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Bioremediating Potentials of Marine Mercury-Resistant Bacteria on Polyaromatic Hydrocarbons Components of Bonny Light Crude Oil

B. O. Uba[1], E. L. Okoye[2*] and I. E. Chukwurah[2]

[1]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.
[2]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BOU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ELO and IEC managed the analyses of the study. Author ELO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the potentials of marine mercury-resistant bacteria isolated from the coastal water of Niger Delta region of Nigeria and examine their ability to tolerate, utilize and biodegrade Bonny light crude oil.
Place and Duration of Study: The study was carried out at Onne light flow terminal sea port located in Eleme Local Government Area of Rivers State, Nigeria between August 2011 and February 2013.
Methodology: The techniques employed for growth and biodegradation studies were total viable count, optical density and gas chromatographic analysis. Several bacterial genera capable of growing at 50 mg/l and higher concentrations of mercury were isolated and include: *Acetobacterium, Arthrobacter, Planococcus, Brevibacterium, Alteromonas, Enterococcus* and *Cupriavidus*.

**Corresponding author: E-mail: ebyemma2006@gmail.com, el.okoye@unizik.edu.ng;*

Results: The result of total viable count, absorbance at 520 nm (OD_{520}nm) and changes in Polyaromatic Hydrocarbons (PAHs) concentrations ranged from 5.88 - 6.45, 5.70 – 6.39 and 5.80 - 6.27 log_{10}cfu/ml; 1.170 - 10.740, 0.880 - 10.210 and 0.460 - 10.090; and 64.779 mg/l - 29.777 mg/l, 128.103 mg/l-74.107 mg/l and 82.085 mg/l-68.809 mg/l respectively after 28 days of incubation period for the consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X), positive control (*Pseudomonas aeruginosa)* and *Cupriavidus necator* MRB X. Thus, *Cupriavidus necator* MRB X showed promising potentials in the bioremediation of crude oil polluted environments and was almost comparable with standard strain *Pseudomonas aeruginosa* but the consortium were comparatively better and potent PAHs degraders of the Bonny light crude oil.

Conclusion: This study has proved the bioremediating potentials of Mercury-resistant bacteria; therefore they could be used as potent bioremediating agents on Polyaromatic Hydrocarbons since their removal has been a challenge to environmental management.

Keywords: Bioremediation; crude oil; hydrocarbon; marine; mercury-resistant bacteria.

1. INTRODUCTION

The Niger Delta area is the centre of petroleum production and development activities in Nigeria. The first commercial oil field was discovered in 1956 at Olobiri in the Niger Delta region of Nigeria and ever since then, frontiers of oil exploration in Nigeria has been expanding, producing medium and light (such as Bonny light) crude oil [1]. This area has a difficult terrain characterized by mangrove swamps and narrow creeks which are inundated by tidal action [2]. The high rate of petroleum – related activities has been associated with frequent oil spills, especially through oil well blowouts, tanker accidents and accidental rupture of oil pipelines. These mishaps results in the release of crude oil and refined petroleum product in the terrestrial and aquatic environments [2].

Polyaromatic Hydrocarbons (PAHs) constitute a large class of organic compounds that are generally described as molecules which consist of three or more fused aromatic rings in various structural configurations [3]. Polyaromatic hydrocarbon (PAH) molecules stability and hydrophobicity are two primary factors which contribute to the persistence of high molecular weight PAH, in the environment [4]. Polyaromatic hydrocarbon released into the environment may come from many sources, including gasoline and diesel combustion, tobacco smoke and discharge of industrial effluents [5]. Areas polluted by organic compounds i.e. fossil fuels or derivatives, pesticides, Polychlorinated Biphenyl (PCBS), Tributylin (TBT) et cetera are often contaminated by some heavy metals.

Mercury (Hg) is a toxic heavy metal with a widespread use in industry. Worldwide many areas are mercury polluted and present a threat to people and environment [6]. Several bacterial species that have been reported with mercury resistance include: *Alcaligenes faecalis*, *Bacillus* spp., *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Brevibacterium iodinium* and *Klebsiella* spp., *Cupriavidus metallidurans*; *Aeromonas hydrophila*; *E. coli* and *Ralstonia metallidurans* [7-11] Mercury–resistance determinants have been found in a wide range of Gram negative and Gram positive bacteria isolated from different environments. They vary in the number and identities of genes involved and are encoded by *mer* operon, usually located on plasmids, Chromosomes; they are often components of transposons and integrons [11-15]. Some studies by Barbieri et al. [16] and Ka et al. [17], have examined mercury resistant bacteria (MRB) and their potential to catabolize toxic xenobiotics. Baldrian et al. [18] studied the influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil. The ability of bacteria to detoxify mercury can be utilized to bioremediate mercury-contaminated waste water and sites as well as other toxic chemicals.

