2SO_4 (148 ml conc. H_2SO_4 in 100ml H_20) is added drop-wise to develop a red colour. Exactly 8 ml of combined reagents made up of 50 ml of 5 N H_2SO_4, 5 ml potassium antimonyl tartrate solution (1.372 g potassium antimonyl tartrate in 500 ml distilled water); 15 ml ammonium molybdate solution (20 g ammonium molybdate crystal in 500 ml distilled water); were added and thoroughly mixed, and allowed to stand for 20 min. The phosphorus content was determined by measuring the absorbance of the sample at 880 nm.

The nitrogen concentration was determined according to the methods of Bremmer and Mulvaney, [17]. One milliliter of the soil sample was introduced into the standard kjeldahl flask containing 1.5 g $CuSO_4$, and 1.5 g Na_2SO_4 as catalyst, alongside concentrated H_2SO_4. The flask was gently heated on a heating mantle, taking care to prevent frothing. The solution was transferred after heating to a 100 ml standard flask and made up to the mark with distilled water. A portion of this digest was pipetted into a semi micro-kjeldahl distillation apparatus and treated with 30 ml of 40% NaOH solution. The ammonia evolved was steam-distilled into a 100 ml conical flask containing 10 ml solution of saturated boric acid to which 4 drops of Tashirus indicator had been previously added. The tip of the condenser was immersed in the boric acid solution and the distillation continued until about two-thirds of the original volume was obtained. The tip of the condenser was finally rinsed with a few milliliters of distilled water. The distillate was then titrated with 0.1N HCl until a purple-pink end point was observed. A blank determination was also carried out in a similar manner without the sample, and the calculation done as follows:

$$\text{Nitrogen (\%)} = \frac{\text{(Real titre – Blank titre)}}{\text{Weight of the sample}} \times 0.1 \times 0.014 \times 100$$

Enumeration of total heterotrophic bacteria (THB)

The THB population in the soil samples was enumerated by adopting the standard plate counts technique using the spread plate method as described by Ogunbayo *et al.,* [18]. These involved spreading aliquots of a serially

diluted 0.1 ml of 10^{-5} dilutions of the soil sample suspension on nutrient agar plates and the plates were incubated at 30 ^{o}C for 24 h. Similar aliquots were also incubated in minimal salt agar plates containing used engine oil as the sole source of carbon and energy. The plates were all incubated aerobically at 30 ^{o}C. The percentages of hydrocarbon-utilizing bacteria (HUB) relative to the total heterotrophic counts were noted.

Enumeration of hydrocarbon utilizing bacteria (HUB)

Oil-utilizing bacteria were isolated from polluted soil samples by enrichment in mineral salt medium (MSM) modified from Okpokwasili and Nwosu, [19] using waste engine oil as a carbon and energy source. The soil samples were sieved using a 2 mm mesh sieve. 10 g of the sieved soil samples was inoculated into 100 ml sterile MSM. 1 ml of the waste engine oil was added to the medium as a sole source of carbon and energy, and the culture was incubated on a rotary shaker at 170 rpm for 1 week.

The enrichment procedure was repeated for three cycles. At the end of each enrichment cycle, 1 ml of the culture was diluted serially 10-fold down the gradient to 10^{-5} and plated. Pure cultures of the isolates were obtained by plating 1 ml of the 10^{-5} dilution of the third enrichment cycle onto MSM agar plates, and incubating at 30 ^{o}C (± 2) for 48 h. Pure cultures obtained by this procedure were stored in slants at 4 ^{o}C until further identification.

Characterization and identification of bacteria

Isolates were identified on the basis of colonial characteristics, Gram's reaction and cell biochemical reactions as described by Cheesbrough [20]. Identification used the taxonomic schemes of Holt *et al*. [21].

Hydrocarbon utilization screening of bacteria

To determine the ability of the isolates to utilize engine oil as the sole carbon and energy source, the growth patterns of isolates in mineral salt medium in the presence of 1% (v/v) of the waste engine oil (5.0 mL in 100 mL MSM) were determined according to Onuoha et al. [22]. Waste engine oil-augmented MSM was dispensed into 250 ml Erlenmeyer, and inoculated with 0.1 ml of 24 h cultures of the bacterial isolates. Incubation was done at 30 ^{o}C for 15 days. Growth patterns were determined monitoring changes in pH, optical density and total viable count at 5-day intervals during the incubation. The pH of the medium was measured using the pH meter (HANNA Instruments). Growth was also monitored by measuring the optical density (OD) at 600 nm using the spectrophotometer (Spectrumlab). Total viable counts of the cultures were obtained by incubation of 0.1 ml of the cultures using the spread plate technique on nutrient agar plates at 30 ^{o}C for 24 h.

