Isolation and Growth Trend of Selected Strains of *Pseudomonas* in Petrochemical Contaminated Soil

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Abstract: Some toxic chemicals from petroleum products constitute environmental pollutants which are considered threats to ecological system. This study was performed to monitor the survival growth of some selected indigenous *Pseudomonas* strains isolated from petrochemical contaminated soil. Standard microbiological procedures and 16S rRNA technique were employed during isolation and identification of the strains. The physiochemical characteristics of the soil sample were conducted using standard laboratory procedure. Optimization of culture conditions were carried out under varying pH concentrations, moisture content, temperature and nutrients (N:P). The identified strains were *P. aeruginosa* PAER4 119, *P. putida* B6-2 and *P. mendocina* NK-01. The result of the soil physiochemical properties showed particle size distribution of 70.60 % for sand, 21.40 % for loam and 8.00 % for clay. The recorded pH value of 5.8, moisture content of 12.5 % and total petroleum hydrocarbons of 7.4 % were observed. The results of the optimization showed highest cell growth at temperature of 30 ^{o}C, pH value of 7, moisture content of 20 %, and nitrogen phosphorus ratio of 10:1. Maximum growth recorded during bioremediation by the strains were 2.90 x 10^{10} cfu/g for *P. putida* B6-2, 2.86 x 10^{10} cfu/g for *P. aeruginosa* PAER4 119 and 2.84 x 10^{10} cfu/g for *P. mendocina* NK-01. Cell growth increased with time. This study revealed the ability of *Pseudomonas* strains to grow and survive in the petrochemical contaminated soil. Hence, this study can be employed for successful bioremediation studies.

Key words: Petrochemicals, contaminated soil, *Pseudomonas* strains.

INTRODUCTION

Petrochemical hydrocarbons are among the environmental pollutants that affect the entire ecosystem (Alexander, 2000). Contamination can be caused by different petroleum fractions released from the petrochemical industries, transportation, and accidental discharge (Shallu *et al*., 2014). Besides petroleum, other sources of petrochemicals could be fossil fuels such as coal or natural gas, or renewable sources (Soccol *et al.,* 2011; Lee *et al.,* 2014).

Petrochemicals contain polyaromatic hydrocarbons (PAHs) of many benzene rings fused in a linear, angular, or cluster arrangements (Mishra *et al.,* 2001). Once discharged into the environment, petrochemicals can be hazardous to the surroundings ecosystems (Rodriguez-Martinez, 2006). Prolonged exposure of petrochemicals may cause severe effects and numerous health problems including liver or kidney diseases and possible damage to the bone marrow (Hadibarata and Tachibana, 2009; Lloyd and Cackette, 2001). The soil contamination causes extensive damage as animals and plant tissue may be destroyed. Accidental oil spills can even cause damage to the sea and shoreline organisms (Rodriguez-Martinez, 2006).

Decontamination of petroleum hydrocarbon contaminated soils by the application of microorganisms is claimed to be an efficient, economic, versatile and attractive in comparison to physicochemical treatments (Karamalidis *et al.,* 2010). Many of such microbes possess the capability to utilize petroleum hydrocarbons as the sole source of carbon thereby transforming them into non-hazardous compounds (Bharathi, and Vasudevan, 2001). There are many factors contributing to microbial growth and degradation of petroleum hydrocarbon including physicochemical properties, contaminants characteristics and bioavailability biological factors (Vidali, 2001; Bharathi, and Vasudevan, 2001). These factors were found to influence the process of microbial growth and survival as well as their bioremediation efficiencies (Vidali, 2001).

Therefore, this study examined the physicochemical characteristics of petrochemical contaminated soil, the capability of different *Pseudomonas* strains to survive at the sole expense of petroleum fractions.

MATERIALS AND METHODS

Sample collection

Five kilograms (5 kg) of soil samples were collected aseptically and randomly from soil surface of petroleum contaminated soil from a depth of 0 – 30 cm from a disposing site of a refining plant of a company which is located at Eleme, Rivers State. Soil samples were taken to the Environmental Microbiology laboratory, Michael Okpara University of Agriculture Umudike, Abia State for studies.

Isolation of *Pseudomonas* strains from the petrochemical polluted soil

Isolation was conducted by serial dilution where 1 g of polluted soil sample was diluted serially with 9 ml of saline water. Inoculum of 1 ml of suspension from dilutions (10^{-4} and 10^{-6}) was added in sterilized Petri plates containing 20 ml of Cetrimide Agar by pour –plate technique. At 30 ^{0}C, the plates were incubated for 48 hours. Bacteria colonies with yellow-green and blue pigments were selected and sub-cultured on nutrient agar plates using streak plate method and incubated at 28±2 ^{0}C for 24 hours. Pure cultures were preserved at 4 ^{0}C for further analyzes (Garrity *et al.,* 2005).

