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Using orthogonal design to determine optimal conditions for biodegradation of phenanthrene in mangrove sediment slurry

Jian Lin Chen[a], Kwai Chi Au[a], Yuk Shan Wong[b], Nora Fung Yee Tam[a,*]

[a] Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China
[b] Department of Biology, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong, China

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ABSTRACT

In the present paper, the effects of four factors, each at three levels, on biodegradation of phenanthrene, a 3-ring PAH, in contaminated mangrove sediment slurry were investigated using the orthogonal experimental design. The factors and levels were (i) sediment types (clay loam, clayey and sandy); (ii) different inoculums (*Sphingomonas* sp., a mixture of *Sphingomonas* sp. and *Mycobacterium* sp., and without inoculum); (iii) presence of other PAHs (fluorene, pyrene, and none); and (iv) different salinities (5, 15 and 25 ppt). Variance analysis based on the percentages of Phe biodegradation showed that the presence of other PAHs had little effect on phenanthrene biodegradation. The kinetics of phenanthrene biodegradation in all experiments was best fitted by the first order rate model. The highest first order rate constant, k value was 0.1172 h^{-1} with 97% Phe degradation; while the lowest k value was 0.0004 and phenanthrene was not degraded throughout the 7-d experiment. The p values of k for the four factors followed the same trend as that for the biodegradation percentage. Difference analysis revealed that optimal phenanthrene biodegradation would take place in clay loam sediment slurry at low salinity (5 to 15 ppt) with the inoculation of both *Sphingomonas* sp. and *Mycobacterium* sp.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) derived from natural sources and human inputs occur widely in coastal environments and accumulate in sediment [1]. The concentrations of reported PAHs ranged from a few $\mu g\,g^{-1}$, in low-level contaminated sediment, to a few hundred $\mu g\,g^{-1}$ in the sediment near industrial sites [2] and have reached as high as a few thousand $\mu g\,g^{-1}$ in some of the seriously contaminated sediment [3]. In Hong Kong Special Administration Region (HKSAR), the concentration of PAHs also differs from place to place, and the highest concentration of PAHs detected in the surface mangrove sediment was 11 $\mu g\,g^{-1}$ [4]. Microbial degradation of PAHs by a variety of naturally occurring soil bacteria is considered to be the major decomposition process in nature and represents a potential solution to remove contaminants [5–7]. Although a variety of bacteria that can use PAHs as the sole carbon and energy source have been isolated from the contaminated environment [8–10], success in the application of bioremediation depends largely on the environmental conditions, including nutrient, salinity, temperature, concentration of contaminant, type of sediment, etc. These factors directly affect the activity of the inoculated and/or the indigenous PAH-degrading bacteria, as well as the bioavailability of PAHs to microorganisms [11,12]. Several suggested important factors on PAH biodegradation, including the ratio of carbon to nitrogen (C: N), salinity, temperature, inoculum size and the concentration of PAHs, have been well studied [13–16]. In addition to the above factors, soil properties (such as soil type) also affect the sorption of PAHs and their bioavailability [17–19], as well as the activity and types of PAH-degrading bacteria [20]. Additionally, since the affected environment is often contaminated by more than one PAH compound, the interaction among different PAH compounds would also affect the rate and extent of biodegradation [21]. Previous research has confirmed that pyrene (Pyr) would co-metabolize in the presence of phenanthrene (Phe) [22,23]; but the effect of the presence of Pyr (a 4-ring PAH) on the biodegradation of Phe (a 3-ring PAH) was less studied.

So far, simultaneously examining the effects of different factors on biodegradation of PAHs is scarce and the optimal condition for an efficient bioremediation in a specific environment is often not understood. One of the reasons is that a full factorial design is traditionally used for this kind of research and every possible combinations of different factors are tested, thus many experiments must be carried out which involves heavy work load and becomes impractical. In the last two decades, the orthogonal experimental design, that aims to achieve operations within one of its components but does not create side-effects to other components, has been used in research areas such as analytical chemistry, chemical engineering and medicine to simultaneously investigate different

* Corresponding author. Tel.: +852 2788 7793; fax: +852 2788 7406.
E-mail address: bhntam@cityu.edu.hk (N.F.Y. Tam).

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Table 1
Experimental conditions for Phe biodegradation in 1:1 mangrove sediment slurry (w/v) based on the orthogonal design form $L_9(3^4)$.