Remediation of polluted systems could be achieved by physical, chemical or biological methods. However, the attendant negative consequences of the physico-chemical methods make the biological alternative of bioremediation more attractive [19]. Interest in the biodegradation mechanisms and environmental fate of PAHs is prompted by their ubiquitous distribution and their potentially deleterious effects or mutagenic, teratogenic or carcinogenic effects on human health [4].

Ojo, [20], reported that the gas chromatographic profiles of degraded Bonny light and Escravos blend by indigenous bacteria showed complete mineralization of Bonny light hydrocarbons and incomplete mineralization of Escravos blend due to the fact that Bonny light consist of more saturated fractions than Escravos blend.

The major problems facing microbiologists are the ability to isolate microbial degraders that are highly efficient within the aquatic or terrestrial ecosystem, in order to enhance our fight against environmental pollution caused by crude oil and other petroleum products [21]. Thus, this study was undertaken to investigate the bioremediating potentials of marine mercury-resistant bacteria on Polyaromatic Hydrocarbons (PAHs) fractions of Bonny light crude oil.

2. EXPERIMENTAL DETAILS

2.1 Materials

2.1.1 Description of the sampling site

The study area was Onne light flow terminal sea port located in Eleme Local Government Area of Rivers State, Nigeria. Onne has two seaport terminals: light and heavy. The sampling site is about 35 km east from Port Harcourt capital city of Rivers State and 4 km from Onne town. It is located between latitude 4°42´25.05˝ N to latitudes 4°44´46.26˝ N and longitude 7°06´55.65˝ E to longitude 7°11´16.72˝ E (I.C Ezeomedo, Department of Environmental Management, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria, Unpublished results. 2011). The water body is bounded by the tributaries from Bonny sea water. Geomorphologically, its location is 5 m above sea level. Environmental survey of the place revealed the presence of human activities such as transportation of petrochemical products through canoes, boats and ships to neighbouring villages, towns, cities, states and nations due to the presence of multinational petrochemical industries such as Chevron Nig. Ltd., Cameron offshore services, Exon Mobil Nig. Ltd., Oando Nig. Ltd, Socotherm Pipecoaters, Beker Hughes Oil Servicing Company e. t. c.

2.1.2 Sample collection

The water sample used for this study was collected from the study area as previously described (Hg concentration: 0.01 mg/L in water and undetected value in dry sediment). A 1 L plastic container and polyethylene bag that were thoroughly washed and rinsed with 5% HCl to avoid the heavy metal binding to the walls of the container and bag were used for both surface water and sediment preliminary analysis of mercury at Ideyi Consults, Port Harcourt.

The sample for bioremediation study was taken aseptically at a depth of 0-15 cm with a 2 L plastic container sterilized with alcohol overnight and brought to the microbiological laboratory and stored at 4℃ in refrigerator [20].

2.1.3 Source of crude oil sample

The Bonny light crude oil used for this study was obtained from Port Harcourt Refining Company; a subsidiary of Nigeria National Petroleum Corporation, Alesa Eleme, Rivers State.

2.1.4 Source of standard strain

The standard organism used in this study as a positive control is *Pseudomonas aeruginosa.* It was obtained from Medical Laboratory Section of the Federal Medical Centre, Asaba, Delta State.

2.2 Methods

2.2.1 Isolation, identification and maintenance of mercury-resistant bacteria

The mercury-resistant bacteria (MRB) were isolated from heavy metal polluted seawater on seawater nutrient agar (SWNA) amended with 10 mg/l of Hg (as $HgCl_2$). A ten- fold serial dilution of the water sample was carried out by pipetting 1 ml of the sample aseptically into sterilized test tubes containing 9 ml of 0.85% of normal saline solution labeled 10^{-1} to 10^{-10} dilution factors with the aid of a sterile pipette in a repeated order. With another sterile pipette, 0.1 ml aliquots of the appropriate dilutions were spread plated on the surfaces of solidified media in triplicates with aid of a glass spreader under aseptic condition (Dilutions are between 10^{-1} and 10^{-4}). Several colonies were picked and streaked on SWNA plates containing 25 mg/l mercury for further purification and maintenance. After incubation, pigmentation and morphological properties such as colour, elevation, edge, surface, optic characteristics, Gram Staining and Spore arrangement were considered. Also, biochemical test such as motility, catalase, oxidase, citrate, indole, hydrogen sulphide production, glucose, sucrose, lactose, mannitol, maltose and saccharose were done [22]. The isolates were

characterized and identified using Bergey's Manual of Determinative Bacteriology after the taxonomic studies were carried out [10,23].

