BIO-REMEDIATION OF REFINERY EFFLUENTS BY STRAINS OF PSEUDOMONAS AERUGENOSA AND PENICILLIUM JANTHINELLUM

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Abstract. A study was conducted to evaluate the potentials of *Pseudomonas aeruginosa* and *Penicillium janthinellum* and their mutants in degradation of crude oil in river Kaduna effluents after two weeks incubation at 30°C, Degradation potentials ranged from partial to good. A mixture of the pure strains and mutants (subjected to 10-minute irradiation) of the two micro-organisms, as well as the pure strains of *Pseudomonas aeruginosa* showed the best degradation. Five and fifteen minute mutants of the two micro-organisms, as well as the pure strains of *Penicillium janthinellum* showed partial degradation. Against pristine and phytane as biomarkers, Carbon 23 (C_{23}) did not appear in the chromatogram of effluents that had undergone partial or good degradation.All consortia were observed to have significant decreases in contents of phenol, oil and grease, phosphates, ammonia, nitrates, and sulphates after two weeks of incubation at 30°C. A comparative analysis of the effluent after two weeks of incubation in relation to FEPA specifications and KRPC treated waste water (TWW) after bioremediation, revealed that, in River Kaduna water sample the phosphate concentration of most consortia were greater than TWW, and FEPA limits, except for consortia G^1 and H^1 that were lower. Other physicochemical parameters showed a lower concentration compared to that of TWW. At the end of experiment, all the consortia except G^1 and H^1 were lower than the FEPA limits for oil and grease. Similar occurrence was observed in phenol concentration for all the consortia.
Keywords: *Biodegradation, hydrocarbons, micro-organisms, physico-chemical characteristics*

Introduction

Nigeria became an exporter of oil when production reached 6000 barrels per day. The current daily production is over two million barrels [5, 13]. Crude oil exploration and production, petroleum refining and marketing operations have several attendant environmental problems [2]. Production effluents consist mainly of produce formation waters emanating continuously from different oil bearing formations together with crude oil and associated gas or condensate [17]. Produce formation waste poses great danger when disposed into fresh waters because of its salinity, heavy metal and Polycyclic Aromatic Hydrocarbons (PAHs) content [21, 20]. Wastewater released by crude oil processing and petrochemical industries are characterized by the presence of large quantities of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulfides, naphthylenic acids and other chemicals [19, 21]. Due to the ineffectiveness of purification systems, wastewaters may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem [7].

Various studies have shown positive correlations between pollution from refinery effluents and the health of aquatic organisms. Previous observations [12], suggested a correlation between contamination of water and sediments with aromatic hydrocarbons from refinery effluents, and compromised fish health. An earlier study [16] demonstrated the accumulation of heavy metals with accompanying histopathology in

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Oreochromis niloticus exposed to treated petroleum refinery effluent from the Kaduna Refinery and Petrochemical Co. Ltd.

Wastewater containing high concentrations of pollutants or having uncomfortable pH is always more difficult and highly expensive to treat [14]. This indicates the need for a more efficient and cheaper secondary method of cleaning up wastewater.

Bioremediation is a new method of oil spill clean up that is far more effective than any of the mechanical methods used [1, 6, 20]. This involves the use of micro-organisms ('Petrophiles') to breakdown complex materials into simple end products. These products exist either naturally in the environment or are artificial ("xenobiotic") products [9]. Biodegradability is important for determining the behaviour of such chemicals in the environment. Within the ecosystem, micro-organisms have evolved a host of enzymes that aid in biodegradation of natural products.

This microbial clean-up method cleans the oil as well as a number of other harmful pollutants and is perhaps the best, most environmentally safe process used today. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source [9]. This makes them singularly qualified for cleaning oil spills and even tanker bottoms containing oil residue. In bioremediation, several different types of these micro-organisms are used. Bioremediation efforts have been hardly, if at all applied in the Nigerian, and possibly West African situation. In view of the fact that Nigeria is now a major player in the oil industry, and with regards to environmental concerns that are increasingly being expressed, it has become more and more important to find solutions to problems of poor environmental management. The objective of this study therefore is to evaluate the efficiency of pure and mutant strains of two micro-organisms (*Pseudomonas aerugenosa* and *Penicillium janthinellum*) in degradation of hydrocarbons and other potentially harmful substances from the Kaduna Refinery and Petrochemical Company

