한국환경농학회지 제23권 제3호 (2004)
Korean Journal of Environmental Agriculture Vol. 23, No. 3, pp. 138-141

Anaerobic Biodegradation of PCP in Japanese Paddy Soils

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(Received July 15, 2004. Accepted August 20, 2004)

ABSTRACT : Seven soil samples were collected from paddy fields located nearby Nagoya city in Japan. All the soils were subjected to flooded condition and incubated with PCP at 30℃ for two months, and their anaerobic PCP degradation have been monitored by checking the PCP concentration of the soils at regular intervals. The degradation of PCP did not occur in the soils autoclaved two times before pre-incubation. On the other hand, all the soils showed significant PCP degradation in non-sterilized condition after 30 days of incubation, except for one soil sample (Yatomi), in which PCP was rarely degraded until 30 days of incubation. This result showed PCP disappearance in the paddy soils was mainly caused by microbiological activity, and depended upon the physicochemical characteristics of the soils.

Key words: degradation, anaerobic, pentachlorophenol (PCP), microbiological activity.

INTRODUCTION

Many environmentally important xenobiotics used for industries, are halogenated and the halogenation is often implicated as a reason for persistence[1)]. Chlorinated aromatic compounds are pollutants of major concern because they often enter the environment in substantial quantities, are toxic and resistant to degradation, and accumulate in sediments and biota[2)]. Dechlorination generally makes xenobiotic compounds less toxic and more readily degradable. Aerobic metabolism of highly chlorinated aromatic chemicals is often restricted ; two adjacent ring positions must be free for hydroxylation, and the chlorine could be removed after ring opening. Thus, the anaerobic dechlorination which occurs prior to ring opening provides a means to overcome the limit of aerobic degradation[3)]. Recently it's been known that anaerobic microbial communities have the ability to degrade a variety of organic pollutants, including chlorinated aromatic compounds[3)]. Also, the research on anaerobic biodegradation of chlorinated aromatic compound is of critical importance because sites contaminated with these compounds include anaerobic environments, such as groundwater, sediments and industrial sludge.

Pentachlorophenol (PCP) is a broad-spectrum biocide that has been used widely as a wood preservative, a preharvest herbicide, a molluscide, and in a variety of other applications. PCP has been used for many years as a herbicide in Japanese paddy fields. But owing to some serious fish kills, its use was restricted to upland fields only in 1971[4)]. The US EPA classified PCP as a priority pollutant because of its carcinogenicity and toxicity in 1993[5)]. It has been reported that many sites including soil, groundwater, and sewage sludge were contaminated with PCP in Finland, Netherlands and Canada. These facts urge the necessity of finding methods to decontaminate PCP from the environment. The paddy field is one of the most familiar anaerobic environments, and its soil has high organic matter content.

The objective of the current research is to analyze the PCP degradation in the soils from various paddy fields and to characterize the relationship between PCP-degrading ability and the properties of each soil.

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MATERIALS AND METHODS

Chemicals

Analytical standard of PCP (purity, 99%) was purchased from Wako pure chemical (Kyoto, Japan). PCP-Na salt (purity, 90%), a commercially marketed form of PCP was also obtained from Wako pure chemical (Kyoto, Japan) and used to obtain desired PCP concentrations of soils. All other chemicals were reagent or HPLC grade as necessary.

Soils

The soil samples were obtained from plow layers of paddy fields located nearby Nagoya city. The fresh soils were sieved through 5 mm sieving and stored in airtight plastic bags at 22℃ for a few days before use.

The physicochemical properties of the soils are listed in Table 1.

Soil conditioning[6]

Before PCP was applied to the soil, soil samples were pre-incubated in order to simulate field conditions. Fifty grams of the soil (dry wt. basis) was placed in a glass sample bottle (12.5 cm × 3 cm i.d.) and submerged with water up to 3 cm deep. The bottle was capped with aluminum foil and pre-incubated at 30℃ in the dark for 2 weeks. In case of sterilized soil samples, each of them was autoclaved at 121℃ for 30 min., two times in consecutive days before starting pre-incubation.

Application of PCP and incubation

An aqueous PCP solution (100 mg/L) was prepared by dissolving 120.3 mg of PCP-Na in distilled, sterilized water and diluting to 1 L. Five milliliters of the PCP solution were mixed well with the pre-incubated soil at a concentration of 10 mg/L on dry soil basis. The soil samples were incubated at 30℃ for 1 hr., 7, 14, 30 and 60 days.

During incubation periods, the water content was restored once a week. The experiments were replicated and the results are reported as the average of duplicates.