Emulsification activity of bacteria

The emulsification index (E_{24}) of the isolates was determined according to the methods of Ganesh and Lin, [23], by adding 1ml of waste engine oil to the same amount of culture media as used for degradation assay, mixing the vortex for 2 min and leaving to stand for 24 h. The percentage of emulsification index was obtained as follows:

$$E_{24} = \frac{\text{Height of the emulsified layer}}{\text{Total height of the liquid column}} \times 100$$

Results

Physicochemical properties of soil samples

The results of the physicochemical analysis of the different soil sample are shown in **Table 1**. The high amounts of organic carbon (5.32 ± 2.65% in MA, 9.79 ± 0.51% in MB and 7.29 ± 3.09% in MC), and THC (2933.76 ± 404.27 mg/kg in MA, 3122.72 ± 131.00 mg/kg in MB and 3202.61± 675.07 mg/kg in MC), compared to the unpolluted soil sample (3.7 ± 2.43% organic carbon content and 39.97± 13.49 mg/kg THC) is indicative of heavy pollution of the mechanical workshop samples with petroleum hydrocarbons. Soil samples from mechanical workshop 1 contained higher amounts of nitrates (0.25 ± 0.03 mg/g), while samples from mechanical workshop 2 contained the highest amounts of phosphates (10.74 ± 0.88 mg/g) and THC (3202.61± 675.07 mg/kg). The pH values of the soil samples indicate all soil samples as moderately acidic to acidic (from pH 5.78 to pH 6.79).

Bacterial count of soil samples

The total heterotrophic bacterial count and hydrocarbon utilizing bacterial count of the original soil samples is shown in **Table 2**. A higher THB count was recorded in polluted soil samples (4.4 ± 1.90 x 10^7 CFU/g from MA sample, 6.0 ± 0.23 x 10^7 CFU/g from MB sample and 4.5 ± 0.03 x 10^7 CFU/g from MC sample) than in the unpolluted soil sample (1.9 x 10^7 CFU/g). Higher THB (6.0 ± 0.23 x 10^7 CFU/g) and HUB (5.2 ± 0.25 x 10^7 CFU/g) counts were observed in the MB sample than in other similar polluted samples indicative of its extent of pollution. Hydrocarbon utilising bacterial counts were slightly lower in all samples than the corresponding heterotrophic bacterial counts.

Characterization and identification of bacteria

The identified bacterial isolates were *Corynebacterium kutscheri, Pseudomonas aeruginosa, Micrococcus agilis, Flavobacterium aquatile, Staphylococcus aureus, Micrococcus luteus, Serratia odorifera* and *Bacillus substilis,* as shown in **Table 3 and 4**.

Hydrocarbon utilization potential of bacteria

Table 5 shows the changes in pH of MSM during growth of bacteria isolates in hydrocarbon. Decreases in pH (to <pH 7.00) were observed in medium containing isolates, *Corynebacterium kutscheri*, *Micrococcus agilis*, *Serratia odorifera* and *Bacillus substilis*, (which, however, increased to above pH 7.00). The greatest decrease in pH occurred in cultures of *Corynebacterium kutscheri* (from 7.00 a.m. on Day 0 to 5.83 ± 0.34 on Day 15). Medium containing other isolates showed slight increases in pH over time. The changes in the total viable counts of bacterial isolates during 15 days of growth in waste engine oil-augmented MSM are shown in **Table 6**. Total viable counts were higher in cultures containing *Corynebacterium kutscheri* (1.94 ± 0.12 x 10^8 CFU/ml on Day 5, 6.73 ± 0.45 x 10^8 CFU/ml on Day 10 and 3.13 ± 0.02 x 10^8 on Day 15). Growth of *Staphylococcus aureus* showed the lowest decreases (0.31± 0.11 x 10^8 on Day

5, 0.44 ± 0.01 x 10^8 on Day 10 and 0.42 ± 0.02 x 10^8 on Day 15).

