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Characterization and biodegradation of polycyclic aromatic hydrocarbons in radioactive wastewater

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ABSTRACT

PAH degrading Pseudomonad and Alcaligenes species were isolated from landfill soil and mine drainage in South Africa. The isolated organisms were mildly radiation tolerant and were able to degrade PAHs in simulated nuclear wastewater. The radiation in the simulated wastewater, at 0.677 Bq/μL, was compatible to measured values in wastewater from a local radioisotope manufacturing facility, and was enough to inhibit metabolic activity of known PAH degraders from soil such as *Pseudomonas putida* GMP-1. The organic constituents in the original radioactive waste stream consisted of the full range of PAHs except fluoranthene. Among the observed PAHs in the nuclear wastewater from the radioisotope manufacturing facility, acenaphthene and chrysene predominated—measured at 25.1 and 14.2 mg/L, respectively. Up to sixteen U.S.EPA priority PAHs were detected at levels higher than allowable limits in drinking water. The biodegradation of the PAHs was limited by the solubility of the compounds. This contributed to the observed faster degradation rates in low molecular weight (LMW) compounds than in high molecular weight compounds.

1. Introduction

The release of new industrial and domestic products such as new forms of paint, detergents, medical and cosmetic products, and pesticides has resulted in the increase of output of hazardous aromatic compounds to the environment. This has raised concerns over accumulative and indeterminate long-term impacts of these compounds to the environment [1–3]. Adding to this problem has been the increase in activities that result in the discharge of radioactive waste containing refractory organics. Waste containing irradiated organics is released from nuclear fuel processing facilities, medical facilities for treating cancer, and scientific research activities [4,5]. Within the waste streams, are found large amounts of tough-to-degrade halogenated compounds such as poly-chlorinated biphenyls (PCBs), chlorophenols, chlorotoluenes, chloropropanes, phosphotyrated organics, and polynuclear aromatic hydrocarbons (PAHs) [6]. The biodegradation process of these compounds is inhibited, not only by the chemical toxicity, but also by the radiotoxicity from radioactive components within the wastewater [7,8].

The difficulty in treating complex organic compounds such as PAHs is not limited to the toxicity and recalcitrance alone. Most of the PAHs are high molecular weight compounds with very low aqueous solubilities, thus, they are not readily bioavailable to microorganisms in the water [7]. But most low molecular weight (LMW) (2–3 rings) PAHs are easily biodegraded by a variety of bacteria from nature [9–12]. The critical mechanism in the organisms that are capable of degrading PAHs and other aromatic compounds is their ability to cleave the aromatic carbon ring (benzene ring) which is responsible for the recalcitrance of the compounds to microbial attack.

Several researchers have investigated biological treatment as a possible cost effective solution for removing PAHs from waste streams [7,13,14]. Conventionally, chemical processes have been used to degrade these compounds. However, most of the chemical treatment technologies produce toxic sludge due to incomplete reactions and formation of undesirable byproducts [15–17]. Biological treatment, on the other hand, offers a more environmentally friendly alternative since the waste compounds may be completely mineralized to CO_2 and H_2O through the biological degradation pathways [18–22].

So far, the impact of radiotoxicity on the biodegradation of organic compounds in radioactive wastewater streams has not been thoroughly investigated. This article reports results on an investigation on the distribution and biodegradability of PAHs in nuclear wastewater streams, as a model for biological treatment of related compounds under radiation exposure conditions.

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Nomenclature

m	number of repetitions required to obtain the target reliability R^*
n	number of experimental units (classes)
R	Pearson's regression coefficient
R_m	interclass correlation coefficient
R^*	target reliability coefficient
s_B^2	the between experimental unit variability (variability among the unit averages)
s_w^2	the pooled within experimental unit variability

