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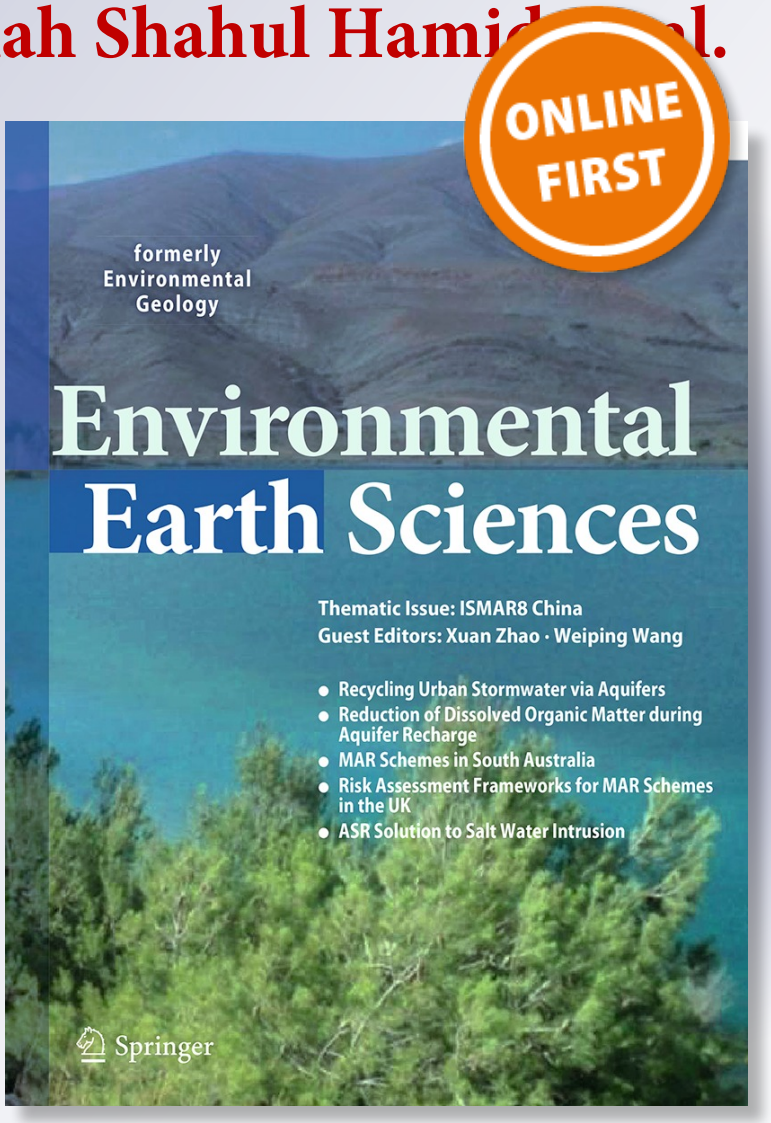

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ORIGINAL ARTICLE

Degradation of polycyclic aromatic hydrocarbons (pyrene and fluoranthene) by bacterial consortium isolated from contaminated road side soil and soil termite fungal comb

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are often present in the environment at concentrations detrimental to both human health and eco-quality. Hence, PAH degradability has been of significant interest, and biological methods seem to be preferred to other options such as chemical oxidation, photolysis and adsorption. Present study was designed to isolate potential PAH-degrading bacteria from termite fungal comb and road side soil with the aim of evaluating the degradation of fluoranthene and pyrene using the isolated microbes. Therefore, 97–99 % pure PAHs (fluoranthene and pyrene) were subjected to biodegradation using bacteria consortiums from soil and the termite fungal comb in separate tests. At varying concentrations (50, 100 and 150 mg l^{-1}) of both PAHs, amendments characterized of *Ralstonia pickettii*, *Burkholderia cepacia* and *Pseudomonas resinovorans* from road side soil reduced fluoranthene more than *Ochrobactrum* sp. and *Pseudomonas* sp. isolated from termite fungal comb. The overall comparison of the PAH degradation showed that the microbial consortium degraded pyrene more than fluoranthene. However, the efficiency of the biodegradation tests on fluoranthene and pyrene was <50 %. The study inferred that isolated bacterial species from termite fungal comb and road side soil when used as consortium can remedy contaminations attributed to more than one PAH. But the degree of degradation by bacteria species may depend on the source of isolation.

