

Anaerobic Degradation of Phenanthrene and Pyrene in Mangrove Sediment

Bea-Ven Chang · I. T. Chang · S. Y. Yuan

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Abstract This study investigated the anaerobic degradation of the polycyclic aromatic hydrocarbons (PAHs) phenanthrene and pyrene in mangrove sediment from Taiwan. The anaerobic degradation of PAH was enhanced by the addition of acetate, lactate, pyruvate, sodium chloride, cellulose, or zero-valent iron. However, it was inhibited by the addition of humic acid, di-(2-ethylhexyl) phthalate (DEHP), nonylphenol, or heavy metals. Of the microorganism strains isolated from the sediment samples, we found that strain MSA3 (*Clostridium pascui*), expressed the best ability to biodegrade PAH. The inoculation of sediment with the strain MSA3 could enhance PAH degradation.

Keywords Anaerobic degradation · Mangrove sediment · Phenanthrene · Pyrene

In recent years, anthropogenic practices such as industrial processing, petroleum spills, and incomplete combustion of fossil fuel have led to an accumulation of polycyclic aromatic hydrocarbons (PAHs) in the environment and PAH contamination is an increasing environmental concern (Simpson et al. 1996). Elevated concentrations of PAH are commonly recorded in marine and coastal sediments near urban and industrial cities (Connell et al. 1998). Mangrove ecosystems, important inter-tidal estuarine wetlands along the coastlines of tropical and subtropical regions, are frequently exposed to anthropogenic contamination by PAH (Kleskowski et al. 1994). Spilled and stranded oils

containing large amounts of PAH penetrate and accumulate in mangrove sediments (Ke et al. 2002).

Microbial degradation is believed to be one of the major processes that clean up PAH-contaminated sediments. The aerobic degradation of PAH in mangrove sediments has been extensively documented (Tam et al. 2002; Guo et al. 2005; Yu et al. 2005), but little is known about the factors that affect the anaerobic degradation of PAH in mangrove sediment. The purpose of this study was to compare the anaerobic degradation of two PAHs in mangrove sediment from five sampling sites, investigate the effects of various factors on the anaerobic degradation of PAH in mangrove sediment, and isolate PAH-degrading bacteria from the sediment.

Materials and Methods

Two PAHs (phenanthrene and pyrene) with 99.0% analytical standards were purchased from the Aldrich Chemical Co. Inc. (Milwaukee, WI). Solvents were purchased from Mallinckrodt, Inc. (Paris, KY); all other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO). Stock solutions of individual PAH dissolved in dimethylsulfoxide were initially established at concentrations of 10,000 mg/L and diluted to 500 mg/L before use.

In July 2005, sediment samples were taken from the Guandu and Zhuwei Mangroves in northern Taiwan. The Guandu Mangrove is at the confluence of the Tanshui River and the Keelung River. The Zhuwei Mangroves Reserve Area in the Tanshui Township of Taipei County is on the north bank of the Tanshui River. Figure 1 shows the locations of the five sample collection sites. Sites A, B, and C were in the Guandu Mangroves, while sites D and E were

B.-V. Chang (✉) · I. T. Chang · S. Y. Yuan
Department of Microbiology, Soochow University,
No 70, Linshin Road, Taipei 11102, Taiwan
e-mail: bvchang@mail.scu.edu.tw

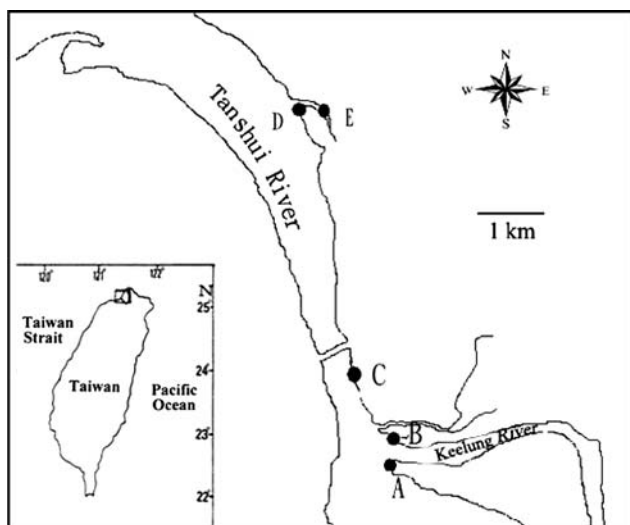


Fig. 1 Mangrove sediment sampling sites in northern Taiwan

in the Zhuwei Mangroves. Deep sediments (5–20 cm) were collected during low tides using a soil core. Sediments were randomly collected, in triplicate, from an area of around 1 m² at the center of each mangrove swamp. Each sediment sample, around 2–3 kg in fresh weight, was actually a composite sample made up by thoroughly mixing sediments collected from several sampling points.

