



Bioremediation Potentials of Hydrocarbonoclastic Bacteria Isolated from Petroleum Refinery Effluent

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To cite this article:

Enimie Endurance Oaikhena, Dominic Bawa Makaije, Samuel Denwe, Muhammad Muktar Namadi, Omolola Elizabeth Fatanmi. Bioremediation Potentials of Hydrocarbonoclastic Bacteria Isolated from Petroleum Refinery Effluent. *Frontiers in Environmental Microbiology*. Vol. 2, No. 6, 2016, pp. 34-37. doi: 10.11648/j.fem.20160206.12

Received: February 8, 2016; Accepted: May 17, 2016; Published: December 9, 2016

Abstract: Petroleum effluent is discharged into River Rido and this poses a threat to the communities that utilize the water downstream of River Rido, the ultimate recipient of the effluent. A total of five hydrocarbonoclastic bacteria were isolated from the petroleum refinery effluent. The isolates were inoculated separately into mineral salt medium supplemented with 1% crude oil to determine their growth rate in a hydrocarbon substrate. All five bacterial isolates manifested no lag phase of growth. *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Streptomyces* sp. exhibited exponential, stationary and death phases of growth while *Pseudomonas aeruginosa* had only exponential and stationary phases of growth on the 16th day of incubation. Mixed culture consortium of all five bacteria isolates had the highest bioremediation potential of 68% as against the single inoculations of *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptomyces* spp. which had 66.4%, 62.4%, 59.7%, 52% and 57% respectively. Hence, the isolated bacteria can be used for bioremediation of petroleum effluent.

Keywords: Bioremediation, Hydrocarbonoclastic, Bacteria, Refinery Effluent

1. Introduction

The petroleum industry is one of the largest industries in Nigeria and the country also obtains most of its revenue from the industry. While the petroleum refinery and petrochemical industries are most desirable for national development and improved quality of life, the unwholesome and environmentally unacceptable pollution effects of the waste from these industries are causes for worry (Nwachi and Warigbani, 2013). Pollutants in the refinery wastewater include aromatic hydrocarbons, phenols, polycyclic aromatics hydrocarbons (PAHs) and heavy metals (Bako *et al.*, 2008). Hydrocarbons are compounds whose structures are made up of hydrogen and carbon atoms.

PAHs present as natural constituents in fossil fuels are formed during the incomplete combustion of organic material and are therefore presents in relatively high concentrations in products of fossil fuels refining (Wang *et al.*, 1999). The

toxicity of hydrocarbon can result in cancer, gastrointestinal infection, heart disease, neurological disorder and respiratory infection (Leachy and Rita, 1990).

The treatment methods used for remediation include physical, chemical and biological processes. Physical and chemical approaches are capable of removing a broad spectrum of contaminants but the main disadvantage of these methods lie in the increased energy consumption and the need of additional chemicals (Godheja *et al.*, 2012). Preferably, the biological method is considered because it is environmental friendly.

Bioremediation is the use of biological agents such as microorganisms (bacteria, fungi and algae) and plants for the removal or detoxification of toxic substances in the environment. Hydrocarbonoclastic bacteria are referred to as bacteria that can biodegrade hydrocarbon. Numerous genera of bacteria are known to be good hydrocarbon degraders and most of them belong to the genera *Pseudomonas*, *Sphingomonas*, *Aeromonas*, *Alcaligenes*, *Staphylococcus*,

Lacticobacillus, *Acinetobacter*, *Arthrobacter*, *Brevibacterium*, *Xanthomonas*, *Mycobacterium*, *Micrococcus*, *Neisseria*, *Enterobacter*, *Rhodococcus* and *Bacillus* species (Plaza *et al.*, 2008). *Achromobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter*, *Flavobacterium*, *Nocardia* and *Corynebacterium* species are also hydrocarbon degraders (Leachy *et al.*, 1990). This research is aimed at isolating hydrocarbonoclastic bacteria from petroleum refinery effluent and also assessing their bioremediating potentials in hydrocarbon cleanup programmes.

