ANAEROBIC DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN PADDY SOILS AMENDED WITH DIFFERENT ELECTRON ACCEPTORS

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Abstract
The anaerobic degradation of two polycyclic aromatic hydrocarbons (PAHs) (biphenyl and naphthalene) was investigated in four uncontaminated paddy soils. The effects of additional electron acceptors, sulfate, nitrate and Fe(III), on the degradation were also tested. Two paddy soils had the capacity degrading biphenyl and naphthalene. The capacity of anaerobic degradation of PAHs in paddy soils may correlate to soil classification and soil pH. Comparing with biphenyl, naphthalene had a higher degradation rate. Addition of electron acceptors resulted in the following order in the naphthalene degradation rate: sulfate ≃ nitrate > without additional electron acceptors > Fe(III). In contrast, highest degradation rate of biphenyl was observed in the soil without any additional electron acceptor.

Introduction
Polycyclic aromatic hydrocarbons such as naphthalene and biphenyl are widely distributed as environmental pollutants. Under aerobic conditions, the biodegradation of less weight PAHs including the pathway of degradation and the isolation of responsible microorganism has been very well-documented. In situ, many contaminated sites contain large anoxic area. Numerous studies have demonstrated that naphthalene and other less weight PAHs can be oxidized under different anaerobic conditions, such as sulfate-reducing, nitrate-reducing, iron-reducing, and methanogenic conditions in recent three decades.[1] 2-napthoic acid, 2-naphthalenol and 2-methynaphalene were reported as initial intermediate metabolites during naphthalene degradation by sulfate-reducing enrichment culture.[2,3,4] *p*-cresol is the only reported metabolite of biphenyl anaerobic degradation in methanogenic condition.[5] Two nitrate-reducing strains and one *proteobacteria* sulfate-reducing strain were successfully isolated as naphthalene degrader.[6,7] However, most of the PAHs anaerobic degradation studies were performed using contaminated sediments as microbial sources. Little is known about the environment fate of PAHs, especially biphenyl, in non-contaminated soils.
Therefore, the purpose of this study is to investigate the degradation fate of biphenyl and naphthalene in uncontaminated anoxic paddy soils and the effect of additional electron acceptors on the PAHs anaerobic degradation.

Materials and methods
Soil samples
Four paddy soils (Kamajima, Kuridashi, Anjo, Togo) without any contamination history were obtained from rice fields located in different places near Nagoya city in Japan. Kuridashi and Kamajima samples belong to gley soil (pH 6.7, 6.6; Total organic carbon (TOC) 0.99%, 2.05%; Total nitrogen (TN) 0.03%, 0.1%; Cl^- (mg/kg) 38.6, 423.8; SO_4^{2-} (mg/kg) 96.5, 191.9; NO_3^- (mg/kg) 0.8, 1.7; Iron oxides (mg/kg) 6.9, 2.3; moisture content 30.7%, 26.1%; respectively). Anjo soil is a yellow highland soil, and Togo soil belongs to gray lowland soil (pH 5, 5.5; TOC 1.15%, 1.34%; TN 0.04%, 0.04%; Cl^- (mg/kg) 26.3, 31.5; SO_4^{2-} (mg/kg) 278.5, 425; NO_3^- (mg/kg) 4.2, 4.1; Iron oxides (mg/kg) 24.2, 23.6; moisture content 16.2%, 25.6%; respectively).[8] Samples were sieved (1 mm mesh), suspended in tap water under room temperature until use.
Anaerobic PAH degradation experiment
Anaerobic degradation experiment was performed in 600 ml serum bottles including 50 g soil and 150 ml anoxic water under a steam of nitrogen gas. The bottles were capped with butyl rubber stoppers and sealed with aluminum crimps. Pure nitrogen gas was flushed to displace the trace oxygen in headspace. Biphenyl or naphthalene was added into each bottle at 0.3mg per bottle as 0.1% of acetone solution. Abiotic controls were provided by adding paraformaldehyde solution (5%, V/V). All the sample bottles were incubated without shaking at 30°C in darkness. After appropriate intervals, the remaining PAH concentration was determined.
Effects of the additional electron acceptors on PAH degradation
Kuridashi soil was chosen to test the effects of electron acceptors. Procedure of sample bottle preparation was

the same as that described above, except that biphenyl and naphthalene crystal was added instead of acetone solution. In addition, 20 mM of amorphous Fe(III) oxide (FeOOH), 10 mM of sulfate or nitrate was added as electron acceptors, respectively. The samples without any additional electron acceptors were also prepared as active controls.

