ANAEROBIC BIODEGRADATION
OF POLYCYCLIC AROMATIC
HYDROCARBONS (PAHS) IN THE
SUBSURFACE SEDIMENT OF
MANGROVE WETLAND

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Anaerobic Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) in the Subsurface Sediment of Mangrove Wetland

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) released into all environmental compartments through natural or anthropogenic activities are toxic, carcinogenic and mutagenic. Mangrove wetlands, located along the coastline of tropical and subtropical regions and close to human activities, are susceptible to PAH contamination. The anaerobic properties of mangrove sediments, especially their vertical changes, will directly affect the PAH contamination and biodegradation; however, studies in this area are limited. Previous related research was mainly focused on monitoring the temporal and spatial changes of PAH concentrations. The effects of anaerobic biodegradation of PAHs in sediment and some important biologically related factors, such as bacterial population and activity, electron acceptors utilization, and anaerobic gas production, on PAH degradation have seldom reported. The present research therefore aims (1) to study the vertical profiles of PAHs and anaerobic properties of the subsurface sediments in Ma Wan, one of the most contaminated mangrove swamps in Hong Kong; (2) to obtain the PAH-degrading anaerobic bacterial consortia from subsurface mangrove sediments under low oxygen (2±0.3% O\textsubscript{2}) and non-oxygen (0% O\textsubscript{2}) conditions; (3) to evaluate the anaerobic biodegradability of PAHs with the amendment of different electron acceptors, including nitrate, manganese Mn(IV), iron Fe(III), sulfate and carbon dioxide (produced by NaHCO\textsubscript{3}).

The vertical distribution of PAHs at different sediment depths, namely 0-2 cm, 2-4 cm, 4-6 cm, 6-10 cm, 10-15 cm and 15-20 cm, in Ma Wan mangrove swamp was investigated. Results showed that the concentrations of total PAHs (summation of 16...
US EPA priority PAHs) increased with sediment depth. The lowest concentration (about 1300 ng g\(^{-1}\) freeze dried sediment) were found in the surface layer (0-2 cm) while the highest value (around 5000 ng g\(^{-1}\) freeze dried sediment) was in the deeper layer (10-15 cm). The percentage of high molecular weight (HMW) PAHs (4 to 6 rings) to total PAHs was more than 89% at all sediment depths. The ratio of phenanthrene (Phe) to anthracene (Ant) was less than 10 while fluoranthene (Flua) to pyrene (Pyr) was around 1. Negative redox potentials (Eh) were recorded in all sediment samples, ranging from -170 to -200 mv, with a sharp decrease at a depth of 6 cm then declined slowly to 20 cm. These findings suggested that PAHs, particularly HMW ones, in Ma Wan sediment were originated from diesel-powered fishing vessels and were mainly accumulated in deep anaerobic layers. Among the electron acceptors commonly used by anaerobic bacteria, sulfate was the most dominant one, followed by Fe(III), nitrate, and Mn(IV) was the least. Their concentrations also decreased with sediment depth. The population size of total anaerobic heterotrophic bacteria increased with sediment depth, reaching the peak number in the middle layer (4-6 cm). In contrast, the aerobic heterotrophic bacterial count decreased with sediment depth. The vertical drop of the electron transport system (ETS) activity under PAH stress suggested that the indigenous bacteria were still active in the anaerobic sediment layer contaminated with PAHs.

Four PAHs, namely Fluorene (Fl), Phe, Flua and Pyr were selected as the target compounds in the enrichment and biodegradation experiments because of their relatively higher concentrations in Ma Wan mangrove sediment. Fresh sediment sample was mixed with mineral salt medium at a salinity of 25 parts per thousand at a ratio of
1:10 (w/v). The sediment slurry was enriched with the mixed PAHs under low oxygen and non-oxygen conditions, and the respective enrichment periods were 30 and 45 days. After three consecutive enrichment periods under each oxygen condition, two enriched consortia showing satisfactory PAH-degrading ability were obtained. A total of six strains of anaerobic PAH-degrading bacteria were isolated, the three from the low-oxygen condition were *Microbacterium*, *Rhodococcus* and *Sphingomonas*, while only one out of three isolates from the non-oxygen condition was identified and it was a *Sphingomonas* species.

A series of biodegradation studies with the inoculation of the enriched consortium and the amendment of different electron acceptors was performed. Results showed that nitrate and sulfate significantly enhanced PAH-degrading ability, while Fe(III) and NaHCO$_3$ did not have any significant effect; Mn(IV) had significantly adverse effect on the biodegradation of PAHs. Under both low-oxygen and non-oxygen conditions, the treatment groups with the inoculation of the enriched PAH-degrading consortium with the addition of electron acceptor had the highest PAHs biodegradation ability, while the control group (just contained the indigenous bacteria) had the lowest biodegradation ability. The 3-ring PAHs showed higher biodegradation percentages than the 4-ring PAHs, irrespective to the oxygen condition and whether the enriched consortia were inoculated or not. The present study was also proved that the bacteria from mangrove sediments could utilize different electron acceptors in the sediment simultaneously.

In summary, the present research revealed that Ma Wan mangrove swamp was contaminated by anthropogenic PAHs, mainly from nearby fishing vessels operated
with diesel. PAHs, especially those with high molecular weights, were accumulated in deep acidic and anaerobic sediments (>10 cm), with pH levels less than 6.0 and Eh at around -200 mv. Two types of enrichment consortia with PAH-degrading ability were obtained from its subsurface sediment, and the anaerobic PAH-degrading bacteria were first time reported. A modified ETS method was successfully applied to evaluate the bacterial activities under PAH stress. The present study demonstrated that the anaerobic biodegradation of PAHs could be improved by the amendment of different electron acceptors, particularly nitrate and sulfate. The biodegradation course was better understood than previous researches as both biological and chemical parameters were monitored.
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