2. Materials and methods

2.1. Culture and media

Candidate PAH degrading bacteria were isolated from contaminated soil from the Chloorkop landfill site [Johannesburg, South Africa (SA)] and from mine water obtained from the Council of Scientific and Industrial Research (CSIR) (Pretoria, SA). Start-up cultures were obtained by inoculating 100 mL of sterile nutrient broth with bacteria from 1 g of soil or 1 mL of mine water. 0.2 g of powdered expired nuclear graphite was added to the medium to serve as a chemically inert radiation source to get a radiation concentration of 1.055 ± 0.067 Bq/μL. The resulting radiation concentration was almost 5000 times higher than the background radiation concentration of natural water sources (0.1 Bq/g) in South Africa [23]. The start-up culture with powdered graphite was incubated in 250 mL Erlenmeyer flasks with shaking in a Labcon SPL-MP 15 Lateral Shaker (Labcon Laboratory Services, South Africa) operated at 120 rpm and 28 ± 2 °C. Enrichment cultures were then obtained by sub-culturing a 2% (v/v) start-up culture in 100 mL mineral salt medium (MSM) with naphthalene as an added carbon and energy source. The enrichment procedure was repeated 3 times to select for radiation tolerant PAH degrading species. MSM used in this study was prepared as the following concentrations of basic minerals in deionised water: 10 mM NH_4Cl, 30 mM Na_2HPO_4, 20 mM KH_2PO_4, 0.8 mM Na_2SO_4, 0.2 mM $MgSO_4$, 50 μM $CaCl_2$, 25 μM $FeSO_4$, 0.1 μM $ZnCl_2$, 0.2 μM $CuCl_2$, 0.1 μM NaBr, 0.05 μM Na_2MoO_2, 0.1 μM $MnCl_2$, 0.1 μM KI, 0.2 μM H_3BO_3, 0.1 μM $CoCl_2$, and 0.1 μM $NiCl_2$, adopted from Roslev et al. (1998) [24]. Preferably, a stock solution of 10 times the concentration of the MSM was prepared and one-tenth of the concentration was transferred to a litre to obtain the required MSN concentration. Commercial media (broth and agar) for start-up culture and plating were prepared by dissolving the prescribed amount indicated on the bottle and autoclaving the solution at 121 °C for 15 min and cooled to room temperature (40–45 °C in the case of agar) before use.

2.2. Chemical reagents

The solvents for HPLC analysis (methanol and acetonitrile) and the salts for MSN preparation were purchased from Merck (Johannesburg, SA). Methylene chloride and ethyl acetate for solid phase extraction (SPE) was also purchased from Merck. Naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, chrysene and indeno(1,2,3-cd)pyrene for degradation experiments were purchased from Sigma–Aldrich (Johannesburg, SA).

2.3. Characterization of compounds in radioactive waste

Environmental samples were collected from a radioisotope processing facility in Cape Town, SA. The samples emitted radiation at 0.677 Bq/μL, enough to inhibit metabolic activity in most mesophilic bacteria from the natural environment [25]. Compounds were extracted in Strata C18 cartridges using ethyl acetate and methylene chloride as solvents. The extracts were concentrated to 1 mL in a Rotavapor® R-210 Dry Vap Concentrator (BUCHI Labortechnik AG, Flawil 1, Switzerland). Pre-concentration during analysis was achieved online using the Turbo Matrix 40 Headspace (Perkin–Elmer, Connecticut, USA) equipped with a solid phase trap. Chemical species were then characterized by GC-MS.

2.4. Microbial characterization

The phylogenetic characterization of cells from the landfill soil and mine water samples was conducted after sub-culturing the cells in nutrient or Luria–Bettani broth. Individual colonies from a serially diluted preparation were carefully examined for colony morphology and cell morphology by Gram-staining. This process, we recognize, could eliminate a wide range of potential PAH degrading bacteria especially anaerobic species in the samples. But at this stage, capturing the full range of obligate anaerobes was not a priority since most anaerobic species employ more complex pathways for catabolism of aromatic compounds; a process which is slower than aerobic and facultative catabolism [26,27].

The purified colonies were streaked on nutrient agar followed by incubating at 30 °C for 18 h in preparation for 16S rRNA gene sequence analysis. Microbial pure cultures were grown from loop-fulls from individual colonies, transferred to fresh media containing low amounts (2–5 mg/L) of naphthalene and acenaphthene. The process was repeated at least three times for each colony type to achieve close to a pure culture of each identified species.

Genomic DNA was extracted from purified colonies according to the protocol described for the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA). 16S rRNA genes were amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer pA corresponds to position 8–27; Primer pH to position 1541–1522 of the 16S gene under the following reaction conditions: 1 min at 94 °C, 30 cycles of 30 s at 94 °C, 1 min at 50 °C and 2 min at 72 °C, and a final extension step of 10 min at 72 °C). PCR fragments were then cloned into pGEM-T-easy (Promega) [Promega Wizard® Genomic DNA Purification Kit (Version 12/2010)]. The 16S rRNA gene sequences of the strains were aligned with reference sequences from phenol and benzoate degrading Pseudomonads using Ribosomal Database Project II programs. Sequence alignment was verified manually using the program BIOEDIT. Pairwise evolutionary distances based on an unambiguous stretch of 1274 bp were computed by using the Jukes and Cantor method [28].

2.5. Degradation of PAHs in simulated nuclear wastewater

Based on the results from radioactive wastewater characterization, a synthetic wastewater with the composition similar to the characterized radioactive wastewater was prepared in the laboratory. PAHs were detected at a range of concentrations as shown in Table 1. 100 mL of simulated nuclear wastewater was then prepared by adding the compounds detected at significantly high concentrations (≥0.2 mg/L) and 0.2 g irradiated graphite as a chemically inert source of radiation. The PAH mixture prepared above was then inoculated with 2 mL of the enriched consortium medium. Cell free controls were used in which sterile mineral salt medium was mixed with only the test compounds. Samples were withdrawn aseptically at intervals and analysed for residual PAH concentration using the methods below.

