Chinese Journal of Oceanology and Limnology
Vol. 22, No. 4, P. 431-435, 2004

Preliminary study on PAH degradation by bacteria from contaminated sediments in Xiamen Western Sea, Fujian, China*

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Received May 14, 2003; revision accepted Jan. 5, 2004

Abstract In order to estimate the biodegradation of three polycyclic aromatic hydrocarbons (PAHs) compounds, bacterial strains were isolated from marine sediments in three heavily contaminated sites (Yuandang Lake, Dongdu Port and Aquacultural zones in Maluan Bay) in Xiamen Western Sea. The results show three bacterial strains, which used pyrene as the sole carbon source, were identified as strains of *Aureobacterium sp.*, *Arthrobacter sp.*, *Rhodococcus sp*. The PAH-degrading bacteria isolated had a strong ability to degrade phenanthrene, fluoranthene and pyrene at different degradation rates. The highest degradation rate was observed when three PAH compounds were mixed with an individual strain in the medium. The three PAHs were degraded after one week with a degradation rate of 89.94 % for phenanthrene and 93.4 % for both of fluoranthene and pyrene. In addition, after 25 days of incubation, the degradation rate was 99.98 % for phenanthrene and 99.97 % for both of fluoranthene and pyrene. Optical density was measured to estimate bacterial growth during the degradation of PAHs. Highest levels of bacterial growth were observed with a three PAH mixture in the culture, suggesting that the concentration of PAHs influenced bacterial growth and the highest levels of degradation for most series were detected after one week of incubation.

Key words: PAHs, biodegradation, bacteria, Xiamen Western Sea

1 INTRODUCTION

Bacterial degradation of PAHs is considered to be a major decomposition process for some natural contaminants and is of great practical interest to bioremediation. Microorganisms capable of degrading PAH compounds are common in soil and sediments contaminated by PAHs. Considerable effort has therefore been made to harness these indigenous microorganisms for use in bioremediation in contaminated sites. Aquatic sediments act as important sinks for PAH derivatives that are discharged either directly or indirectly into aquatic environment. The field of biodegradation is now witnessing some interesting developments (Cerniglia, 1992, 1993; Wilson and Jones, 1993) such as isolation of bacteria capable of utilizing the four-ring-aromatics fluoranthene and pyrene as carbon and energy sources (Boldrin et al., 1993; Bouchez et al., 1995; Geiselbrecht et al., 1996; Kelley and Cerniglia, 1991; Walter et al., 1991; Zheng et al., 2001; Maskaoui et al., 2003).

PAHs, like other classes of hydrocarbon compounds, are series of homologous compounds, interactions between these compounds are likely to play an important role in biodegradation process. Several environmental variables impacted upon organic pollutant degradation in the various terrestrial and aquatic systems examined. These include temperature, pH and salinity. The most important factors however, were compound-related factors such as the size of the microbial population used, organic pollutant concentration, pre-exposure time, and concentration (Bauer and Capone, 1985). Bio-degradation in the marine environment and especially in contaminated sites could be utilized to reduce damage caused by oil spills and general water quality improvement schemes.

* Supported by the NSFC (No.30070157, 40206015), and the Scientific Fund of Quanzhou City (No. Z200234).
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The objective of this study is to enhance understanding of the role of microorganisms in biodegradation of PAHs and provide an explanation for the degradation of PAHs by bacterial strains isolated from marine sediment in the heavily polluted sites in Xiamen Western Sea.

2 MATERIALS AND METHODS

Sediments were collected from heavily contaminated sites on May 12th, 1999 in Xiamen Western Sea. Bacterial strains were isolated from the sediment samples and used one of the following PAH (phenanthrene, fluoranthene and pyrene) as sole carbon and energy source.