2. Materials and Methods

2.1. Collection of Sample

Refinery effluents were aseptically collected into sterile bottles from five different sites of Kaduna petrochemical refinery company (Kaduna, Nigeria) in the month of January, 2014. Samples were placed in a cool aid box and transported to the Department of Biological Science Laboratory, Nigerian Defence Academy (NDA), Kaduna for isolation of bacteria.

2.2. Isolation of Hydrocarbonoclastic Bacteria

Bushnell-Haas Agar was used to culture the crude oil degrading bacteria. The agar was supplemented with 1% crude oil before 0.1ml of the 10-fold diluted effluent samples from the contaminated sites were inoculated and incubated at room temperature for 5 days. The bacteria were isolated and sub cultured into nutrient agar plates to obtain pure cultures of each tolerant strain (Thavasi *et al.*, 2007).

2.3. Identification of Hydrocarbonoclastic Bacteria

The pure isolated bacteria strains were identified morphologically and on the basis of their biochemical reactions in the Department of Biological Sciences, NDA. The results obtained from the identification were compared with Bergey Manual of Bacteriology (Holt, 1994).

2.4. Evaluation of Crude Oil Degrading Potentials of the Bacteria through Optical Density Determination

For evaluation of the change in optical density of crude oil, ten millimeters of Mineral salt broth was supplemented with 1% v/v crude oil. Twenty microliters (20µl) of each isolated bacterium was inoculated into the broth and incubated for 16 days at room temperature. The broth's optical density was

taken using a spectrophotometer at 600nm. The samples were measured against a blank containing broth with 1% crude oil at day 0, 2, 4, 6, 8, 10, 12, 14 and 16 respectively (Jyothi *et al.*, 2012).

2.5. Evaluation of Bioremediation of Hydrocarbon in Wastewater by Determination of Chemical Oxygen Demand (COD)

Nutrient broth and effluent were added in a ratio of 4:1 by mixing 120ml of nutrient broth and 30ml of sterilized effluent into seven flasks, separately. Seven sets of 250ml flasks were set for the work. An uninoculated flask served as the control and the others were inoculated separately with 20µl of *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptomyces* sp. and with the mixed consortium of the five organisms. The experimental set up was left to stand for ten days at room temperature and the COD was measured using reflux digestion method at two days intervals (Usman *et al.*, 2012).

3. Results

Five possible hydrocarbonoclastic bacteria from the refinery effluent were identified morphologically and biochemically as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Streptomyces* sp. (Table 1) and their respective degradative capabilities (Figure 1). None of the bacterial isolates exhibited a lag phase in their growth performances. All five isolates exponentially grew up to the 8th day of cultivation. Except for *Pseudomonas aeruginosa* whose optimal growth was attained from the 10th to 16th day, the other four bacterial isolates reached stationary and death phases after the 8th to 14th day of degradation of the crude oil (Table 2). The Chemical Oxygen Demand (COD) was reduced from 250 to 84mg/L with *Pseudomonas aeruginosa*, 250 to 94 mg/ml with *Serratia marcescens*, 250 to 100.8mg/ml with *Klebsiella pneumoniae*, 250 to 120mg/L with *Proteus vulgaris*, 250 to 117mg/L with *Streptomyces* sp. and 250 to 79mg/L with Mixed Culture Consortium (MCC). The percentage reduction of the organic components in treatments having *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptomyces* spp. and MCC were 66.4%, 62.4%, 59.7%, 52%, 57% and 68% respectively (Figure 2).

Table 1. Biochemical and Morphological Characterizations of the Five Hydrocarbonoclastic Bacteria Isolated from Refinery Effluent.