Table 1. The physicochemical properties of soil samples

Soil	pH	EC (mS/m)	Total-C (%)	Total-N (%)
Kuridashi	6.78	4.77	0.99	0.03
Nagakute B-10	6.63	1.54	1.05	0.04
Nagakute B-13	6.93	2.39	0.80	0.04
Yatomi	6.75	2.43	1.30	0.06
Anjo	6.93	2.24	1.34	0.04
Togo	7.22	34.80	2.05	0.10
Kamashima	6.73	1.29	1.15	0.04

Analysis of PCP

For PCP determination, soil samples were extracted and cleaned up following the method of Secchieri et al[7]. Five milliliters of 50% aqueous solution of sulfuric acid were added to the incubated sample and mixed well. The soil slurry was transferred into a 500 mL centrifuge bottle, and 80 mL of acetonitrile was added. The bottle was shaken for 30 min and centrifuged at 9,000 rpm/20 min. The supernatant was collected and stored. This extraction procedure was repeated twice, and the supernatants were pooled. The acetonitrile portion was distilled off using a rotary evaporator. The residual water was extracted twice with 80 mL of n-hexane in aseparatory funnel. The combined n-hexane layer was concentrated to about 2 mL and applied to a preconditioned Bakerbond CN column (500 mg). The columns were washed with 2 bed volumes of n-hexane and dried 5 min, and PCP was eluted twice with 1 mL aliquots of acetonitrile. The combined eluent was concentrated to dryness under the flow of nitrogen gas, and dissolved in mobile phase (2 mL). PCP was quantified by high-performance liquid chromatography (HPLC) using a Shimadzu HPLC system equipped with a Puresil C-18 cartridge column (4.6 × 250 mm) and a UV detector at 254 nm. The mobile phase was acetonitrile/water/acetic acid (65:34:1 v/v %), at a flow rate of 1.0 mL/min[8].

Analyses of sulfate, nitrate and ferrous iron

The air-dried soil (2 g) was extracted with 80 mL of distilled water[9]. The water extract was filtered through a membrane filter with pore size of 0.2 μm. Sulfate and nitrate ions were measured by a Shimadzu ion chromatograph PIA-1000 equipped with a column IC-A3(S) and a electroconductivity detector. The analytical condition was according to the manufacturer's instruction The mobile phase was composed of 8 mM of p-hydroxybenzoic acid and 3.2 mM of bis(2-hydroxyethyl)iminotris(hydroxymethyl) methane. The flow rate was 0.2 mL/min, and the injection volume was 10 μL. The detection limits in this procedure were 4 μg/g-soil for nitrate and 8 μg/g-soil for sulfate, respectively.

The free iron oxides in the soils were determined by an Asami-Kumada method[10]. The air-dried soil, 2 g, was mixed with 3 g of sodium dithionite and 100 mL of 0.02 M of EDTA. The soil mixture was maintained at 70℃ for 15 min. The extract was filtered through a No. 6 Advantec (Toyo) filter paper. The residue soil was washed with 1% NaCl solution three times. The extract and washed solutions were combined. An aliquot was mixed with 1 mL of 5% hydroxylamine hydrochloride, 2 mL of 0.1% o-phenan-

throline and 1.5 mL of 3.5 M acetic anhydride-acetate buffer (pH 6). After 30 min, the absorbance of the solution was measured at 508 nm.

RESULTS AND DISCUSSION

The patterns of PCP degradation in the seven soils were shown in Fig. 1 and 2.

In non-sterilized condition, the PCP was found to be highly degraded in all of the soils except Yatomi after 30 days, however the degradation rate was changed depending on the soil samples. From this graph, it is clear that all soils except Yatomi soil begin to degrade PCP after 14 days of incubation. Biodegradation of toxic substances in the environment is often preceded by an acclimation period (lag phase). It has been reported that the length of the lag phase prior to PCP degradation in the anaerobic cyclone fermentor (lag phase=9.4 days) increased substantially when compared to a 0.8-day lagphase in the aerobic one[11].

PCP degradation in sterilized soils was not significant

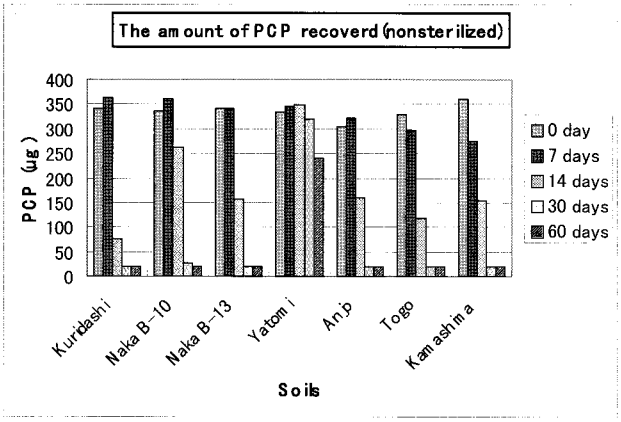

Fig. 1. Anaerobic biodegradation of PCP in non-sterilized paddy soils according to the incubation periods.