Materials and methods

Study site

The Kaduna Refinery and Petrochemical Company (KRPC) occupies a land area of 2.89 square kilometres approximately 15km Southeast of Kaduna city [11] (See map; Fig 1). Its location has an elevation of approximately 615m above mean sea level [8]. Kaduna Refinery was constructed by the Chiyoda Chemical Engineering and Construction Company (now Chiyoda Corporation) and was commissioned in 1980 with an initial capacity of 100,000 BPSD as the third Refinery in Nigeria in order to cope with the tremendous and growing demand for petroleum products [11]. In December 1986, the design capacity of the fuels plants of the Refinery was successfully increased by an additional 60,000 BPSD to the initial 50,000 BPSD, bringing the total refinery installed capacity to 110,000 BPSD [11].

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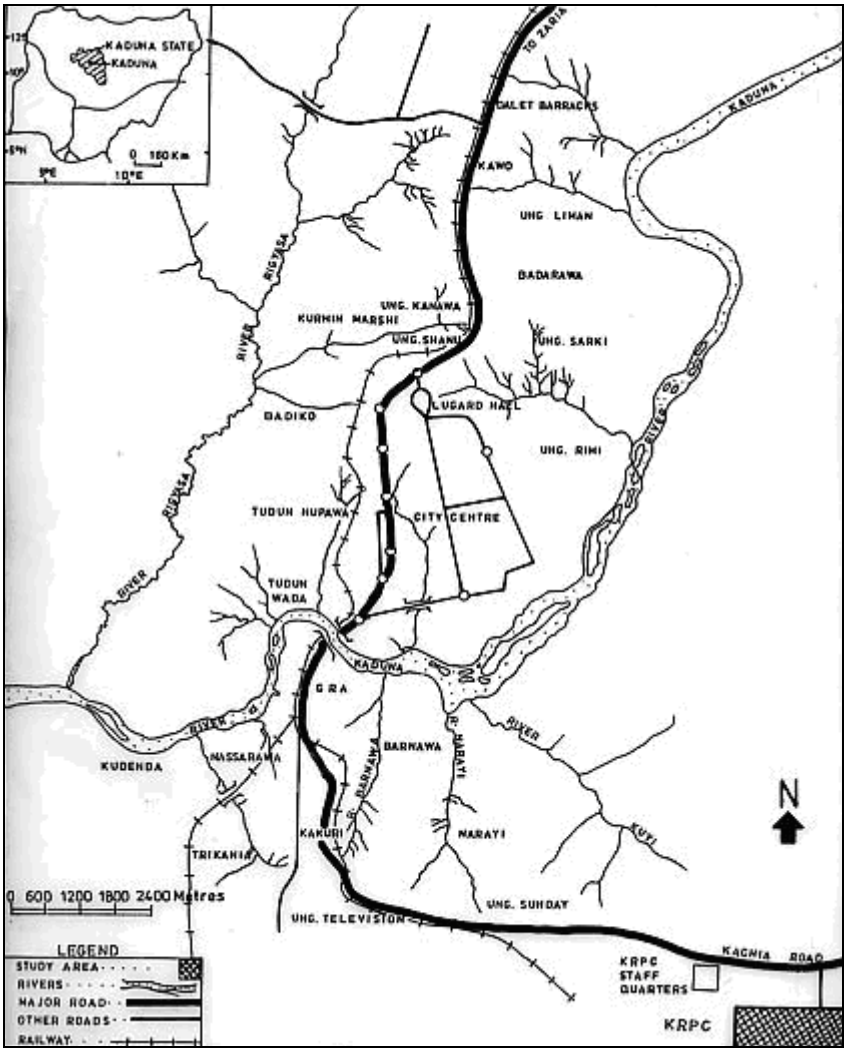

Figure 1. *Map of Kaduna city*

The Refinery was designed to process two types of crude oils: the imported heavy crude and Nigerian light crude into fuels and lubes products [11]. The Nigerian light crude which is basically naphthenic is reserved for the manufacture of fuel products and the imported heavy crude, that is paraffinic is on the other hand, used mainly for the production of lubricating oils, waxes and asphalts [11]. Consequently, the refinery has two process sections: the Fuel section and the Lubricating Oil, wax and Asphalt section [8].

Microorganisms

Pseudomonas aerugenosa and *Penicillium janthinellum* were obtained from the stock culture of the Department of Pharmaceutical Sciences and Department of Microbiology respectively.