Table 1
Concentration of PAHs in nuclear wastewater samples collected from a radioisotope manufacturing facility.

Compound name	Concentration (mg/L)	Error of determination ± value	Error of determination %
Naphthalene	1.654	0.016	0.967
Acenaphthylene	0.001	0.000	0.016
Acenaphthene	25.101	0.239	0.952
Fluorene	0.942	0.008	0.849
Phenanthrene	0.390	0.004	1.026
Anthracene	0.695	0.006	0.863
Fluoranthene	0.000	0.000	0.000
Pyrene	0.014	0.002	14.286
Benzo(a)anthracene	0.019	0.001	5.263
Chrysene	15.305	0.146	0.954
Benzo(b)fluoranthene	0.057	0.012	21.053
Benzo(k)fluoranthene	0.005	0.000	0.990
Benzo(a)pyrene	0.048	0.002	4.167
Dibenzo (ah)anthracene	0.047	0.001	2.128
Benzo(ghi)perylene	0.006	0.000	0.825
Indeno(1,2,3-cd)pyrene	0.438	0.006	1.370

2.6. Analytical methods

2.6.1. GC-MS analysis

Characterization of the PAHs in the environmental samples was performed in a Clarus 600T GC/MS (Perkin–Elmer, Connecticut, USA) equipped with a Perkin–Elmer Elite – 5MS capillary column (30 m × 0.25 mm ID × 0.5 μm fixed phase) with helium as carrier gas. The GC detectors were bypassed in this method. Sample extraction was achieved online by Turbo Matrix 40 Headspace equipped with a trap. PAHs were extracted by boiling at a headspace oven temperature of 80 °C for 40 min at a vial pressure of 30 psi and desorbed onto the GC column at a pressure of 15 psi. The GC oven was programmed at a temperature gradient of 40 °C/min, ramped at 6 °C/min to 150 °C and at 15 °C/min to 250 °C. Degradation intermediates were identified by searching for closest matches in the Wiley Mass Spectral Libraries.

2.6.2. HPLC analysis

HPLC analysis was performed using a Waters 2695 separation module with Waters 2998 Photodiode Array Detector (Waters Corporation, Massachusetts, USA). A Waters PAH C_{18} symmetry column (250 mm × 4.6 mm ID × 5 μm fixed phase) was used (Waters Corporation). The samples were first filtered with a 5 mL syringe filter (0.45 μm pore size), the injection volume was 10 μL and the detector wavelength was 254 nm. The mobile phase was 70% acetonitrile and 30% deionised water and the dosing pumps were operated at a run-time pressure of 1684 psi. Calibration curves were prepared from standards of known concentration in order to quantify individual compounds in simulated and actual wastewater.

2.6.3. Radiation measurement

Radiation sources within samples were simulated by a radioactive graphite powder obtained from the Nuclear Energy Commission of South Africa (Pelindaba, North West Province, SA). The powdered graphite served as the source of radiation. However, the graphite did not react chemically and was completely non-degradable by the microorganisms in solution. Radiation levels were determined by a RadEye Lab-0 Giga-counter (Thermo Electron 'Erlangen' GmbH, Germany).