2.2.2 Development of resistance to the mercuric compound

In order to establish their resistance to the mercuric compound, the isolates were exposed to increasing concentrations of mercury from 25 mg/l, 50 mg/l, 100 mg/l, 500 mg/l, 1000 mg/l and 1500 mg/l for screening for the most highly resistant strain as adopted by Rahman et al. [24] and Sharma and Fulekar [25].

2.2.3 Crude oil adaptation test

According to the method of Wang [26], the marine bacterial isolates were then adapted for crude oil utilization in 9.9 ml mineral salt medium containing 0.1 ml of the crude oil as the carbon source [27]. This mixture is then agitated manually and incubated at room temperature (28±2℃) for all isolates. These were then incubated for 10 days. Loopfuls of the adapted culture organisms were then transferred into Nutrient Agar and incubated at room temperature (28±2℃) for 24 – 48 hrs. Single discrete colonies of the isolates were later transferred into slants incubated and stored in the refrigerator at 4℃ for further use [28].

The obtained cultures labeled *Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X were found with the three highest absorbance values and then screened for PAHs biodegradation using Bonny light crude oil as the m*ode*l substrate.

2.2.4 Preparation of test and control cultures

The sea water nutrient broth was used for the preparation of inoculants. Sterile 50ml of the medium in test tubes were inoculated with a loop each of the test organisms and incubated room temperature (28±2℃) for 18 – 24 hrs.

2.2.5 Biodegradation at 30℃ for 4 weeks

The mineral salt medium stated below (K_2HPO_4, 1.8 g; K_2HPO_4, 4.0 g; NH_4Cl, 0.2 g; $MgSO_4$, $7H_2O$, 0.1 g; NaCl 0.1 g; $FeSO_4$. $7H_2O$, 0.1 g and distilled water 1 litre) excluding agar was autoclaved and amended with sterilized 1 ml crude oil [21]. 99 ml of medium were dispensed into nine (9) sterile 250 ml flasks. 5 ml of the developed inocula as previously described were used to inoculate the nine (9) 250 ml flasks of three sets each. The flasks were incubated at room temperature (28±2℃) with manual shaking of 100 strokes per minutes for 30 minutes [26] for a sampling period fixed for 0,7,14, 21 and 28 days [29].

2.2.6 Enumeration of crude oil degrading bacteria

Four sets of 250 ml flasks each were used for the estimation of total viable count (TVC) of *Cupriavidus necator* MRB X, positive control (*Pseudomonas aeruginosa*), negative control and consortium. Suspensions were serially diluted to 10^{-4} and 0.1 ml was inoculated using spread-plate technique. This was carried out for 0, 7, 14, 21 and 28 days and the plates were incubated at room temperature (28±2℃) [19]. Similarly, using the same four sets of 250 ml flasks, the optical density for *Cupriavidus necator* MRB X, *Pseudomonas aeruginosa*, negative control and consortium were measured at 520 nm wavelength. This was carried out for 0, 7, 14, 21 and 28 days sampling period by adopting the method described by Ekpo and Ekpo [21].

2.2.7 Extraction and analyzing of the residual oil

10ml of water sample each were transferred to a separation funnel and the measuring cylinder used for transferring the solution into separation funnel was rinsed with 1 ml of dichloromethane (organic solvent). The sample(s) and organic solvent were shaken to mix properly, so as to have all available organic material extracted into the organic solvent. They were rinsed further with 1 ml of dichloromethane so as to ensure that no traces of organic material are left unextracted. The organic extract or crude oil was collected into receiving container (sample vial), by passing the organic extract through an extraction column packed with glass-wool, silica-gel, and anhydrous sodium sulphate. The silica gel aids the clean-up of the extract by disallowing the passage of debris and impurities of other compounds that are not PAH's (Polycyclic Aromatic Hydrocarbons) while the anhydrous sodium sulphate acts as a dehydrated agent to rid the organic extract of every form of moisture/water contained in the sample(s). The concentrated clean extracts/ fractions were added 1 ml of dichloromethane to dissolve them and were transferred into labeled glass vials with Teflon or rubber crimp caps for gas chromatography (GC) analysis. The residual oil was measured and quantitative changes in the polyaromatic hydrocarbon profiles of the oil were

analyzed using Hewlett Packard gas chromatography(HP 5890 Series II) [29].