Morphological and biochemical characterization of *Pseudomonas* isolates

Colony morphologies and microscopic observations were conducted and the biochemical tests were carried out to characterize and identify isolates (Holt, *et al.,* 1998). Other biochemical tests were also conducted for species characterization. Identification was based on Bergeys Manual of Systematic Bacteriology (Brenner *et al.,* 2005).

Molecular Identification of *Pseudomonas* strains

Confirmation of *Pseudomonas* isolates were characterized by DNA extraction, agarose gel electrophoresis, amplification by polymerase chain reaction (PCR), and full length sequencing of the 16S rRNA gene.

The method of cetyl trimethylammonium bromide (CTAB) was employed for the genomic DNA extraction whose size detected by 1 % agarose gel electrophoresis (Raieta *et al.,* 2015). The forward used primer was 27R-(5'-ACGGCTACCTTGTTACGACT-3') and the reverse primer was 1502F-(5'-AGAGTTTGATCCTGGCTCAG-3') (McPherson and Muller, 2000).

For the PCR reaction, conditions were as follows: DNA templates (70 ng/µl) 2.5 µl; dNTP mixture (10 mM) 0.5 µL; 27 F (10 ρmol/L) 0.4 µL; 1,495 F (10 ρmol/L) 0.4 µL; 10x PCR Buffer (2.5) with $MgCl_2$ (50 mM) 1 µL; Taq DNA polymerase (5 U/µl) 0.3 µL; bringing up ddH_2O 17.4 µL (Madueno *et al.,* 2011). The PCR amplification conditions were as follows: force-degeneration at 95 ºC for five minutes, degeneration at 95 ºC for one minute, annealing at 60 ºC for 30 seconds and at 72 ºC for 35 seconds, 30 cycles, with final extension at 72 ºC to five minutes (Madueno *et al.,* 2011). After purification, the PCR products were sent for sequencing at International Institute for Tropical Agriculture (IITA), Ibadan. The Basic Local Alignment Search Tool (BLAST) method was used for resemblance analysis of the 16S rDNA sequence through the GenBank database (Sambrook and Russell, 2001).

Determination of physicochemical properties of petrochemical contaminated soil

The pH was measured with the aid of a HACH Mini Digital Pen Type pH Meter (Santa Fe, NM 87505, USA) in a 1:25 (w/v) soil to water ratio. Soil texture was analyzed with Soitest 152H (Bouyocous Scale hydrometer, USA) using the Bouyocous hydrometer method (Kotler *et al.* 2001). Soil moisture content was performed with Kenton Electric Oven (Kenton International Co., Ltd, China) using the gravimetric oven drying method (APHA, 2004).

The soil colour was identified visually from the spectral reflectance data of Bray-1 spectrophotometric method (APHA, 2004). Total Petroleum Hydrocarbons (TPH) of the contaminated soil was determined by ultrasonic treatment of soil extracted in 1:1 (v/v) mixture of hexane and acetone (extraction method EPA 3550b) as 5 g of the soil was mixed with 2 g anhydrous Na_2SO_4. Then extracted at 20 ^oC in 15 mL of the solvent with the aid of an ultrasound device delivering 250 W (Branson M8800). The resulting suspension was centrifuged (10,000 g, 5 min) to remove soil particles and the procedure was repeated. The solvent was evaporated using a concentrator (Eppendorf vacufuge plus) and the residual TPH amount was determined gravimetrically (Liu *et al.,* 2012).

Optimization of *Pseudomonas* strains culture conditions.

Optimization for culture conditions of the three selected *Pseudomonas* isolates was performed using mineral salt medium (MSM) which consisted of (g/l): 4 g/l $NaNO_3$, 1.5 g/l KH_2PO_4, 0.5 g/l Na_2HPO_4, 0.2 g/l $MgSO_4.7H_2O$, 0.0011 g/l $FeSO_4.H_2O$, and 0.01 g/l $CaCl_2$. The medium was adjusted to different pH of 5.0, 6.0, 7.0 and 8.0 using 0.1 N HCl and 0.1 N NaOH. Isolates were inoculated and incubated at 37 ^0C for 48 hrs.