Keywords PAHs · Degradation · Bacterial consortium · Contaminated road side soil · Soil termite fungal comb

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one among many pollutants that are indiscriminately found in the environment. To a large extent, human exposure to PAHs appears highly unavoidable (Sen and Field 2013). PAHs are products that emanate from incomplete/partial combustion of fossil fuels in cars, industries and our kitchen. The presence of PAHs in the environment is an increasing concern due to the associated toxicity, mutagenicity and carcinogenicity (Sato and Aoki 2002; Lee et al. 2013; Man et al. 2013; Kim et al. 2013). Despite the fact that PAHs in the environment undergo chemical oxidation, photolysis, bioaccumulation, volatilization and adsorption, yet microbial degradation and transformation have been identified as the principal processes for the pollutants' removal (Zeng et al. 2010).

Recent studies have focused on bioremediation as a tool for the restoration of contaminated soil at low cost, due to its promising capacity (Lu et al. 2012; Shahsavari et al. 2013). The use of bacteria in the degradation of PAHs has been widely reported (Haritash and Kaushik 2009) and various bacteria that have the ability to degrade individual PAHs had been isolated and identified (Cerniglia 1993; Safekordi and Yaghmaei 2001). However, there is limited report on bacteria with the capability to degrade a broad

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range of PAHs (Shafiee et al. 2006). In fact, the following genera of microbes are known to degrade PAHs especially low molecular weight PAHs; *Acinetobacter, Aeromonas, Agmenellum, Alcaligenes, Bacillus, Berjerinckia, Burkholderia, Corynebacterium, Cyclotrophicus, Flavobacterium, Micrococcus, Moraxella, Mycobacterium, Nocardioides, Pseudomonas, Lutibacterium, Rhodococcus, Streptomyces, Sphingomonas, Stenotrophomonas, Vibrio, Paenibacillus* (Kim et al. 2005; Juhasz and Naidu 2000; Daane et al. 2002; Samanta et al. 2002; Van Hamme et al. 2003; Xia et al. 2005).

Another study (Fritsche and Hofrichter 2008) mentioned that despite the fact that a single microbe can degrade organic pollutants, still microbes optimally metabolize pollutant when introduced as consortium. In addition, the study found that a complex mixture of pollutants or organic compounds requires microbial communities to work together in order to efficiently degrade the pollutant, since a combination of genetic information among the group of organisms give the best potential for degradation. However, the source of the microbes required for degradation of the pollutants is important, especially when considering the level of microbial diversity and the associated ecological relevance. Furthermore, it is important to note that soil and macro-organisms are integral parts of the ecosystem, and both are the reservoir and enhancers of metabolic activities for vast number of microbes. Though normal soil is a serene habitat for numerous microorganisms, yet, contaminated/polluted soils are known to harbor some microbes considered to be persistent organisms (Carlot et al. 2002; Megharaj et al. 2003; Adesomoye et al. 2006). Hence, some macro-organisms like termites are known to harbor and enhance microbial activities. Soils termites are also known as "soil engineers" and are one of the main macro-invertebrate decomposers in arid and semi-arid environments. The termites exert additional impacts to the soil by building biostructures that alter the physical and chemical properties of the soil. Termites are not alone when it comes to bioremediation capability, rather bacteria that live in the termite and its surrounding were also found to have the capability to degrade pollutants. In fact, Ngugi et al. (2005) found that *Macrotermes michaelseni*, isolated from the intestinal tract of a fungi-cultivating termite had the capability to degrade resorcinol, a phenolic compound produced naturally or through human activities (Hans 1994). *Macrotermes michaelseni* has also been found to degrade both phenol and benzoic acid. However, no research has been carried out to test the pyrene and flouranthene degradation ability of bacteria isolated from the fungus comb of *Macrotermes gilvus.*

Therefore, this study was designed to isolate potential PAH-degrading bacteria from termite fungal comb and road side soil with the aim of evaluating degradation of fluoranthene and pyrene using the isolated microbes. It intends to elucidate the characteristics of isolated bacteria when used as consortium than when utilized in discrete forms as reported by previous studies. This is because the rate and extent of the degradation does not depend only on the type of bacteria and various environmental factors including temperature, pH, and the presence of nutrient sources (Haritash and Kaushik 2009), but also on interactions that may exist among the microbes in a particular environment (Emenike et al. 2013).