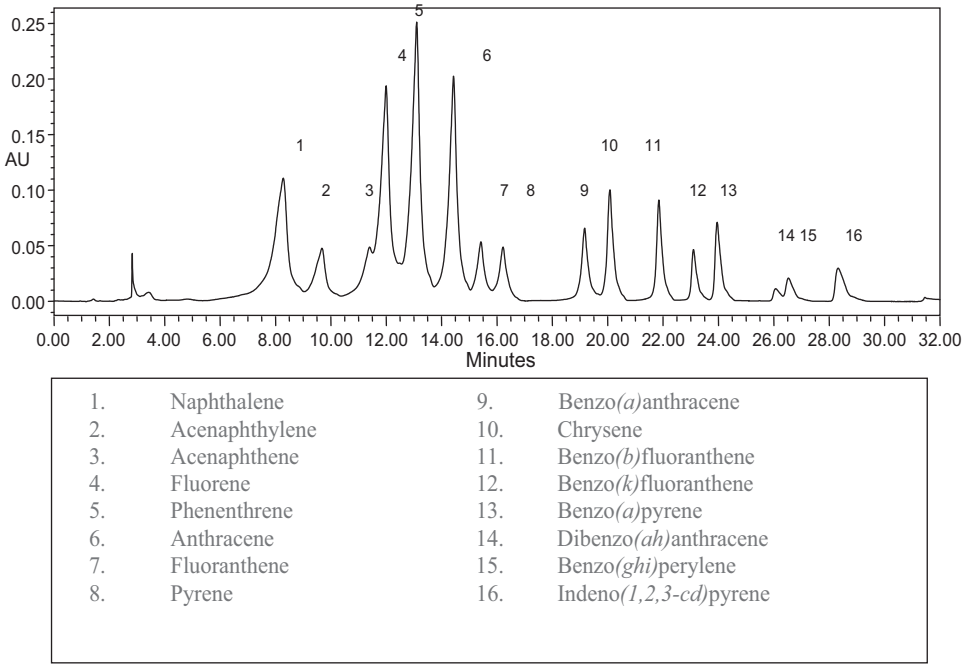

Fig. 1. Chromatographic determination of the 16 US EPA priority PAHs using HPLC equipped with a photodiode array detector (HPLC-PDA).

Table 2
Characterization of naphthalene degrading bacteria isolated from landfill site.

16S rRNA ID	% Identity
Microbacterium esteraromaticum	98%
Achromobacter xylosoxidans	99%
Alcaligenes sp.	89%
Pseudomonas aeruginosa	99%
Pseudmonas pseudo alcalegenes	99%

Table 3
Characterization of naphthalene degrading bacteria isolated from mine water.

16S rRNA ID	% Identity
Stenotrophomonas sp., *Stenotrophomonas maltophilia* strain KNUC285	96%
Bacillus sp.	99%
Pseudomonas putida, *P. taiwanensis*	99%

2.6.4. Statistical reliability

The required number of determinations for each sample was established using the Statistical Reliability Test as described by Sawyer et al. (2003) [29]. A grid of three determinations by five different operators (classes) was obtained for each method and the reliability factor R_m was determined from variances using the equation below:

$$R_m = \frac{s_B^2 - s_w^2}{s_B^2 + (n-1)s_w^2} \quad (1)$$

where R_m = interclass correlation coefficient, n = number of experimental units (classes), s_B^2 = between experimental unit variability (variability among the unit averages), and s_w^2 = pooled within experimental unit variability. The required number of determination to achieve a target reliability of 95% ($R^* = 0.95$) was then obtained by factoring the reliabilities based on the power test:

$$m = \frac{R^*(1-R_m)}{R_m(1-R^*)} \quad (2)$$

where m = number of repetitions required to obtain the target reliability R^*, R^* = target reliability coefficient, R_m = calculated (interclass) reliability coefficient (Eq. (1)). For the PAH solution, triplicate determinations were required to achieve a reliability factor of 0.95.

3. Results and discussion

3.1. Characterization of radioactive wastewater

3.1.1. Standard solutions and calibration

Calibration curves were obtained using a series of varying concentrations (5–20 mg/L) of a multi-component standard containing each of 16 PAHs. The baseline separation of the target compounds was obtained in a time of less than 35 min. These results are illustrated in Fig. 1. The calibration curves of the compounds were all linear with $R^2 = 0.999$.

3.1.2. Identification and quantification of PAHs in actual waste stream

Sixteen (16) EPA priority PAHs in the actual polluted wastewater were detected in the concentration range 0.001–25.1 mg/L (Table 1). The lower molecular weight compounds in the samples (2–3 ring group of PAHs), namely, naphthalene, acenapththylene, acenaphthene, fluorene, phenanthrene and anthracene, were detected at concentrations covering the full spectrum of detected concentrations, 0.001–25.1 mg/L, with acenaphthene (detected at 25.1 mg/L) as the most abundant. The high molecular weight (4–6 ring) PAHs, namely, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene varied in concentration from 0 to 15 mg/L with chrysene as the most abundant in this group. Notably, fluoranthene was the only PAH that was not detected in the environmental samples (from site) – the value reported was below the detection limit of the measurement methods. PAHs by ring size were predominated by 2- and 3-ring PAHs. On average, the 2–3 ring PAHs consisted of about 64.4% of 16 EPA priority PAHs, whereas 6–4 ring PAHs only accounted for the remaining 35.6%.

Table 4
Performance of isolates and other known PAH degrading bacteria under irradiation conditions (1.055 Bq/L) in batches containing 23.1 ± 0.6 mg/L naphthalene.