For sediment samples from sites A, B, C, D, and E, the organic carbon was 1.18%, 2.54%, 1.78%, 0.89% and 1.89%, respectively; the pH, determined in sediment/water mixtures (1:1, w/v), was 7.3, 7.4, 7.1, 6.5, and 7.6, respectively; the anaerobic bacterial count was 5.1×10^5 , 6.4×10^6 , 4.6×10^5 , 6.4×10^4 , and 6.4×10^5 CFU/g, respectively; phenanthrene concentration was 0.20 µg/g, 0.56 µg/g, not detected (ND), ND and ND, respectively; and the concentration of pyrene was ND, 0.28 µg/g, ND, ND and ND, respectively. The experimental medium consisted of (all concentrations in g/L): NH₄Cl, 2.7; MgCl₂ · 6H₂O, 0.1; CaCl₂ · 2H₂O, 0.1; FeCl₂ · 4H₂O, 0.02; K₂HPO₄, 0.27; KH₂PO₄, 0.35; and resazurin, 0.001. The pH was adjusted to 7.0 following autoclaving; 0.9 mM titanium citrate was added as a reducing reagent.

Experiments were performed using 125 mL serum bottles containing 45 mL of medium, 5 g of mangrove sediment, and 50 µg/g of a mixture of the two PAHs (phenanthrene and pyrene). PAH degradation was first measured in the sediment samples from sites A, B, C, D, and E. The following factors were added to study their effects on the degradation of PAH: 20 mM sodium acetate, 20 mM sodium lactate, 20 mM sodium pyruvate, 1% (w/v) sodium chloride, 5 g/L humic acid, 0.96 mg/L cellulose, 10 g/L zero-valent iron, 5 mg/L di-(2-ethylhexyl) phthalate (DEHP), 5 mg/L nonylphenol, 2 mg/L heavy metals (Cd, Cu, Pb, and Zn), 20 mM sodium hydrogen carbonate,

20 mM sodium sulfate, 20 mM sodium nitrate, 50 mM BESA, 50 mM vancomycin, or 50 mM sodium molybdate-2-hydrate. We also inoculated the sediment with the PAH-degrading bacteria. Inoculated control samples were treated with non-sterile sediment and were shaken prior to incubation at 30°C and pH 7.0 in the dark. Sterile controls were autoclaved at 121°C for 30 min on two consecutive days.

All experiments were conducted in an anaerobic glove box (Forma Scientific, model 1025 S/N, USA) filled with N₂ (85%), H₂ (10%), and CO₂ (5%) gases. Bottles were capped with butyl rubber stoppers and wrapped in aluminum foil to prevent photolysis, then incubated without shaking at 30°C in the dark. The bottle contents were periodically sampled in order to measure residual PAH concentration. All experiments were performed in triplicate.

PAH extraction and analysis were performed as described by Yuan et al. (2001). Recovery percentages were 96.5% and 97.5% for phenanthrene and pyrene, respectively. Detection limits were 100 µg/L for both compounds. The purified bacterium was identified as described by Chang et al. (2007a). The PAH degradation data collected for this study fit well with first-order kinetics: $S = S_0 \exp(-k_1 t)$, $t_{1/2} = \ln 2/k_1$, where S_0 is the initial concentration, S the substrate concentration, t the time period and k_1 the degradation rate constant. Significant differences were calculated using a standard variance F -test.

Results and Discussion

We first compared anaerobic degradation rates in mangrove sediment samples collected from five sampling sites (Table 1). The results showed that the degradation rates were higher for phenanthrene than for pyrene. The molecular weights for phenanthrene and pyrene are 178

Table 1 Anaerobic degradation rate constants (k_1) and half-lives ($t_{1/2}$) of phenanthrene and pyrene in mangrove sediment samples collected from five sampling sites

Sampling site	Phenanthrene			Pyrene		
	k_1 (1/day)	$t_{1/2}$ (days)	r^a	k_1 (1/day)	$t_{1/2}$ (days)	r^a
A	0.010	69.3	0.98	0.007	99.0	0.89
B	0.014	49.5	0.96	0.009	77.0	0.95
C	0.008	86.6	0.93	0.006	115.5	0.91
D	0.006	115.5	0.92	0.004	173.3	0.95
E	0.005	138.6	0.96	0.003	231.0	0.94

All treatment figures were significantly different at $p < 0.05$

^a r , correlation coefficient

and 202 g/mol, respectively. PAH with high molecular weights are more resistant to biotransformation than those with low molecular weights (Yu et al. 2005). We did not detect PAH degradation products at any stages. The high-to-low order of PAH degradation rates in the five sediment sampling sites was B → A → C → D → E. The results showed that samples from site B had higher phenanthrene and pyrene degradation rates than those from the other sampling sites. It was also found that sediment samples from site B had higher phenanthrene and pyrene concentrations than those from the other sampling sites. The sediment samples from site B were collected from the Guandu Mangrove, where they have been affected by vehicle exhaust deposition and the discharge of industrial, livestock and household waste and wastewater. We will restrict our discussion to results from the sediment sample from site B in the next experiment.

