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Biodegradation of Indeno (1, 2, 3 - c, d) Pyrene and Dibenzo (A, H) Perylene by Aerobic Heterotrophic Bacteria and Cyanobacteria in Brackish Water

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Authors' contributions

This work was carried out in collaboration between all authors. Author IT designed the project in collaboration with the co-authors. He planned and led the research to execution, analysed data and effected all the corrections as required by the reviewers. Author AS assisted in the design, execution and also contributed in the literature search and statistical analysis. Author EMA was responsible for recording of values during tests and also contributed in literature search. All authors read and approved the final manuscript.

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ABSTRACT

Biodegradation of indeno (1, 2, 3- c, d) pyrene and dibenzo (a, h) perylene; high molecular weight PAHs by isolates of aerobic heterotrophic bacteria, cyanobacteria and its consortium from Boodo Creek characterized with brackish water was monitored for 56 days using GC- MS. The initial concentration of indeno (1, 2, 3- c, d) pyrene in treatment with aerobic heterotrophic bacteria (AHB) was 0.09 mg/l, cyanobacteria (CB) was 0.08 mg/l; AHB+CB was 0.3 mg/l and the control, C was 0.12. Dibenzo (a, h) perylene had AHB 0.10 mg/l; CB 0.07 mg/l; AHB + CB 0.21 mg/l and C 0.23 mg/l. The quantity of the PAH's monitored reduced to 0 on day 56 in all the treatment options though fluctuation in the quantity of the HMW PAH's was observed throughout the period monitored for biodegradation. Biodegradation of indeno (1, 2, 3- c, d) pyrene and dibenzo (a, h) perylene did not vary with time ($p > 0.05$).

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are said to be ubiquitous in the environment, mainly due to anthropogenic activities such as combustion or heat related processes that involve hydrocarbons such as coal gasification and other processes like waste incineration [1,2]. PAHs are both persistent in the environment and toxic to humans and other living organisms with several reported to be carcinogenic and mutagenic [3,4]. Environmental Protection Agency has classified indeno (1, 2, 3-c, d) pyrene as a probable human carcinogen among others whereas benzo (g, h, i) pyrene is known for its carcinogenic, mutagenic and teratogenic properties [5].

The solubility of PAHs in water is low hence its bioavailability has made them recalcitrant to microbial attack. In the aquatic environment, PAHs tend to absorb onto the particle phase due to their high hydrophobicity and solid water distribution ratios [6,7,8]. The distribution of PAHs in the aquatic environment is greatly affected by aquatic particulates that act as aggregates of many complicated organic materials [9].

Biodegradation has become the most common remediation method for removing PAH and other pollutants from natural environments [10]. The process is undertaken by a wide variety of bacteria and other microorganisms including cyanobacteria which utilises PAHs as sources of carbon for energy and as part of detoxification process [1].

Several studies have been carried out on the biodegradation of PAHs by indigenous bacteria in natural waters. Degradation of pyrene by bacterial has been reported by a Mycobacterium sp isolated from sediment [11]. Microbial communities in the gulf of Mexico have been reportedly thought to be responsible for intrinsic bioremediation of crude oil released by deep water horizon oil spill causing rapid proliferation of bacterial taxa which decomposes oil hydrocarbons [12,13]. Degradation of a mixture of PAH's; naphthalene, fluorine and benzo(ά) pyrene a highly recalcitrant PAH was carried out and results indicated that all the PAHs studied disappeared from samples collected from the study sites at Orange beach [14]. It was also reportedly degraded by Mycobacterium sp strain PYR-1 in sediment – water microcosms [15,16]. Previous studies in marine environments have implicated Cycloclasticus as a major PAH degrader among other bacteria [17,18]. Furthermore, most of the literature studies focused on low molecular weight PAHs such as phenanthrene and pyrene, and little is known about the influences of aquatic particles on the biodegradation of high molecular weight PAHs such as chrysene, benzo(a)pyrene and benzo(g,h,i)perylene. It is known that the biodegradation of high molecular weight PAHs, especially those PAHs with more than 4-rings, are always degraded by means of co-metabolism [19]; low molecular PAHs such as phenanthrene could serve as a cometabolic substrate to enhance the biodegradation processes of high molecular weight PAHs [20].

