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Bioremediation of Refinery Effluent by Consortium of Bacteria Isolated from Abattoir Waste

Urhibo Voke Owarieta[1], Odesiri-Eruteyan Eunice Asiboroko[1], Emecheta Onyeka Kingsley[1] and Nnaji Praise Tochukwu[2*]

[1]Department of Environmental Management and Toxicology, Federal University of Petroleum Resources Effurun, Delta State, Nigeria.
[2]Department of Microbiology, University of Nigeria Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EOK actively participated in sample collection and analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research was conducted to examine the efficacy of bioremediation of refinery effluent by a consortium of bacterial organisms from abattoir waste.
Study Design: The experimental and analytical research designs were adopted for the study.
Place and Duration of Study: The study was conducted in a laboratory in the Department of Environmental Management and toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria. The research was conducted within three months.
Methodology: Standard methods were adopted for sample collection, microbiological analysis and determination of physicochemical properties.
Results: Out of eight bacterial species isolated from abattoir waste, *Pseudomonas, Proteus* and *Bacillus* species recorded high hydrocarbon utilization potential. There was a significant difference between the physicochemical property of the control and the refinery effluents. The effluents had a mean Biochemical Oxygen Demand (BOD) of 19 mg/l, Dissolved Oxygen (DO) of 3 mg/l, Total

**Corresponding author: E-mail: nnajipraisetochukwu@gmail.com;*

Dissolved Solids (TDS) of 35 ppm, pH of 6.67, Electrical Conductivity (EC) of 71 μs/cm and temperature of 29.5°C. Samples subjected to 0 – 10 days biodegradation test by *Pseudomonas, Proteus* and *Bacillus* species recorded a mean increment in EC, reduction in pH, increase of TDS and reduction in Total Petroleum Hydrocarbon (TPH) with *Proteus* species being the best. The percentage of degradation by *Proteus* was 69.7%, *Bacillu*s 61.56% and *Pseudomonas* 53.19%.
Conclusion: The use of abattoir waste bacteria for bioremediation purposes are very safe, cost-effective and aids the environment from accumulating more burdens of unsafe compounds that render it harmful for living organisms including man.

Keywords: Bioremediation; bacteria; refinery; effluents; abattoir waste; petroleum.

1. INTRODUCTION

The petroleum industries play a major role in the economy of many oil-producing countries in the world. They yield several benefits and as well constitute a major source of pollution to the environments. The emission of volatile compounds, heavy metals and Persistent Organic Pollutants ($POP_{(S)}$) all contribute to the pollution of the environment [1]. The cooling of equipment with water, condensates from steam, water from desalting of crude oil, and all other activities associated with the use of water in the oil and gas industry results in the generation of effluents [2]. Abattoir wastes also constitute major environmental challenges in many countries of the world. This waste supports the growth of certain microbes (more especially bacteria) because of the rich nutrient content which is necessary for the growth of those organisms [3]. Abattoir wastes consist of animal blood, animal faeces, paunch manure, wastewater, bones, horns etc. Their chemical properties are similar to that of municipal sewage [4]. Animal blood has approximately 375,000 mg/l COD and constitutes a major dissolved pollutant in abattoir waste [3].

The use of living organisms like microbes to breakdown or remove contaminants, pollutants and toxins in the environment is known as Bioremediation. Environmental problems like the oil spill, contaminated groundwater etc can be properly addressed through such an environmental friendlier approach. Microbial enzymes play a key role in the biodegradation processes [5]. The inefficient treatment of effluents from refineries is one of the greatest challenges to the environment. Biological treatments of refinery effluents are safer due to their environmental friendliness compared to chemical treatments [6,7,8].

It is worthy to note that the term petroleum does not refer to only liquid hydrocarbon like the premium motor spirit (PMS), Dual-purpose kerosene (DPK) etc., but also includes natural gas and viscous solids known as bitumen [9]. Petroleum is called crude oil when it is released from the ground. It is made up of complex mixtures of varying hydrocarbons. Effluents from petroleum refineries are often characterized by the presence of petroleum products [10,11].

Wastewater and effluents most times contains organic materials that are decomposed by aerobic microorganisms [12]. Some significant amount of oxygen which is referred to as Biochemical Oxygen Demand (BOD) is needed by the microorganisms for this breaking down processes to take place. The reduction in the available oxygen can result in the relocation, weakness or even the death of many living organisms in water bodies [13]. It is true that most refineries subject their wastewaters to some physiochemical and a few biological treatments, however, these pollutants still find their way into natural habitats such as the soil and nearby water bodies [14].