Table 2 shows the effects of various substrates on the anaerobic degradation of PAH. PAH degradation was enhanced compared to the inoculated control following the addition of acetate, lactate, or pyruvate. A possible explanation is that these carbon sources promote the growth of methanogen, which results in faster degradation (Oremland 1988). The metabolizing of lactate or pyruvate by acidogen produces either hydrogen or hydrogen carbonate, both of which enhance methanogen production (Oremland 1988).

Table 2 Effects of the addition of various substrates on phenanthrene and pyrene anaerobic degradation rate constants (k_1) and half-lives ($t_{1/2}$) in the mangrove sediment samples

Treatment	Phenanthrene			Pyrene		
	k_1 (1/day)	$t_{1/2}$ (days)	r^a	k_1 (1/day)	$t_{1/2}$ (days)	r^a
Inoculated control ^b	0.014	49.5	0.96	0.009	77.0	0.95
Acetate	0.021	33.0	0.95	0.014	49.5	0.93
Lactate	0.028	24.8	0.98	0.012	57.8	0.95
Pyruvate	0.026	26.7	0.97	0.016	43.3	0.97
Sodium chloride	0.018	38.5	0.96	0.010	69.3	0.89
Humic acid	0.010	69.3	0.94	0.008	86.6	0.93
Cellulose	0.026	26.7	0.96	0.010	69.3	0.96
Zero-valent iron	0.028	24.8	0.94	0.019	36.5	0.94
DEHP	0.011	63.0	0.98	0.008	86.6	0.97
Nonylphenol	0.010	69.3	0.98	0.007	99.0	0.93
Cd	0.009	77.0	0.97	0.007	99.0	0.96
Cu	0.007	99.0	0.94	0.005	138.6	0.94
Pb	0.012	57.8	0.98	0.009	77.0	0.95
Zn	0.008	86.6	0.94	0.006	115.5	0.95

All inoculated control and treatment figures were significantly different at $p < 0.05$

^a r , correlation coefficient; ^b Inoculated control: pH 7.0, 30°C, PAH 50 µg/g

It is also possible that the action of sulfate-reducing bacteria on lactate produces pyruvate plus two electrons and that the action of pyruvate produces acetate plus two electrons; both of these processes would result in faster degradation (Legall and Fauque 1988).

The addition of 1% sodium chloride enhanced the anaerobic degradation of PAH. The optimum salinity for the degradation of phenanthrene was around 1–2%; high salinity (3.5%) has an inhibitory effect on the degradation of phenanthrene (Tam et al. 2002). The addition of humic acid inhibited the anaerobic degradation of PAH. Humic acid plays an important role in the sorption and binding of PAH and PAH metabolites (Conte et al. 2001). The addition of cellulose enhanced the anaerobic degradation of PAH because it stimulates anaerobic respiration (Quantin et al. 2005). The addition of zero-valent iron enhanced the anaerobic degradation of PAH. Zero-valent iron can serve as the electron donor and was capable of efficiently removing PAH (Chang et al. 2007b).

Liu et al. (2000) found PAH, DEHP, nonylphenol, and heavy metals in samples collected from various Taiwanese rivers and we therefore studied the effects of these pollutants on the anaerobic degradation of PAH in mangrove sediment. The data in Table 3 also show that PAH anaerobic degradation was inhibited by the addition of DEHP, nonylphenol, or heavy metals. It may be that DEHP and nonylphenol are also used as carbon sources, thus inhibiting PAH anaerobic degradation. Furthermore, the toxicity of heavy metals such as Cd, Cu, Pb, and Zn are known to decrease microorganism activity (Giller et al. 1998).

Table 3 indicates that the anaerobic degradation of PAH was enhanced under methanogenic and sulfate-reducing conditions and inhibited under nitrate-reducing conditions. The order of anaerobic degradation rates of PAH in mangrove sediment under these conditions was observed to be sulfate-reducing conditions > methanogenic conditions > nitrate-reducing conditions. Table 4 shows that the anaerobic degradation of PAH was inhibited by the addition of molybdate (a selective inhibitor of sulfate-reducing bacteria), BESA (a selective inhibitor of methanogen), or vancomycin (a selective inhibitor of eubacteria) (Lovely and Philips 1988; Distefano et al. 1992). The results obtained under the three anaerobic conditions and in the presence of the three microbial inhibitors indicate that sulfate-reducing bacteria, methanogen and eubacteria are involved in PAH degradation; sulfate-reducing bacteria may play a major microbial role in the PAH degradation, but methanogen and eubacteria are also involved. Coates et al. (1996) found phenanthrene oxidation to be due to the effects of sulfate-reducing bacteria.

