

Anaerobic Degradation of Aromatic Compounds by Microorganisms in Paddy Field

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Abstract

Consortia demonstrated the high capacities of anaerobic degradation of various aromatic compounds, which were successfully enriched from gley paddy soils under different conditions. Phenol and cresol was decomposed anaerobically using nitrate, ferric oxide or sulfate as electron acceptors. Biphenyl was degraded to CO₂, especially without addition of external electron acceptor. Alkylphenols with middle length of alkyl chain, were co-metabolically degraded with the presence of hydroxybenzoate as the co-substrate under nitrate reducing conditions. The microorganisms responsible for the anaerobic co-metabolism was *Thauera* sp. Reductive dechlorination activity was also observed for polychlorophenols, fthalide, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins with the presence of lactate, formate or H₂ as electron donor. The fthalide dechlorinator was classified as *Dehalobacter* sp. Coupling of two physiologically-distinct anaerobic consortia, aromatic ring degrader and reductive dechlorinator, resulted in the mineralization of pentachlorophenol under anaerobic conditions. These results suggested that gley paddy soils harbored anaerobic microbial community with versatile capacity degrading aromatic compounds under anaerobic conditions.

Key words: anaerobic degradation, reductive dechlorination, iron-reducing condition, sulfate-reducing condition, combination of consortia

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Introduction

Reductive dechlorination has been observed in various chlorinated organic chemicals under anaerobic conditions of paddy soils (Figure 1). Anaerobic microbial dechlorination of DDT produced dichlorobenzophenone via the intermediate dichlorodibenzodichloromethane (DDD) (Focht 2003). In the case of hexachlorocyclohexane (HCH), reductive dechlorination produced trichlorobenzene. Anaerobic

dechlorination of thiobencarb (herbicide) in paddy soils produced desmethyl thiobencarb which caused growth inhibition in rice plant (Moon and Kuwatsuka 1984).

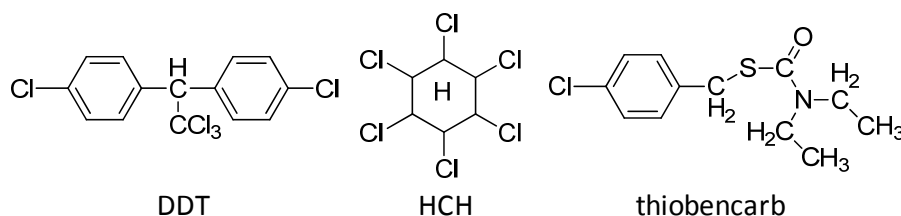


Fig. 1. Examples of pesticides dechlorinated under anaerobic paddy soil conditions.

On the other hand, the degradation of aromatic ring of the compounds has been considered mainly degraded aerobically at the soil surface under flooded conditions because the degradation under aerobic conditions have been reported much higher than under anaerobic conditions. Therefore, the biodegradation of chlorinated aromatics in agricultural environment has been considered to need firstly the anaerobic dechlorination producing less-chlorinated intermediate and then aerobic decomposition converting the intermediate metabolite into CO₂; That is coupled anaerobic-aerobic process (Armenante *et al.* 1999; Ehlers and Rose 2006). However, the coupled process is costly and requires a large amount of energy, so that not implemented as remediation method. Since 1990s, studies have demonstrated that anaerobic microorganisms anaerobically oxidized aromatic ring of benzene, toluene, ethylbenzene and decomposed to CO₂ via benzoyl-CoA pathway (Holliger and Zehnder 1996). These finding suggested that coupled anaerobic-anaerobic processes, that is reductive dechlorination and anaerobic aromatic ring decomposition, is possible and may be happening in paddy field.

Thus, in this study, we have examined anaerobic biodegradation of aromatic ring and the coupled anaerobic-anaerobic processes for complete decomposition of chlorinated aromatic compounds in paddy soil.

Materials and Methods

Seven paddy soils were collected from Kuridashi, Yatomi, Kamajima, Nagakute B-10 and C-16, Togo and Anjo, Aichi Prefecture, Japan (Kim *et al.* 2004). The soil was sieved (2mm) and stored under flooded condition at room temperature until use. Kuridashi and Kamajima gley soils showed high activities for both anaerobic degradation of aromatic ring and the dechlorination. These two soils have been used.