To test the influence of temperature; culture media were incubated at 24 ^0C, 30 ^0C, 37 ^0C 45 ^0C at pH 7 for 48 hrs (Hamzah *et al.,* 2010). The effect of moisture content (12 %, 16 %, 20 % and 24 %) on the growth of the three isolates was determined after the incubation at pH of 7 for 48 hrs (Rajasekar *et al.,* 2007). The effect of nitrogen phosphorus ratio (4:1, 6:1, 8:1 and 10:1) were also determined in the culture medium with the same culture conditions (Lee *et al*., 2006). The inoculum density of each of the isolates was also determined using standard plate count technique (Adeyemi *et al.,* 2010).

Inoculums and Treatment Preparations

Growth trend of the *Pseudomonas* strains was performed in mineral salt medium (MSM) as the isolates were cultivated overnight in mineral broth and cells were centrifuged, washed and suspended in normal saline. Soil sample was sterilized by autoclaving thrice at 121 °C for 15 minutes prior to study. Three uniform sterilized plastic microcosms of 3 cm x 3 cm x 3 cm containing 100 g of soil sample each were used. About 50 ml of the suspension of each strain were separately sprayed on the 100 g sterilize soil and mixed in the plastic microcosm within the laboratory. The plastic microcosm was incubated for the period of 28 days at 37 °C. An approximate moisture content of 20 % was also maintained by addition of sterilized water at appropriate intervals.

Monitoring of the Growth Trend of *Pseudomonas* strains

One gram of the soil sample from each of the plastic microcosms was routinely sampled at seven days intervals. Serial dilution was performed and appropriate diluents were inoculated into mineral salt medium using pour plate technique and incubated at 37 °C for 48 hrs. The growth trend of *Pseudomonas* strains was monitored by determining the *Pseudomonas* counts using standard plate count technique (Adeyemi *et al.,* 2010).

RESULTS

Characterization and identification of bacteria isolates.

The growth in all the three isolates after 48 hours incubation showed different colony morphologies. Gram staining showed that these isolates were Gram negative bacteria which appeared as rods and motile (Table 1). The selected isolates morphologically and biochemically resembled *Pseudomonas aeruginosa*, *Pseudomonas mendocina* and *Pseudomonas putida* (Table 1). The identities of the selected isolates were further confirmed with their accession numbers using molecular methods as shown in Table 2.

Table 1. The morphological and biochemical identification of *Pseudomonas* isolates

S/N	Morphological and biochemical tests	*Isolate P_1*	*Isolate P_2*	*Isolate P_3*
1	Colony character	Blue to red pigment	Yellowish pigment	Yellow to brown, green pigment
2	Cell type	Rod	Rod	Rod
3	Motility	Motile	Motile	Motile
4	Gram reaction	-	-	-
5	Gluconate	+	-	-
6	Gelatin hydrolysis	+	-	-
7	Oxidase	+	+	+
8	Casein	+	-	V
9	Urease utilization	+	-	-
10	Arginine dihydrolase	+	+	+
11	Indole production	-	-	-
12	Glycerol	+	-	+
13	Catalase	+	+	+
14	Ammonuim salts “sugars” Ethanol	+	-	+
15	Glucose	+	+	+
16	Manitol	+	-	-
17	Citrate	+	+	+
18	Sucrose	-	-	- or V
19	Starch hydrolysis	-	-	-
20	Identified bacteria	***P. aeruginosa***	***P. mendocina***	***P. putida***

Key: (+) means positive, (-) means negative and (v) means variable

Table 2. Molecular identification of *Pseudomonas* isolates using DNA sequencing

Isolate Code	Description of Organisms	Accession Number	Identification
Isolate P_1	*Pseudomonas aeruginosa* strain PAER4 119, 16s ribosomal RNA gene partial sequence.	NZ CP013113.1	96 %
Isolate P_2	*Pseudomonas mendocina* strain NK-01, complete sequence	NZ CP017290.1	100 %
Isolate P_3	*Pseudomonas putida* strain B6-2, complete genome	NZ CP015202.1	99 %

Optimization for growth conditions for *Pseudomonas* strains.

The effects of the pH, temperature, moisture content and nitrogen phosphorus ratio on the inoculum density of the *Pseudomonas* strains are illustrated in Fig. 1 to 4.