When oxygen is measured in its dissolved form it is referred to as dissolved oxygen (DO) [13]. Electric conductivity (EC) and the total dissolved solids (TDS) are major parameters that are used to determine water quality. These parameters are indicators of salinity level and are correlated. The measure of liquid capacity to conduct electric charge is called EC. It is important to state that EC depends on the dissolved ion concentration, temperature and ionic strength [15].

2. MATERIALS AND METHODS

2.1 Study Site and Sampling

The site of sampling petroleum effluent was Warri Refining Petrochemical Company (WRPC) Ekpan Delta State, Nigeria. It's Global Positioning System (GPS) is N 5º33'48.930'' and E 5º42'58.476. Abattoir waste was sampled from Refinery Junction Abattoir in Uvwie Local

Government Area of Delta State Nigeria with a GPS of N 5º34'12.336'' and E 5º46'54.270'' Samples (petroleum effluent) were collected using sterilized bottles. Abattoir waste was collected using sterile containers. Both Petroleum effluents and abattoir wastewater were .transported in different ice packaged bag for further laboratory analysis.

2.2 Physiochemical Analysis of Refinery Effluent

All physiochemical analysis ranging from pH analysis, Electrical conductivity (EC), Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD) and Dissolved Oxygen (DO) were conducted using Winkler method as illustrated in [16,17].

2.3 Total Petroleum Hydrocarbon

The total petroleum hydrocarbon was determined by the gravimetric method as explained by [18].

2.4 Microbiological Analysis

2.4.1 Serial dilution, culture and Identification of hydrocarbon utilizing bacteria on mineral medium

The culturable bacteria were counted using standard plate dilution method. One ml was suspended in 9 ml of sterile seawater (Ph 7.2) and vortexed for 1min at low-speed aliquots of 100 µL. Thereafter 10 fold serial dilutions were done and dilutions 6-10 were spread on a Mineral Salt Agar (MSA) with the following constituents KH_2 PO_4 (1 g), K_2 HPO_4 (1 g), NH_4 NO_3 (1 g), $MgSO_4$ (0.2 g), FeCl $_2$ (0.05 g), CaCl (0.02 g), agar-agar (15 g) and incubated at 30°C for 7 days.

2.4.2 Characterization and identification of bacterial Isolates

Isolates were characterized and identified by their morphological and biochemical characteristics according to the Bergey's manual of system bacteriology. The biochemical tests that aided in the identification of bacteria species includes; Gram staining, Motility test, Indole test, Citrate utilization test, Catalase test, Oxidase test, Triple Sugar Iron (TSI), Methyl red test (Voges-Proskauer test), Standardization of bacterial isolates and Turbidity test to Screen hydrocarbon degraders were also performed.

2.4.3 Biodegradation experiment

The standard method of biodegrading analysis according to the method of Ogbonna [19] was adopted for the studies.

2.5 Study Design and Data Analysis

The experimental and analytical research designs were adopted for this study. Both refinery effluents and abattoir waster were randomly sampled. Both effluents and abattoir waste were allocated groups (ABW and EFF as indicated in Table 1) after initial exposure to carefully controlled treatments during transportation from the site and in the laboratory. The relationship between two factors (the intervention of bacterial isolates from abattoir waste on refinery effluents) was determined. The rate of outcome was determined by comparison of the outcome on refinery effluents and control (distilled water). The data generated from the research was presented in graphs and tables. The values were analyzed using, measures of central tendencies, Analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Physiochemical Analysis

The results of the physicochemical analysis indicated in the methodology are hereby represented in tables and graphs as illustrated Table 1.

Table 1. The table below illustrates the result of the physicochemical analysis that was conducted on the refinery effluent samples and distilled water (control) (p=0.05)

Parameter	Polluted	Control
Biochemical oxygen demand (mg/l)	19	5
Dissolved oxygen (mg/l)	3	6.5
Total dissolved solids (ppm)	35	179
pH	6.67	7.89
Electrical conductivity(µs/cm)	71	255
Temperature(^{0}C)	29.5	29.7

3.2 Biochemical Analysis

Represented below are the results of the biochemical analysis that was conducted on the culturable bacteria to aid in their identifications.

3.3 Screening Test for the Utilization of Petroleum Hydrocarbon by Bacterial Isolates

The identified bacteria were screened to determine their ability to utilize petroleum hydrocarbon. The results generated are represented in the table.

The Isolates that exhibited heavy and moderate growth rates were used for the biodegradation experiments.