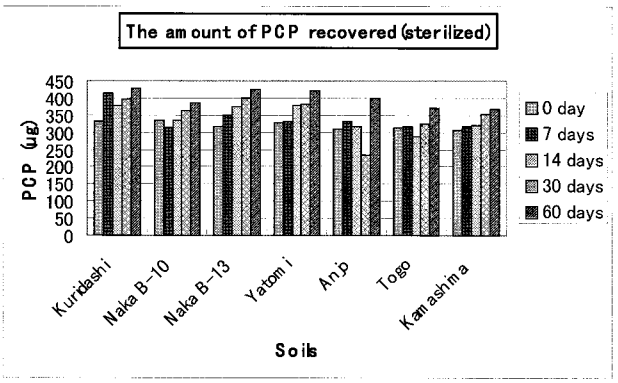

Fig. 2. Anaerobic biodegradation of PCP in sterilized paddy soils according to the incubation periods.

during the incubation periods. This result suggests that PCP degradation is related to the microbial activity in the soil. Ide et al[12] found PCP was decomposed within a few weeks after its application in rice fields. And they pointed out a reductive dechlorination was revealed as one of decomposition pathways, in which some microorganisms play a dominant role. However, little is known about the microorganisms, which are responsible for the anaerobic biodegradation of PCP in the paddy soils.

The PCP incubated with the seven paddy soils under flooded condition was degraded as a first-order reaction. Degradation rate constants of PCP in the seven soils were calculated using first-order reaction kinetics and shown in Table 2. Time course data for all the seven soils approximated well with first-order kinetics as shown in high r^2 values.

The half-life ($t_{1/2}$) of PCP in the seven paddy soils were calculated by a mathematical model illustrating pseudo-first order kinetics $C=C_0e^{-Kt}$, where C_0 is the initial concentration of PCP (μg/g), C is the concentration at time (μg/g), t is the incubation time (days), and K is the PCP rate constant (day^{-1}). Half-life, $t_{1/2}$, was obtained from ln2/K. All the tested soils except Yatomi soil showed similar PCP-degradation rates.

Reductive dechlorination involves the removal of a chlorine substitute from a molecule with concurrent addition of electrons to the molecule. Generally this reaction is an initial step in metabolism under highly reduced conditions, and requires a source of reducing equivalents as an electron donor, with the chlorinated compound serving as a concomitant electron acceptor. Reductive dechlorination can yield energy for the microorganisms involved; however, it is known that this process is often inhibited by other electron acceptors, such as sulfate, nitrate or Fe(III)[13]. The levels of these electron acceptors were determined in the sample soils, and the results were shown in Table 3.

Table 2. First-order rate constants of PCP degradation in the seven paddy soils

Soil	K (day^{-1})	$r^{2a)}$	Half-life ($t_{1/2}$,days)
Kuridashi	0.103	0.93	6.7
Nagakute B-10	0.090	0.87	7.7
Nagakute B-13	0.101	0.94	6.9
Yatomi	0.006	0.87	126.0
Anjo	0.097	0.90	7.1
Togo	0.099	0.96	7.0
Kamashima	0.100	0.96	6.9

[a)]Coefficient of determination.

Table 3. The concentrations of electron acceptors in the seven paddy soils

Soil	SO_4^{2-}(μg/g)	NO_3^-(μg/g)	Fe^{3+}(μg/g)
Kuridashi	81.79	-[a)]	10.10
Nagakute B-10	40.06	-	2.06
Nagakute B-13	34.00	-	6.99
Yatomi	79.19	29.85	14.02
Anjo	60.45	-	23.62
Togo	40.06	-	2.33
Kamashima	62.54	-	24.16

[a)]Below the detection limit(4 μg/g soil)

As was expected, the nitrate concentration was very high in Yatomi soil compared with other soils, and this factor seems to have contributed to the persistency of PCP in Yatomi soil. Similar results were reported in an experiment with river sediments, in which trifluralin degradation was delayed in the presence of nitrate[14].

In conclusion, all the paddy soils tested here showed significant PCP degradation under flooded condition except Yatomi soil, in which PCP rarely degraded until 30 days of incubation. On the other hand, this degradation patterns were not observed in sterilized condition, suggesting that PCP degradation was caused primarily by the action of anaerobic microorganisms. The relatively high level of nitrate in Yatomi soil seems to have inhibited PCP biodegradation pathway of reductive dechlorination. It seems that further study is required for a better understanding of the basic mechanisms underlying anaerobic biodegradation of PCP, and for the identification of responsible microbial consortium.

ACKNOWLEDGEMENT

This work was supported by the Post-doctoral Fellowship Program of Korea Science & Engineering Foundation (KOSEF).

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