Ideno (1,2,3-cd) pyrene and benzo (g, h, i) perylene are classified as Carcinogenic PAHs among others [21]. Ideno (1, 2, 3 - c,d) pyrene is a polycyclic aromatic hydrocarbon which has six fused benzene rings and has been included in the 16 PAHs considered by USEPA as priority pollutant as a result of its acute toxicity, carcinogenicity and teratogenicity [22]. Biodegradation of ideno (1, 2, 3-c,d) pyrene was previously monitored in soils bioaugmented with reeds and immobilized cells of strains and showed high leve of degradation within 40 days in an estuarine reed wetlands stimulator [22]. [23] implicated bacterial cultures of *Stenotrophomonas* sp, *Pandoraea* sp and *Pseudoxanthomonas mexicana* for efficient degradation of ideno (1, 2, 3-c, d) pyrene for 25 days incubation period.

2. MATERIALS AND METHODS

The study aimed at determining the biodegradation potential of aerobic heterotrophic bacteria and cyanobacteria resident in Bodo creek, a brackish water ecosystem to biodegrade benzo (g, h, i) perylene and indeno (1, 2, 3-c,d) pyrene; high molecular weight PAHs.

In the study, molecular characterization of aerobic heterotrophic bacteria and cyanobacteria isolated from petroleum hydrocarbon contaminated Bodo creek was carried out. Investigation of benzo (g, h, i) perylene and ideno (1, 2, 3-c,d) pyrene degradation potential of the isolates was done. To achieve this, different treatment options comprising of aerobic heterotrophic bacteria (AHB), aerobic heterotrophic bacteria and Cyanobacteria (AHB + CB), Cyanobacteria (CB) and the control, (C) were set up in aqueous medium, spiked with known volume of petroleum hydrocarbons and monitored for biodegradation using agilent 7890 model of GC-MS for 56 days as reported in Ichor et al. [1,24]. We hypothesized that the isolated aerobic heterotrophic bacteria and Cyanobacteria from crude contaminated Bodo creek may have adapted to exposure of petroleum hydrocarbons having remained viable and may possess the inherent capability for petroleum hydrocarbon degradation.

3. RESULTS

The results of biodegradation study for indeno (1, 2, 3 – c, d) pyrene by aerobic heterotrophic bacteria, Cyanobacteria, consortium of aerobic heterotrophic bacteria and Cyanobacteria and the control are as presented in Fig. 1. Its initial