Analysis

For measuring the remaining PAH concentration, 0.5ml of sample slurry was taken periodically during incubation after the bottles had been heavily shaken by hand. Samples were analyzed by a high performance liquid chromatography with a UV detector after acidification with HCl and extraction with 0.5ml of acetonitrile.

Results and discussion

Anaerobic biphenyl and naphthalene biodegradation in paddy soils

Anaerobic degradation of naphthalene and biphenyl was observed in Kamajima and Kuridashi paddy soils without any acclimation period. In the comparison with the abiotic controls, apparent decrease of naphthalene and biphenyl in both active samples clearly suggested that microbial action was responsible for the degradation (Fig. 1). The anaerobic degradation of these two PAHs fitted the first-order kinetics. The biodegradation rate constants and half-lives were calculated and listed in Table 1. Kuridashi soil had higher capacity degrading biphenyl (0.0062 1/day) and naphthalene (0.008 1/day) than Kamajima soil (0.0036 and 0.0064 1/day). Although the biphenyl and naphthalene degradation rates in our study were not very high, the results indicated a potential that natural attenuation of PAHs can occur even in some non-contaminated anoxic paddy soils.

In both Kamajima and Kuridashi soils, biphenyl showed slower degradation rate than naphthalene. On one hand, it may because lower water solubility of biphenyl (4 mg/l) than that of naphthalene (30 mg/l) limited biphenyl bioavailability to microorganisms in soil.[9] On the other hand, more stable chemical structure of biphenyl may be another factor affecting anaerobic degradation rate.

In contrast to Kamajima and Kuridashi grey soils, Togo gray lowland soil and Anjo yellow highland soil showed no microbial capacity degrading biphenyl up to 255 days incubation (Fig. 1). Similarly, no naphthalene degradation was found in both of Togo and Anjo soils, which suggested that soil classification may be very important factor for the microbial capacity of the PAH degradation under anaerobic conditions. In addition, Togo and Anjo soils had a lower pH which may inhibit the microbial activity and decrease the potential degrading PAHs.[10]