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Induction of mutant strains

A Phillip's germicidal Lamp (of 254nm short wavelength) was used to induce mutations in three out of 4 batches each of *Pseudomonas aerugenosa* and *Penicillium janthinellum* grown in Petri dishes. Three batches from each species were irradiated for 5, 10, and 15 minutes. Mutants were immediately transferred into fresh Petri dishes and allowed to grow for 8 days before they were used in the experiment.

Crude oil, refinery effluent and River Kaduna samples

Samples of effluent and river Kaduna water were respectively collected separately using 2L, 4L and 50L sterile polyethylene vessels at KRPC refinery, Kaduna and River Kaduna, while crude oil was collected from fuels laboratory, KRPC. Samples of KRPC effluents and River Kaduna water were transported in ice chests and analysed for some physicochemical parameters when the temperature of samples had normalized within 2 weeks of cultivation.

Inocula development

Colonies of Pseudomonas aerugenosa (Ps) and Penicilium janthinellum (Pe) and their mutants (Ps5, Ps10, Ps15, Pe5, Pe10, Pe15) in different combinations of Ps alone, Pe alone, PsPe, Ps5Pe5, Ps10Pe10, and Ps15Pe15 were transferred respectively from agar plates to 6 containers containing 1.5g of fertilizer, 100ml of wastewater and 400ml of River Kaduna samples. These media were manually shaken and cultivated for 24 hours at room temperature. Of these, grown cultures were used to inoculate fresh culture media, which were cultivated at the same conditions for 2 weeks.

Culture medium

One hundred milligrams (100ml) of waste water samples was supplemented with 1.5g of fertilizer containing 660ppm of NH_4NO_3:460 ppm of PO_4^{-2} (1:4:1) in 1L of water from river Kaduna water.

The control experiment

Control I: 100ml of Treated Waste Water samples + 1.5g of fertilizer + 1L of Distilled water. Control I: 100ml of Treated Waste Water samples + 1L of water from river Kaduna. Control III: 100ml of Treated Waste Water samples + 1.5g of fertilizer + 1L of water from river Kaduna.

Experimental set up

Thirty six (36) polyethylene containers (3L each) with caps were used throughout the course of this experiment. Nine (9) of these containers were used for inocula development, 18 containers for the culture medium (i.e. 6 containers with 3 replicates each), while the remaining 9 served as the control (i.e. 3 containers with 3 replicates each). These set ups were arranged in a Randomized Complete Block Design at room temperature of 25 – 30°C in the Laboratory.

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Gas/liquid chromatographic determination of oil biodegradation

Nine set ups each of three replicates for the investigations were: (1). Pseudomonas aerugenosa, (2) Penicillium janthinelum, (3): Pseudomonas aerugenosa and Penicillium janthinelum,(4) Pseudomonas aerugenosa and Penicillium janthinelium Irradiated with 254nm UV lamp for 5 minutes, (5) for 10 minutes, (6) for 15 minutes, (7) Control I, (8) Control II, (9) Control III. Each set up was replicated three times.

Ten milliliters (10ml) of water from river Kaduna was added to each test tube. Next, 1ml of crude oil was added to each test tube and allowed to form a thin layer over the water surface. Control experiments were (7) Control I: 5ml of Distilled water + 1ml of Crude Oil; (8) Control II: 10ml of River Kaduna + 1ml of Crude Oil; (9) Control III: 10ml of River Kaduna + 0.5g of Fertilizer. With a sterile pipette, Set-up No. 1 was inoculated with 2ml of the Pseudomonas aerugenosa, Set-up No. 2 was inoculated with 2ml of Penicilium janthinellum, Set-up No. 3 was inoculated with 1ml of both microbes, and Set-ups Nos. 4, 5, and 6 were inoculated with 1ml of UV irradiated strains both microbes. A cap was placed on each tube and each was inverted several times to allow the micro-organisms to mix with the crude oil. Caps were loosened one-half turn and the set-ups were incubated at 30^{o}C. Each tube was observed and inverted every 24 hours for 16 days. Each set up was subjected to a Gas/Liquid Chromatographic analysis.

Results

Table 1 presents a summary of the results for the degradation of crude in river Kaduna effluents after two weeks incubation at 30°C, by *Pseudomonas aeruginosa* and *Penicillium janthinellum* and their mutants in different consortia. Degradation potentials ranged from partial to good. A mixture of the pure strains and mutants (subjected to 10-minute irradiation) of the two micro-organisms, as well as the pure strains of *Pseudomonas aeruginosa* showed the best degradation. Five and fifteen minute mutants of the two micro-organisms, as well as the pure strains of *Penicillium janthinellum* showed partial degradation.