3.4 Biodegradation Set-Up

The figure is a representation of pH of refinery effluent inoculated with *Proteus, Bacillus and Pseudomonas* species (from abattoir waste) in comparison with the control (distilled water) during the biodegradation analysis.

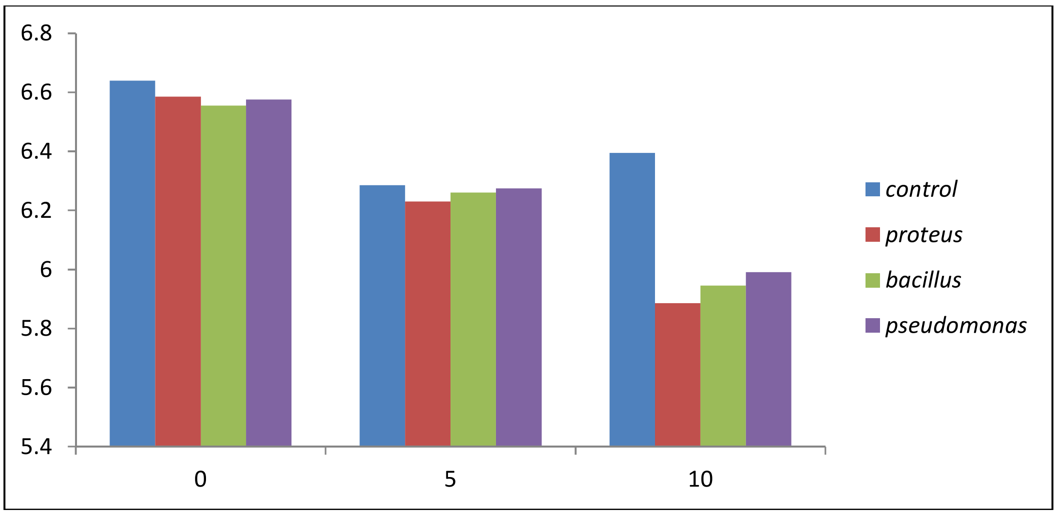

Fig. 1. pH changes during the study

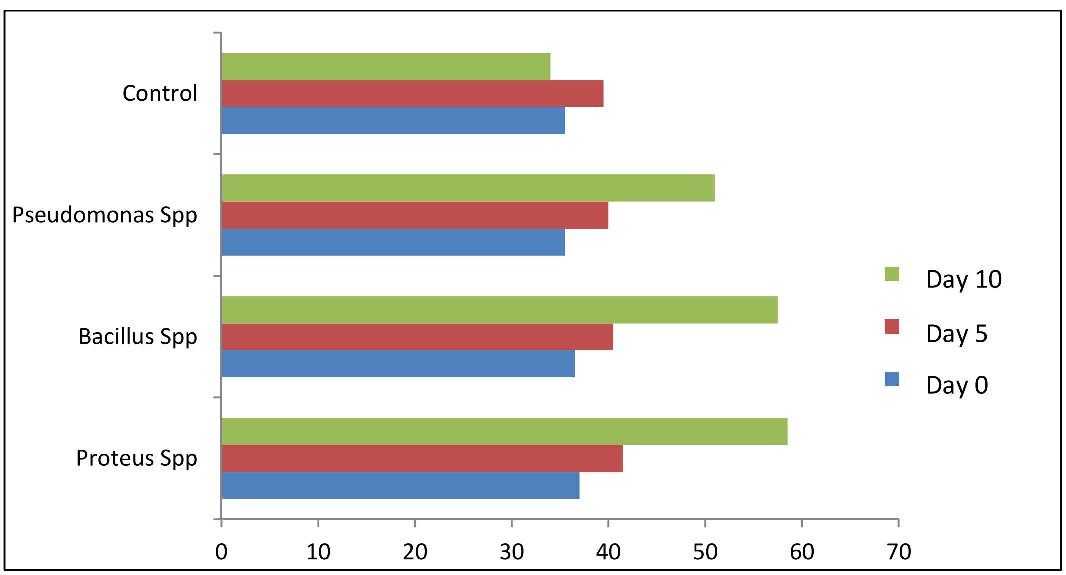

Fig. 2. Electrical conductivity (EC) in μs/cm

The histogram is a representation of Electrical conductivity (EC) (µs/cm) of biodegradation setup with *Proteus, Bacillus and Pseudomonas* species isolated from abattoir waste.

The figure is a graphical representation of Total dissolved solids (TDS) during the biodegradation test by *Proteus, Bacillus and Pseudomonas species*.