Name of bacteria	Source of bacteria	Irradiation exposure (Bq/μL)	Cell count after 24 h (CFU/mL)	Remaining concentration after 24 h (mg/L)	NP removal after 24 h (%)
Non-irradiated controls					
Microbacterium esteraromaticum	Landfill	0.001	1.33×10^7	16.31	29.4
Achromobacter xylosoxidans	Landfill	0.013	1.42×10^7	14.60	36.8
Alcaligenes sp.	Landfill	0.002	1.41×10^7	0.00	100.0
Pseudomonas aeruginosa	Landfill	0.013	2.03×10^6	2.30	90.0
Pseudmonas pseudo alcalegenes	Landfill	0.013	2.11×10^7	0.23	99.1
S. maltophilia strain KNUC285[a]	Mine water	0.012	1.88×10^8	3.51	84.8
Bacillus sp.	Mine water	0.000	1.01×10^7	8.83	61.9
Pseudomonas putida, *P.taiwanensis*	Mine water	0.001	2.33×10^6	0.84	96.5
Pseudomonas putida DMP-1[b]	Soil	0.001	3.67×10^8	0.01	100.0
Irradiated batches					
Microbacterium esteraromaticum	Landfill	1.055	1.64×10^3	19.61	15.2
Achromobacter xylosoxidans	Landfill	1.054	1.31×10^2	21.65	6.5
Alcaligenes sp.	Landfill	1.066	1.32×10^6	0.45	98.3
Pseudomonas aeruginosa	Landfill	1.043	3.33×10^6	1.42	93.9
Pseudmonas pseudo alcalegenes	Landfill	1.067	3.12×10^5	0.33	98.7
S. maltophilia Strain KNUC285[a]	Mine water	0.766	4.33×10^4	16.31	29.4
Bacillus sp.	Mine water	0.687	2.64×10^3	19.32	16.5
Pseudomonas putida, *P.taiwanensis*	Mine water	1.052	2.41×10^4	3.33	85.7
Pseudomonas putida DMP-1[b]	Soil	1.033	2.13×10^3	21.83	5.6

[a] *Stenotrophomonas maltophilia* strain KNUC285.
[b] From Shen and Wang (1995) [30].

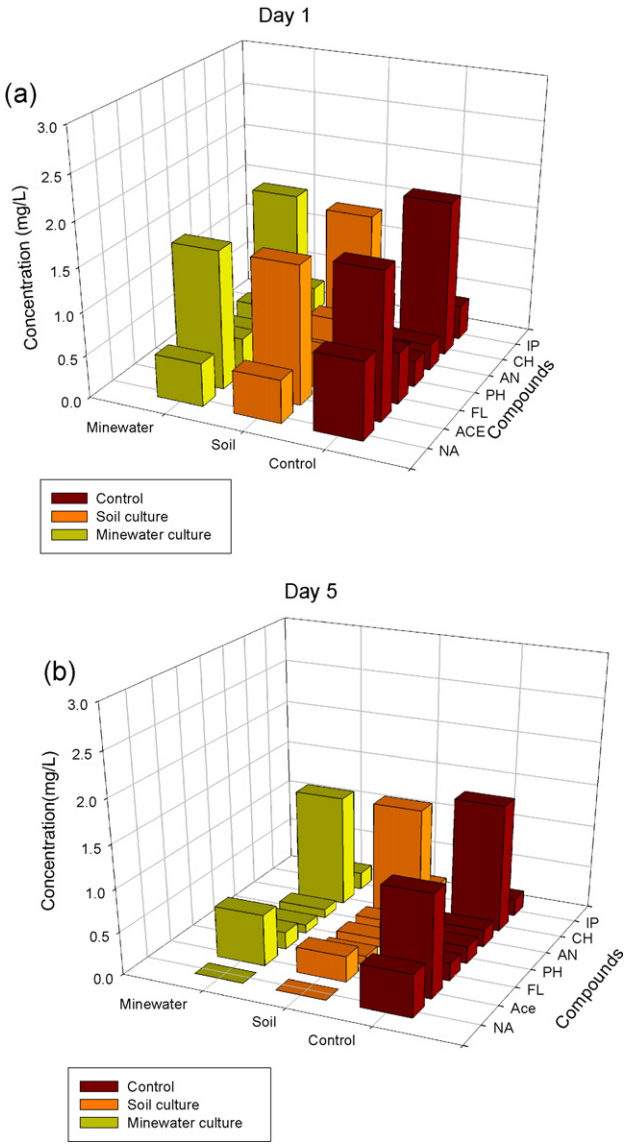

Fig. 2. PAHs degradation after during incubation of a mixed compound sample with bacteria (a) after 1 day of inoculation (b) after 5 days of inoculation.