Figures 2-5 show typical chromatographic profiles of the effluents under no inoculation, no degradation, partial, and good degradation conditions after two of weeks incubation at 30°C respectively. Against pristine and phytane as biomarkers, Carbon 23 (C_{23}) did not appear in the chromatogram of effluents that had undergone partial or good degradation.

All consortia were observed to have significant decreases in contents of phenol, oil and grease, phosphates, ammonia, nitrates, and sulphates after two weeks of incubation at 30°C (Tables 2-7).

A comparative analysis of the effluents after two weeks of incubation (Table 8) in relation to FEPA specifications and KRPC treated waste water (TWW), revealed that, the phosphate concentration of most consortia were greater than TWW, River Kaduna and FEPA limits, except for consortia G^1 and H^1 that were lower. Other parameters showed a lower concentration compared to that of TWW. At the end of experiment, all the consortia except G^1 and H^1 were lower than the FEPA limits for oil and grease. Similar occurrence was observed in phenol concentration for all the consortia.

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Table 1. *Gas/liquid chromatographic analysis of crude oil in a bioremediation scheme using Pseudomonas aerugenosa and Penicillium janthinellum and their mutants*

Consortia	Degradation rating
A^1 (E + Rk + N + *Pseudomonas aerugenosa*)	D
B^1 (E + Rk + N + *Penicillium janthinellum*)	C
C^1 (E + Rk + N + PePs)	D
D^1 (E + Rk + N + Pe_5Ps_5)	C
E^1 (E + Rk + N + $Pe_{10}Ps_{10}$)	D
F^1 (E + Rk + N + $Pe_{15}Ps_{15}$)	C
G^1 (E + Dt)	B
H^1 (E + Rk)	B
I^1 (E + Rk + N)	C

Note: B: No Degradation, C: Partial Degradation, D: Good Degradation. E: Effluent, Rk: Water from river Kaduna, N: N.P.K fertilizer, PePs ($_{5,10,15}$): *P. aerugenosa* and *P. janthinellum* irradiated at 5, 10 and 15 minutes of UV (254nm).

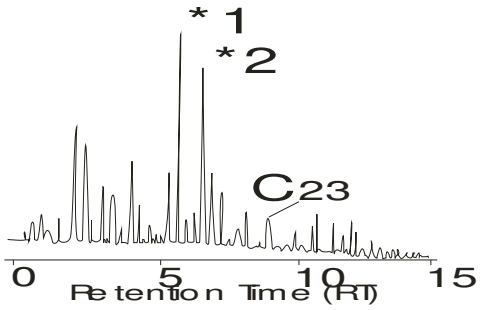

Figure 2. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates without inoculation (Sterile, A) with Pseudomonas aerugenosa and Penicillium janthinellum after two weeks at 30°C.*

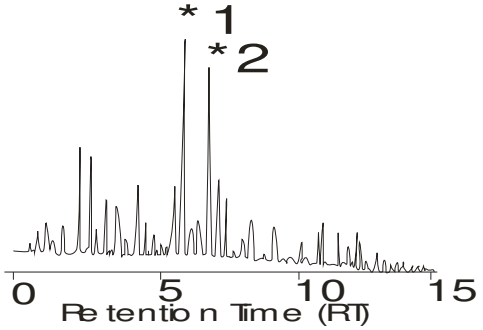

Figure 3. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates showing no degradation (B) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

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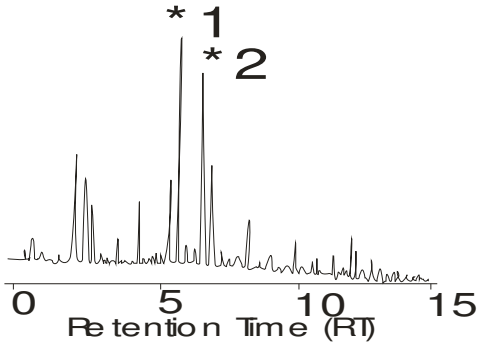

Figure 4. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates partial degradation (C) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

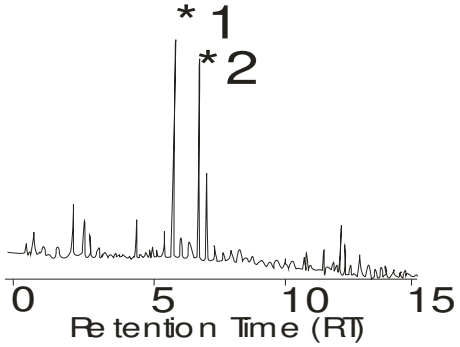

Figure 5. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates showing good degradation (D) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

