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Research Article

Isolation and Characterization of some Hydrocarbon Utilizing bacteria from Refinery Effluents

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Abstract

The present work was undertaken to isolate and characterize the hydrocarbon degrading bacteria associated with effluent samples collected from Kaduna refinery and petrochemical company (KRPC). The samples were analyzed microbiologically using standard microbiological techniques. Bacillus cereus and Pseudomonas aeruginosa were successfully isolated from the refinery effluents. However, following treatment before the effluents are discharged, the levels of most parameters were brought within permissible limits. From the study, it was apparent that Bacillus cereus and Pseudomonas aeruginosa have the ability to grow and survive in refinery effluents.

Key words: Hydrocarbons, exploration, pollution, physicochemical, effluents, *Bacillus cereus* and *Pseudomonas aeruginosa*

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Introduction

In Nigeria, industrial growth is identified as a major tool for economic development (Adeyeye 2002). The production of useful consumer products is the indicators of this growth. Nevertheless, waste production is also a function of every industrial process. Ironically, these industrial corporations do not consider adequately the functionality of their waste treatment plant so as to accommodate basic treatments such as sedimentation, sand filtration, oil and grease traps and precipitators for gaseous emissions (Adebayo 2007). So, the indiscriminate discharge of solid, liquid and gaseous wastes into the land and municipal drains remains the only way of disposing off their waste. Sometimes, farmers dam the flow of this waste water (industrial effluents) and use for irrigation purposes due to advantageous presence of potassium, nitrogen, phosphorus and other essential elements present in them (Niroula 2003). However, the use of dams polluted by industrial effluents from textiles, shoes, cosmetics, plastics, and other household cum industrial consumables has its negative consequence on the plants through the alteration of the physico-chemical properties of the receiving water body. The aquatic habitats are killed by the toxic chemicals with the resultant disruption of the aquatic ecosystem and its food chain. The decomposition of the organic materials by micro-organisms in the aquatic ecosystem leads to the lowering of the level of dissolved oxygen, which in turn inhibits the growth or cause the death of the aquatic habitats (Onuegbu *et al* 2008).

Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon and energy for metabolic activities and these microorganisms are omni present and widely distributed in the nature. The microbial utilization of hydrocarbons depends on the chemical nature of the compounds within the petroleum mixture and on environmental determinants (Adeline *et al*., 2009). Hydrocarbons enter into the environment through waste disposal, accidental spills, as pesticides and via losses during transport, storage, and use. Hydrocarbon (petroleum)- degrading bacteria are reportedly ubiquitous in the environment and were widely distributed in marine, freshwater, soil habitats and their use in bioremediation of hydrocarbon-contaminated soils, which exploits their ability to degrade and/or detoxify organic contaminants, has been established as an efficient, economical, versatile and environmentally sound treatment (Atlas, 2011; Austin *et al*., 2007). Due to extensive increase in environmental pollution, numerous biodegradative bacteria have been isolated in the past, and their physiology, biochemistry, and genetics have been intensively studied**.** Biodegradation, which is the destruction of organic compounds by microorganisms, is carried out largely by diverse bacterial populations, mostly by *Pseudomonas* species (Boboye *et al*, 2010; Dubey, 2009).

The extensive use of petroleum products leads to the contamination of almost all compartments of the environment, and biodegradation of the hydrocarbons by natural populations of microorganisms has been reported to be the main process acting in the depuration of hydrocarbon-polluted environments Chaillan *et al.*,2004), the mechanism of which has been extensively studied and reviewed. The fuel is a complex mixture of normal, branched and cyclic alkanes, and aromatic compounds obtained from the middle-distillate fraction during petroleum separation.

The aim of the study was to isolate and characterize *Bacillus cereus* and *Pseudomonas aeruginosa* and to analyze some physicochemical parameters from Kaduna refinery effluents

Materials and methods

Collection and handling of refinery effluents

Effluent samples were collected between October, 2015- March, 2016 from Kaduna Refining and Petrochemical Company (KRPC).The samples were collected in sterile wide mouth bottles. The containers were rinsed with the effluents at the points of collection.

The samples were collected by lowering the bottles (with a weighted rope tied to its neck) into the bottom of the well mixed section of the water body, 30 cm deep, and allowed to over flow before withdrawing. After collection, the body of the containers was rinsed thoroughly with water. To avoid deterioration, the samples were transported in ice chest to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria for analysis.