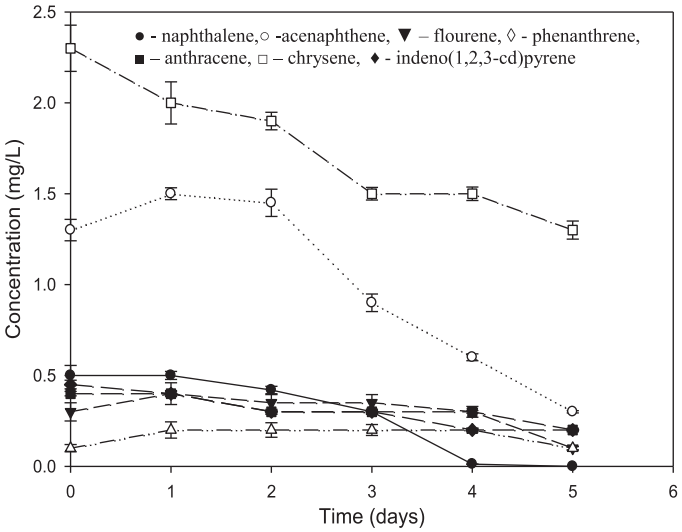

Fig. 3. Degradation of simulated wastewater by soil culture from culture with 30 mg/L added PAHs and a solution radiation of 0.677 Bq/μL showing insignificant degradation in high molecular weight compounds phenanthrene, flourene, and indeno(1,2,3-cd)pyrene.

3.2. Culture characterization

From the 16S rRNA fingerprinting and a BLAST search of recognisable putative rRNA identities, a total of 5 PAH tolerant species were found in the landfill soil samples and 3 species were isolated from mine water (Tables 2 and 3). The isolated species were regarded as candidate species for PAH degradation due their PAH toxicity tolerance. Hits were scored with 96% confidence and above, except for *Alcaligenes* sp. at 89% identity.

3.3. Screening Test–PAH degradation under irradiation conditions

The results on relative PAH degradation activity of the isolates and standard cultures under irradition conditions (Table 4) showed that *Pseudomonas* sp. and *Alcaligenes* sp. from the landfill soil were the most resistant to radiation. These two species suffered less than one log kill rate during incubation under 1.067 Bq/μL radiation. PAH removal was highest in these cultures with up to 98.7% removal observed in *Pseudomonas pseudo alcaligenes* under 1.067 Bq. The control culture was the phenol/naphthalene degrading species, *Pseudomonas putida* DMP-1 from Shen and Wang (1995) [30] which were severely impacted by the radiation. The non-aromatic ring cleaving bacteria in the soil samples, *Microbacterium esteraromaticum*, *Achromobacter xylosoxidans*, and *Bacillus* sp., although mildly resistant to radiation, achieved only low conversion rates of PAHs (Table 4).

3.4. Biodegradation of simulated waste

The effect of molecule size on the biodegradability of the compounds using the isolated organisms was evaluated using a simulated wastewater of known composition. Although the studies on PAH degradation have been conducted by several researchers, most of the existing information comes from experiments conducted with highly purified cultures. Moreover, biodegradation of the aromatic compounds under irradiation conditions has not been the main focus of previous investigations. This research targeted the bioremediation of nuclear waste. The development of mixed-culture was favoured to take advantage of possible cooperative interactions of different species in the consortium. In such a system, we hope to derive a self optimising community of microorganisms.

Experiments were performed using a mixture of 7 PAHs namely, naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, chrysene and indeno(1,2,3-cd)pyrene. Initial results showed that low molecular weight (2–3 ringed) compounds were degraded faster than high molecular weight compounds (4–6 rings) (Fig. 2). Approximately 67% removal was achieved in the 6 ring PAH (indeno(1,2,3-cd)pyrene) as opposed to 100% in the lighter molecule (naphthalene) (Fig. 2 and Table 5). Interestingly, all PAHs showed a certain amount of removal including the largest compound in the system, indeno(1,2,3-cd)pyrene (Figs. 3 and 4).

Compared to controls, the degradation of total PAHs in the experiments inoculated with live cultures showed a significant removal after 5 days of incubation (Figs. 3 and 4). Some removal of the low and medium range PAHs was also observed under the control conditions (without bacteria), which was attributed to the stripping action during culture incubation under continuous shaking (Fig. 5).