2.1 Sediment sampling and treatment

Sediment samples from the contaminated zones were collected using sterilized glass bottles and then transferred to the laboratory shortly. Microorganisms were isolated from the sample by mixing 1 g of sediment with 10 ml of sterile sodium pyrophosphate solution ($Na_2P_2O_7$, 2.8 g/L) and 3-g glass beads (3 mm diameter) in a 50 ml plastic centrifugation tube. The tube was closed and shaken for 2 hours in a horizontal position on a rotary shaker (350 r/min). The solid particles were allowed to settle for 30 min, and aliquots of the supernatant were used as inoculums. Dilutions of the inoculums were prepared with pyrophosphate solution. The suitable dilute (0.1 ml) was plated on the media which, were either blank (reference plates) or contained one PAH compound as a sole carbon source. The plates were incubated for up to 2 weeks at 25 °C, and monitored regularly for growth or zone formation.

2.2 Isolation and purification of single strain

The isolation of single strains was initiated by a repeated streaking of individual zone-forming colonies on solidified mineral medium containing PAHs. Colonies that were still able to grow and to form clearing zones on PAH were extracted again and checked for purity using microscopes. Purification steps were repeated several times. The strains were identified by using the biology microbial identification system.

2.3 Culture conditions and degradation experiment

Batch cultivation was carried out in shake flasks. The composition of the mineral salt medium (Mueller et al., 1989) was as follows: $(NH_4)_2SO_4$ (1000 mg/L), K_2HPO_4 (800 mg/L), KH_2PO_4 (200 mg/L), $MgSO_4$ (1000 mg/l), $CaCl_2$-H_2O (100 mg/L), $FeCl_3$-$6H_2O$ (Weissenfelds et al., 1990), and $(NH_4)_6Mo_7O_2$-$4H_2O$ at pH 7.2. For the growth and degradation of PAH components, a 100 ml-conical flask containing 30 ml of medium was placed in a shaker at 26°C at 140 r/min. This process was carried out in darkness. The final concentration of PAH (single or a mixture of two or three), used during these experiments, was 50 mg/L in acetone. A flask control containing only bacteria without PAH was treated in parallel. The bacteria used for biodegradation was a mixture of the three bacterial strains identified later as *Aureobacterium sp.*, *Arthrobacter sp.* and *Rhodococcus sp*. Samples were taken from experimental systems at certain periods (0, 7, 13, 18, 25 day) for extraction by Solid-Phase Extraction (SPE) method followed by analysis of residual PAH by gas chromatography with mass spectroscopy (GC/MS). Growth of the cells was monitored by measuring optical density at 600 nm.

3 RESULTS AND DISCUSSIONS

When phenanthrene, fluoranthene and pyrene were provided in one culture, simultaneous degradation of all the hydrocarbons occurred (Fig.1). Similar results were observed when mixtures of two or three PAHs were investigated (Figs. 2, 3 and 4). Although phenanthrene, fluoranthene and pyrene were degraded simultaneously in mixtures, degradation occurred with different kinetics compared to single compounds (Figs. 4, 5 and 6).

Lower molecular weight PAHs degrade rapidly in sediments, while higher molecular weight PAHs, such as benzo(a)anthracene, chrysene or benzo (a)pyrene, are quite resistant to microbial attack. This means that the potential biodegradation rates for PAHs are higher in PAH-contaminated sediments than in pristine sediments (Cerniglia, 1992). In this study, phenanthrene had a very high degradation rate (99.98 %) especially in the presence of fluoranthene and pyrene. The degradation rate of phenanthrene reached 89.94 % after only one week of incubation. In the presence of fluoranthene or pyrene, the degradation rate after 25 days of incubation was similar (99.5 and 99.66 % respectively) and also higher. When phenanthrene was used as sole carbon source in the medium, however, the degrada-

tion rate was lower than that in the presence of other PAHs, and also lower than that of fluoranthene and pyrene when they were used separately as substrate in the culture medium (Figs. 4, 5 and 6).