All the three isolated *Pseudomonas* strains were capable to grow at different pH of 5, 6, 7 and 8 while higher growth activity was observed at pH range of 6 to 8 (Fig. 1). Generally, *Pseudomonas* strains at different pH showed inoculum density ranging from1.03 x 10^7 cfu/g to1.94 x 10^7 cfu/g. At pH 7, *Pseudomonas* strains showed highest growth with corresponding inoculum density of 1.94 x 10^7 cfu/g for *P. mendocina* NK-01, 1.82x 10^7 cfu/g for *P. aeruginosa* PAER4 119 and 1.78 x 10^7 cfu/g for *P. putida* B6-2. Among the three *Pseudomonas* strains, *P. mendocina* NK-01 recorded best growth.

The *Pseudomonas* strains were able to grow at different temperature conditions of 24 ^{0}C, 30 ^{0}C, 37 ^{0}C and 45 ^{0}C as the maximum growth was observed at 35 ^{0}C for all the three strains (Fig. 2).

The highest inoculum densities recorded were 1.98 x 10^7 cfu/g for *P. putida* B6-2, 1.94 x 10^7 cfu/g for *P. aeruginosa* PAER4 119 and 1.89 x 10^7 cfu/g for *P. mendocina* NK-01 at 37 ^{0}C. There was clear connection between the temperature and the inoculum density for the strains at 37 ^{0}C. As shown in Fig. 2, *P. aeruginosa* PAER4 119 showed highest inoculum density comparing to the other strains at 37 ^{0}C. The inoculum density for each of the strains is different at 37 ^{0}C.

The result also showed a corresponding increase in inoculum density of each of the strains with an increase in the nitrogen phosphorus ratio (N:P) as shown in Fig. 3. The three isolated *Pseudomonas* strains were able to grow at different nitrogen phosphorus ratio conditions. The highest inoculum density recorded by each strains was 2.01 x 10^7 cfu/g for *P. putida* B6-2, 1.73 x 10^7 cfu/g for *P. aeruginosa* PAER4 119 and 1.94 x 10^7 cfu/g for *P. mendocina* NK-01 at N:P ratio of 10:1. Among the three isolated strains, *P. putida* B6-2 showed highest growth. However, *P. putida* B6-2 and *P. mendocina* NK-01 were able to utilize nitrogen phosphorus source better than *P. aeruginosa* PAER4 119 at N:P ratio of 10:1.

In the present study, all the *Pseudomonas* strains were able to grow at 12% to 24% range of moisture content while the highest growth was observed at 20 % by *P. putida* B6-2 (Fig 4). There was a positive connection between the moisture content and the inoculum density for the strains at moisture content of 20 %. At different moisture contents, the inoculum density for the three strains ranged from1.20 x 10^7 cfu/g to 2.00 x 10^7 cfu/g. The highest inoculum density recorded by each of *Pseudomonas* strains was 2.00 x 10^7 cfu/g for *P. putida* B6-2, 1.85 x 10^7 cfu/g for *P. aeruginosa* PAER4 119 and 1.94 x 10^7 cfu/g for *P. mendocina* NK-01 at moisture content of 20 %. However, the inoculum density of each of three *Pseudomonas* strains is different at moisture content of 20 %.