Exp. number	Sediment[a]	Inoculum[b] (MPN g^{-1} sediment)	Concentration of PAH(s)[c] (mg kg^{-1} air-dried sediment)	Salinity (ppt)
1	HC	None	Phe (100)	5
2	HC	*Sphingomonas* sp. (10^6)	Phe + Fl (50 + 50)	15
3	HC	*Sphingomonas* sp. + *Mycobacterium* sp. ($0.5 \times 10^6 + 0.5 \times 10^6$)	Phe + Fl + Pyr (50 + 25 + 25)	25
4	KLH	None	Phe + Fl (50 + 50)	25
5	KLH	*Sphingomonas* sp. (10^6)	Phe + Fl + Pyr (50 + 25 + 25)	5
6	KLH	*Sphingomonas* sp. + *Mycobacterium* sp. ($0.5 \times 10^6 + 0.5 \times 10^6$)	Phe (100)	15
7	MP	None	Phe + Fl + Pyr (50 + 25 + 25)	15
8	MP	*Sphingomonas* sp. (10^6)	Phe (100)	25
9	MP	*Sphingomonas* sp. + *Mycobacterium* sp. ($0.5 \times 10^6 + 0.5 \times 10^6$)	Phe + Fl (50 + 50)	5

[a] HC, KLH and MP were surface sediment collected from the mangrove swamp of Ho Chung, Kei Ling Ha and Mai Po, respectively;
[b] None means no inoculum was added into the sediment slurry;
[c] Phe: phenanthrene; Fl: fluorene; Pyr: pyrene.

factors and optimize the condition [24–26]. Recently, our group has demonstrated that the orthogonal design could be applied to determine significance of some environmental factors including C:N ratio, inoculum size, nutrient addition, temperature and salinity on biodegradation of Phe in sediment slurry by *Sphingomonas* sp., a PAH-degrading isolate from mangrove sediment [15]. The present study, a continuation of our previous work, aims to further explore the application of the orthogonal design in investigating the effects of four factors including sediment types, inoculums, the presence of other PAHs and salinities, each at three levels (Table 1), on biodegradation of Phe in mangrove sediment slurry.

2. Materials and methods

2.1. Chemicals and PAH-degrading bacterial isolates

Standards of phenanthrene (Phe, 96%), fluorene (Fl, 99%) and pyrene (Pyr, 98%) were purchased from Sigma Chemicals, USA. The stock solution of each PAH compound was prepared by dissolving an appropriate amount of the standard in distilled acetone, with a final concentration of 5000 mg L^{-1}. All solutions were kept in a brown bottle at 4 °C and wrapped with aluminum folds to avoid any light exposure prior to use. Other chemicals used in the present study were analytical grade. *Sphingomonas* sp. and *Mycobacterium* sp., two bacterial strains enriched and isolated from surface mangrove sediment were used as the inoculums. The former isolate was found to have preference to degrade Phe while the latter one was a Pyr-degrader [27,28].

2.2. Mangrove sediment

Surface sediment (0–2 cm) collected from three typical mangrove swamps in HKSAR, namely Ho Chung (HC), Mai Po (MP) and Kei Ling Ha (KLH), were filtered through a 2 mm sieve and kept moist at 4 °C not more than one day before the experiment. The soil texture was analyzed by the sieving and pipette technique following the standard method described by Gee and Bauder [29]. The total organic matter (TOM) in sediment was determined by loss of ignition method (at 550 °C for 6 h); while the total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were measured by the Flow Injection Analyzer (Lachat QuikChem® 8000, Lachat Instruments, USA) after the Kjeldahl acid digestion method [30]. The 16 USEPA priority PAHs in the sediment were extracted and analyzed according to Tam et al. [4], details are described in Section 2.4.