Materials and methods

Chemicals and media

Fluorene, acenaphthene, fluoranthene, chrysene, pyrene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd) pyrene were acquired from Sigma–Aldrich (Selangor, Malaysia). The purity of the PAHs used was 97–99 %. PAH stock solutions used were made at 500 mg l^{-1} in acetone and were diluted to 50, 100 and 150 mg l^{-1}. HPLC grade of other solvents and chemicals were also utilized. The mineral salt medium (MSM) used has been described in previous studies (Zajic 1972; Abioye et al. 2012). The contents (per liter) are detailed in Table 1. In the event that a solid medium was required, 20 g of agar was added to produce the solid medium. The pH of the mixture was maintained at 7.4.

Road side soils and soil termite fungal comb sampling

Road side soil samples (0–5 cm in depth) were collected from the Chan Saw Lin Industrial area (Kuala Lumpur, Malaysia). The samples were taken using a stainless steel soil auger and consequently transferred to the laboratory for immediate use. The soil termite fungal comb used in this study was obtained from the termite nest near the Chemistry Department building, University of Malaya (Kuala Lumpur, Malaysia). Sample collection and experimental setup were replicated three times to ensure

Table 1 Chemicals and the amount used for preparing the liquid medium

Chemical	Amount (g)
K_2HPO_4	1.80
NH_4Cl	4.00
$MgSO_4{\cdot}7H_2O$	0.20
KH_2PO_4	1.20
$FeSO_4{\cdot}7H_2O$	0.01
NaCl	0.10

accuracy and accommodate variability across generated parameters/components deemed relevant to the study.

Growth test on individual PAHs

Two (2) grams of contaminated road side soil and termite fungal comb samples were separately weighed and placed in a pre-cleaned beaker containing 100 ml of saltwater. The saltwater was prepared by adding 10 g of sodium chloride (NaCl) into 100 ml of distilled water. Both mixtures were then shaken for 30 min to extract the supernatant from the samples. 1 ml of each prepared extract was then placed in separate test tubes that were pre-filled with 9 ml of distilled water to obtain a solution with an initial dilution factor of 10. The solution was then serially diluted until a 10^5 dilution was obtained. After the serial dilution, 1 ml of each dilution was spread on dried agar plates layered with individual PAHs in two replicates. The concentration of the PAHs used in this study was 100 mg l^{-1}. The inoculated plates were then incubated in dark conditions at 35 °C for 7 days. The growth of bacteria was visually measured by comparing the incubated plates with the controls (un-inoculated plate, and/or a plate without PAHs).

Concurrently, the growth of the bacteria used in this study was monitored using a liquid medium. 10 ml of 500 mg l^{-1} of individual PAH was added to 250-ml pre-cleaned conical flask which was gently shaken to evaporate the solvent used (acetone). The flask was then filled with 90 ml of fresh MSM medium and 10 ml of the obtained supernatant aliquot. The mixture was then shaken in an incubator at 150 rpm and 35 °C in dark conditions. The growth was monitored daily by measuring the optical density using a spectrophotometer at 600 nm. Other than fluoranthene and pyrene, the growth test was also done with fluorene, acenaphthene, chrysene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene.

Enrichment and isolation of the bacteria

In the enrichment process, 10 ml of the culture was mixed with a new 90 ml MSM medium in a conical flask with pre-dried fluoranthene or pyrene. The mixture was then continuously shaken for 7 days, before 10 ml of it was taken out and mixed in a new 250-ml conical flask pre-treated with fluoranthene or pyrene. The same steps were repeated for 5 times. At the end of the enrichment process, bacterial strains in the culture were isolated by spreading the serially diluted consortium on agar plates coated with a layer of fluoranthene or pyrene on the surface, and incubated at 35 °C for 24 h. Bacteria colonies that produced clear zones were picked up from the plates and sub-cultured to obtain pure individual isolates. The pure cultures were then subjected to an identification step using a Biolog automated system (Biolog Microstation System). The system was based on the MicroStation™ System/ Microlog User's Guide (Biolog 2009). To ensure the authenticity of identified organisms, the microbial identification protocol was duly repeated (Bochner 1989a, b; Spiegelman et al. 2005).