Determination of physicochemical parameters

Physicochemical parameters of the refinery effluents were determined using ASTM (American Standard Methods) (2002) as adopted by the Chemical Laboratory, KRPC, Kaduna. The parameters analyzed were: Temperature, pH, Turbidity, Conductivity, Total Dissolved Solids (TDS), Total Suspended Solids (TSS),Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), chemical oxygen demand (COD), Oil and grease, *Chloride, Total* nitrogen, Sulphate, Phosphate, Lead (Pb) and Chromium (Cr).

Isolation and characterization of *Bacillus* and *Pseudomonas* species from refinery effluent

Ten milliliter (10ml) of the collected refinery effluents was transferred into 90ml of sterile distilled water in a test tube using a sterile pipette to obtain a tenfold dilution. Using sterile 1ml pipette, 0.1ml of the prepared dilutions from 10^{-3}-10^{-6} were aseptically transferred onto the surface of solidified mannitol egg yolk polymyxin agar and centrimide agar for the isolation of *Bacillus* and *Pseudomonas species* respectively and spread evenly using a sterile bent glass rod. Plates were prepared in triplicates. The inoculated plates were incubated at 37^0C and observed for bacterial growth after 24hours (Anon, 2007).

Different colonies observed on the incubated plates were purified by repeated streaking of each distinct colony on nutrient agar until pure colonies were obtained. Purified bacterial isolates were transferred on sterile nutrient agar slants and stored at 4^0C for further identification.

Characterization of the bacterial isolates

Characterization of the isolates was achieved using biochemical tests (Cheesebrough, 2004). A 24 hrs pure culture of each of the isolates was used to determine their Gram's reaction (using the primary and secondary dyes, crystal violet and safranin). The following biochemical tests were carried out: catalase, oxidase, indole, motility, aerobic growth, anaerobic growth, fluorescent pigmentation, starch hydrolysis, gelatin Liquefaction, o-nitro phenyl beta galactosidase (ONPG), and Voges proskauer tests respectively. The isolates identified using conventional biochemical tests were further authenticated using microgen identification system (*Wang et al*., 2012).

Data analysis

Data obtained from the study was analyzed using statistical package for social sciences (SPSS) version 21. Statistically, significant association between time and increase in bacterial cell numbers was determined and analyzed using the two way analysis of variance (ANOVA). The ANOVA decision criterion was employed using 95% confidence interval with $P < 0.05$ as statistically significant

Results and Discussion

Table 1: Physico-chemical properties of raw, treated and discharge point refinery effluent used as source of the bacterial isolates

	Effluent			Permissible
Parameters (FEPA)	Raw	Treated	Discharge	limits
Temperature 0^{0}C	29^{0}C	27^{0}C	28^{0}C	40^{0}C
pH	6.21	6. 61	6.63	6-9
Turbidity (NTU)	70.1	6.33	6.28	5.82
Conductivity (µs/cm)	103.2	97.2	96.6	400
Total Dissolved Solids (mg/l)	260.0	180.0	178.4	2000
Total Suspended Solids (mg/l)	210.0	200.0	193.0	30
Dissolved Oxygen (mg/l)	150.0	2.3	3.6	4.0
Biochemical Oxygen Demand (mg/l)	30.0	1.3	2.50	50
Chemical Oxygen Demand (mg/l)	400.0	170.0	168.0	40
Oil and Grease (mg/l)	540.0	40.0	23.0	10
Chloride (mg/l)	12.50	8.0	7.2	10
Total Nitrogen (mg/l)	1.15	1.97	2.40	10
Phosphate (mg/l)	5.56	6.96	6.98	5.0
Lead	8.13	0.01	0.01	0.03
Chromium	5.04	0.01	0.01	0.01

FEPA = Federal Environmental Protection Agency

The observations made in this study indicate that all the physicochemical properties of the raw refinery effluents, with the exception of temperature and pH, far exceed the limit that could permit the survival and growth of some microorganisms. For instance, the low level of dissolved oxygen (DO), and high levels of biological oxygen demand (BOD), Chemical oxygen demand (COD) and Oil and Greases could pose a serious challenge to the survival and growth of microorganisms (Obayori *et al*. 2009; Barathi and Vasudevan, 2010). Under such conditions, only microorganisms with the requisite adaptations could survive and grow in it. Organisms capable of surviving and growing in an environment provided by the raw refinery effluents, therefore, should be adapted to the general toxic properties of the refinery effluents (Barathi *et al*. 2010). In addition such organisms should be able to depend on hydrocarbons as their sole source of carbon and energy for cellular growth (Debajit and Yadav, 2014). It is conceivable therefore that, such an environment as provided by the refinery effluents, is most likely to be populated by hydrocarbon degrading strains of bacteria (Okerentugba and Ezeronye 2003; Ojo, 2006; Idise *et al*. 2010; Obinna *et al*. 2014). This assertion is strongly supported by the drastic reduction in the level of DO, BOD, COD as well as oil and greases observed following effluent treatment strategy wholly dependent on the natural flora employed by the Kaduna Refinery.