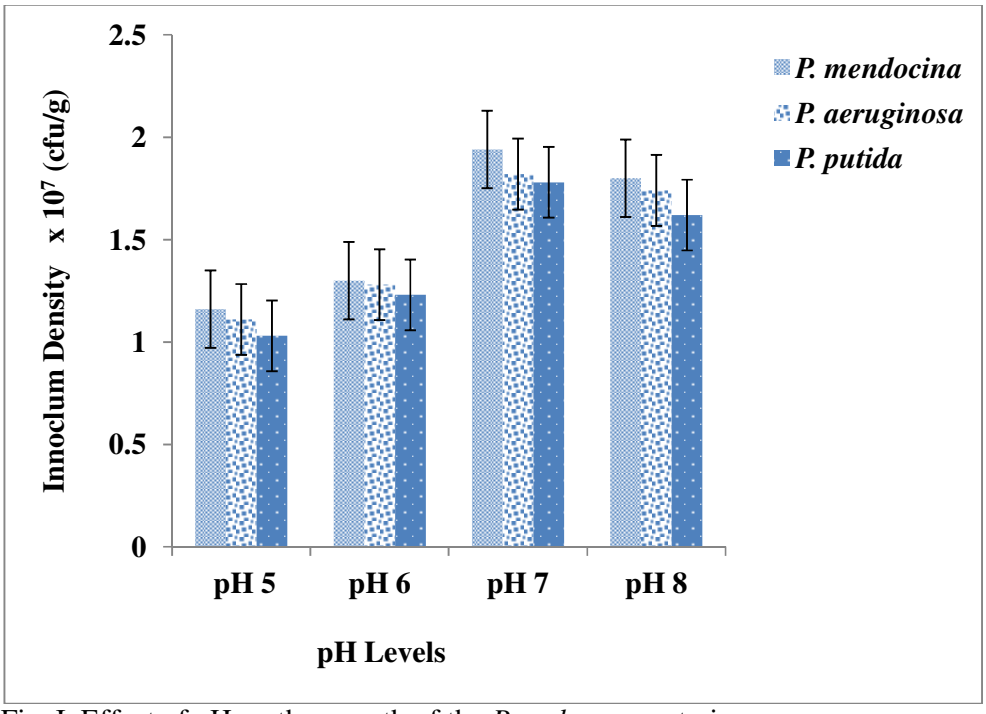

Fig. I. Effect of pH on the growth of the *Pseudomonas* strains

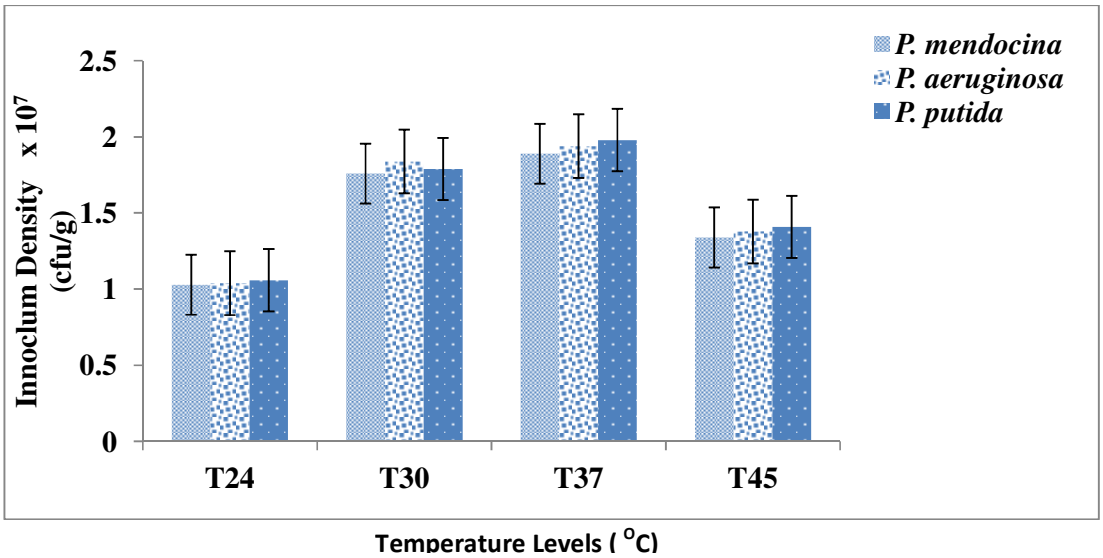

Fig. 2. Effect of temperature on the growth of the *Pseudomonas* strains

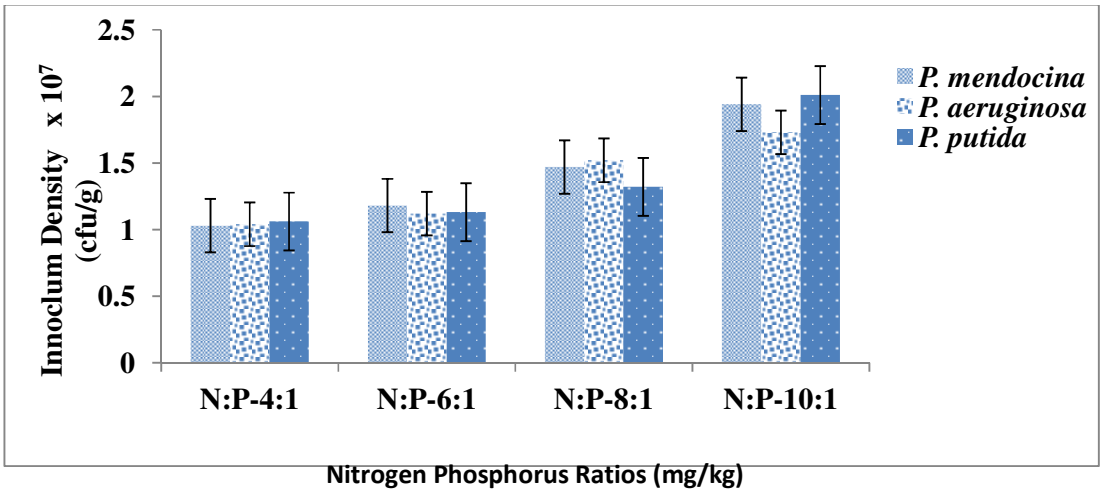

Fig. 3. Effect of nitrogen phosphorus ratio on the growth of the *Pseudomonas* strains