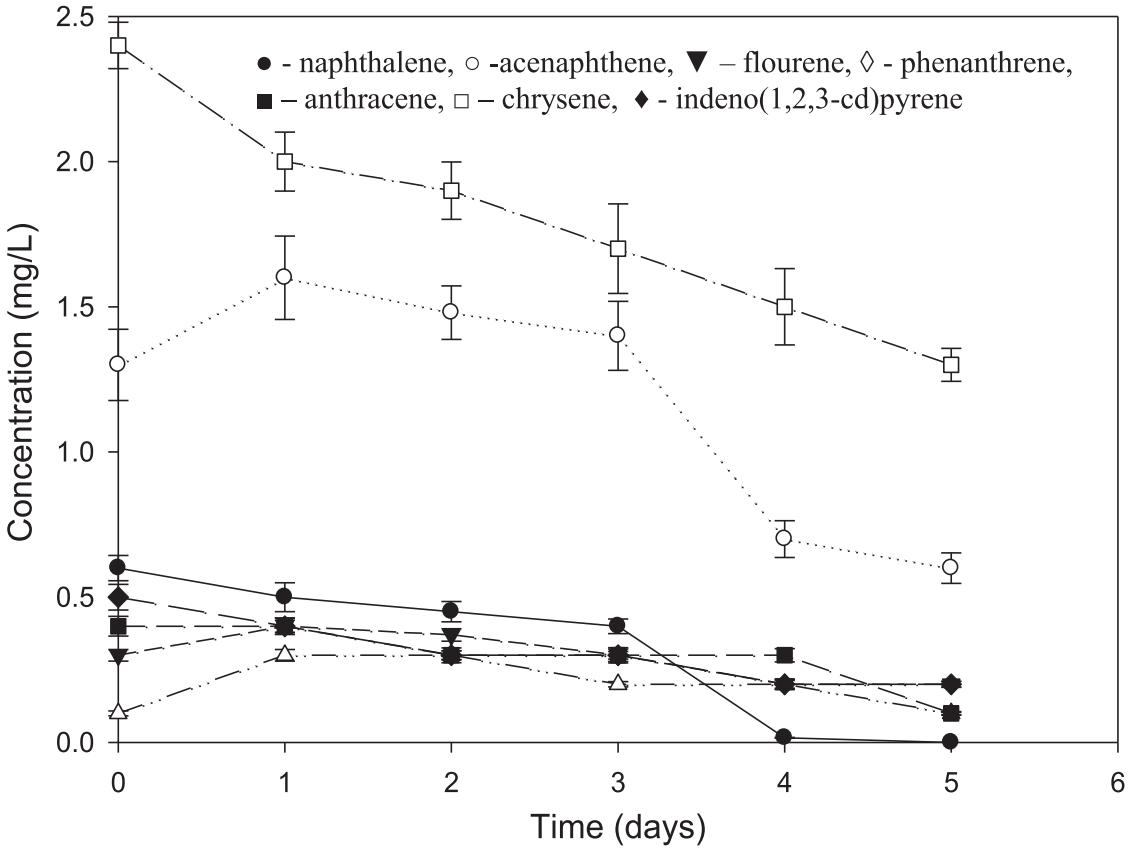

Fig. 4. Degradation of simulated wastewater by mine water culture from culture with 30 mg/L added PAHs and a solution radiation of 0.677 Bq/μL showing lower performance of this culture in degrading PAHs. The degradation rates are further decreased for the higher molecular weight compounds chrysene and acenaphthene. The highest degradation rate is observed in naphthalene.

3.5. Comparative performance of the two culture

Comparative performance was conducted based on the degradation rate of individual compounds by the two consortia: landfill bacteria and mine water bacteria. The biodegradation percentages of acenaphthene and fluorene by the landfill soil bacteria were 92.5% and 90%, respectively. In contrast, the mine water bacteria achieved 85% and 80% removal, respectively, much lower than the removal by the landfill soil consortium. For the high molecular weight compounds—phenanthrene, anthracene, chrysene and indeno(1,2,3-cd)pyrene—all cultures achieved similar removal rate percentages: approximately 80%, 90%, 66.5% and 60%, respectively (Table 5).

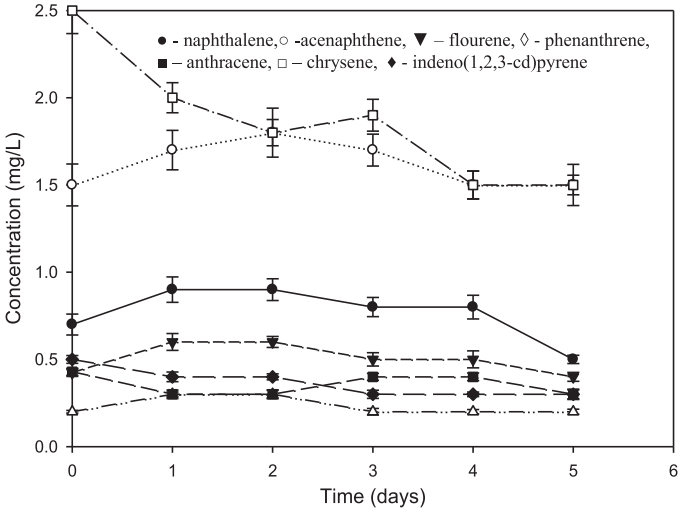

Fig. 5. Concentration profiles of simulated wastewater without bacteria (control) showing some removal by air stripping during aeration of the batches. A characteristic decrease is observed for chrysene. The rest of the compounds including naphthalene do not show significant reduction in the absence of the microorganisms.