The Percentage degradation of Total Petroleum Hydrocarbon by *Proteus*, *Bacillus* and *Pseudomonas* species are as follows: *Proteus*=69.7%, *Bacillus*=61.56% and *Pseudomonas*=53.19%.

3.5 Discussion

Environmental pollution by refinery effluent poses a serious challenge to aquatic and terrestrial organisms. On this note, it is very necessary that refinery effluents are treated properly to reduce the high chances of becoming a potential risk to the environment. In this study microbes isolated from abattoir waste was used for bioremediation of refinery effluent from the Warri refinery and petrochemical company. As presented in Table 2, *Escherichia coli, Pseudomonas* species, *Streptococcus* species, *Proteus* species, *Enterobacter* species, *Bacillus* species, *Micrococcus* species *and Lactobacillus* species were isolated and identified from the abattoir waste. Out of these bacterial organisms *Proteus* species, and *Bacillus* species had the highest petroleum hydrocarbon utilization potential followed by *Pseudomonas* species as indicated in Table 3.

Considering the analysis of variance conducted to determine the level of difference in Total Petroleum Hydrocarbon (TPH) degradation by the identified bacteria, it was observed that at p=0.05, there is a significant difference. Therefore we can accept the alternative hypothesis and reject the null hypothesis. It is important to say that the increment in EC, reduction in pH, increase of TDS and reduction in TPH of effluents inoculated with *Pseudomonas, Proteus* and *Bacillus species* within the time of 0 – 10 days is a confirmation of their bioremediation abilities. However, from the consortium of bacterial isolates from the abattoir waste, *Proteus* species with 69.7% efficiency recorded the highest bioremediation power besides *Bacillus* and *Pseudomonas* which recorded 61.56% and 53.19% efficiencies respectively.

The study actually proofed that the refinery effluent was a pollutant that can reduce and pose serious hazards to the environment. Considering the difference in biochemical oxygen demand between the effluent (19 mg/l) and the control (distilled water) (5 mg/l), such effluent can cause the relocation, weakness or even death of aquatic lives.