2.3 Statistical Analysis

The results were expressed as mean±standard deviation (mean±s.d.) of three different replicate. Statistical analysis was performed on data generated from the study using Microsoft excel and SPSS soft ware. One way analysis of variance (ANOVA) was used to compare differences in mean result of the different sample groups.

3. RESULTS AND DISCUSSION

The result of the colonial morphology of the marine mercury bacterial isolates obtained using sea water nutrient agar (SWNA) is presented in Table 1, while that of the biochemical reactions is shown in Table 2. The predominant shape, colour, margin, elevation, optic and surface colonial characteristics are irregular, creamy, rhizoidal/ convex/entire, raised/flat opaque and smooth. The predominant cell morphology and arrangement were Gram-positive rod shaped bacteria followed by coccus and coccibacillus that appeared singly or in pairs. Most of them are spore, indole, H_2S and oxidase negative; catalase positive and ferment maltose.

Table 3 presents the screening test for adaptation to crude oil at absorbance of 520 nm for the eleven marine bacterial isolates obtained. *Cupriavidus necator* MRB X was found to be the highest utilizer (3.58), while *Planococcus citreus* MRB IV was the least utilizer (2.64).

Fig. 1 shows the curves and absorbance values of *Cupriavidus necator* MRB X, *Pseudomonas aeruginosa*, consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) and negative control for 28 days biodegradation. The consortium had the highest optimal growth, followed by the *Pseudomonas aeruginosa* and the *Cupriavidus necator* MRB X ranging from 1.171, 0.885 and 0.461 to 10.75, 10.21 and 10.05. The negative control without cell had no remarkable increase ranging from 2.510 to 3.940. Initially, the absorbance was low and later increased dramatically after 28 days incubation period though the consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) utilized the crude oil more than *Pseudomonas aeruginosa* and *Cupriavidus necator* MRB XII. In day 0, the organisms had not fully adapted to the new environment and were producing new enzymes; after they had acclimatized in day 7, they began to grow exponentially though at a slight increase in days 21 and 28.

Table 4 presents the total number of viable cells of the test organisms and negative control for the 28 days biodegradation study. The consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis MRB VII and Cupriavidus necator MRB X) had the highest mean count, followed by Pseudomonas aeruginosa and Cupriavidus necator* MRB X ranging from 5.88, 5.70 and 5.80 to 6.45, 6.39 and 6.27 $\log_{10}$cfu/ml. The negative control had very neglible growth. The total viable cells increased significantly in the first seven days and then decrease in the day14 and 21 and later increase in day 28. The maximum mean microbial count for consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) is 6.23 followed by *Pseudomonas aeruginosa* which is 6.11 and *Cupriavidus necator* MRB X having 6.0 counts all expressed in Log_{10}cfu/ml.

Table 1. Colonial morphology of marine mercury bacterial isolates

Isolates	Colour	Shape	Margin	Elevation	Optic	Surface
MRB I	Creamy	Round	Convex/Entire	Raised	Transparent	Smooth
MRB II	Milky	Irregular	Rhizoidal/ Convex	Raised	Opaque	Smooth
MRB III	Grey	Round	Entire	Flat	Transparent	Dry/Rough
MRB IV	Milky	Round	Entire	Raised	Opaque	Smooth
MRB V	Creamy	Irregular	Rhizoidal/ Convex	Flat	Opaque	Rough/Dry
MRB VI	Creamy	Irregular	Convex	Flat	Translucent	Smooth
MRB VII	Creamy	Irregular	Rhizoidal	Flat	Opaque	Smooth
MRB VIII	Milky	Irregular	Convex	Flat	Translucent	Smooth
MRB IX	Yellow	Round/circular	Entire	Raised	Translucent	Smooth
MRB X	Milky	Irregular	Convex/Entire	Raised	Transparent	Smooth/mucoid
MRB XI	Creamy	Irregular	Convex	Flat	Opaque	Smooth