4. Discussion

The presence of large molecular weight compounds in the nuclear wastewater is of major concerns especially benzo(a)pyrene (BaP) and indeno (1,2,3-cd)pyrene. The level of benzo(a)pyrene in the environment serves as the basis for the development of environmental evaluation and cleanup regulations in the developed world [31]. In this study, the benzo(a)pyrene was detected at 0.048 mg/L, much higher than its disposal regulation limit of 0.05 μg/L. Surprisingly, the largest PAH, indeno(1,2,3-cd)pyrene was also detected in higher values than expected in most industrial effluents. Normally, aqueous concentrations of indeno(1,2,3-cd)pyrene are very low due to its known low aqueous solubility. The concentration of these compounds in the effluent from the actual wastewater stream is worrying knowing that these compounds are the most toxic among the wider spectrum of PAHs.

Naphthalene is used widely as the model compound to investigate the ability of bacteria to degrade PAHs. In this study, high removal rates were achieved in low molecular weight PAHs including naphthalene, with naphthalene as the most degradable (Fig. 3). Degradation of the higher molecular weight (HMW) compounds in the simulated waste was much slower in the cultures with

Table 5
Percent removals of PAHs during biodegradation of mixed PAHs.

Compound	Initial concentration (mg/L)	Final concentration[a]	Removal[a] %	Final concentration[b]	Removal[b] %
Naphthalene	2	0	100%	0	100%
Acenaphthene	4	0.3	92.5%	0.6	85%
Fluorene	1	0.1	90%	0.2	80%
Phenanthrene	0.5	0.1	80%	0.1	80%
Anthracene	1	0.1	90%	0.1	90%
Chrysene	4	1.3	66.5%	1.3	66.5%
Indeno(1,2,3-cd)pyrene	0.5	0.2	60%	0.2	60%

[a] Landfill soil culture.
[b] Mine water culture.

bacteria from mine water than in the landfill soil bacteria. This suggests that more diverse culture of the landfill soil bacteria achieved better results. Diverse cultures tend to supply complimentary functions from species favouring different niches at different times as was suggested earlier by Li et al. (2002) [32]. The advantage of wider diversity in a culture is expected to be greater as the compounds become larger and more complex – requiring more complex biodegradation pathways.

Generally, the values of high molecular weight PAHs reported here surpassed the values encountered in most conventional effluents. Anyakora et al. (2005) [33] detected all the PAHs from sediment samples at concentrations ranging from 0.1 to 28 μg/kg. The 2–3 rings group of PAHs was detected at higher values on average than the rest of PAHs. The solubility if the main contributing factor of the high observed aqueous concentrations of the 2–3 ring compounds.

Results in Tables 1 and 2 are consistent with literature observations with respect to the prominence of Psuedomonads and Alcaligenes among PAH tolerant species [34–38]. The other species, *Achromobacter* sp., *Janibacter* sp. and *Stenotrophomonas* sp. have also been known to degrade complex aromatic compounds as demonstrated by Walczak et al. (2001) [39], Zhang et al. (2009) [40], Juhasz et al. (2000) [41], and other researchers. However, *Janibacter* sp. and its variants was the only group not detected in the study. Biological application in actual treatment systems has been attempted both in bioreactors and *in situ*. The ability of bacteria to utilize PAHs as growth substrates has been reported extensively by others over the past few decades [42–44]. Biodegradation of complex mixed hydrocarbons usually requires the co-operation of more than a single species. Individual microorganism can metabolize only a limited range of compounds to a certain extent. In mixed cultures, each species plays a different metabolic role, bringing together an overall broad enzymatic capacity otherwise unachievable by a single species.

The results from this study suggest that the addition of an enriched consortium could enhance the efficiency of PAH degradation. Similar results are reported from previous studies [10,18,45] where enriched consortia achieved higher removal rates of mixed PAHs. However, results in this study were obtained under significantly irradiated conditions, in an environment hostile to most mesophilic bacteria. The described culture and method thus represents a significant contribution towards the decontamination efforts in environments contaminated with radioactive waste.

5. Conclusion

Microorganisms isolated in this study degraded PAHs in simulated nuclear wastewater with a background radiation of 1.067 Bq. The culture was predominated by the *Pseudomonas aeruginosa*, *Alcaligenes* sp. *Pseudmonas pseudo alcalegenes* which are known phenolic degrading organisms. Also present in the landfill soil samples were *Microbacterium esteraromaticum* and *Achromobacter xylosoxidans* which participate in the degradation of cellulotic materials in landfills. The bacteria degraded lower molecular weight compounds easily but were slow in degrading higher molecular weight compounds. The slow removal of the HMW compounds was attributed to the low bioavailability due to low solubility. Although a wide range of organisms capable of degrading PAHs and other aromatic compounds have been studied widely, information on the behaviour and performance of such organisms under radioactive conditions is still rare. This study, demonstrates successfully that radiation tolerant consortia can be trained to degrade recalcitrant compounds under radiotoxic conditions.

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