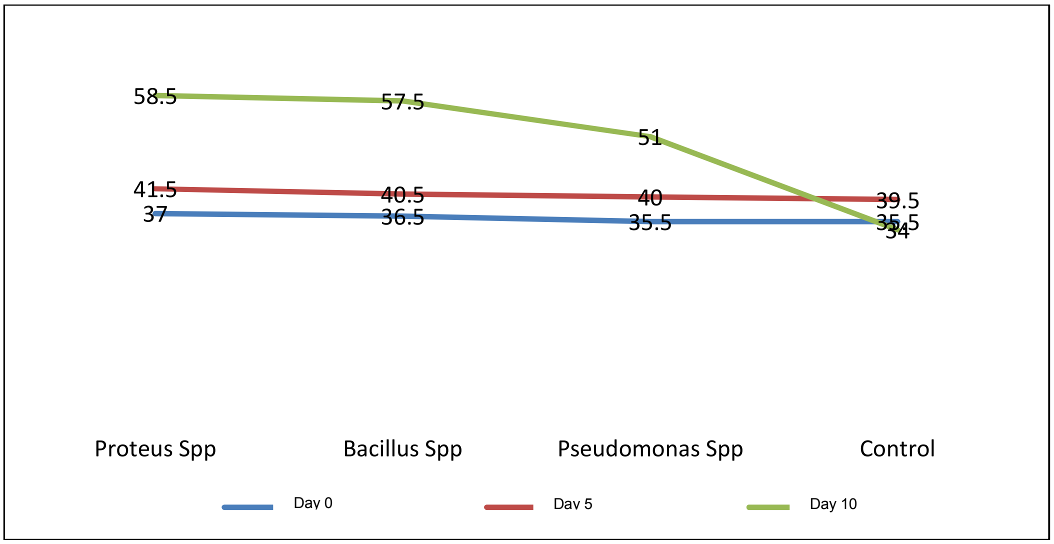

Fig. 3. A graphical representation of total dissolved solid (TDS) in ppm

Table 2. Results of biochemical analysis conducted on the isolate

Isolates	Gram	Shape	Motility	Catalase	Oxidase	Glucose	Sucrose	Gas PR	Acid	Indole	Citrate	H_2S	V.prosk Prosk	Methyl Red	Urease	Probable Isolate
EFF 1	-	R	+	+	+	+	-	-	-	+	-	-	+	-	+	*Pseudomonas* species
ABW 1	+	C	+	+	-	+	+	+	+	+	-	+	-	-	-	*Bacillus* species
ABW 2	-	R	+	+	-	+		+	+	+	-	+	-			*Enterobacter* species
ABW 3	-	R	+	+	+	+	+	+	+	+	+	-	-	+	-	*Escherichia coli*
ABW 4	-	R	+	+	+	+	+	+	+	+	+	-	-	+	-	*Escherichia. coli*
ABW 5	-	R	+	+	-	+		-	+	+	+	-	+	+	+	*Proteus* species
ABW 6	-	R	+	+	-	+	-	-	+	+	+	-	+	+	+	*Proteus* species
ABW 7	-	R	+	+	-	+	-	-	-	+	-	-	+	-	-	*Pseudomonas* species
EFF 2	+	C	+	+	+	+	-	+	-	+	-	-	+	-	-	*Micrococcus* species
EFF 3	+	R	+	-	-	+	+	+	+	+	+	-	-	-		*Lactobacillus* species
EFF 4	+	C	+	+	-	+	+	+	+	+	-	-	-			*Streptococcus* species

Key: EFF=Effluent; ABW =Abattoir waste; +=Positive; - =Negative, R=Round; C=Curved

Table 3. The table below shows the results for petroleum hydrocarbon utilization by bacteria isolate

S/N	Isolate's code	Identified bacterial	growth rate
1	ABW 7	*Pseudomonas* species	**
2	ABW 1	*Bacillus* species	***
3	ABW 5	*Proteus* species	***
4	ABW 2	*Enterobacter* species	*
5	ABW 3	*Escherichia coli*	*
6	EFF 2	*Micrococcus* species	*
7	EFF 3	*Lactobacillus* species	*
8	EFF 4	*Streptococcus* species	*

*Key: ***= heavy growth; **= Moderate growth; *= Little growth*

Table 4. TPH (mg/l) values during monitoring

Day	Control	Proteus	Bacillus	Pseudomonas
0		0.4665		
5	0.389	0.304	0.3125	0.358
10	0.378	0.174	0.212	0.293

Table 5. Analysis of variance (ANOVA) ANOVA was conducted at p=0.05, to determine if there is a significant difference in Total Petroleum Hydrocarbon (TPH) for the degradation setup from the assay

			Summary			
Groups	**Count**	**Sum**	**Average**	**Variance**		
Control	3	1.2335	0.411167	0.002327		
Proteus	3	0.478	0.239	0.00845		
Bacillus	3	0.5245	0.26225	0.00505		
Pseudomonas	3	0.651	0.3255	0.002112		
			Anova			
Source of variation	**SS**	**df**	**MS**	**F**	**P-value**	**F critical**
Between Groups	0.085646	4	0.021411	4.62635	0.047931	4.533677
Within Groups	0.027769	6	0.004628			
Total	0.113415	10				

Key: SS= Sum of square; MS= Mean square

The DO level of 3 mg/l was lower compared to the control which had 6.5 mg/l (Table 1). The low TDS could have also given room for a low EC according to Anna [15]. The findings of the studies are in agreement with [20]. *Pseudomonas* species and *Bacillus* species which was among the major hydrocarbon-degrading bacteria that were isolated from the abattoir waste was also named among the nine organisms which were known to be degraders of hydrocarbon [20].

4. CONCLUSION

The importance of cleaning up of the environment by means that do not pose a threat to other constituents of the environment cannot be overemphasized. It is worthy to note that the use of abattoir waste for bioremediation purposes is cheap when compared to the use of other methods. With the increased risk of hydrocarbon components been identified as members of carcinogens and neurotoxic organic pollutants, the choice of embracing the use of organism form natural waste (like abattoir waste) from our daily anthropogenic activities is a cost-effective approach to bioremediation of refinery effluents. This will save the environment from accumulating more burdens of unsafe volatile compounds and deposit of POP that renders it harmful for living organisms including man.

The prohibition and high cost of disposal of pollutants by incineration and burial in some part of the world is a credit to the adoption of a friendlier and available bioremediation option like this. From the studies and other findings [20,21], it is acknowledged that bioremediation removes hydrocarbon from contaminated sites more than mechanical and chemical methods which have limited effectiveness.

5. RECOMMENDATIONS

The use of abattoir waste for bioremediation should be encouraged by the various institutions of society. Abattoir wastes are readily available and accessible even for industrial usage regardless of geographical location. The uses can also help in the cubing of possible environmental menace that could be caused by the excessive concentration of such waste.

The government and Petroleum industries should release more grant for the research of wastes that can support the growth of microorganisms with high potentials for bioremediation. Further studies can consider a metagenomic analysis of other environmental samples to get organisms with high bioremediation profile.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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