Colonial Variables	Isolates and their Cultural Characteristics				
	IST 1	IST 2	IST 3	IST 4	IST 5
Shape	Rod	Rod	Rod	Rod	Rod
Colony colour	green pigment	Yellow	Gray	White	red pigment
Colony Form	Circular	Circular	Irregular	Filamentous	Circular
Colony Elevation	Convex	Raised	Raised	Lobate	Flat
Gram reaction	-	-	-	+	-
Motility	+	+	+	-	+
Biochemical Reactions of Isolates					
Indole	-	+	-	-	-
Methyl red	-	+	-	-	-

Colonial Variables	Isolates and their Cultural Characteristics				
	IST 1	IST 2	IST 3	IST 4	IST 5
Voges Proskauer	+	+	+	-	+
Citrate	+	-	+	-	+
Urease	-	+	+	+	-
Catalase	+	+	+	-	+
Oxidase	+	-	-	-	-
Ornithine Decarboxylase	-	-	-	-	+
Mannitol	+	-	+	-	+
Nitrate reduction	+	+	+	+	+
H ₂ S Production	-	+	-	-	-
Glucose	-	+	+	+	+
Lactose	-	-	+	+	-
Sucrose	-	+	-	-	-
Gas Production	-	+	+	-	-
Probable Bacteria Isolates	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>K. pneumoniae</i>	<i>Streptomyces</i> spp.	<i>Serratia marcescens</i>

Key: + Positive, - Negative, Spp- species, *P. aeruginosa* - *Pseudomonas aeruginosa*, *P. vulgaris*- *Proteus vulgaris* and *K. pneumoniae*- *Klebsiella pneumoniae*.

Table 2. Optical Density Readings for the Bioremediation Potentials of Each Hydrocarbonoclastic Bacteria.

ORGANISMS	DAYS OF INCUBATION								
	0	2	4	6	8	10	12	14	16
<i>Pseudomonas</i> sp.	0	0.071	0.168	0.281	0.385	0.489	0.642	0.645	0.66
<i>Serratia</i> sp.	0	0.069	0.158	0.264	0.344	0.401	0.425	0.428	0.396
<i>Klebsiella</i> sp.	0	0.055	0.17	0.274	0.361	0.468	0.476	0.456	0.392
<i>Proteus</i> sp.	0	0.075	0.151	0.301	0.404	0.414	0.391	0.358	0.326
<i>Streptomyces</i> sp.	0	0.074	0.161	0.245	0.391	0.441	0.454	0.391	0.295

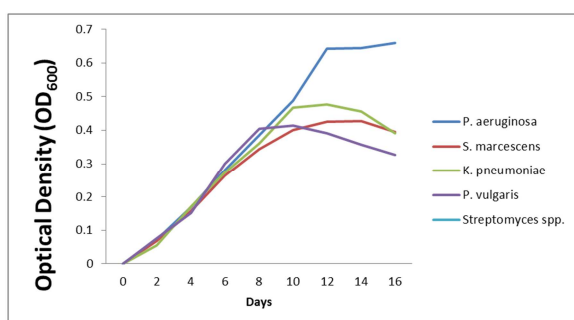


Fig. 1. In Vitro Crude Oil Degradative Abilities of the Five Bacteria Isolated from Refinery effluent.

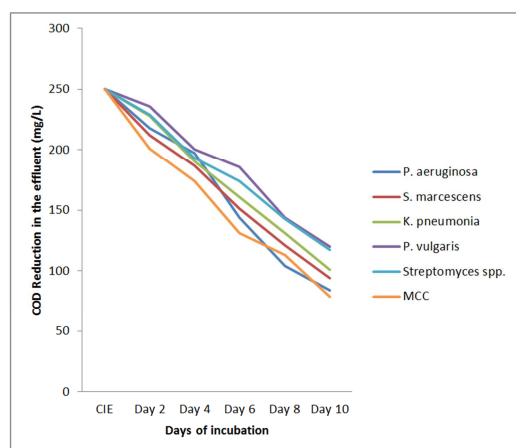


Fig. 2. COD reduction by bacterial isolates from Refinery effluent.

KEY

MCC- Mixed Culture Consortium (Consisting of all five isolates), COD- Chemical Oxygen Demand, CIE- COD in Effluent

4. Discussion

The five bacteria isolates with hydrocarbonoclastic potentials were isolated and identified as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Streptomyces* sp. (Table 1). This finding is similar to the study carried out by Kafizadeh *et al.* (2010) at Lake Parishan where the isolates included *Providencia* sp., *Enterobacter* sp., *Serratia* spp., *Escherichia coli*, *Citrobacter* sp., *Shigella* sp., *Klebsiella* sp., *Acinetobacter* sp. and *Pseudomonas* sp.