2.3. Experimental design

The orthogonal design form $L_9(3^4)$, selected from the design module of Statistica 6.0 (SoftStat Inc., 1984–2001, USA), was used. The form, with a total of nine experiments, was used to investigate the effect of four factors, including sediment types, inoculums, presence of other PAHs and salinities (Table 1). An appropriate amount of stock Phe solution (Section 2.1) was added to each 250 mL conical flask to yield the designed initial concentrations. The acetone in each conical flask was allowed to evaporate. The sediment slurry was then prepared by mixing 100 g fresh sediment with 100 mL distilled water in the conical flask. The flasks were shaken at 180 rpm at 30 °C. Another set of flasks containing the sterilized sediment slurry (by autoclaving the sediment and water at 121 °C and 0.1 MPa pressure for 20 min to prevent any microbial activity), prepared in the same method, without bacterial inoculation, was also prepared to determine any abiotic loss of PAHs. All experiments were carried out at pH 6.8, and each was in triplicate. The sediment slurry (10 mL) was collected from each flask at 1 h (day-0), day-3 and 7. All samples were immediately freeze-dried and used for analysis of residual PAHs.

2.4. Extraction and analysis of PAH

Each freeze-dried sediment sample was transferred to a 250 ml pre-ash conical flask with the addition of m-terphenyl, as the internal standard (prepared in acetone with concentration of 1000 mg L^{-1}). The sediment was extracted by 100 mL of dichoromethane and methanol (2:1, v/v) followed by sonication in an ultrasonic bath for 15 min. The extract was then filtered into a separating funnel using No. 541 Whatman filter paper. A second extraction was conducted with 100 mL dichoromethane and

Table 2
General properties of surface sediment collected from three mangrove swamps.

Properties	Mangrove sediment		
	HC	KLH	MP
Texture (%)			
Sand	59.25 ± 1.85^a	88.07 ± 1.29^b	0.77 ± 0.06^c
Silt	22.77 ± 1.54^a	4.47 ± 0.03^b	45.07 ± 1.43^c
Clay	20.56 ± 1.37^a	8.07 ± 1.29^b	64.59 ± 0.64^c
Nutrient (%)			
TOM	6.44 ± 0.22^b	1.08 ± 0.25^c	9.30 ± 0.71^a
TOC	2.25 ± 0.07^a	0.32 ± 0.05^b	3.31 ± 0.12^a
TKN	0.024 ± 0.002^a	0.001 ± 0.000^b	0.068 ± 0.009^a
TP	0.010 ± 0.001^a	0.010 ± 0.001^a	0.066 ± 0.012^a
Concentration of PAHs (ng g^{-1} freeze-dried sediment)			
16 PAHs	239.8 ± 70.3^a	151.7 ± 51.9^b	579.9 ± 224.5^c
Fl	51.66 ± 12.23^a	5.72 ± 1.76^b	122.52 ± 24.54^c
Phe	32.35 ± 5.91^a	20.27 ± 3.24^b	47.34 ± 10.25^c
Pyr	38.44 ± 9.81^a	20.50 ± 7.53^b	20.49 ± 7.98^b

HC: Ho Chung; KLH: Kei Ling Ha; MP: Mai Po; TOM: total organic matter; TOC: total organic carbon; TKN: total Kjeldahl nitrogen; TP: total phosphorus; Fl: fluorene; Phe: phenanthrene; Pyr: Pyrene; The mean and standard deviation values of triplicates are shown, and the values in the same row followed by different letters (a, b, c) in the superscript position indicate they are significantly different according to one-way ANOVA at $p \leq 0.05$.

Table 3
Biodegradation percentages of three PAHs at the end of 7-d experiment and the first order rate constants (k) of Phe biodegradation under different experimental conditions based on the orthogonal design form $L_9(3^4)$ as described in Table 1.

Exp. number	Final biodegradation %			k of Phe (h^{-1})	R^2
	Fl	Phe	Pyr		
1	NA	99.29	NA	0.0755	0.992
2	97.62	96.97	NA	0.1172	0.984
3	93.33	96.15	93.75	0.0702	0.976
4	28.57	0.15	NA	0.0004	0.837
5	97.14	98.37	0.01	0.0705	0.971
6	NA	99.49	NA	0.0806	0.989
7	23.53	23.02	22.58	0.0021	0.924
8	NA	41.47	NA	0.0033	0.953
9	85.71	96.95	NA	0.0519	0.955

Mean of three replicates are shown; R^2: regression coefficient of fitting the experimental data of Phe biodegradation to the first order rate model ($n = 3$, critical $R_1^2 = 0.920$); NA: not available (no corresponding PAH spiked).

methanol (1:2, v/v). The two extracts were combined and 90 mL Milli-Q water was added to discard the methanol phase. The funnel was shaken thoroughly for 15 min and the dichloromethane phase was poured into a 250 mL conical flask (Quickfit). The dichloromethane phase was concentrated to 1 mL at 19 °C by a rotary evaporator.