Preparation of the bacteria consortium

The fluoranthene-degrading bacteria from the contaminated road side soils were named RSS-F1, RSS-F2 and RSS-F3 while the pyrene-degrading bacteria were named RSS-P1, RSS-P2 and RSS-P3. Similarly, the fluoranthene-degrading bacteria from the termite fungal comb were named T-F1, T-F2 and T-F3 while pyrene-degrading bacteria from the same source were named T-P1, T-P2 and T-P3. To further grow the bacterial consortium for use in the degradation studies, three colonies of each bacteria species were selected and inoculated in a conical flask with 100 ml of MSM medium that was pre-treated with fluoranthene or pyrene. The mixtures were then subjected to the same enrichment steps for five more times before they were used in the degradation studies.

Degradation study

The degradation of PAHs was performed by allowing the bacteria consortium to utilize a single PAH as the sole source of both carbon and energy. The degradation study as performed in a liquid culture, was prepared by adding 10 ml of 500 mg l^{-1} single PAH, diluted in acetone, into a sterile empty 250-ml flask to obtain a PAH solution (50 mg l^{-1}). The acetone was then evaporated by gently shaking the flask. MSM medium (90 ml) was subsequently added to each flask with the dried PAH and 10 ml of the previously enriched bacteria consortium were inoculated into the flask before incubation of the cultures at 150 rpm and 35 °C.

In this degradation study, three different conditions were tested. (i) Medium + PAHs + bacterial consortium; (ii) medium + PAHs; and (iii) medium + bacterial consortium, with (ii) and (iii) serving as controls. All treatments including the controls were in triplicates and 10 ml of the cultures were taken on a weekly basis. These were extracted with 3 × 10 ml dichloromethane using a separation funnel (liquid–liquid extraction), concentrated and injected into gas chromatography–mass spectroscopic (GC–MS). All steps in the degradation studies were repeated for all the varying PAH concentrations (50, 100 and 150 mg l^{-1}). Degradation of PAHs was determined by calculating the remaining concentration of PAHs in the broth culture.

Instrumental analysis

The determination of the PAHs and their degradation by-products were performed via the use of GC–MS QP2010 Plus (Shimadzu, Japan) equipped with RTX-5MS (Crossbond 5 % diphenyl/95 % dimethyl polysiloxane) column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium with the purity of 99.999 % was used as the carrier gas, with an average velocity of 40 cm/s. The temperature of the GC oven was initially set as 60 °C for 2 min and subsequently increased to 280 °C at the rate of 6 °C min^{-1} and maintained for 2 min. The temperatures of the injection port and the transfer line were maintained at 290 and 300 °C, respectively. The data used in the quantitative analysis were acquired in the electron impact mode (70 eV) and scanning range of 50–550 amu at 1.5 s $scan^{-1}$.

Results and discussion

The result showed that bacteria growth was found only in the medium where fluoranthene and pyrene served as the sole carbon sources. Therefore, only these two individual PAHs were used in further enrichment and the degradation studies.

Identification of the PAH-degrading strains

The Programmed Microbial Identification System from Biolog has been proven to be useful in the characterization and classification of bacteria (Weissenfels et al. 1990). Table 2 revealed the microbial distribution in the two samples (termite fungal comb and contaminated road side soil). It was observed that high similarity existed at both the genus and species levels of the bacteria distribution in the termite fungal comb for both carbon sources. *Pseudomonas* sp. as identified, usually have the potential to degrade PAHs; hence in this study, *Pseudomonas putida biotype B* and *Pseudomonas aeruginosa* were expected to degrade fluoranthene and pyrene. However, the degradation effect of *Ochrobactrum* sp. will expectedly vary based on the carbon sources. On the other hand, more bacterial diversity was observed in the degradation of fluoranthene and pyrene using the contaminated road side soil isolates. *Ralstonia pickettii* was common at the utilization of either of the carbon sources. *Burkholderia* sp. and *Pseudomonas* sp. were only found in fluoranthene-induced media while *Ochrobactrum* sp. and *Cornynebactrium* sp. were found when pyrene was the carbon source.