Table 2. Biochemical reactions for bacteria identification

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
G. reaction	+	+	+	+	-	-	+	-	+	-	+
Shape	R	C	R	C	R	R	R	R	C	CB	C
Order	P	S	P	P	ST	ST	S	ST	S	S	P
S. staining	-	-	-	-	-	-	-	-	-	-	+
Catalase	-	+	+	+	-	-	+	-	+	+	-
Oxidase	-	-	-	-	+	+	-	+	-	+	-
Indole	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+	+
Motility	+	-	-	-	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-
Glucose	A/G	-/-	-/-	-/G	-/-	-/-	A/G	-/-	-/-	-/G	A/G
Sucrose	A/G	-/G	-/-	-/G	-/-	-/G	A/G	A/-	-/-	A/G	A/G
Lactose	-/-	-/G	-/-	A/G	-/-	-/-	A/G	-/-	-/-	A/G	A/G
Saccharose	A/G	-/G	-/-	-/G	-/-	-/G	A/G	A/-	-/-	A/G	A/G
Mannitol	-/G	-/G	-/-	-/G	-/-	-/-	A/G	A/-	-/-	A/G	A/G
Maltose	A/-	-/-	-/-	A/G	-/-	-/-	A/-	A/-	-/-	A/-	A/-

I-Acetobacterium woodii MRB I; II- Planococcus citreus MRB II; III- Brevibacterium casei MRB III; IV-Planococcus citreus MRB IV; V- Alteromonas colwelliana MRB V; VI- Alteromonas colwelliana MRB VI VII- Arthrobacter globiformis MRB VII; VIII- Alteromonas nigrifaciens MRB VIII IX- Planococcus citreus MRB IX; X-Cupriavidus necator MRB X; XI – Enterococcus casseliflavus MRB XI
N.B: S. Staining = Spore staining, G. reaction = Gram reaction, Order= arrangement
+ = Positive, - = Negative, A= Acid Production, G= Gas Production,
H_2S = Hydrogen Sulphide Production, R= Rod, C= Coccus, P= Pairs, S= Singly, ST= Straight, CB= Coccobacillus

Table 3. Bonny light crude oil adaptation test of the marine isolates

Isolates	Absorbance (520nm)
Acetobacterium woodii MRB I	3.540
Planococcus citreus MRB II	2.960
Brevibacterium casei MRB III	2.980
Planococcus citreus MRB IV	2.640
Alteromonas colwelliana MRB V	3.060
Alteromonas colwelliana MRB VI	3.380
Arthrobacter globiformis MRB VII	3.520
Alteromonas nigrifaciens MRB VIII	3.180
Planococcus citreus MRB IX	3.240
Cupriavidus necator MRB X	3.580
Enterococcus casseliflavus MRB XI	3.200

Absorbance (520 nm) = Optical density at 520 nanometre wavelength; Values above represent the mean of three different replicate at 1:10 dilution and factor of 1500

Table 5 presents the changes in PAHs concentrations during the crude oil degradation by the test organisms for 28 days laboratory study. The consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) had the highest degrading effect followed by *Pseudomonas aeruginosa* and *Cupriavidus necator* MRB X ranging from 64.779 mg/l, 128.103 mg/l and 82.085 mg/l to 29.777 mg/l, 74.107 mg/l and 68.809 mg/l. Figs. 2-7 also show the chromatographic analyses of initial and final peaks of the PAHs chromatograms after 28 days of biodegradation. The consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) degraded the PAHs faster than the *P. aeruginosa* and *Cupriavidus necator* MRB X ranging from very severely degraded to moderately degraded members of the PAHs biodegraded Bonny light crude oil sample.

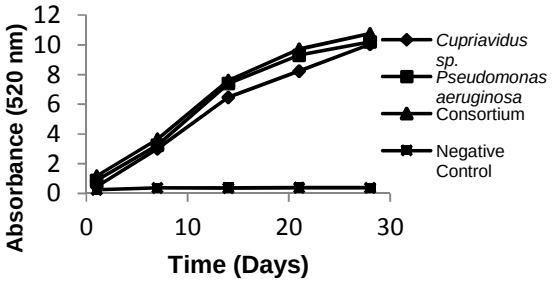

Fig. 1. Growth curves of *Cupriavidus necator* MRB X, *Pseudomonas aeruginosa,* consortium and negative control after 28 days biodegradation

OD_{520} = Optical density at 520 nanometre wavelength
Values above represent the mean of triplicate determination at 1:10 dilution and factor of 1500
Consortium = Acetobacterium woodii MRB I, Arthrobacter globiformis MRB VII and Cupriavidus necator MRB

Industrial use of mercury led to the pollution of the environment. Consequently, mercury removal is a challenge for environmental management [30]. Lower costs and higher efficiency at low

metal concentrations make biotechnological processes very attractive in comparison with physico-chemical methods for heavy metal removal [31]. The use of bacteria for removing metal from contaminated environments is a promising technology. The potential for bioremediation applications of the microbial *mer* operon has been long recognized; consequently, *E. coli* and other wild and genetically engineered organisms for the bioremediation of Hg^{2+} contaminated environments have been assayed by several laboratories [30].