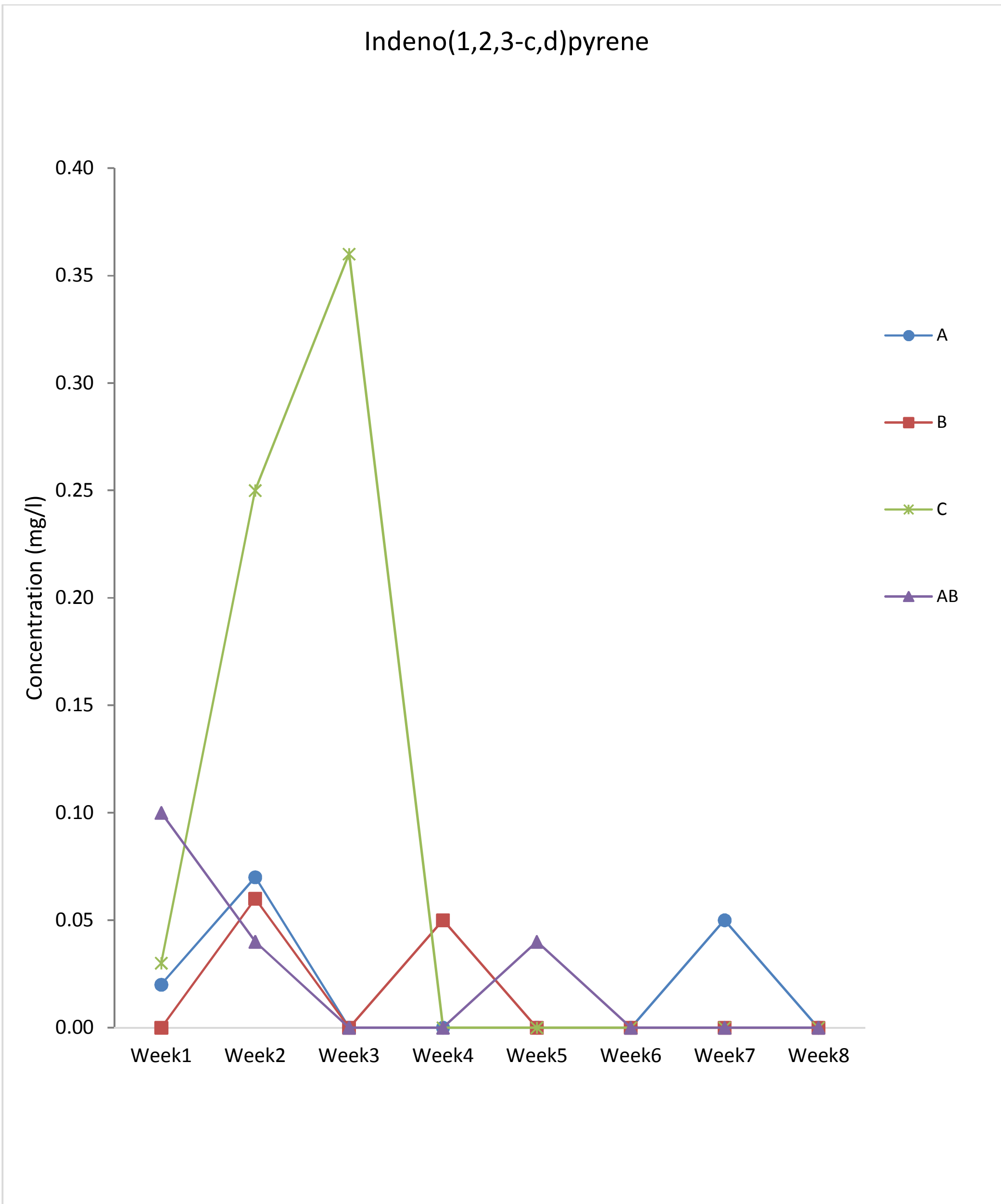

Fig. 1. Biodegradation of Indeno (1, 2, 3- c, d) pyrene

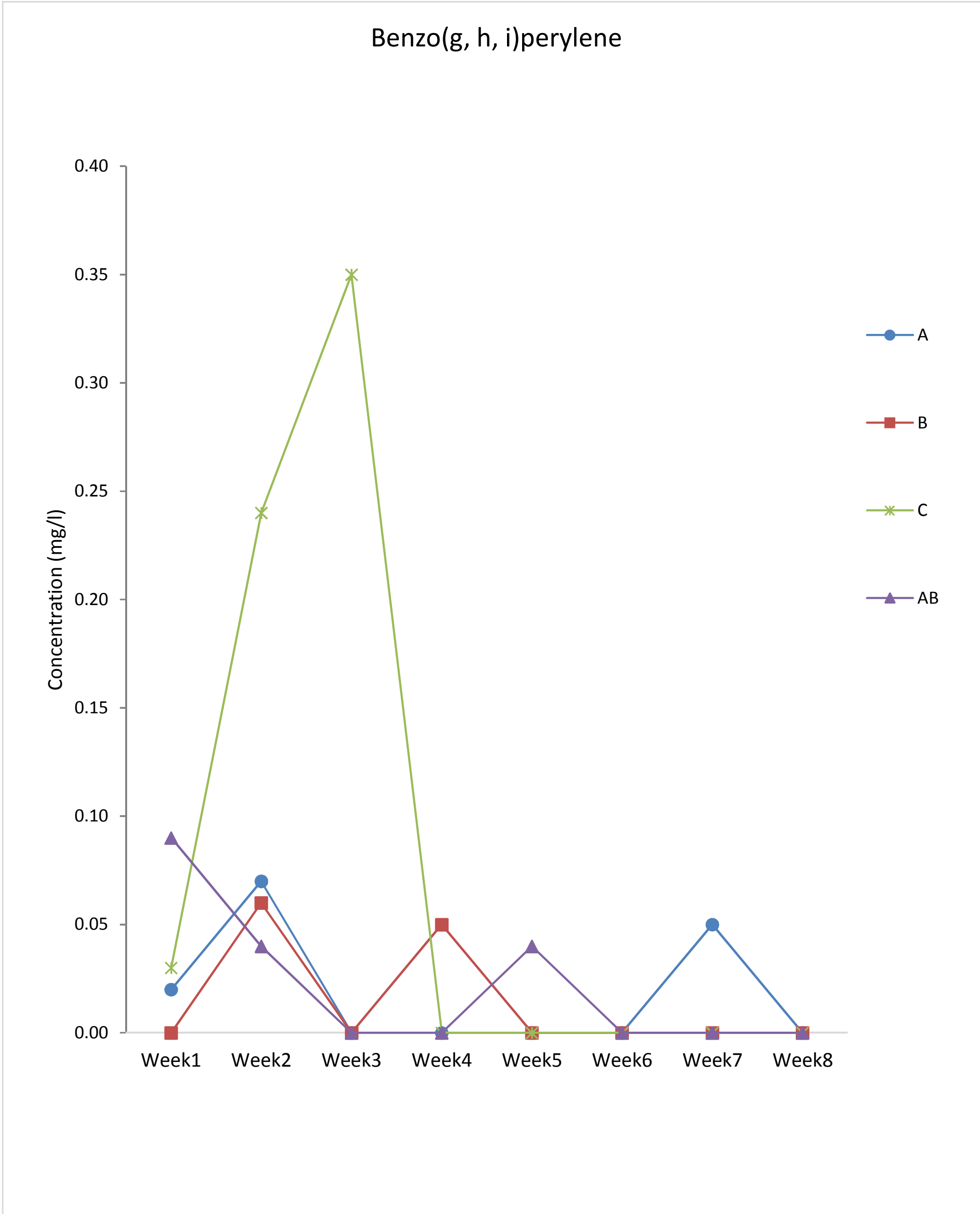

Fig. 2. Biodegradation of Benzo (g, h, i) Perylene

quantity in treatment option AHB was 0.09 mg/l but reduced to 0.02 mg/l and 0.07 mg/l on the first and second week monitored respectively. It reduced to 0 but increased to 0.05 mg/l in week 7 and 0 in week 8 the last day monitored. For CB, the initial quantity was 0.08 mg/l but reduced to 0 on week1 and increased to 0.06 mg/l, reduced to 0 and rose to 0.05 mg/l in week 2,3 and 4 respectively and further reduced to 0 throughout the remaining weeks monitored. The consortium of AHB+CB had 0.3 mg/l as the initial quantity, 0.1 mg/l and 0.04 mg/l on week 1 and respectively but reduced to 0 and reappeared in week 5 with 0.04 mg/l but reduced and maintained at 0 throughout the remaining period monitored. In the control, C, the initial quantity of ideno (1, 2, 3 – c, d) pyrene was 0.12 mg/l but reduced to 0.03 mg/l and increased to 0.25

mg/l and 0.36 mg/l in week 1, 2 and 3 respectively. It was however reduced to 0 in week 4 and remained at 0 throughout the period monitored.

Fig. 2 shows biodegradation of benzo(g, h, i) perylene by the treatment options employed. The initial quantity for treatment AHB was 0.10 mg/l which was degraded to 0.02 mg/l and 0.07 mg/l on week 1 and 2 but reappeared to 0.05 mg/l on week 7 and reduced to 0 in week 8. For CB, the initial concentration of was 0.07 mg/l which reduced to 0 in week 1 but rose to 0.06 mg/l in week 2. Fluctuation was observed but remained at 0 from week 5 throughout the period monitored. The initial concentration of benzo (g, h, i) perylene for treatment AHB+CB was 0.21 mg/l but was mineralized to 0.09 and 0.04 mg/l in week 1 and 2 respectively but reduced to 0 in week 3 and 4. It rose to 0.04 in week 5 and reduced to 0 throughout the remaining period monitored. The control had initial concentration of benzo(g, h, i) perylene to be 0.23 mg/l which reduced to 0.03 mg/l in week 1 but rose to 0.24 mg/l and 0.35 mg/l in week 2 and remained at 0 throughout the period monitored.