The above may imply that certain PAHs, especially fluoranthene and pyrene can be degraded by *Ochrobactrum* sp. just like the *Pseudomonas* sp. This may support the reason for the several studies that have been conducted to isolate, characterize and identify microorganisms which have ability to degrade a wide range of PAHs compounds (Yuan et al. 2000; Dean-Ross et al. 2001; Wong et al. 2002; Prabhu and Phale 2003; Santos et al. 2007; Zhao et al. 2009; Rani et al. 2009). *Pseudomon*as sp., a gram-negative bacterium, has been reported as the main bacteria species that functions as a degrader of PAHs. It has the ability to degrade both low and high molecular weight PAHs if an appropriate growth medium is supplied (Yuan et al. 2000; Juhasz and Naidu 2000; Dean-Ross et al. 2001). Other than a PAH degrader, genus *Pseudomon*as has also been reported to have the capability to degrade other organic recalcitrant pollutants (Auger et al. 2012; Plociniczak et al. 2013; Zhang 2008).

Although other genus of bacteria found in this study were not characterized as the main degrader of PAHs, previous researches have proved that the bacteria from genus *Ochrobactrum, Ralstonia, Burkholderia* and *Corynebacterium* were also capable and have a promising potential to degrade PAHs (Ghosal et al. 2013; Kim et al.

Table 2 Identification of the bacteria from either the termite fungal comb or the contaminated road side soil

Samples	Source of carbon	Organism	Identity
Termite fungal comb	Fluoranthene	T-F1	*Ochrobactrum intermedium*
		T-F2	*Pseudomonas aeruginosa*
		T-F3	*Pseudomonas putida biotype B*
	Pyrene	T-P1	*Pseudomonas putida biotype B*
		T-P2	*Ochrobactrum tritici*
		T-P3	*Pseudomonas aeruginosa*
Contaminated road side soils	Fluoranthene	RSS-F1	*Ralstonia pickettii*
		RSS-F2	*Burkholderia cepacia*
		RSS-F3	*Pseudomonas resinovorans*
	Pyrene	RSS-P1	*Ralstonia pickettii*
		RSS-P2	*Ochrabactrum anthropi*
		RSS-P3	*Corynebacterium appendicis*

2003; Chavez-Gomez et al. 2003; Seo et al. 2009; Othman et al. 2011; Fernández-Luqueño et al. 2011). Hence, some of the bacteria used in this study have been previously identified as PAH degraders, it is believed that this is the first time that these bacteria were isolated from a different source (termite fungal comb) for the purpose of degrading PAHs. It is also believed that this is the first time that their capability to degrade PAHs in a consortium was identified.

Degradation studies

PAH researches in recent years focused more on the degradation of high molecular weight PAHs. The studies included the isolation and identification of several microorganisms that can mineralize and grow on four-ring PAHs when used as the sole carbon and energy sources (Bouchez et al. 1995; Boonchan et al. 1998; Juhasz et al. 1997; Kastne et al. 1994; Mueller et al. 1990; Walter et al. 1991; Yuan et al. 2000). Some of these isolates have been used to identify the biochemical pathways involved in the catabolism of the PAHs (Mrozik et al. 2003). An isolated bacteria consortium can only degrade PAHs in the soluble form.

Degradation of fluoranthene by the bacteria consortium isolated from the termite fungal comb and the contaminated road side soil

Table 3 shows the mean reduction in concentration of fluoranthene due to its degradation by the bacteria consortium from the termite fungal comb and the bacteria consortium from the road side soil. The degradation trends are shown in Fig. 1. The results indicated that the bacteria consortium isolated from the road side soil has a greater ability to degrade fluoranthene than the bacteria consortium from the termite fungal comb. When the bacterial consortium from the road side soil was used, 28 ± 16, 32 ± 3 and 36 ± 14 % total degradations of fluoranthene were obtained for the 50, 100 and 150 mg l^{-1} concentrations, respectively. These values are about 10 % higher than those produced by the bacterial consortium from the termite fungal comb under similar conditions.