Anaerobic biodegradation experiments were performed using 60 to 600 ml-volume of serum bottles containing from 3 to 30g of soils with 20 to 50ml of water or medium. For the degradation

experiment of phenol, alkylphenols and biphenyl, sodium nitrate, one of the two electron acceptors, sodium sulfate, or ferric iron oxide (FeOOH), was introduced. The condition without any addition of external electron acceptor was also provided. For the dechlorination experiment of pentachlorophenol (PCP), fthalide, polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxin, lactate, formate, or hydrogen gas has been utilized as external electron donor. The medium compositions were described previously (Yoshida *et al.* 2007). All the bottles were incubated in the dark at 30°C for the appropriate intervals. When the degradation of dechlorination was observed, 5 to 20 % of the soil culture was transferred to a new bottle prepared in the same condition using steam-sterilized paddy soil.

During or after the incubation, chemicals were extracted with acetonitrile and ethyl acetate after the acidification, dried over Na₂SO₄ anhydrous, and measured by a gas chromatography/mass spectrometry. Chloride and sulfate ions were determined by an ion chromatography. Ferrous iron was measured calorimetrically using phenanthroline method. Gas composition of the head space gas was analyzed by a gas chromatography equipped with thermal conductivity detector and flame ionization detector. Detailed procedures were described previously (Baba and Katayama 2007b; Shibata *et al.* 2007a; Yang *et al.* 2009). Mineralization of pentachlorophenol and biphenyl was confirmed using uniformly ¹⁴C radio-labeled compounds (Yang *et al.* 2009; Yang *et al.* 2008). Characterization of the microbial community structure of the consortia was performed by analyses of respiratory quinone profile and PCR (polymerase chain reaction)-DGGE (denaturing gradient gel electrophoresis) targeting 16S rRNA genes. The procedures were described in details previously (Yoshida *et al.* 2007).

Results and Discussion

Phenol and p-cresol degradation

Kamajima and Kuridashi paddy gley soils degraded phenol and p-cresol under anaerobic conditions. Consortia were enriched with and without electron acceptor. Electron acceptors successful to maintain the degradation activity were nitrate, sulfate and ferric iron as shown in Table

Stoichiometric relation was observed between phenol degradation and the consumption of sulfate or ferric iron, suggesting the complete degradation of phenol.

Sulfate-reducing conditions: $2\text{C}_6\text{H}_6\text{O} + 7\text{SO}_4^{2-} + 2\text{H}^+ + 6\text{H}_2\text{O} \rightarrow 12\text{HCO}_3^- + 7\text{H}_2\text{O}$

Iron-reducing conditions: $\text{C}_6\text{H}_6\text{O} + 28\text{Fe}^{3+} + 17\text{H}_2\text{O} \rightarrow 6\text{HCO}_3^- + 28\text{Fe}^{2+} + 34\text{H}^+$

Major respiratory quinone was menaquinone-6, suggesting the predominance of *Deltaproteobacteria* in the phenol-degrading consortia both under sulfate-reducing and iron-reducing conditions. The PCR-DGGE analysis indicated the presence of *Syntrophorhabdus*-like bacterium in the sulfate-reducing

conditions, *Geobacter*-like bacteria in the iron-reducing conditions (Yang *et al.* 2007).

Table 1. Anaerobic degradation of phenol and cresol with different electron acceptors

Electron acceptor	Phenol	Cresol	Biphenyl
Nitrate	+++	+++	+
Sulfate	+++	Not examined	+
Ferric iron	+++	+++	+
None*	+++	Not examined	++

*Fermentative or syntrophic degradation

Although [U - ^{14}C]- labeled biphenyl was also degraded anaerobically with electron acceptors (Table 1), the higher activity was observed without electron acceptor. About 1% of biphenyl was converted into CO_2 and 5% into water-soluble metabolites (Yang *et al.* 2008).

Alkylphenol was not degraded with any electron acceptor. However, the addition of 4-hydroxybenzoate into the culture enabled the anaerobic oxidation of alpha-carbon in alkyl group of the alkylphenol as shown in Figure 2. When the alkyl group of alkylphenols are shorter than C_3 , the degradation proceeded further, but not in longer than C_4 , and the corresponding alkylphenone became dead-end product. From the enriched consortium, the phenol-degrading nitrate-reducing *Thauera* sp R5 has been isolated, which oxidized alpha-carbon of alkyl group with the reduction of nitrate to nitrite. p-Cresol, 4-ethylphenol, and 4'-hydroxyacetophenone were also utilized as co-substrate by *Thauera* sp R5 (Shibata and Katayama 2007b).

