Remedial Effects of *Cylindrotheca Closterium* on Polycyclic Aromatic Hydrocarbons in Sediments

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Abstract. *Cylindrotheca Closterium* was implanted on the surface of spiking sediments to study the remedial effects of *C. closterium* on polycyclic aromatic hydrocarbons (PAHs) in sediments. The result showed that *C. closterium* changed redox potential, microorganism community structure and quantity of sediment. *C. closterium* could promote the degradation of phenanthrene in sediments by affecting microbial communities.

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbons with two or more benzene rings and carcinogenic, mutagenic and toxic. Because of PAHs’ high partition coefficients, sediment becomes an important sink of that in the environment [1]. The pollution of PAHs in coastal areas is particularly serious [2,3]. Hence, removal of PAHs from environment is gaining increasing scrutiny by researchers. Bacteria, fungi and algae in the environment have the ability to degrade PAHs [4]. Grosser (1991) found that degradation rate of PAHs was increased in sediments by adding bacteria [5]. Microphytobenthos (MPB) is the main contributor to marine primary productivity and the foundation of marine ecosystem. MBP could release oxygen by photosynthesis and change the oxygen environment of the sediment surface. Therefore, MPB could change redox processes and chemical cycle of compounds [6,7].

In this study, we added phenanthrene and pyrene to the sediments as the target pollutants and implant *Cylindrotheca Closterium* in the surface of spiking sediments to study the remedial effects.

2. Experimental

2.1. Materials
Phenanthrene and pyrene were obtained from Sigma. All organic solvents used for the analysis were of analytical grade and purchased from Tianjin Chemical Reagent Factory. *C. closterium* was purchased from the Institute of Oceanology of the Chinese Academy of Sciences.

2.2. Bioremediation experiment
The initial concentration of phenanthrene and pyrene were 17.45 mg/kg and 16.92 mg/kg, respectively. The following treatments were made: (1) with *C. closterium* on the sediment surface at an initial density of $2 \times 10^5$ cells cm$^{-2}$ (treatment C); and (2) without *C. closterium* on the sediment surface.
The experimental containers were plexiglass columns with 2-cm-thick sediment and 2-cm-thick artificial seawater. The culture temperature was 25°C, under a 16:8 light:dark cycle at light intensity 3200 Lux. The experiment lasted 12 d.

Phenanthrene and pyrene concentrations in sediments were analysed as follows: 5 g of sediment sample was Soxhlet extracted, purified and concentrated to 1 mL. Internal standard (hexamethyl benzene) was added before GC-MS analysis. The extracts were analysed by GC-MS (Agilent, Santa Clara, USA) in SIM mode. The GC-MS system consists of an Agilent 6890 GC equipped with an Agilent 5975C MS detector and a HP-5 MS capillary column (30 m × 0.25 mm i.d.) coated with 0.25-μm film of 5% phenyl methyl siloxane. Injector and detector temperatures were both set at 250 °C. Helium was used as a carrier gas at a flow rate of 1.0 mL min⁻¹. The temperature program started at 100 °C, increased to 280 °C at a rate of 20 °C min⁻¹, and held for 2 min. The total time was 11 min. The MS conditions for EI ionization were as follows: the ion energy at 70 eV and the ion source temperature at 230 °C.

The redox potential (Eh) in sediments was measured by Unisense puncture microelectrode (Danish Unisense company).

PLFAs were extracted in three steps using a modified procedure [8].

3. Result and Discussion

3.1. Eh and MPB

At the end of the experiment, in treatment C, the chlorophyll a content of sediments in the depth of 0-4 mm was 8.3 μg/g. It was significantly higher than that in the deeper layer (<3.1 μg/g). Fig. 1 showed that Eh decreased with the increase of sediments’ depth in two treatments. Eh of treatment C was significantly higher than that of treatment NC in all sites. Under the influence of C. closterium, Eh decreased rapidly in the range of 3-8 mm in sediments. With the increase of sediment depth, the depth of oxygen released from C. closterium was gradually decreased. It indicated that C. closterium could improve the oxygen environment on the surface of the sediments by releasing oxygen [6].