According to Mrozik et al. (2003), fluoranthene placed in a liquid culture is harder to degrade than fluoranthene placed on a plate. In their study, an average of 6 and 12 % of fluoranthene samples (100 mg l^{-1}) were degraded in a 2-week incubation time by two *Mycobacterium* strains namely NJS-1 and NJSP. On the contrary, both bacteria degraded phenanthrene (100 mg l^{-1}) 100 % during the same incubation period. Another study (Zhou et al. 2008) indicates that there are some differences between the degradation in a liquid culture and on a plate.

Degradation of pyrene by the bacteria consortium isolated from the termite fungal comb and the contaminated road side soil

The degradation of pyrene by bacterial consortium isolated from both termite fungal comb and road side soil are detailed in Table 4 and Fig. 2. Early studies on the bacteria degradation of pyrene were carried out by Heitkamp et al. (1988); since then, various pyrene-degraders have been successfully isolated. Most of them belong to genus *Sphingomonas* (Leys et al. 2004; Cunliffe and Kertesz 2006), *Mycobacterium* and *Rhodococcus* (Miller et al. 2004;

Table 3 Degradation of fluoranthene by the bacteria consortiums isolated from termite fungal comb and road side soil

Bacteria's sources	Time	50 mg l^{-1}		100 mg l^{-1}		150 mg l^{-1}	
		Remaining conc. (mg l^{-1})	Reduction (%)	Remaining conc. (mg l^{-1})	Reduction (%)	Remaining conc. (mg l^{-1})	Reduction (%)
Termite fungal comb	Day 0	50.0 ± 0.00	0.00	100.0 ± 0.00	0.00	150.0 ± 0.00	0.00
	Day 1	48.1 ± 1.01	0.44	99.0 ± 0.13	0.21	145.3 ± 4.5	0.90
	Week 1	44.0 ± 2.78	8.32	96.8 ± 2.03	2.47	132.1 ± 6.00	9.79
	Week 2	44.3 ± 1.90	8.83	91.3 ± 7.42	8.01	114.4 ± 12.72	21.90
	Week 3	40.7 ± 3.23	15.61	79.2 ± 8.24	20.20	114.0 ± 10.23	22.61
	Week 4	39.8 ± 3.13	17.63	77.6 ± 7.77	21.80	111.0 ± 3.12	24.30
Road side soil	Day 0	50.0 ± 0.00	0.00	100.0 ± 0.00	0.00	150.0 ± 0.00	0.00
	Day 1	48.4 ± 1.31	0.41	98.7 ± 1.20	0.38	147.2 ± 2.00	1.18
	Week 1	46.9 ± 1.36	3.53	90.1 ± 6.43	9.04	113.1 ± 15.31	24.42
	Week 2	46.8 ± 0.77	3.62	86.8 ± 8.91	12.41	109.2 ± 10.10	26.53
	Week 3	43.8 ± 2.86	9.84	72.7 ± 9.31	26.60	101.1 ± 10.21	32.21
	Week 4	35.0 ± 2.02	28.01	67.2 ± 6.95	32.20	95.0 ± 6.42	36.32