Characterization of the isolates from Kaduna refinery and petrochemical company (KRPC) effluent presented in Tables 1 and 2 identified the isolates to be *Bacillus cereus* and *Pseudomonas aeruginosa*.

Table 2: Cultural, morphological and biochemical characteristics of the isolates

Characteristic/Test	Isolate 1	Isolate 2
Colony Shape	Spherical	Round Spherical
Colony Colour	Brown	Fluorescent greenish
Shape of cell	Rod	Rod
Motility	+	+
Gram's staining	+	-
Aerobic growth	+	+
Anaerobic growth	+	-
Fluorescent Pigmentation	-	+
Catalase	+	+
Oxidase	+	+
Indole	-	+
Starch hydrolysis	+	-
Gelatin liquefaction	+	+
Voges proskauer	+	-
Identity	*Bacillus cereus*	*Pseudomonas aeruginosa*

KEY: + = Positive; **-** Negative

Physico-chemical analysis of the samples of refinery effluents revealed only slight differences between the temperature and pH of the raw, treated and discharge point samples (Table 3). It was observed that, the levels of turbidity, Total dissolved solids (TDS), Total suspended solids (TSS), Dissolved oxygen (DO), Biological oxygen demand (BOD), Chemical oxygen demand (COD) and Oil and Greases (O&G) were relatively high in the raw refinery effluents as expected of all untreated industrial waste waters.

However, marked decreases were observed in the Turbidity, DO, COD, BOD and Oil and Greases at the effluent treatment and discharge points (Table 3). On the other hand, slight increases in the total nitrogen (N) and Phosphate contents were observed in the effluent treatment and discharge points (Table 3).

The isolation of hydrocarbon utilizing strains of *B. cereus* and *P. aeruginosa* from the refinery effluents is considered as evidence that these strains are active hydrocarbon degraders in that environment (Chikere and Ekwubu 2014).

The preponderance of wild strains of *Bacillus cereus and Pseudomonas aeruginosa* in the refinery effluents observed in this study agrees with the reports of Idise *et al*., (2010). This observation is not unexpected in view of many earlier reports to the effect that, consistent exposure of non- hydrocarbon degrading strains indigenous to hydrocarbon polluted sites often result in the emergence of Hydrocarbon degrading strains (Nilanjana and Prethy, 2014).

To be able to survive such an environment, these bacteria must have developed enzymatic and physiological responses that allow them to utilize the hydrocarbons as substrates (Ojo 2006).It also indicates that, the refinery effluents contain appreciable populations of indigenous active hydrocarbon degrading strains of these bacteria (Chikere and Ekwubu, 2014).

Conclusion

In conclusion, hydrocarbon utilizing bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) were isolated from Kaduna refinery effluent. The observation made in this study strongly suggests that, refinery effluents could provide a rich source of hydrocarbon degrading bacteria and the proper identity of such isolates should be thoroughly confirmed using techniques other than their biochemical profiles.

Table 3: Biochemical characterization and identification of *Bacillus cereus* and *Pseudomonas aeruginosa* using Microgen Bacillus Strip (BAC 1 & 2), GNA and GNB-ID system

	Bacillus Strip 1 (BAC 1)												**Bacillus Strip 2 (BAC 2)**													
Isolate Code	**ARA**	**CEL**	**INO**	**MAN**	**MNS**	**RAF**	**RHA**	**SAL**	**SOR**	**SUC**	**TRE**	**XYL**	**ADO**	**GAL**	**MDM**	**MDG**	**INU**	**MLZ**	**IND**	**ONPG**	**ARG**	**CIT**	**VP**	**NIT**	Octal code	Identity
01	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	00260013	*Bacillus cereus* (99.11%)
	G N A Wells												**G N B Wells**													
02	**Lysine**	**Ornithi**	**H2S**	**Glucose**	**Mannito**	**Xylose**	**ONPG**	**Indole**	**Urease**	**V. P.**	**Citrate**	**TDA**	**Gelatine**	**Malonat**	**Inositol**	**Sorbitol**	**Rhamno**	**Sucrose**	**Lactose**	**Arabino**	**Adonito**	**Raffinos**	**Salicin**	**Arginin**	00510022	*Pseudomonas aeruginosa* (87.09%)
	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-		

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