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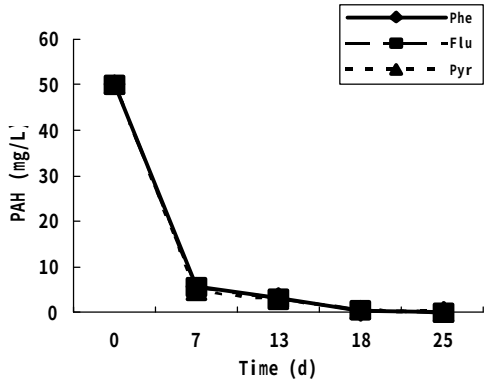

Fig.1 Degradation of three PAHs mixture by the three bacterial strains

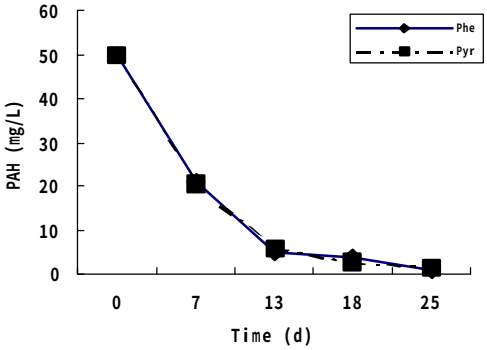

Fig.2 Degradation of two PAHs mixture by the three acterial strains

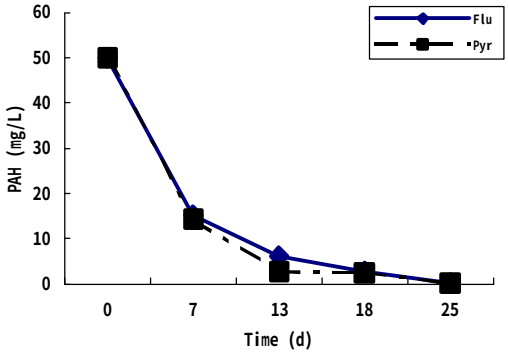

Fig.3 Degradation of two PAHs mixture by the three bacterial strains

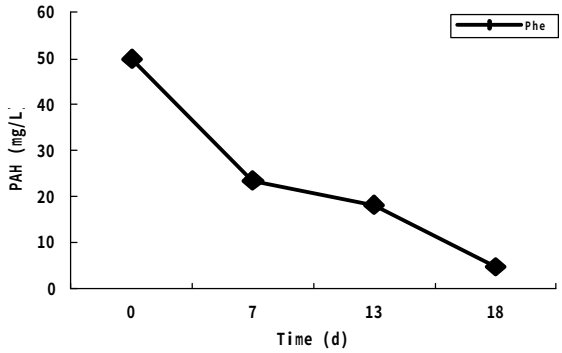

Fig.4 Degradation of phenanthrene by the three bacterial strains

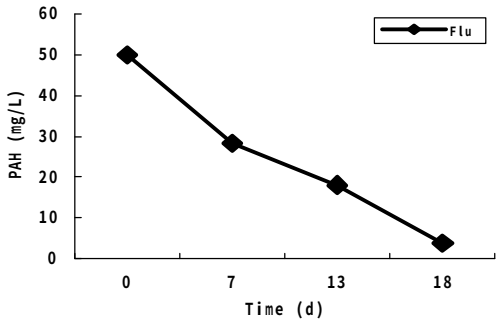

Fig.5 Degradation of fuoranthene by the three bacterial strains

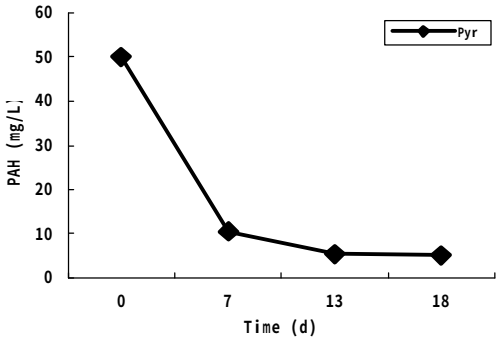

Fig.6 Degradation of pyrene by the three bacterial strains

In this study, phenanthrene had a very high degradation rate (99.98 %) especially in the presence of fluoranthene and pyrene. The degradation rate of phenanthrene reached 89.94 % after only one week of incubation. In the presence of fluoranthene or pyrene, the degradation rate after 25 days of incubation was similar (99.5 and 99.66 % respectively) and also higher. When phenanthrene was used as sole carbon source in the medium, however, the degradation rate was lower than that in the presence of other PAHs, and also lower than that of fluoranthene and pyrene when they were used separately as substrate in the culture medium (Figs. 4, 5 and 6).