Data are presented as mean ± standard deviations for the replicates

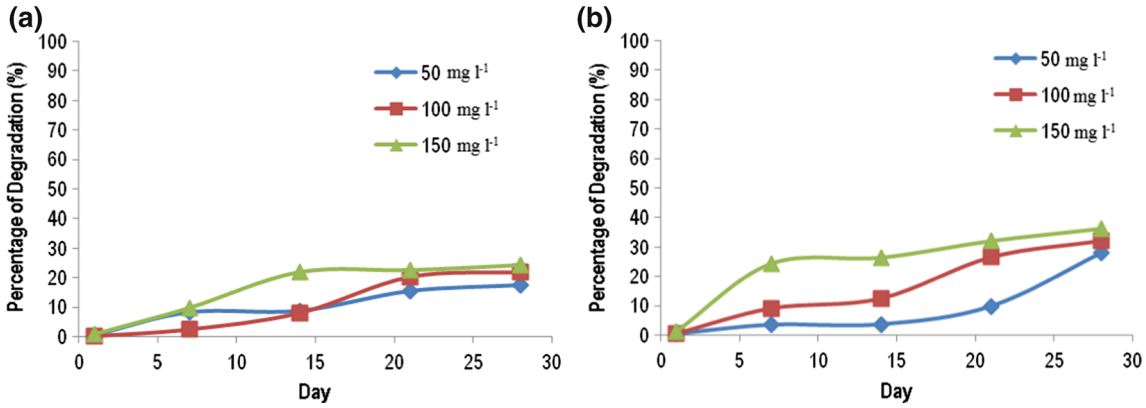

Fig. 1 The degradation trend of fluoranthene in percentage (%) by **a** the bacterial consortium isolated from the termite fungal comb, **b** the bacteria consortium isolated from the contaminated road side soil

Table 4 Degradation of pyrene by the bacteria consortiums isolated from the termite fungal comb and the road side soil

Bacteria's sources	Time	50 mg l^{-1}		100 mg l^{-1}		150 mg l^{-1}	
		Remaining conc. (mg l^{-1})	Reduction (%)	Remaining conc. (mg l^{-1})	Reduction (%)	Remaining conc. (mg l^{-1})	Reduction (%)
Termite fungal comb	Day 0	50.0 ± 0.00	0.00	100.0 ± 0.00	0.00	150.0 ± 0.00	0.00
	Day 1	47.3 ± 2.01	4.31	96.9 ± 1.39	1.21	138.1 ± 5.20	0.64
	Week 1	47.1 ± 1.12	4.70	70.3 ± 5.53	28.32	124.2 ± 5.17	11.12
	Week 2	43.1 ± 1.33	12.71	66.7 ± 3.52	32.01	117.1 ± 5.90	16.01
	Week 3	40.4 ± 8.21	18.21	65.7 ± 862	33.04	92.2 ± 5.29	33.62
	Week 4	31.9 ± 3.77	35.43	59.5 ± 8.17	39.41	81.1 ± 1.11	41.60
Road side soil	Day 0	50.0 ± 0.00	0.00	100.0 ± 0.00	0.00	150.0 ± 0.00	0.00
	Day 1	49.4 ± 0.21	0.38	96.6 ± 1.12	1.63	148.4 ± 1.81	0.58
	Week 1	47.6 ± 1.19	4.02	84.6 ± 3.86	13.82	120.1 ± 3.26	19.74
	Week 2	42.4 ± 4.47	14.50	80.2 ± 2.84	18.31	93.1 ± 5.45	37.50
	Week 3	41.3 ± 3.03	16.72	61.1 ± 10.52	37.81	86.3 ± 2.23	42.41
	Week 4	30.2 ± 5.11	39.22	58.8 ± 3.18	40.12	82.2 ± 6.04	44.80