All the five bacteria isolates with potentials for hydrocarbon degradation did not exhibit lag phase of growth (Fig 1) which might be attributed to their previous association with the hydrocarbon contaminated environment of the effluent drains from the refinery. This finding is similar to the observation made by Ojumuo *et al.* (2004) and Usman *et al.* (2012). *Pseudomonas aeruginosa* exhibited two phases of growth which are exponential and stationary phases while the other isolates exhibited three phases of growth which are exponential, stationary and death phases. The outcome corresponds with the findings of Usman *et al.* (2012) whose isolates manifested exponential, stationary and death phases for *Micrococcus luteus*; while *Bacillus* sp. had exponential and stationary phases only.

According to Ornston and Yeh (2010), the utilization of hydrocarbon by bacteria is feasible as they possess catabolic enzymes for hydrocarbon metabolism and more importantly they have an immense capacity for adaptive change. The dominant groups of bacteria isolated from KRPC drains were gram negative bacteria for possible hydrocarbon degradation (*S. marcescens*, *P. vulgaris*, *K. pneumoniae* and *P. aeruginosa*). This result corroborates with the report of

Kaplan and Kitts (2004). In general, the gram negative bacteria have been reported as the most effective group of hydrocarbon degrading bacteria. Lipopolysaccharide produced in the bacterial membranes of gram negative bacteria supports the formation and stabilization of oil in aqueous system and contributes by increasing the attack surface on the pollutant for subsequent assimilation (Van Hamme *et al.*, 2003).

The percentage COD reduction rate for *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Streptomyces* sp. were 66.4%, 62.4%, 59.7%, 52% and 57% respectively for pure culture isolates (Fig 2). This result implies that the gram negative bacteria are more effective for degradation of organic compounds than the gram positive bacteria. Hamza *et al.* (2009) did a similar research on petroleum refinery wastewater and observed that COD reduction rate was 82.9% by *Bacillus subtilis* and 76.5% by *Micrococcus luteus*. Although, maximum efficacy were observed using gram positive bacteria which is contrary to our report of gram negative bacteria. Mixed Culture Consortium (MCC) consisting of all five bacteria isolates had higher percentage (68%) of COD reduction. The probable reason why MCC was more effective than the pure isolates might be as a result of the synergistic effect of enzymatic reactions from various bacteria isolates on the refinery pollutant or the composite effect of enzymes of the five isolates acting more on metabolites than enzymes of individual isolates.

The results obtained is relatively lower than that of Abdullahi *et al.* (2012) who reported 97% COD reduction by a mixed culture consortium. Thenmozhi *et al.* (2001) stated that a consortium was more effective for bioremediation than pure culture isolates. The isolate with the best COD reduction capability is *P. aeruginosa* while the least of them is *P. vulgaris* as pure culture isolates. Therefore, these isolates can be referred to as hydrocarbonoclastic bacteria because of their possession of catabolic enzymes for hydrocarbon degradation. The differences in the rate of hydrocarbon degradation between isolates may be due to the difference in types of catabolic enzymes elaborated by individual types in the degradation of hydrocarbons (Majid *et al.*, 2008).

5. Conclusion

The study involves the isolation of hydrocarbonoclastic bacteria from refinery effluent. Five hydrocarbonoclastic bacteria isolated were *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Streptomyces* sp. Bioremediation potentials showed that the mixed culture consortium (MCC) is more preferable for bioremediation than the pure culture of individual isolates. Among the pure isolates, *Pseudomonas aeruginosa* had the highest bioremediation potential than the other four isolates and can be represented in decreasing order as *Pseudomonas aeruginosa* > *Serratia marcescens* > *Klebsiella pneumoniae* > *Streptomyces* sp. > *Proteus vulgaris*.

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