In this present study, seven genera of marine mercury resistant bacteria were isolated from mercurypolluted marine water and are presented in Tables 1 and 2 respectively. The genera of bacterial isolates include *Acetobacterium, Arthrobacter, Planococcus, Brevibacterium, Alteromonas, Enterococcus* and *Cupriavidus.* Three of these organisms, *Acetobacterium woodii* MRB I*, Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X were used for the biodegradation and detoxification of crude oil and mercury studies. Members of these genera have been studied and documented [10,16,32].

Fig. 1 shows the growth curves of the test organism, consortium and negative control without cells. The result showed that consortium growth increased significantly at P (< 0.05) than *Pseudomonas aeruginosa* and *Cupriavidus necator* MRB X. The differences in the results could be associated with the differences in enzymatic capabilities of degrader. The results agrees with the observation of Ilori and Amund [33], that the abilities of these organisms to utilize both low and high molecular weight PAHs are an indication that they likely possess the ring fission enzymes. The absorbance in the control flask was irregular with very slight increase and decrease and vice versa. The result of viable cells recovered after 28 days of incubation period as presented in Table 4 showed that consortium growth increase and decrease significantly at P (< 0.05) than *Pseudomonas aeruginosa* and *Cupriavidus necator* MRB X. The differences in the results especially in Days 14 and 21 may be attributed to membrane toxity of these lipophilic hydrocarbons. These agree with the observation of Sikkema et al*.* [34], that the accumulation of lipophilic hydrocarbons in the membrane lipid bilayer may lead to loss of membrane integrity, increase in permeability to protons and consequently dissipation of the proton motive force and impairment of intracellular pH homeostasis. Cell numbers in the control flask remained practically unchanged throughout the 28 days monitoring period.

Table 4. Total viable count for 28 days biodegradation (Log_{10} cfu/ml)

Time (Days)	*C. necator* MRB X	*P. aeruginosa*	Consortium	Negative control
0	5.80±0.01	5.70±0.01	5.88±0.01	n.d.
7	6.11±0.01	6.32±0.01	6.39±0.01	n.d.
14	6.00±0.10	6.11±0.01	6.23±0.01	n.d.
21	5.75±0.01	5.80±0.01	5.93±0.01	n.d.
28	6.27±0.01	6.39±0.01	6.45±0.01	n.d.

Consortium= Acetobacterium woodii MRB I, Arthrobacter globiformis MRB VII and Cupriavidus necator MRB X; n.d. = not determine = Too low to count (TLTC) i.e. Mean values above are below standard limit: 30-300 colonies; Values are mean±S.D. of triplicate determination. Log_{10}cfu/m = logarithm to base 10 of the mean values of colony forming unit per millimeter

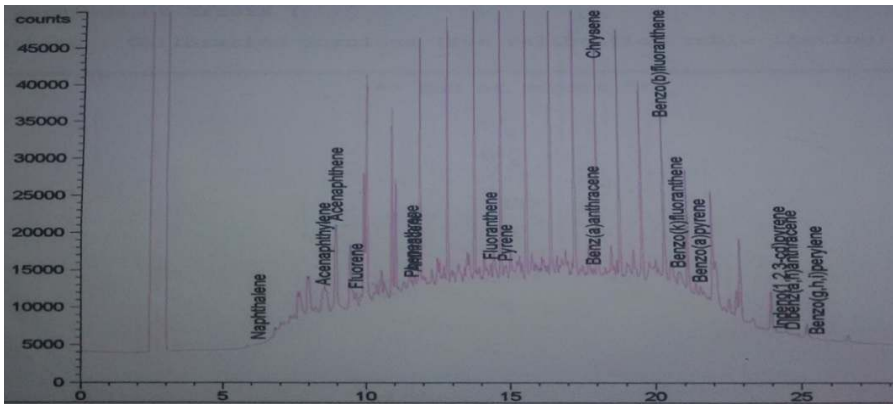

Fig. 2. Chromatographic profile of *Cupriavidus necator* MRB X on day 0

Table 5. Effect of biodegradation on polycyclic aromatic hydrocarbons (PAHs) of bonny light crude oil (mg/l)

Time (Weeks)	*C. necator* MRB X	*P. aeruginosa*	Consortium
0	82.09±0.01	128.10±0.03	64.78±0.01
2	78.16±0.01	109.74±0.01	41.06±0.01
4	68.80±0.01	74.11±0.01	29.77±0.01

Values are mean±S.D. of triplicate determination. Consortium= Acetobacterium woodii MRB I, Arthrobacter globiformis MRB VII and Cupriavidus necator MRB X Mg/l = Concentration of PAHs in milligram per litre