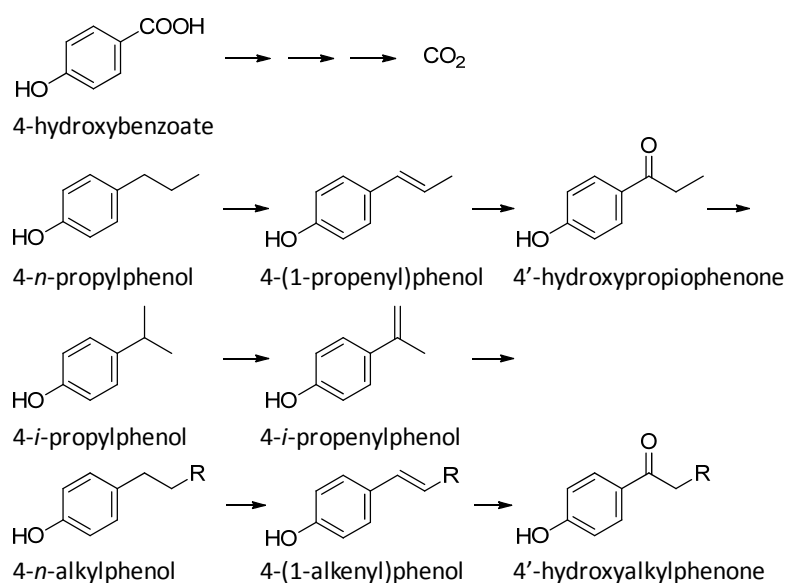


Fig. 2. Possible co-metabolic pathways of alkylphenols with 4-hydroxybenzoate as co-substrate.

PCP degradation activity was observed in all the examined paddy soils (Kim *et al.* 2004). The strong activity was observed in two gley soils, Kuridashi and Kamajima. The dechlorinating consortium enriched from Kamajima soil dechlorinated PCP completely to phenol, which has not been observed in isolates previously reported (Bouchard *et al.* 1996; Madsen and Licht 1992; Mohn and Kennedy 1992). As the electron donor, acetate and pyruvate and hydrogen have been utilized by the consortium instead of lactate. Effect of specific inhibitor, respiratory quinone profile and PCR-DGGE analysis for 16S rRNA genes suggested the predominance of *Firmicutes* in the consortium.

Coupling of two anaerobic consortia, PCP-dechlorinator and one of the phenol-degraders (either sulfate-reducing or iron-reducing consortium) resulted in the mineralization of PCP to CO₂ and CH₄, which was evidenced by the stoichiometric release of chloride ion and ¹⁴CO₂ and ¹⁴CH₄ production of the tracer experiment (Figure 3) (Li *et al.* 2010b; Yang *et al.* 2009).

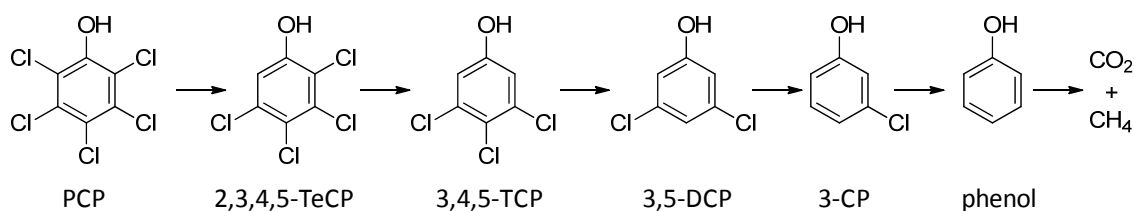


Fig. 3. Anaerobic PCP mineralization by using reductive dechlorinating and phenol oxidizing anaerobic consortia.

For the dechlorination reaction, lactate was supplied as electron donor, and for the phenol degradation, sulfate or ferric iron as electron acceptor, respectively. The simultaneous introduction resulted in the unwanted consumption. After the depletion of sulfate in the coupled process utilizing the phenol-degrading sulfate-reducing consortium, the degradation of PCP slowed down but continued. This reaction was considered to be carried out by the syntrophic microbial reaction.