The concentration of Phe in the sediment was determined by a Hewlett Packard 5890 gas chromatography equipped with HP-5MS fused silica capillary column and connected to a flame detection detector (GC-FID). The fused column was 30 m long, 0.32 mm internal diameter coated with methyl silicone and 0.2 μm thickness film (95% dimethyl, 5% diphenyl-polysiloxane). The injector and detector temperatures were set at 280 °C and 300 °C, respectively. Helium was used as the carrier gas. The oven temperature program was from 80 °C (for 2 min) to 120 °C at a rate of 10 °C/min and from 120 °C to 300 °C at a rate of 4 °C/min and held at 300 °C for 15 min. The identification and quantification of Phe, Fl and Pyr were based on matching the retention time (±5%) with the internal standard (m-terphenyl) and the respective PAH standard solution.

2.5. Statistical analyses

The differences in general properties and concentrations of PAH among the three types of mangrove sediment were compared by one-way analysis of variance (ANOVA) followed by Tukey test if the ANOVA result is significant at of $p \leq 0.05$ (SPSS 13.0, SPSS Inc. Illinois, 1989–2004, USA). The biodegradation kinetics of Phe, Fl and Pyr was described by the first order rate model and the first order rate constant k was calculated [15]. The factorial ANOVA of the design module of Statistica 6.0 (SoftStat Inc., 1984–2001, USA) was used to determine which factor was significant in affecting the percentage and rate of Phe biodegradation in the mangrove sediment slurry.

3. Results

3.1. General properties of mangrove sediment

The sediment types varied among mangrove swamps, with sandy sediment in KLH (88.07% sand), clay loam sediment in HC (59.25% sand, 22.77% silt and 20.56% clay) and clayey sediment in MP (64.59% clay) (Table 2). The nutrient content including TOM, TKN and TP were highest in MP sediment while the lowest was in KLH sediment. The C: N: P ratios in the sediment of KLH, HC and MP were 31:0.13:1, 200:2:1 and 47:1:1, respectively. The concentrations of total PAHs (summation of 16 USEPA priority PAHs) in the surface sediment varied from 151 to 579 ng g^{-1} freeze-dried sediment, with the highest concentration in MP, around four times