Table 7 shows the results of turbidity measurement (OD_{600}) of the medium during incubation. The highest increases in turbidity were recorded in cultures with *Corynebacterium kutscheri*, (0.189 ± 0.04 on Day 0 to 0.301± 0.11 on Day 15), and *Bacillus substilis*, (0.165 ± 0.04 on Day 0 to 0.341± 0.02 on Day 15). The lowest turbidity was observed with *Staphylococcus aureus* (0.20 ± 0.04 on Day 0 to 0.14 ± 0.02 on Day 15).

Emulsification activity of bacteria

Figure 1 shows the emulsification index (E_{24}) of the bacterial isolates in waste engine oil. *Corynebacterium kutscheri* had the highest emulsification index (52%). The lowest emulsification index was observed for *Staphylococcus aureus* (8%).

Table 1: Physicochemical properties of different soil samples contaminated with waste engine oil.

Parameters	Unpolluted (Control)	Polluted		
		MA	MB	MC
pH	6.79 ± 0.24	6.18 ± 0.13	5.78 ± 0.03	6.44 ± 0.07
Nitrate (mg/g)	0.14 ± 0.02	0.25 ± 0.03	0.23 ± 0.02	0.20 ± 0.02
Phosphate (mg/g)	6.65 ± 1.72	6.92 ± 2.92	10.74 ± 0.88	8.49 ±1.9
Moisture content (%)	32.25 ± 9.30	43.09 ± 0.65	45.13 ± 7.02	27.72 ± 9.09
Organic carbon content (%)	3.7 ± 2.43	5.32 ± 2.65	9.79 ± 0.51	7.29 ± 3.09
THC (mg/kg)	39.97 ±13.49	2933.76 ± 404..27	3202.61± 675.07	3122.72±131.00

Values represent means of triplicate determinations ± SD. MA = Mechanic Workshop 1, MB = Mechanic Workshop 2, MC = Mechanic Workshop 3. THC = Total hydrocarbon content.

Table 2: Bacterial count of soil samples contaminated with waste engine oil.

Parameter	Unpolluted (Control)	Polluted		
		MA	MB	MC
THB (CFU/g)	1.9 ± 0.02 x 107	4.4 ±1.90 x 107	6.0 ± 0.23 x 107	4.5 ± 0.03 x 107
HUB (CFU/g)	6.5 ± 0.04x 106	3.2 ± 0.05 x 107	5.2 ± 0.25 x 10 7	1.9 ± 0.10 x 106

Values represent means of triplicate determinations ± SD. MA = Mechanic Workshop 1, MB= Mechanic Workshop 2, MC= Mechanic Workshop 3.

Table 3 : Biochemical characteristics of spore (Sp), catalase (Ca), motility (Mo), oxidase (Ox), litmus reaction (Lr), urease (Ur), gelatin (Ge), citrate (Ci), glucose (Gl), maltose (Mal), mannitol (Man), lactose (La), sucrose (Su), arabinose (Ar) and xylose (Xy) for studied bacterial isolates from soil contaminated with waste engine oil.

Isolates	Isolate code	Sp	Ca	Mo	Ox	Lr	Ur	Ge	Ci	Gl	Mal	Man	La	Su	Ar	Xy
1	MA3, MB1, MC4	-	+	-	-	-	-	-	+	A	A	A	-	-	-	AG
2	MA2, MB2	-	+	+	+	+	-	+	-	-	A	A	A	-	A	A
3	MA1	-	+	-	-	-	-	-	-	A	-	A	-	-	AG	AG
4	MA4	-	+	-	+	+	-	-	-	A	A	-	A	A	-	-
5	MB3, MC1, MC2, MA5	-	+	-	-	+	-	-	-	-	-	-	-	A	-	-
6	MA6	-	+	-	+	-	-	-	-	-	-	A	-	A	-	-
7	MC3	-	+	+	-	-	+	+	+	A	A	A	A	A	A	-
8	MB4, MC5	+	+	+	-	+	-	+	+	A	A	A	-	A	A	AG

Keys: AG = acid and gas production; A = acid production only; + = positive reaction; - = negative.

Table 4: Phenotypic characteristics of bacterial isolates from soil contaminated with waste engine oil.