Fthalide-dechlorinating bacterium was also enriched from Kamajima soil with lactate as electron donor and identified in the consortium. The difference in PCR-DGGE for 16S rRNA genes between fthalide-spiked and non-spiked cultures suggested that the bacteria involved in the fthalide-dechlorination were *Dehalobacter* FTH1 and FTH2. *Dehalobacter* FTH1 and FTH2 increased in the population with the increase in chloride ion, suggesting that these *Dehalobacter* species are novel strain dechlorinating chlorinated aromatics (Yoshida *et al.* 2009a), in contrast with the previous reports on *Dehalobacter* as dechlorinator of chlorinated aliphatics (Holliger *et al.* 1998; Sun *et al.* 2002; Wild *et al.* 1997). Possible metabolic pathway was determined by the analysis of metabolites at different times as shown in Figure 4. The metabolic pathway was different

from that observed in soil, where fthalide was dechlorinated to 4,6-dichlorophthalide (diCPH) via 4,6,7-triCPH (Ishida and Nambu 1975).

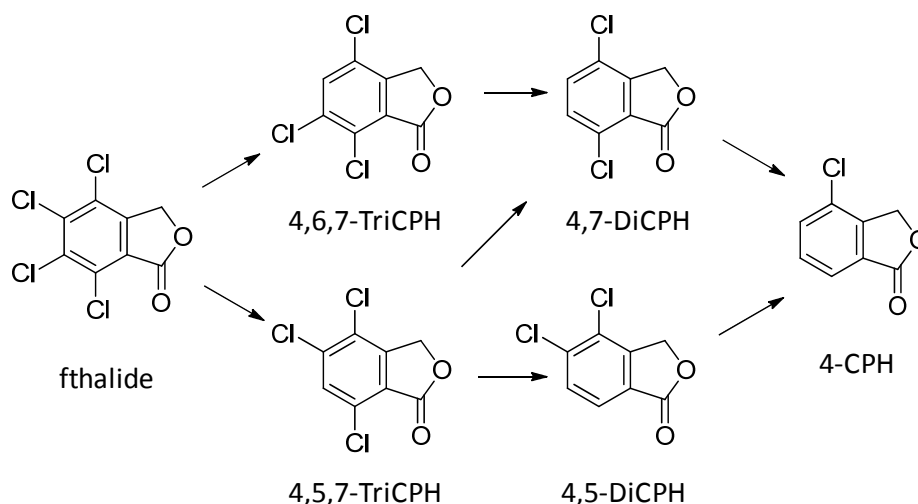
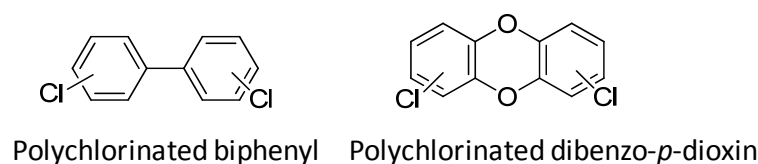


Fig. 4. Possible metabolic pathway of fthalide in *Dehalobacter* FTH1 and FTH2 containing culture.

The *Dehalobacter*-containing consortium also dechlorinated 2,3,4-trichloro and 2,3,4,5-tetrachloro-biphenyls and 1,2,3-trichlorodibenzo-*p*-dioxin (Yoshida *et al.* 2009b). A wider spectrum for PCB dechlorination was observed in the enrichment from Kuridashi gley soil using acetate and lactate as electron acceptors, where most of PCB congeners in Kanechlor 300 and 400 were dechlorinated (Baba and Katayama 2007b; Baba *et al.* 2007a; Baba *et al.* 2007c).



Conclusions

The anaerobic consortia were enriched from gley paddy soils. The consortia demonstrated the versatile capacity of degradation: anaerobic degradation of phenol, *p*-chresol, biphenyl, anaerobic co-metabolism of alkylphenol, dechlorination of PCP, fthalide, PCB and trichlorodibenzo-*p*-dioxin. The coupling process of PCP dechlorination and phenol degradation enabled to mineralize PCP under totally anaerobic conditions. This suggested that multiple microbial populations can work synergistically in the paddy soil environment. It is very important to identify the microorganism responsible for the targeting reactions for the understanding the coupling processes in paddy soil.

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