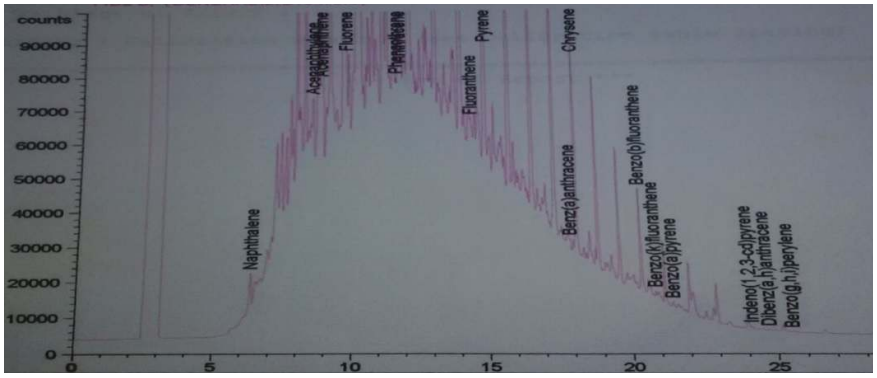

Fig. 3. Chromatographic profile of *Pseudomonas aeruginosa* on day 0

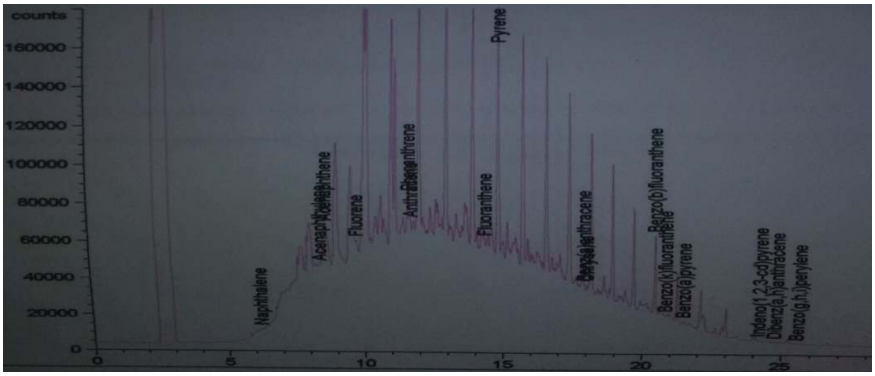

Fig. 4. Chromatographic profile of consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) on day 0