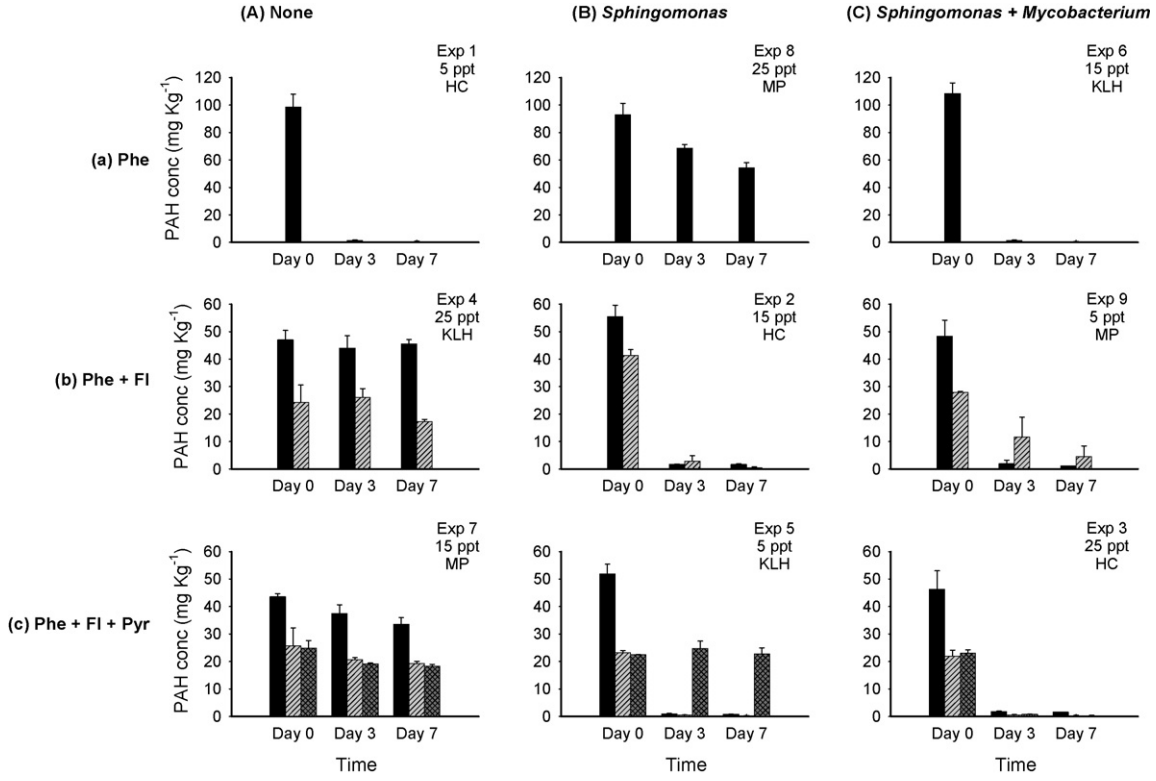

Fig. 1. Concentrations of residual PAHs during 7-d experiment under condition (A) without inoculum, (B) with the inoculation of *Sphingomonas* sp. and (C) with inoculation of *Sphingomonas* sp. and *Mycobacterium* sp. (Mean and standard deviation values of triplicates are shown; solid bar: Phe, shaded bar: Fl, hatched bar: Pyr; Phe, Fl and Pyr: phenanthrene, fluorene and pyrene, respectively).

Table 4
The ANOVA results showing the effect of four factors on Phe biodegradation in mangrove sediment slurry based on $L_9(3^4)$ orthogonal design.

Factors	First order rate constant (k)		Final biodegradation percentage (%)	
	F	p-value	F	p-value
Sediment types	4.003	0.032	3.922	0.038
Inoculums	4.326	0.020	4.737	0.017
Presence of other PAHs	1.313	0.153	1.725	0.197
Salinities	4.189	0.028	4.439	0.022

of the lowest value in KLH sediment. The MP sediment also had the highest concentrations of Fl and Phe (Table 2). These results suggested that MP was the most polluted mangrove swamp.

3.2. Biodegradation of phenanthrene

The changes of Phe in the sterilized sediment slurry, without any microorganisms, were minimal (0.7–1.8%), indicating its abiotic loss in the present study was negligible. The biodegradation of Phe was well fitted by the first order rate model, $C = C_0 e^{-kt}$, where C (mg kg^{-1} air-dried sediment) is the concentration of Phe at time t, C_0 (mg kg^{-1} air-dried sediment) is the initial Phe concentration and k (h^{-1}) is the first order rate constant, with R^2 values larger than 0.9 except Exp. 4 that had the lowest Phe biodegradation (Table 3). When no inoculum was added, just the indigenous microorganisms, the rate and extent of Phe biodegradation by natural attenuation in KLH and MP sediment at 25 ppt salinity was minimal (Exps. 4 and 7) except the HC sediment slurry at a salinity of 5 ppt (Exp. 1), suggesting that only the indigenous microbes in HC at low salinity were effective in degrading Phe. For KLH and MP, the residual concentrations of Phe in the sediment slurry at the end of 7-d experiment were still maintained at around 40 mg kg^{-1} (Fig. 1), and almost no Phe was degraded in KLH sediment slurry.

When the Phe-degrader, *Sphingomonas* sp., was added, Phe biodegradation in MP sediment slurry increased to 42% at a salinity of 25 ppt; while more than 96% of Phe were degraded in both HC and KLH sediment slurries (Fig. 1) and the k values were 0.12 h^{-1} (the highest k value) and 0.07 h^{-1}, respectively (Table 3). When the two PAH-degraders were inoculated together, more than 96% Phe was removed at the end of 7-d experiment for all sediment slurries, and the k values varied from 0.05 to 0.08 h^{-1}. These results suggested that the presence of two PAH-degraders, *Sphingomonas* and *Mycobacterium* sp., enhanced the biodegradation of Phe in mangrove sediment slurry.

3.3. Factors affecting biodegradation of phenanthrene and optimal condition

The significant factors affecting the biodegradation kinetics of Phe, the first order rate constants (k) and the biodegradation percentages at the end of 7-d experiment in mangrove sediment slurry were salinity, inoculums and sediment types but the presence of Fl and/or Pyr had little effect (Table 4). Based on the results depicted in Fig. 2, the optimal condition for Phe biodegradation was clay loam sediment (HC) at low salinity (5 to 15 ppt), and with the inoculation of both *Sphingomonas* sp. and *Mycobacterium* sp.