The degradation of pyrene was influenced by the presence of other PAHs. When this hydrocarbon was added together with phenanthrene and fluoranthene, a faster degradation of pyrene was observed and the degradation of these PAHs was almost complete. The isolated individual strains were able to degrade all the three PAHs after 7 days of incubation period (Fig.1). The time course of pyrene degradation was influenced by the presence of phenanthrene since the PAH with low molecular weight was degraded faster than that with lower molecular weight. A similar degradation rate of both pyrene and fluoranthene was observed when these compounds were mixed in the same culture (99.72% and 99. 66 % respectively). When pyrene was used as sole source carbon, the degradation rate was 89.82 %, lower than that in the presence of other PAHs and its degradation time was significantly longer than that if it was not mixed with other PAHs. Fluoranthene had the similar properties to those of pyrene but its degradation rate was 90.36 % when it was used alone in the medium culture. After 18 days of incubation, the rate was higher than that of pyrene (89.82%).

Bacterial growth was monitored during the degradation of PAHs by measuring the optical density of the culture. As illustrated in Fig.7, higher bacterial growth was detected when phenanthrene, fluoranthene were provided in one culture especially after one week of incubation. In other studies (Cerniglia, 1992), microbial degradation of PAHs in aquatic and terrestrial ecosystems was found to be strongly influenced by a wide variety of abiotic and biotic factors including: temperature, pH, soil type, aeration, nutrients, depth, diffusion, microbial adaptations, bioavailability, previous chemical exposure, water availability, sediment toxicity, physico-chemical properties of the PAH, concentration of the PAH and other seasonal factors. Thus, concentration of PAH in the culture was a critical factor in these series of experiments since the bacterial growth had higher levels with significant amount of PAHs (phenanthrene + fluoranthene + pyrene=150 mg/L). In a culture where two PAHs were mixed in one media, the bacterial growth was higher for (phen. + pyr.) mixture than that for (fluo. + pyr.) after 13 days after incubation. Bacterial growth was similar when each PAH was used as the sole carbon source.

Higher bacterial growth was observed with a three PAH mixture in the culture medium, suggesting that the concentration of PAHs influences the bacterial growth and the highest growth for most degradation series was detected after one week of incubation.

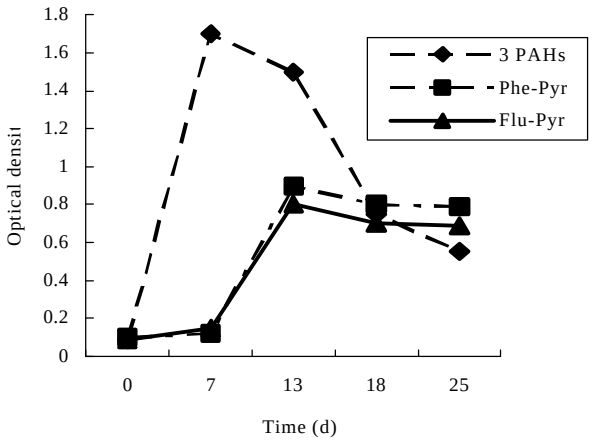

Fig.7 Changes in cell density of the three bacterial strains

Isolation and characterization of PAH-degrading bacteria was a preliminary step toward understanding the microbiology and fate of PAHs in marine sediments. However, the true biodiversity of PAH-degrading marine strains is still largely unknown. Prior to this study, only marine bacteria were used in the degradation of PAHs. Bacteria isolated from sediment samples containing polycyclic aromatic hydrocarbons (PAHs: pyrene, phenanthrene and fluoranthene) were quantified and characterized. The three strains isolated from contaminated zones with pyrene as the sole carbon source, were identified as strains of *Aureobacterium sp*., *Arthrobacter sp*. and *Rhodococcus sp*. respectively.