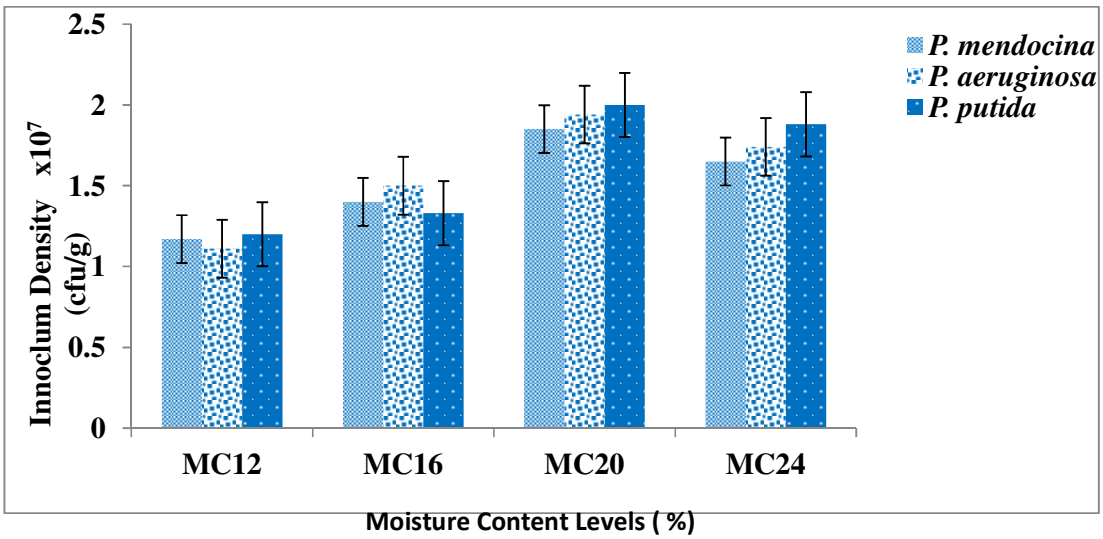

Fig. 4. Effect of moisture content on the growth of the *Pseudomonas* strains

Physicochemical analysis of petrochemical contaminated soil.

The physicochemical characteristics of the soil sample are shown in (Table 3). Particle size distribution of the soil sample was 70.60 % for sand, 21.40 % for loam and 8.00 % for clay while the soil colour was recorded as brown. The soil sample showed a pH value of 5.8 and a moisture content of 12.5 %.The percentage concentration of the total petroleum hydrocarbons (TPH) of the contaminated soil sample was 7.4 %.

Table 3. Physiochemical Properties of the Petrochemical contaminated soil sample.

S/N	Parameter	Contaminated soil
1.	Textural Class	Sand Clay loam
(a)	Sand	70.60 %
(b)	Clay	8.00 %
(c)	Loam	21.40 %
2	Soil Colour	Brown
3	Moisture content	12.54 %
4	Soil pH	5.80 %
5	TPH	7.40 %

Key: TPH=total petroleum hydrocarbons

Growth trend of the three *Pseudomonas* strains

All the *Pseudomonas* strains after inoculation into the treatment soil showed similar growth trend in relation to each other as shown in fig 5.

The *Pseudomonas* strains were able to grow and survive in the petrochemical contaminated soil for a period of 28 days. The results showed increasing population density from day 7 to day 14 and subsequently decreased from day 14 to day 28. The population density of the strains ranged between 2.66 x 10^{10} cfu/g and 2.90 x 10^{10} cfu/g.

The maximum growth recorded by each of *Pseudomonas* strains were 2.90 x 10^{10} cfu/g for *P. putida* B6-2, 2.86 x 10^{10} cfu/g for *P. aeruginosa* PAER4 119 and 2.84 x 10^{10} cfu/g for *P. mendocina* NK-01 on 14th day. There was no difference in the *Pseudomonas* counts of *P. mendocina* NK-01and *P. aeruginosa* PAER4 119 on day 7, day 14 and day 28 as shown in fig. 5.