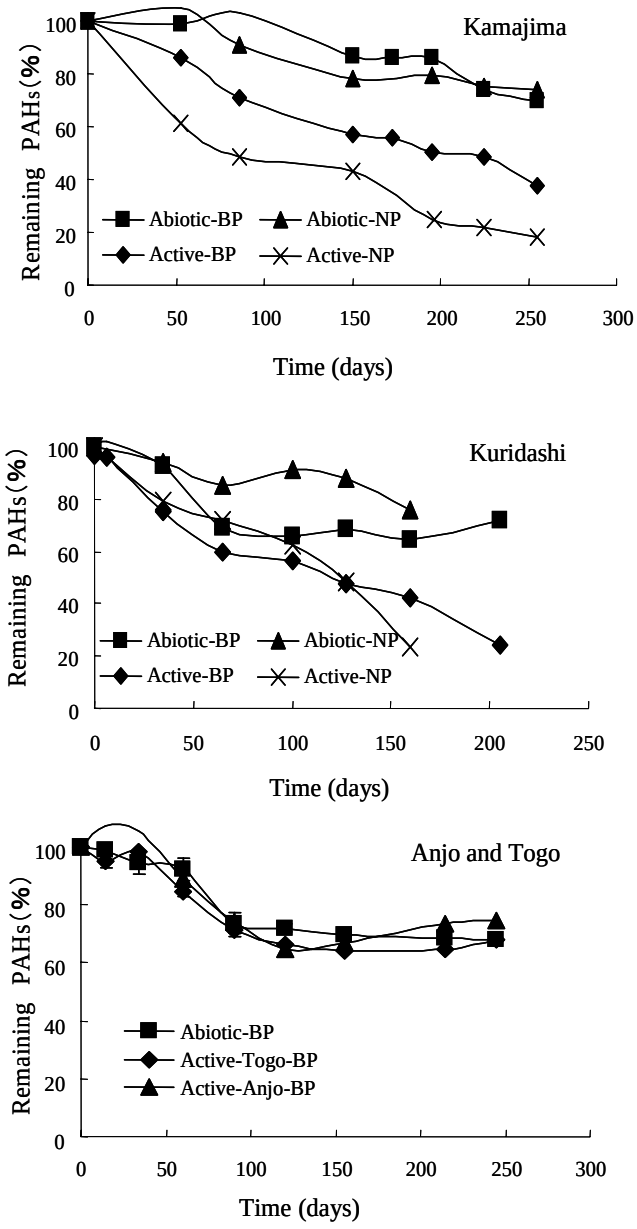

Fig. 1 Anaerobic degradation of biphenyl (BP) and naphthalene (NP) in paddy soils

Table 1. Anaerobic PAH degradation rate constants and half-lives

Sample	Degradation rate constants (1/d)		Half-lives(d)	
	Naphthalene	Biphenyl	Naphthalene	Biphenyl
Kuridashi	0.008	0.0062	86	112
Kamajima	0.0064	0.0036	108	193

Effects of electron acceptors on anaerobic degradation of biphenyl and naphthalene in Kuridashi soil

The effects of electron acceptors on the anaerobic degradation of naphthalene in Kuridashi soil are shown in Fig. 2. After 160 days of incubation, about 80% of initial naphthalene was degraded in samples amended with sulfate or nitrate. Around 40% and 50% of naphthalene was degraded in samples amended with Fe(III) and without any additional electron acceptor, respectively. The results indicated that sulfate and nitrate enhanced naphthalene degradation and addition of sulfate and nitrate can be used as an effective treatment strategy to clean up anoxic soils contaminated with naphthalene. A few reports showed PAH biodegradation under iron-reducing condition and Fe(III) was considered to be the most abundant potential electron acceptor for organic matter oxidation in a wide variety of sub-surface environments. However, the addition of Fe(III) in our paddy soil did not enhance biphenyl or naphthalene degradation.

The highest anaerobic degradation rate of biphenyl was observed in the samples without any additional electron acceptor. In contrast to naphthalene, the addition of sulfate had an inhibitory effect on biphenyl degradation (Fig. 2). This illustrated that microbial population degrading naphthalene and biphenyl were different in anoxic paddy soil. Ambrosoli et al, for the first time, have demonstrated that biphenyl and some other PAHs biodegradation in uncontaminated paddy soil might be partly attributed to the fermentation activity with the presence of acetate or glucose.[11] Further work is needed to characterize the responsible microorganisms in anoxic paddy soils for degrading PAHs.

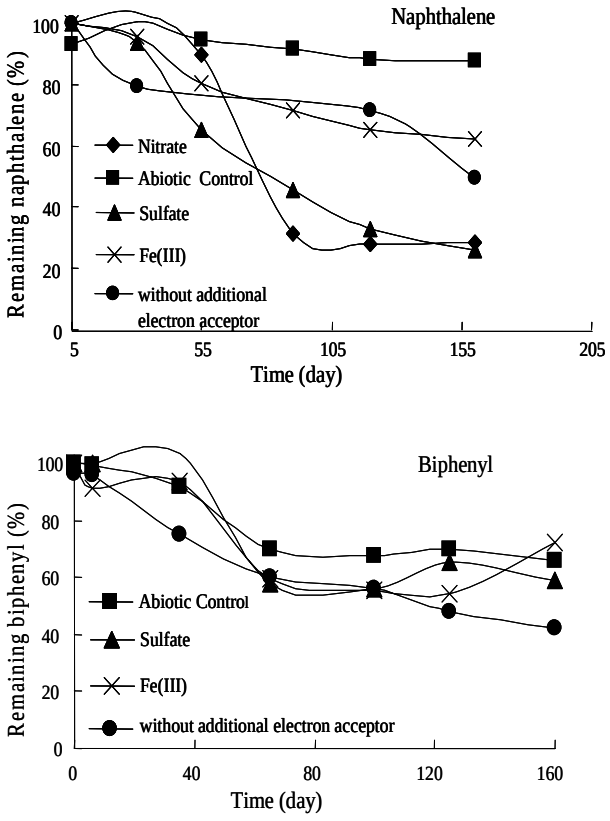

Fig. 2 The effects of electron acceptors on the anaerobic degradation of naphthalene and biphenyl.

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