3.2. PAHs concentration

Table 1 showed that the residual of phenanthrene in the system was increased with the increase of sediment depth in two treatments. The same characterization of pyrene was the opposite. In the same depth of sediment, the residue of phenanthrene in treatment C was lower than that in treatment NC. The same characterization of pyrene was not significant. This might be due to the molecular weight effect of the compounds. For PAHs, the larger the molecular weight was, the more difficult it was to be used by microbes. C. closterium reduced the residue of phenanthrene in sediments.

3.3. Microbial community

Fig. 2 showed that the number of aerobic bacteria was decreased with the increase of sediment depth in two treatments. In the same depth of sediment, the number of aerobic bacteria in treatment C was great higher than that in treatment NC. However, when the depth of sediment was greater than 4 mm, the number of aerobic bacteria was sharply reducing. This situation was consistent with the trend of Eh. The number of anaerobic bacteria was increased with the increase of sediment depth in two treatments. It indicated that C. closterium improved the oxygen environment on the surface of sediments by releasing oxygen and the influence decreased with the decline of oxygen permeability. C. closterium inhibited the growth of anaerobic bacteria by improving the oxygen environment. The degree of inhibition decreased with the decrease of oxygen permeation. C. closterium changed the community structure and quantity of microbes and affected the degradation of phenanthrene in sediments [9].

| Depth(mm) | With C. closterium | Without C. closterium |
|-----------|---------------------|-----------------------|
|           | Phenanthrene | pyrene | Phenanthrene | pyrene |

Table 1. PAHs concentration of sediments at 12 d
|        | (mg/kg) | (mg/kg) | (mg/kg) | (mg/kg) |
|--------|---------|---------|---------|---------|
| 0-4    | 7.8     | 12.8    | 8.8     | 13.0    |
| 4-8    | 8.5     | 10.9    | 9.9     | 11.0    |
| 8-12   | 9.2     | 10.8    | 10.6    | 10.6    |
| 8-16   | 9.5     | 10.7    | 10.8    | 10.7    |

Figure 1. The redox potential of sediments at 12 d

Figure 2. (a) Aerobic bacteria and (b) anaerobic bacteria content of sediments at 12 d

4. Conclusion

*C. closterium* could improve the oxygen environment on the surface of sediments by releasing oxygen. It created favourable conditions for the growth of aerobic bacteria and inhibited the growth of
anaerobic bacteria. The degradation of phenanthrene in sediments was promoted by implanting C. closterium.

References
[1] Waigi, M. G., Kang, F., Goikavi, C., et al. (2015) Phenanthrene biodegradation by sphingomonads and its application in the contaminated soils and sediments: a review. International Biodeterioration & Biodegradation, 104: 333-349.
[2] Horii, Y., Ohura, T., Yamashita, N., et al. (2009) Chlorinated polycyclic aromatic hydrocarbons in sediments from industrial areas in Japan and the United States. Archives of environmental contamination and toxicology, 57(4): 651-660.
[3] Sanctorum, H., Elskens, M., Leermakers, M., et al. (2009) Sources of PCDD/Fs, non-ortho PCBs and PAHs in sediments of high and low impacted transboundary rivers (Belgium–France). Chemosphere, 85(2): 203-209.
[4] Cerniglia, C. E. (1992) Biodegradation of polycyclic aromatic hydrocarbon: A review. Biodegradation, 3: 351-368.
[5] Grosser, R. J., Warshawsky, D., Vestal, J., R. (1991) Indigenous and enhanced mineralization of pyrene, benzo[a]pyrene, and carbazole in soils. Applied and Environmental Microbiology, 57(12): 3462-3469.
[6] Denis, L., D., Desreumaux, P. (2009) Short-term variability of intertidal microphytobenthic production using an oxygen microprofiling system. Marine and Freshwater Research, 60: 712-726.
[7] Racchetti, E., Longhi, D., Ribaudo, C., et al. (2017) S Nitrogen uptake and coupled nitrification–denitrification in riverine sediments with benthic microalgae and rooted macrophytes. Aquatic Sciences, 1-19.
[8] He, Y., Xu, J. M., Lv, X. F., et al. (2009) Does the depletion of pentachlorophenol in root-soil interface follow a simple linear dependence on the distance to root surfaces. Soil Biology & Biochemistry, 41(9): 1807-1813.
[9] Chekol, T., Vough, L. R., Chaney, R. L. (2004) Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. Environmen International, 30(6): 799-804.