Isolates	Isolate code	Gram reaction/Cell shape	Probable Bacteria
1	MA3, MB1, MC4	Gram-positive Rods	*Corynebacterium kurtscheri*
2	MA2, MB2	Gram-negative Rods	*Pseudomonas aeruginosa*
3	MA1	Gram-positive Cocci	*Micrococcus agilis*
4	MA4	Gram-negative Rods	*Flavobacterium aquatile*
5	MB3, MC1, MC2, MA5	Gram-positive Cocci	*Staphylococcus aureus*
6	MA6	Gram-positive Cocci	*Micrococcus luteus*
7	MC3	Gram-negative Rods	*Serratia odorifera*
8	MB4, MC5	Gram-positive Rods	*Bacillus substilis*

Table 5 : pH of the medium during the growth of bacterial isolates in hydrocarbon medium.

Isolates	Days			
	0	5	10	15
Micrococcus agilis	7.00	6.51 ± 0.01	6.38 ± 0.03	6.04 ± 0.05
Pseudomonas aeruginosa	7.00	7.06 ± 0.07	7.78 ± 0.01	7.71 ± 0.06
Corynebacterium kutscheri	7.00	6.51± 0.01	6.38 ± 0.00	5.83 ± 0.34
Flavobacterium aquatile	7.00	7.31± 0.23	7.50 ± 0.16	7.17 ± 0.28
Micrococcus luteus	7.00	6.13± 0.07	7.23 ± 0.03	7.37 ± 0.06
Staphylococcus aureus	7.00	7.23± 0.03	7.31± 0.03	7.23 ± 0.07
Serratia odorifera	7.00	7.09 ± 0.01	6.73 ± 0.04	6.42 ± 0.07
Bacillus substilis	7.00	6.85 ± 0.13	6.78 ± 0.18	7.24 ± 0.02

Data represent means of triplicate determinations ± SD.

Table 6: Total viable count (TVC) of bacterial isolates during growth in hydrocarbon medium (x 108 CFU/ml).

Isolates	Days			
	0	5	10	15
Micrococcus agilis	0	0.63 ± 0.03	2.46 ± 0.02	0.87 ± 0.06
Pseudomonas aeruginosa	0	0.54 ± 0.02	2.13 ± 0.16	0.45 ± 0.02
Corynebacterium kutscheri	0	1.94 ± 0.08	5.27 ± 0.07	3.18 ± 0.07
Micrococcus luteus	0	0.95 ± 0.01	2.49 ± 0.03	1.43 ± 0.03
Flavobacterium aquatile	0	1.94 ± 0.12	6.73 ± 0.45	3.13 ± 0.02
Staphylococcus aureus	0	0.31± 0.11	0.44 ± 0.01	0.42 ± 0.02
Serratia odorifera	0	0.54 ± 0.03	2.23 ± 0.25	0.48 ± 0.04
Bacillus substilis	0	1.39 ± 0.41	4.08 ± 0.65	2.47 ± 0.07

Data represent means of triplicate determinations ± SD.

Table 7: Turbidity measurement of bacterial growth in hydrocarbon medium (OD600).

Isolates	Hours			
	0	5	10	15
Micrococcus agilis	0.97 ± 0.01	0.154 ± 0.01	0.209 ± 0.12	0.201± 0.03
Pseudomonas aeruginosa	0.033 ± 0.001	0.035 ± 0.10	0.182 ± 0.10	0.097± 0.05
Corynebacterium kutscheri	0.189 ± 0.04	0.252 ± 0.07	0.386 ± 0.04	0.301± 0.11
Flavobacterium aquatile	0.085 ± 0.01	0.153 ± 0.08	0.196 ± 0.07	0.227± 0.05
Micrococcus luteus	0.040 ± 0.02	0.083 ± 0.06	0.162 ± 0.03	0.183± 0.04
Staphylococcus aureus	0.20 ± 0.04	0.053 ± 0.10	0.084 ± 0.05	0.014± 0.02
Serratia odorifera	0.40 ± 0.02	0.172 ± 0.11	0.121 ± 0.001	0.096± 0.01
Bacillus substilis	0.165 ± 0.05	0.204 ± 0.01	0.413 ± 0.05	0.341± 0.04

Data represent means of triplicate determinations ± SD.