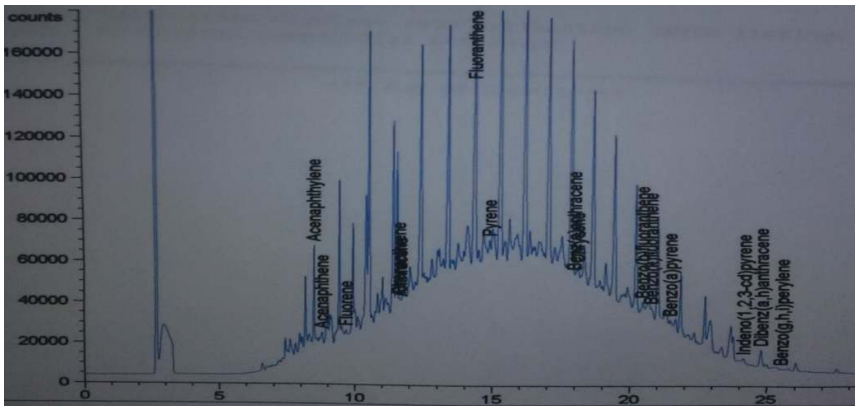

Fig. 5. Chromatographic profile of *Cupriavidus necator* MRB X on day 28

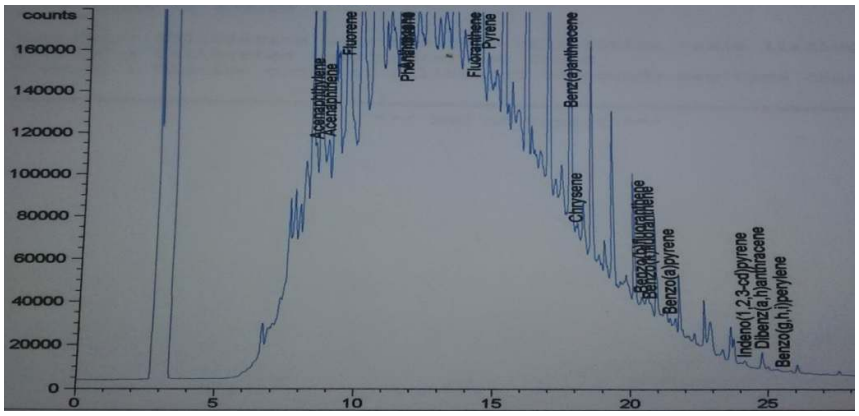

Fig. 6. Chromatographic profile of *Pseudomonas aeruginosa* on day 28

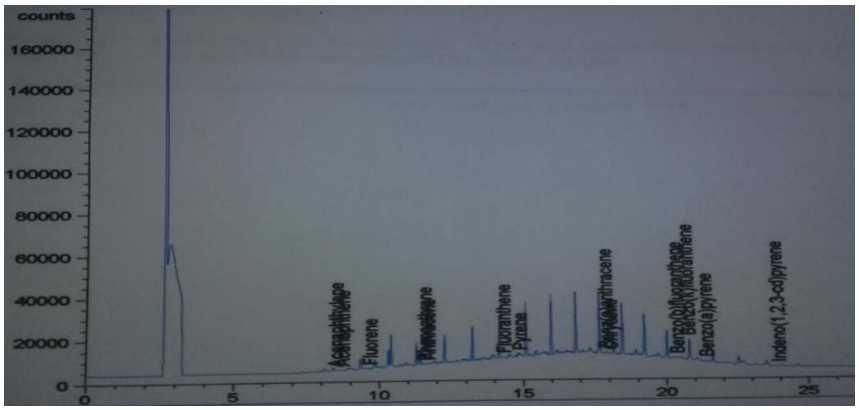

Fig. 7. Chromatographic profile of consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) on day 28

Changes in PAHs concentrations during the crude oil degradation by the test organisms and consortium presented in Table 5 showed that degradation of the oil by the consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) increased significantly at P (< 0.05) than *Pseudomonas aeruginosa* and *Cupriavidus necator* MRB X. The results here showed the possible reaction when marine mercury resistant bacteria (MRB) are used to bioremediate PAHs pollutants in the environment. The fact that these bacteria degraded a large proportion of the PAHs in the crude oil even more than positive control (*Pseudomonas aeruginosa*) which has been implicated by these workers [35,36] for its PAHs degrading ability may explain that these isolates have similar metabolic profiles with most hydrocarbon –degraders. The chromatographic profile of PAHs of the test organisms and consortium as previously mentioned are shown Fig. 2 - 7. In the Fig. 2 – 4 of day 0, there was little reductions in the number and sizes of the peaks in the PAHs component of the Bonny light crude oil by the test organism and consortium. These reductions in number and sizes of the peaks increase and finally lead to very severely degraded peaks for consortium, severely degraded for positive control (*Pseudomonas aeruginosa*) and moderately degraded for *Cupriavidus necator* MRB X after 28 days incubation period as shown in Figs. 5 - 7. These chromatographic observations were clear proofs of biodegradation loss of some of the volatile Bonny light crude oil PAHs components especially in the consortium chromatograms. From this, it appears that preference for a particular hydrocarbon might be associated with the genetic make-up of the organism. This observation was supported by the studies done by Singer and Finnerty, [37] and Okpokwasili et al. [38], in which they stated that the degradation of hydrocarbons was mediated by microorganisms with plasmid-borne genes.

The whole results revealed that the Onne light flow terminal seaport harboured a number of highlymercury- resistant bacteria which are capable of growing or thriving at different concentrations of divalent mercury (Hg^{2+}).

Statistically significant differences were detected in the growth and degradation parameters measured between the test organism *Cupriavidus necator* MRB X, the positive control organism (*Pseudomonas aeruginosa)* and consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X). The differences have shown that both the test organisms and their consortium have higher utilization of Bonny light crude oil though the consortium (*Acetobacterium woodii* MRB I, *Arthrobacter globiformis* MRB VII and *Cupriavidus necator* MRB X) is comparatively better and potent PAHs degrader.

4. CONCLUSION

In this study, the test organism *Cupriavidus necator* MRB X showed plausible biodegradation potential which was almost comparable with the standard strain *Pseudomonas aeruginosa*. The combination of the test organism with other organisms (Consortium) even gave a better result which may be attributed to synergistic reactions between the organisms. Thus, *Cupriavidus necator* MRB X has shown promising bioremediating potentials and could be used alone or in combination with other organisms in the bioremediation of both aquatic and terrestrial environments contaminated with Mercury and Polyaromatic Hydrocarbons.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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