Table 2. *Oil and grease (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	**A[1]**	**B[1]**	**C[1]**	**D[1]**	**E[1]**	**F[1]**	**G[1]**	**H[1]**	**I[1]**
0	12.48^{c}	10.80^{c}	11.40^{c}	12.50^{c}	12.60^{c}	12.00^{c}	12.30^{c}	11.40^{c}	11.80^{b}
4	10.23^{b}	8.47^{b}	7.49^{b}	10.48^{b}	8.59^{b}	10.75^{c}	12.20^{c}	11.20^{c}	9.30^{b}
8	9.74^{b}	6.37^{a}	6.91^{b}	10.15^{b}	6.45^{a}	10.25^{b}	12.01^{c}	11.01^{c}	7.01^{b}
12	9.48^{b}	4.75^{a}	5.30^{a}	9.13^{b}	4.41^{a}	9.67^{b}	11.50^{c}	10.60^{c}	5.52^{a}
16	9.25^{b}	3.45^{a}	4.80^{a}	7.87^{b}	3.74^{a}	8.95^{b}	11.01^{c}	10.20^{b}	3.61^{a}

Values with similar superscripts do not have any significant differences within columns.**Key: A[1]:** Rk+Ef+N+Pe**, B[1]:** Rk+Ef+N+Ps, **C[1]:** Rk+Ef+N+PePs, **D[1]:** Rk+Ef+N+Pe_5Ps_5, **E[1]:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F[1]:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G[1]:** Rk+Ef, H^{1}: Dt+Ef, **I[1]:** Rk+Ef+N.

[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 3. *Phenol concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	3.21^c	1.94^b	3.04^c	2.78^c	3.10^c	2.48^b	1.48^b	2.14^b	1.79^b
4	0.90^a	0.00^a	0.71^a	0.87^a	0.30^a	2.01^b	1.24^a	1.94^b	0.68^a
8	0.67^a	0.00^a	0.50^a	0.51^a	0.20^a	1.49^b	1.41^b	1.97^b	0.71^a
12	0.49^a	0.00^a	0.30^a	0.41^a	0.16^a	1.18^a	0.80^a	1.60^b	0.50^a
16	0.30^a	0.00^a	0.10^a	0.30^a	0.10^a	0.80^a	0.50^a	1.08^a	0.20^a

Values with similar superscripts do not have any significant differences within columns **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N. [River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 4. *Ammonia concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	1.48^c	1.24^c	1.35^c	0.89^b	1.41^b	0.59^b	0.57^b	1.08^c	1.44^c
4	1.34^c	1.18^c	1.21^c	0.82^b	1.02^b	0.54^b	0.56^b	1.04^b	1.21^c
8	1.14^c	0.84^b	0.75^b	0.67^b	0.52^a	0.50^a	0.50^a	0.87^b	0.87^b
12	1.04^b	0.52^a	0.65^b	0.57^b	0.48^a	0.44^a	0.50^a	0.84^b	0.51^a
16	1.02^b	0.20^a	0.50^a	0.50^a	0.45^a	0.31^a	0.50^a	0.92^b	0.20^a

Values with similar superscripts do not have any significant differences within columns.
Key: A^1: Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.
[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 5. *Nitrate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	1.00^c	0.84^c	0.53^b	0.88^c	1.01^c	0.94^c	0.49^b	0.61^b	0.98^c
4	0.80^b	0.61^b	0.48^b	0.80^b	0.84^c	0.91^c	0.48^b	0.60^b	0.67^b
8	0.67^b	0.42^b	0.39^b	0.72^b	0.61^b	0.74^b	0.46^b	0.58^b	0.61^b
12	0.54^b	0.24^a	0.26^a	0.68^b	0.50^b	0.52^b	0.46^b	0.52^b	0.55^b
16	0.50^a	0.10^a	0.20^a	0.64^b	0.50^b	0.54^b	0.42^b	0.46^b	0.48^b