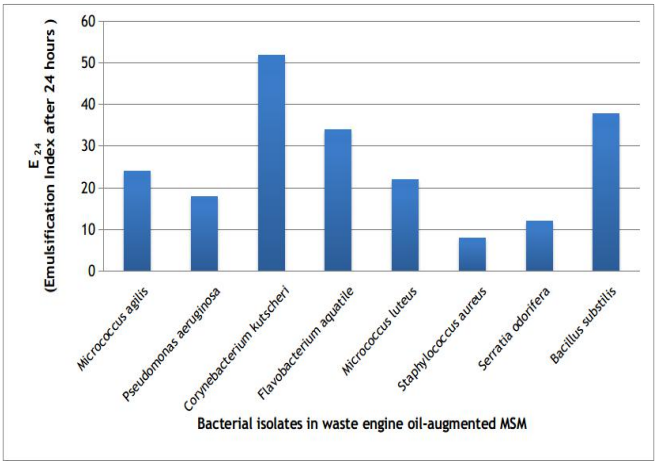

Figure 1 : Emulsification activity (E24) of bacterial isolates.

Discussion

This study on the assessment of hydrocarbon utilizing the potential of bacteria isolated from waste engine oil-polluted soil on hydrocarbons reveals the presence of hydrocarbon-utilizing bacteria in waste engine oil-polluted soil environment as well as the potential of one of these isolates *Corynebacterium kutscheri* to degrade hydrocarbons under a variety of experimental conditions.

The results of the physicochemical analysis of the soil samples, as presented in **Table 1** showed higher levels of properties in the polluted soil samples when compared with the unpolluted soil sample. The results support the results of Chikere [24], and Chikere and Ekwuabu [25], which showed high physicochemical parameters in polluted soil samples compared to unpolluted samples determined and indicated previous exposure of polluted samples to hydrocarbon contamination with traces of other organic and inorganic contaminants.

The high bacterial counts recorded in polluted soil samples, as presented in **Table 2**, compared with the unpolluted control samples could be attributed to the myriad of nutrients, high organic matter concentration and other ecological factors that influence the survival of soil microorganisms that play important roles in the decomposition and recycling of nutrients [26]. Luepromchai *et al*. [27] has reported an increase in the numbers of hydrocarbon degraders in soil in the presence of PAHs, without any impact on the overall bacterial numbers. The difference between THB and HUB counts was observed to be minimally insignificant, which suggests that most of the micro-organisms present in various polluted sample sites are

hydrocarbon degraders that can withstand the concentrations of hydrocarbons and use them as a source of carbon [25].

The investigation of the morphology and type of bacterial colonies obtained from the oil-polluted soil showed seven bacterial species, which are *Corynebacterium kutscheri, Pseudomonas aeruginosa, Micrococcus agilis, Flavobacterium aquatile, Staphylococcus aureus, Micrococcus luteus*, *Serratia odorifera,* and *Bacillus substilis*. This shows the majority of bacteria isolated as being Gram-positive species. Some researchers have, however, reported oil-polluted soils as being dominated by Gram-negative bacteria [25]

The results of the emulsification test as shown in **Figure 1** demonstrates that the isolates produced emulsifying compounds. A large variety and number of biosurfactant producers have been isolated from hydrocarbon-impacted sites [28], although they have also been identified from soils, which are unconnected to hydrocarbon contamination [29]. *Corynebacterium kutscheri* showed the highest emulsification (52%) at 1% waste engine oil, while the least was *Staphylococcus aureus* (8%). *Corynebacterium* sp has also been reported by Onuoha *et al.* [22] with high emulsification ability among three hydrocarbon-degrading bacterial species isolated from soil.

Decrease in the pH of the medium (as shown on **Table 5**), increase in total viable counts (**Table 6**), as well as in turbidity of the medium (**Table 7**) were regarded as indicators of degradation. The correlation between the pH of the medium and cell growth with hydrocarbon utilization has been previously reported by Patila *et al.,* [30]. The preliminary estimation of the isolate's degradative ability during growth in hydrocarbon supplemented medium suggested the effectiveness of growth in hydrocarbon medium by *Corynebacterium kutscheri*, and thus its potential as a candidate for further biodegradation studies.

Conclusion

The result of this study indicates that the contaminated environments possess indigenous populations within them, as seen in bacterial count results of microbiological growth of mineral salt cultures with waste engine oil as the sole carbon source. These indigenous bacteria isolated in this study show promising, ability to degrade petroleum hydrocarbons with *Corynebacterium kutscheri* as a potential candidate for further biodegradation efficiency studies.

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