Data are presented as mean ± standard deviations for the replicates

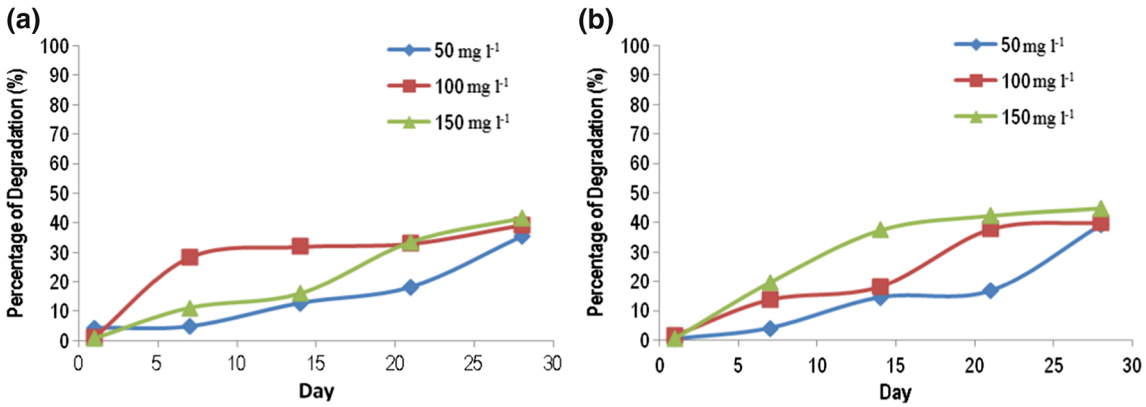

Fig. 2 The degradation trend of pyrene in percentage (%) by **a** the bacterial consortium from the termite fungal comb, **b** the bacterial consortium from the road side soil

Hennessee et al. 2009). In this experiment, a combination of *R. pickettii, O. anthropi* and *Corynebacterium appendicis* as the bacteria consortium from the road side soil was found to degrade pyrene better than the bacteria consortium from the termite fungal comb, which consisted of *P. putida biotype B*, *O. tritici* and *P. aeruginosa*. The mean differences in the total reduction between the pyrene degraded by the bacteria consortium from the road side soil and the pyrene degraded by the termite fungal comb at 50, 100 and 150 mg l^{-1} concentrations were 4, 1 and 3 %, respectively. These results show that the differences were small compared to the degradation of fluoranthene by the two isolates. Yet, the results indicate that the overall degradation of pyrene was higher when compared with fluoranthene degradation independently of the bacterial consortium used.

From the degradation results of fluoranthene and pyrene, it can be seen that the bacteria combination of *R. pickettii, O. anthropi* and *C. appendicis*, which were isolated from the road side soil, had a higher capability to degrade fluoranthene and pyrene than the bacteria consortium with the combination of *P. putida biotype B*, *O. tritici* and *P. aeruginosa* that was isolated from the termite fungal comb. Sequel to this, the isolated *Pseudomonas* sp. appears to be a significant degrader than any other microbe in this study considering its degree of occurrence.

The reduction in concentration of both fluoranthene and pyrene increased with time. Comparing with the studies conducted earlier (Sayara et al. 2009), the mean reductions recorded are considered poor since the measured total reductions for all samples were averagely <50 % in the 4-week experiment. Moreover, it was identified from the studies that the degradation rate of both fluoranthene and pyrene increased with increasing PAH concentrations. This same situation was observed by (Sayara et al. 2009). The degradation efficiency was found to decrease with the decrease in concentrations of PAH model molecule. The reason for such trend may be associated to metabolic activities of the microbes which are assumed to be very dependent on the model molecules as carbon sources. Hence, higher concentration of fluoranthene and pyrene seem to encourage more pronounced extra-cellular activities among the microbes.

Generally the observed degradation trend (Figs. 1, 2) seems to point towards some kinetic irregularities across the weeks of experimental monitoring. Such trend could be attributed to microbial behavior and interaction. Despite the fact that the PAHs served as the carbon source, yet, individual microbes possess the ability to compete for nutrient and space whenever they exist in a combined state. Therefore, competition among the microbes alongside their individual extra-cellular potentials could have influenced the kinetic of the degradation process. Also environmental stress pattern can induce responses that are slightly or significantly different from the response nature of some microbes while being in an unpolluted environment. Therefore, induced response adaptation of the microbes may invariably affect the doubling time; hence their ability to recover and overcome stress may significantly influence a steady or distorted kinetic trend. Similarly, enzymatic activities of the microbes could have caused varied degrees of volatilization and adsorption which are known to contribute to PAH degradation in contaminated environment (Haritash and Kaushik 2009).

Conclusion

The present study showed that bacteria species that possess PAH degradation potential can be isolated from termite fungal comb and contaminated road side soil. It has also been shown that some microbes may have PAH degradation potential, but such potential can be dependent on the source of isolation; consortium from contaminated road side soil are more efficient in the degradation of PAHs than isolates from termite fungal comb. Similarly, the study also infers that pyrene degradation was more pronounced than fluoranthene when exposed to the introduced microbes. However, the measured degradation levels as induced by the consortiums is generally low, but may be enhanced by further manipulation of the identified microbes.

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Conflict of interest The authors have declared no conflict of interest.

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