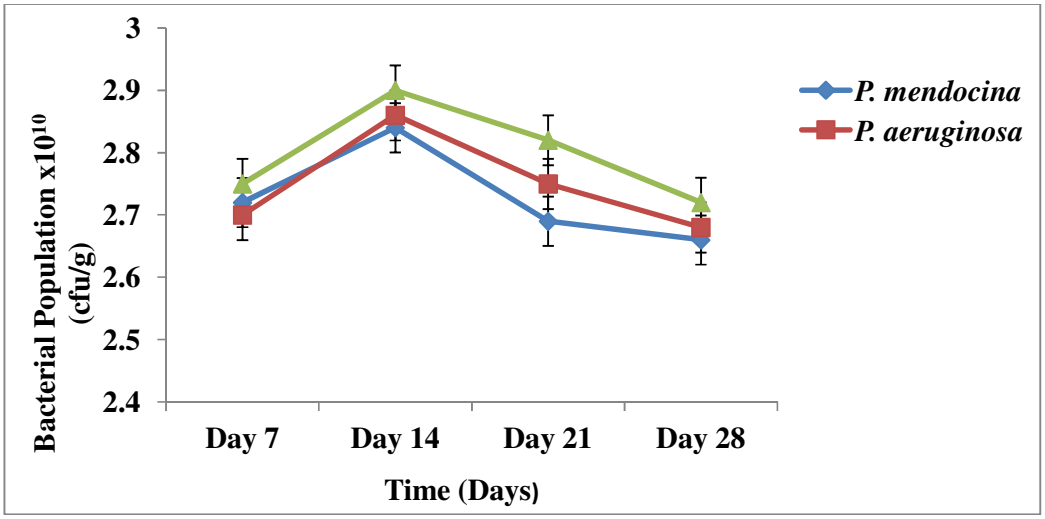

Fig. 5. Growth trend of *Pseudomonas* strains in Petrochemical contaminated Soil.

DISCUSSION

The results from molecular analysis revealed similarities of the isolated strains of *Pseudomonas mendocina*, *Pseudomonas putida*, and *Pseudomonas aeruginosa*. This is supported by Yergeau *et al.,* (2012), which also showed the relative abundance of *Pseudomonas* species in petroleum polluted area. Genus *Pseudomonas* are usually referred to as oil eating bug in petroleum industry due to their metabolic versatilities to use wide variety of carbon sources as nutrients and ability to adapt to adverse environmental conditions (Kilbane, *et al.,* 2000). Shkidchenko *et al.,* (2004) similarly reported the capability of *Pseudomonas* isolates to grow and survive during biodegradation study of petrochemical contaminated soil.

The addition of hydrocarbon to an ecosystem due to accident oil spills may selectively increase or decrease size of microbial population depending on certain factors (Bharathi and Vasudevan, 2001). These factors include composition of the contaminant, species of microorganism present within the microbial community and environmental factors such as of the temperature, pH, moisture content, chemical particular ecosystem etc (Khashayar and Mahasa, 2010).

Variation in pH of a culture medium is as a result of accumulation of metabolic waste products by the bacterial cells which strongly affects its growth. Therefore, it is very important to maintain optimum pH condition for bacterial growth medium (Rajasekar *et al.,* 2007). The maximum cell growth obtained by the strains at pH 7 for different buffers in this study is similar to findings of Luo *et al*. (2013) who reported that at pH level of 7, *Pseudomonas* sp. F4 showed efficient diesel oil degradation potential. However, higher growth activity was observed at pH range of 6 to 8. The results showed by Zouaoui and Bouziane, (2012) revealed that *P. aeruginosa* was able to grow at pH range from 6 to 8 and reached lipase maximum activity 38.5 U/ml at pH 7.

The effects of temperature for growth of the three isolated strains were evaluated. It was found that temperature is an important factor that affects the growth of bacteria in petrochemical contaminated environment. The results revealed that all the *Pseudomonas* strains were able to grow at different temperature condition for 24 ^{o}C, 30 ^{o}C, 37 ^{o}C and 45 ^{o}C. Similar study of Shukor *et al.,* (2009), revealed the ability of bacterial strains from hydrocarbon contaminated soil to grow in a wide range of temperature. Maximum cell growth was also observed for each of the *Pseudomonas* strains at temperature of 37 ^{o}C,. Similarly, study of Luo *et al*. (2013) reported that the diesel oil-degrading ability of *Pseudomonas* sp. F4 was observed to be at 37 ^{o}C. Also, *Pseudomonas putida* MTCC 102 and *Pseudomonas aeruginosa* NCIM 102 were capable of decolorizing the dye over at 37 ^{o}C with a good efficiency. Kalme *et al*. (2007) also reported similar finding where at 37 ^{o}C and pH of 6.8, majority of the azo dye reducing bacterial species were able survive and reduce the dye.