3.4. Biodegradation of fluorene and pyrene

The changes of Fl and Pyr in the sterilized sediment slurry, without any microorganisms, were minimal (less than 1.8%), indicating that abiotic losses of these two PAHs in the present study were negligible. With the inoculation of *Sphingomonas* sp. alone or together with *Mycobacterium* sp., the residual concentrations of Fl decreased gradually with time. However, such decline of Fl was not observed in the sediment slurry without inoculum (Fig. 1) and <30% Fl was degraded (Table 2), indicating the indigenous microorganisms in mangrove sediment had little ability to degrade Fl. Similarly, significant pyrene biodegradation only took place in the slurry with the inoculation of both *Sphingomonas* and *Mycobacterium*, with the highest degradation of 94% in HC sediment (Exp. 3) (Table 3). The first order rate models were also used to calculate the k constants for Fl and Pyr. The R^2 for Fl biodegradation varied from 0.71 to 0.96, and the higher value was found with the experiment with higher Fl biodegradation. The k values for Fl, ranging from 0.017 to 0.0258. For Pyr, the R^2 values were 0.850 and 0.788 for Exps. 3 and 7, respectively, and the respective k were 0.0220 and 0.0017. The k values of Pyr were significantly lower than that for Phe, indicating that the 4-ring PAHs were more difficult to degrade than Phe.

4. Discussion

4.1. Experimental design

The orthogonal design combined with statistical approach has been applied in research areas to optimize the conditions for improving the production of primary and secondary metabolites in fermentation processes [24], and enhancing the biodegradation of phenol in wastewater treatment [31]. The present study demonstrated that the significant factors and the optimal condition for biodegradation of Phe in contaminated sediment slurry were identified by the orthogonal design with only nine experiments. If a traditional full factorial design is employed to examine the effects of four factors, each at three levels, a total of 64 (4^3) experiments have to be conducted. It is obvious that the orthogonal design can significantly reduce the experimental work load. However, the orthogonal design has two fundamental limitations, one is that it only considers first order effects and the second one is that it does not account for the interaction among factors [26]. As the kinetics of Phe biodegradation in mangrove sediment slurry has proved to follow the first order rate model in the present study and in our previous work [15], the limitations mentioned above are insignificant. In the present study, the optimal range of salinity identified was the same as that reported in our previous work using the tra-

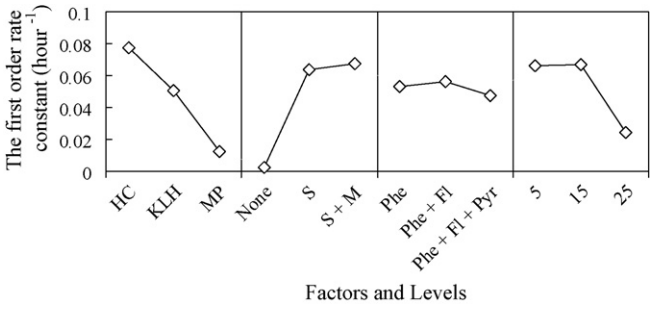

Fig. 2. Level effects of each factor on the first order rate constant of Phe biodegradation (k). Each factor had three levels and the values for each level are shown in Table 1 (HC, KLH and MP: sediment collected from Ho Chung, Kei Ling Ha and Mai Po, respectively; None, *S* and *M*: no inoculum, *Sphingomonas* sp. and *Mycobacterium* sp., respectively; Phe, Fl and Pyr: phenanthrene, fluorene and pyrene, respectively; 5, 15 and 25 were salinities at ppt).

ditional approach [32]. The highest first order rate constant for Phe obtained in the present study ($k = 0.1172\ h^{-1}$) with the presence of other PAH was also comparable to the k value reported by Chen et al. [15] for the optimal biodegradation of single Phe contaminant. The missing interactions, if present, can be handled by running further experiments. It is therefore feasible to employ the orthogonal design to determine the significance of different environmental factors and identify the optimal condition for PAH biodegradation in contaminated sediment.