Values with similar superscripts do not have any significant differences within columns. **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 6. *Sulphate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	20.80^d	18.70^c	19.48^d	21.04^d	20.70^d	24.00^e	5.05^a	6.19^a	21.00^d
4	20.10^d	16.60^c	15.92^b	20.14^d	18.50^c	23.03^e	5.02^a	6.10^a	18.60^c
8	19.50^d	15.20^b	15.10^b	19.70^d	16.60^c	21.13^d	5.00^a	5.98^a	17.22^c
12	18.30^c	13.70^b	14.50^b	18.90^c	15.50^b	21.00^d	5.00^a	5.96^a	16.63^c
	17.40^c	12.60^b	13.70^b	18.01^c	14.40^b	20.60^d	4.90^a	5.94^a	15.20^b

Values with similar superscripts do not have any significant differences within columns.**Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 7. *Phosphate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	50.00^d	50.40^d	50.00^d	50.40^d	50.00^d	50.77^d	0.10^a	0.50^a	49.70^e
4	47.20^d	44.30^d	46.10^d	49.20^d	47.00^d	48.90^d	0.10^a	0.40^a	46.70^e
8	40.30^c	36.50^c	39.70^c	41.30^c	38.70^c	41.80^d	0.10^a	0.40^a	39.00^d
12	33.10^c	29.70^c	29.90^c	31.80^c	30.40^c	38.40^c	0.10^a	0.30^a	31.50^d
16	26.70^b	21.20^b	23.50^b	29.40^c	22.70^b	35.50^c	0.10^a	0.30^a	27.90^c

Values with similar superscripts do not have any significant differences within columns. **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.
[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 8. *Comparative physico-chemical quality [mg/L]of KRPC treated wastewater (TWW), River Kaduna water and consortia after bio-remediation*

	Oil and grease	Phenol	Ammonia	Nitrate	Sulphate	Phosphate
TWW	13,50	3,45	1,48	0,50	18,80	5,80
River Kaduna water	0,05	<0.1	0,78	0,05	2,02	0,01
FEPA Limitation Guideline (1991)	10,00	0,50	0,20	0,20	NI	NI
A^1	9,25	0,30	1,02	0,50	17,40	26,70
B^1	3,45	0,00	0,20	0,10	12,60	21,20
C^1	4,80	0,10	0,50	0,20	13,70	23,50
D^1	7,87	0,30	0,50	0,64	18,01	29,40
E^1	3,74	0,10	0,45	0,50	14,40	22,70
F^1	8,95	0,80	0,31	0,54	20,60	35,50
G^1	11,01	0,50	0,50	0,42	4,90	0,10
H^1	10,20	1,08	0,92	0,46	5,94	0,30
I^1	3,61	0,20	0,20	0,48	15,20	27,90

Discussion

The synergy of microbial systems was effective in the degradation of pristane and phytane (biomarkers) as well as other hydrocarbons in the consortia.The highest percentage (70%) break down of oil and grease was observed in C^1 . This explains the synergistic potentials of the combined culture of the bacterium (*P. aerugenosa*) and fungus (*P. janthinellum*) in this bioremediation. Consortia I^1 was also very effective in oil and grease biodegradation. FEPA [10] requirements for oil and grease in wastewater release/discharge were met by most microbial systems except for the controls, which had very low biological activities.

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Significant decreases in phenol concentration of all consortia (especially the controls G^1 and H^1) agree with a report (15) that there was decrease in phenol in consortia with low biological activities because of the volatility of phenol (21). Weathering/evaporation may have contributed to the loss of phenol in these consortia (15). The efficacy of consortium B^1 that contained effluent, River Kaduna water sample, NPK fertilizer and *Pseudomonas aerugenosa* in this bioremediation scheme, was confirmed by the 100% degradation of phenol. This bacterium has often been cited as one of the most efficient phenolytic micro-organisms [16] due to its capability to transport and metabolize phenols and other hydrocarbons [7]. All consortia except consortia F^1, G^1 and H^1 met the FEPA requirements/guideline for treated wastewater.

The effectiveness of bio-augmentation, bio-stimulation and/or synergistic potentials of combined microbial cultures in bioremediation was observable in the cases of Nitrates, Ammonia, and Sulphates. In the case of phosphates only the colonies irradiated at 254 nm for 10 minutes showed significant biodegradation. Mutants responsible for the biodegradation of sulphates and phosphates may be responsible for the breakdown in these physicochemical parameters. Phosphate is a growth-limiting factor of micro-organisms (3). This could be the reason for the early removal of phosphates in consortia like D^1 and I^1 with low biological activities at the commencement of the experiment. However, very high phosphate concentration was observed as compared to effluents without fertilizer. This could therefore be due to the addition of fertilizer.

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