These three strains were later used separately in further research focusing particularly on degradation of pyrene. Pure culture of *Rhodococcus sp*. was capable of degrading pyrene, phenanthrene and fluoranthene (50 mg/L) to very low levels within 3 weeks. At a concentration of 50 mg/L, pyrene caused the highest growth of the strain and had the

highest degradation rate. However, pyrene degradation by this strain was not observed at concentration up to 200 mg/L. There was a close relationship between the biomass of the bacteria and the degradation of PAHs. Co-metabolism may play an important role in the degradation of PAHs. 3% NaCl and pH of 7.0 was the optimal condition for degradation by *Aureobacterium sp.* on pyrene. Nitrogen rather than phosphorus was found to be a limiting factor in the degradation.

This work confirms earlier reports that low molecular weight can be degraded easily in marine sediments contaminated by PAHs. Despite being focused on marine sediment, this research is presently limited to Xiamen Western Sea.

4 ACKNOWLEDGEMENT

The author would like to thank the Key Laboratory of Marine Ecology & Environmental Science, Institute of Oceanology, Chinese Academy of Sciences and Key Lab. of Marine Environmental Science, China Ministry of Education (Xiamen) for supporting this project. The author thanks Ms. Guo Chuling and Ms Hong Liyu for their helps.

References

Bauer, J. E. and G. D. Capone, 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intrtidal marine sediments. *Applied and Environmental Microbiology* **50**: 81-90.

Boldrin, B., A. Tiehm and C. Fritzsche 1993. Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by *Mycobacterium* sp. *Applied and Environmental Microbiology* **59**: 1927-1930.

Bouchez, M., D. Blanchet, J-P. Vandecasteele, 1995. Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain associations: inhibition phenomena and cometabolism. *Applied Microbiology Biotechnology* **43**: 156-164.

Cerniglia, C. E., 1992. Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation* **3**: 351-368.

Cerniglia, C. E., 1993. Biodegradation of polycyclic aromatic hydrocarbons. *Curr. Opin. Biotechnol.* **4:** 331-338.

Geiselbrecht, A. D., R. P. Herwig, J. W. Deming and J. T. Staley, 1996. Enumeration and phylogenetic analysis of polycyclic aromatic hydrocarbon-degrading marine bacteria from Puget Sound Sediments. *Applied and Environmental Microbiology* **62**: 3344-3349.

Kelly, I. and C. E. Cerniglia, 1991. The metabolism of fluoranthene by a species of *Mycobacterium*. *J. Ind. Microbiolgy and Biotechnology* **7:** 19-26.

Maskaoui, K., T. L. Zheng, H. S. Hong, Z. M. Yu, Z. Hu, T. Yun and L. Z. Cai, 2003. Current status of bacteriological parameters and DOC/POC in Xiamen coastal waters. *Acta Oceanol. Sin.* **22** (3): 443-451.

Walter, U., M. Beyer, J. Klein, H. J. Rehm, 1991. Degradation of pyrene by *Rhodococcus sp.* UW1. *Applied Microbiology Biotechnology* **34**: 671-676.

Wilson, S. C. and K. C. Jones, 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. *Environmental Pollution* **81**: 229-249.

Wessenfelds, W. D., M. Beyer and J. Klein 1990. Degradation of phenanthrene, fluorene and fluoranthene by pure bacterial cultures. *Applied Microbiology Biotechnology* **34**: 528-535.

Zheng, T. L., T. C. Zhuang and L. Z. Cai, 2001. The role of microbes in the bioremediation of marine polluted environment. *J. Xiamen University* (Natural Science) **40**(2):524-534.