4. DISCUSSION

The study monitored biodegradation of Benzo (g, h, i) Perylene and Indeno (1, 2, 3-c,d) pyrene from crude contaminated waters of Bodo creek; a moderate salt aquatic environment. The aerobic heterotrophic bacteria and cyanobacteria isolates used were reported in Ichor et al. [25]. The isolates and the consortium of the bacteria and cyanobacteria effectively degraded the high molecular weight PAH's used in the study though fluctuations were observed in the course of monitoring the degradation due to novel synthesis as reported in Ichor et al. [24,1]. The result of the present study revealed higher rates of biodegradation of the HMW PAH's tested by the consortium of aerobic heterotrophic bacteria and cyanobacteria compared to other treatments. The overall result provides sufficient evidence on the capability of the resident microbial flora from Bodo creek to efficiently remove HMW PAH's. Mineralization of phenanthrene, fluoranthene, benzo(b) fluoranthene and benzo(k)fluoranthene, anthracene, benzo (a) anthracene and dibenzo(a,h) anthracene by thesame isolates of aerobic heterotrophic bacteria and cyanobacteria interaction in crude oil contaminated brackish water of Bodo creek has been reported previously [24,25,26].

Biodegradation of PAH's in marine environment by bacteria has been reported in previous studies. For example, *Cycloclasticus* has been frequently detected in marine PAH's degradation [27,28,29,30,18]. At San Diego Bay sediment, bacteria isolates from the genera *Cyclosclasticus, Pseudoalteromonas, Marinobacter, Vibrio, Marinomonas and Halomonas* grew on chrysene and phenanthrene (28); *Porticoccus hydrocarbonoclasticus* has been isolated as a PAH degrader [31] and strains of *Porphyrobcter* and *Microbacterium* were isolated on benzo (a) pyrene. Degradation of benzo (a) pyrene from marine enrichment was carried out by strains of *Stenotrophomonas*, *Ochrabactrum* and *Pseudomonas* spp [32]. [33] found *Sediminicola* as a major microbe found in PAH and oil contaminated Liaodong Bay of Bohai Sea in China. *Bacillus subtilis* BMT4i and *Mycbacterium* sp PYR degraded benzo (a) pyrene respectively [34,35]; *Geobacillus stearothermophilus* (AAP7919) was implicated in biodegradation of anthracene [36]; Mycobacterium vanbaalenii PYR-1 degraded phenanthrene, pyrene and dimethylbenz(a) anthracene [37,38]; *Pseudomonas putida* P15, BS3760 biodegraded Pyrene, phenanthrene, benz(a)anthracene and chrysene [39,40]. 23 (2015) monitored degradation of pyrene and indeno (1, 2, 3 – cd) pyrene by bacterial cells bioaugmented with reeds in an estuarine reed wetland stimulator in small scale natural conditions within 40 days. Its success was linked to its ability to immobilize and enrich PAH degraders, initiate co – metabolism, and ensure PAH bioavailability [41].

Results from previous studies has demonstrated the capability of indigenous cyanobacteria in saline water to degrade PAH's. [42] implicated Agmenellum quadruplicatum in phenanthrene biodegradation; [43] reported 80% removal of phenanthrene by *Aulosira fertilissima*. [44] reported biodegradation of between 34 to 100% of pyrene by six strains in 7 days from different genera which include; *Chlorella*, *Selenastrum*, *Chlamydomonas*, *Scenedesmus* and *Synechocytis*. [45] found rapid degradation in cyanobacterial mats though pure cultures of it could not degrade hydrocarbons. Previous research findings however attributed biodegradation of hydrocarbons to associated heterotrophic bacteria [46,47,48,49] which varies with the result of our present study where pure cultures of the isolated cyanobacteria degraded indeno (1, 2, 3-cd) pyrene and dibenzo (a, h) perylene.

5. CONCLUSION

The study has shown the capability of indigenous microbial community of Bodo creek to biodegrade indeno (1, 2, 3 – c, d) pyrene and dibenzo (a, h) perylene in moderate salt concentrations without bioaugmentation, nutrient amendment and biostimulation. The findings the study has further provided insight in to efficient and effective degradation of high molecular weight PAH's in actual petroleum hydrocarbon contaminated sites and can serve as a model study for stimulating the resident microflora for effective, efficient, quicker bioremediation and restoration of the crude oil polluted aquatic ecosystems of the Niger Delta region of Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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