In the present study, *Pseudomonas* strains were able to grow at different nitrogen phosphorus ratio conditions. The organic carbon content in petroleum hydrocarbon contaminated site is found to be very high as a result of constant input of hydrocarbons (Rodriguez-Martinez, 2006). In addition to a readily degradable carbon source, microorganisms require mineral nutrients such as nitrogen, phosphate and potassium (N, P and K) for cellular metabolism and therefore successful growth. Hence, it is important practice to supplement contaminated site with nutrients, generally nitrogen and phosphates to stimulate the microbial community and therefore enhance bioremediation (Atagana *et al.* 2003). However, the three isolated *Pseudomonas* strains showed maximum cell growth at a nitrogen phosphorus ratio (N:P) of 10:1. Leys *et al.,* 2005) in a similar report revealed that growth of hydrocarbon-degrading bacteria and hydrocarbon degradation can be strongly enhanced by fertilization with inorganic N and P. In majority of the treatments, the C:N:P ratio is maintained as 120:10:1.

Moisture is very vital for all biological processes to help transport nutrients, foods, and waste products in and out of the microorganisms (Potin *et al.* 2004). Some level of moisture must be present for cell growth and biodegradation to occur especially in soil environments. However, too much water can impede the aeration of the soil, and the process may turn anaerobic (Potin *et al.* 2004; Shallu *et al.* 2014). Therefore, in this study, the effect of moisture content on the population density of each of the isolated strains was investigated. This study showed maximum cell growth for the isolated strains at moisture content of 20 %. The result correlates with the study of Potin *et al.* (2004) which reported that the optimum ratio of moisture ranged from 12-32 % during the biodegradation of hydrocarbons in soil. However, the optimum ratio of moisture will depend on the climate and soil type.

It was observed that an increase in viable counts of the isolates correlated to the number of bioremediation period. Similar results were reported by Shkidchenko *et al.,* (2004) where bacterial isolates were able to grow and survive with high bacterial counts (cfu/g) during biodegradation study of petrochemical contaminated soil. The growth trend observed by the *Pseudomonas* strains with respect to the inoculum selection procedure could be due to their physiological characteristics which determine to a great extent their fate and activities in soil (Darine and Ridha, 2013). Furthermore, the optimization of growth conditions for the three selected bacterial isolates might have contributed to the sustained growth and survival in the petrochemical contaminated soil (Mahalingam and Nithya, S. 2014). There was initial progressive growth rate from day 14 to day 21st during the study. The corresponding increase in *Pseudomonas* viable cell counts recorded could be due to availability of essential growth factors such as nutrients, moisture, oxygen etc. which is normal during exponential phase of microbial growth.

Physicochemical characteristics of soil are important for successful hydrocarbon biodegradation (Renner, R. 1999). Soil texture affects permeability, water content and the bulk density of soil. The relative survival of the *Pseudomonas* strains in sandy loam soil could be due to high permeability property of sandy loam soil which supports cell growth and sustain biodegradation by enhancing transportation and the distribution of water, nutrients and oxygen in the soil (Renner, R. 1999). Also, the effect of soil condition showed relative growth trend. The growth trend observed with respect to the initial soil treatment (sterilized soil) could be attributed to the absence of predation, competition and bacteriorvory in the sterilized soils as a result of the elimination of indigenous organisms in the process of sterilization (Razavi, D.S. and Lakzian, A. 2007).

However, it was observed that there was a decrease in viable counts after a period of time. The reason could be as a result of depleted nutrients, oxygen and presence of accumulated toxic waste by products in the system (Prescott *et al.,* 2005). Also, the growth variation observed among each strain may be explained on the basis that these viable counts were on the whole contaminant (petrochemicals) not on specific component of the petrochemical as a substrate. Another reason for the variation in the growth trend as observed from the results may be due to the biodegradation capability of the individual *Pseudomonas* strains in the contaminated soil (Prescott *et al.,* 2005 and Shkidchenko *et al.,* 2004).

CONCLUSION

This study has successfully demonstrated the ability of the three isolated *Pseudomonas* strains to grow and survive in the petrochemical contaminated soil. It was also observed that proper examination of the population dynamics, bioavailability, physiological as well as physicochemical factors of any contaminated environment is vital for enhancing bacterial growth in order to achieve a successful bioremediation processes. Hence, this study can be employed for successful bioremediation studies.

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