4.2. Multi-factors on bioremediation of PAH-contaminated sediment

Bioremediation of sediment contaminated with PAHs depends on the ability of microorganisms to use PAHs as the sole carbon and energy source and degrade them to less toxic intermediates and eventually to carbon dioxide under aerobic conditions [6]. A variety of PAH-degrading bacteria in the genera of *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhodococcus, Mycobacterium* and *Sphingomonas* have been isolated from the environment, especially the contaminated sites [27,33–37]. Their ability to degrade PAHs was commonly conducted in culture medium and the inoculum was the only microorganism, therefore, the results were difficult to apply to the real sediment or sediment slurry condition. In the present study, the two bacterial isolates, *Sphingomonas* and *Mycobacterium*, were reported to have the ability to degrade 3- and 4-ring PAHs in culture medium, but their roles in contaminated sediment with the presence of the indigenous microbes are questionable [27,28]. The interactions between the inoculums and the indigenous microorganisms, dependent on specific environmental condition, may affect the survival and degradation ability of the inoculums. The efficacy of bioaugmentation was debatable. Some studies showed that the biodegradation of PAHs was enhanced by the inoculation of PAH-degrading microbes in PAH-contaminated soil/sediment slurry [27,34,38,39]; while others reported that the inoculum had limited potential in improving PAH bioremediation due to its poor adaptability and the keen competition from the indigenous microorganisms [37,40,41]. The present study revealed that the two PAH-degrading bacterial isolates, especially when inoculated together, significantly enhanced biodegradation of Phe, Fl and Pyr.

Variations in sediment properties such as soil texture, concentrations of organic matter, nutrients and C: N: P ratio etc., are common in natural environments. The types of sediment would affect the adsorption of organic compounds onto soil particles as well as their bioavailability; the higher the bioavailability, the faster the biodegradation [19,42]. Amellal et al. [14] showed that Phe biodegradation in sandy sediment slurry was higher than that in clayey slurry due to the clay aggregates had higher Phe adsorption capacities. In the present study, more than 93% of Phe, Fl and Pyr in clay loam sediment slurry (HC) at a salinity of 25 ppt were degraded at the end of 7-d experiment, while only 41.47% was biodegraded in the MP clayey sediment slurry at the same salinity; and KLH sandy sediment slurry had the least biodegradation ability. Lahlou et al. [20] found that the microbial community structure and composition varied among sediment types. The Phe-degrading bacteria were present at a higher density in clayey than in sandy sediment slurries [14]. The clay or clay-like particles in sediment might provide more protective microhabitats to the bacteria from predation by protozoans, thus enhancing the chance of bacterial survival [43,44]. Heijnen et al. [45] also reported that increasing the content of bentonite clay favoured the survival of bacteria added to the soil. These studies suggested that biodegradation of PAHs in clayey sediment, due to its stronger adsorption and lower bioavailability, was less than in sandy sediment; but the degraders may have higher survival in clayey sediment. Clay loam sediment with soil texture somewhere between clayey and sandy sediment would have the highest biodegradation, explaining why the optimal sediment type for Phe biodegradation was the HC sediment slurry.

Another factor that affects Phe biodegradation in sediment and its persistence is the interaction between different PAH compounds in the contaminated environment [46,47]. Guha *et al.* [48] found that the degradation of Pyr was enhanced when Nap and Phe were present, but Phe biodegradation was improved in the presence of Nap. In this study, the presence of Fl and/or Pyr had little effect on the biodegradation of Phe. Phe, a low molecular weight PAH, was easier to be degraded than the high molecular weight PAH such as Pyr, and the mangrove sediment always harbored Phe-degrading bacteria [23,27,28,37].

5. Conclusions

- The types of sediment showed significant effect on Phe biodegradation with the highest biodegradation in the HC sediment slurry and the lowest in the clayey MP sediment slurry.
- Salinity and the inoculation of PAH-degrading bacterial isolates also played important roles in Phe biodegradation. The Phe biodegradation ability of *Sphingomonas* sp. was slightly enhanced with the inoculation of the other PAH-degrader, *Mycobacterium* sp., indicating a positive interaction between the two inocula.
- The presence of Fl (a 3-ring PAH) and Pyr (a 4-ring PAH) did not have any significant effect on Phe biodegradation.
- The optimal phenanthrene biodegradation would take place in contaminated clay loam sediment slurry at low salinity (5–15 ppt) with the inoculation of both *Sphingomonas* sp. and *Mycobacterium* sp.

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