We also isolated pure bacterial strains from the mangrove sediment samples and found six bacterial strains capable of anaerobically metabolizing PAH as carbon

Table 3 Comparison of the phenanthrene and pyrene anaerobic degradation rate constants (k_1) and half-lives ($t_{1/2}$) in mangrove sediment samples following the addition of three microbial inhibitors

Treatment	Phenanthrene			Pyrene		
	k_1 (1/day)	$t_{1/2}$ (days)	r^a	k_1 (1/day)	$t_{1/2}$ (days)	r^a
Inoculated control ^b	0.014	49.5	0.96	0.009	77.0	0.95
Methanogenic conditions	0.018	38.5	0.95	0.013	53.3	0.97
Sulfate-reducing conditions	0.021	33.0	0.96	0.016	43.3	0.94
Nitrate-reducing conditions	0.010	69.3	0.97	0.007	99.0	0.96

All inoculated control and treatment figures were significantly different at $p < 0.05$

^a r , correlation coefficient; ^bInoculated control: pH 7.0, 30°C, PAH 50 µg/g

Table 4 Comparison of the phenanthrene and pyrene anaerobic degradation rate constants (k_1) and half-lives ($t_{1/2}$) in mangrove sediment samples following the addition of three microbial inhibitors

Treatment	Phenanthrene			Pyrene		
	k_1 (1/day)	$t_{1/2}$ (days)	r^a	k_1 (1/day)	$t_{1/2}$ (days)	r^a
Inoculated control ^b	0.014	49.5	0.96	0.009	77.0	0.95
BESA	0.010	69.3	0.91	0.007	99.0	0.92
Molybdate	0.008	86.6	0.95	0.005	138.6	0.95
Vancomycin	0.007	99.0	0.89	0.004	173.3	0.92

All inoculated control and treatment data were significantly different at $p < 0.05$

^a r , correlation coefficient; ^bInoculated control: pH 7.0, 30°C, PAH 50 µg/g

sources. The isolate showing the greatest degrading power was strain MSA3. Phenanthrene and pyrene degradation rates for the strain are shown in Table 5. These results showed the utilization of phenanthrene and pyrene as carbon sources for growth by the strain MSA3. The strain was relatively close to *Clostridium pascui*, with a similarity of 99.0%; the strain was gram-positive, rod-shaped and formed white colonies. *Clostridium* spp. capable of degrading PAH has been described (Yuan et al. 2007). *Clostridium* spp. may present frequently in the Taiwan aquatic environment.

As shown in Table 5, PAH degradation in sediment-free culture samples was higher than that in the sediment samples. One possible explanation for this significant difference is that the tendency of PAH to adsorb to sediment particles may reduce the degrading effectiveness of microorganisms by reducing the bioavailability of PAH, thus retarding the biodegradation process (Yuan et al. 2001). Table 5 also shows that the highest PAH degradation rate was found in the non-sterile sediment with the inoculation strain MSA3, whereas the non-sterile sediment without any inoculation had a lower degradation rate. These results suggested that the inoculation of sediment with the strain MSA3 could enhance PAH degradation. This is consistent with the findings of Yu et al. (2005), who reported that the degradation of PAH was enhanced by a bacterial consortium enriched from mangrove sediments.

In summary, the mangrove sediment samples used in these experiments were all capable of anaerobically degrading PAH. The addition of acetate, lactate, pyruvate, sodium chloride, humic acid, cellulose, zero-valent iron, DEHP, nonylphenol, or heavy metals affected the anaerobic degradation of PAH in mangrove sediment. The results support the feasibility of the removal of PAH in mangrove sediment by anaerobic degradation. Our next goal is to characterize the structure of the microbial community involved in the anaerobic degradation of PAH in mangrove sediment.

Table 5 Comparison with or without sediment of the addition of the strain MSA3 on phenanthrene and pyrene anaerobic degradation rate constants (k_1) and half-lives ($t_{1/2}$)

Treatment	Phenanthrene			Pyrene		
	k_1 (1/day)	$t_{1/2}$ (days)	r^a	k_1 (1/day)	$t_{1/2}$ (days)	r^a
Sediment-free with strain MSA3	0.032	21.7	0.97	0.017	40.8	0.91
Sterile sediment with strain MSA3	0.016	43.3	0.92	0.010	69.3	0.95
Non-sterile sediment without strain MSA3	0.014	49.5	0.96	0.009	77.0	0.95
Non-sterile sediment with strain MSA3	0.018	38.5	0.89	0.012	57.8	0.94

All treatment figures were significantly different at $p < 0.05$

^a r , correlation coefficient

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