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Anaerobic degradation of chlorothalonil in four paddy soils

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ABSTRACT

Degradation of Chlorothalonil (CTN) was investigated in four different paddy soils under anaerobic conditions. The CTN biodegradation is strongly affected by the properties of the paddy soils. Soils associating with rich total carbon (TC), repeated CTN application, and neutral pH have shown the high capacity to biodegrade CTN. Additionally, anaerobic CTN biodegradation was accompanied by the methane generation and a drop of oxidation–reduction potential (ORP). The initial CTN concentration had a significant effect on CTN removal efficiency, and increase in the initial CTN concentration resulted in the decreasing of CTN removal percentage. However, it is believed that the inhibitory effect on anaerobic biodegradation of CTN is negligible in natural environment due to the much lower concentration of CTN in natural environment (at ng g^{-1} or pg g^{-1} level) than the one (10 μg g^{-1}) investigated in this study. The 4-hydroxy-2,5,6- trichloroisophthalonitrile (HTI), one of the major metabolites of CTN degradation, has shown the significant inhibitation to the anaerobic CTN biodegradation when its residual level is over 0.1 μg g^{-1}.

1. Introduction

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, CTN), one of the most popular fungicides, belongs to the group of halogenated benzonitriles. It is well known that CTN works by contacting and further inhibiting cell respiration enzymes related to glutathion (Arvanites and Boerth, 2001). Over the past two decades, the CTN has been widely applied to control many fungal diseases in agriculture (Cox, 1997). However, residual CTN has been often detected in vegetables, crops, soils, and environmental water (Andersson and Bergh, 1991), and the toxicity of CTN and its effect on human health have been extensively studied (Sherrard et al., 2003). Caux et al. (1996) have reported CTN is highly toxic to fish, birds, and aquatic invertebrates in environment. The CTN has been considered as a moderately persistent fungicide in soil, and the half-life of CTN in soil has been reported in the range from 4 days to 6 months (Singh et al., 2002). The variation of reported CTN half-life data could be due to the different experimental conditions (Gambacorta et al., 2005). In addition, the repeated application times have a significant effect on degradation of CTN (Walker et al., 1988; Van der Pas et al., 1999). Singh et al. (2002) reported that the half-life of CTN for the first dose in soil was 8.6 days and this was extended to 21.5 days for the third treatments. In the top layer of soil, 4-hydroxy-2,5,6-trichloroisophthalonitrile (HTI) is the primary breakdown metabolite of CTN in the presence of water, and it is more acutely toxic, persistent, and mobile in soil than the CTN itself (Cox, 1997). Moreover, the HT1 may cause inhibition of the CTN degradation due to its inhibitory effect on microorganisms (Motonaga et al., 1996). Meanwhile, Other CTN metabolites, including dechlorinated or substituted forms of CTN, such as 2,5,6-trichloro-4-methoxyisophathalonitrile, 3-dicarbamo-yl-2,4,5,6-tetrachlorobenzene, 2,4,5-tri- chloroisophthalonitrile, 1,3-dicyanobenzene, etc. have been reported (Gustavo and Damia, 1998). The aerobic metabolism has been considered as the most suitable pathway for CTN microbiological degradation (Katayama et al., 1997). However, some of metabolites from the group of halogenated benzonitriles have been reported (Regitano et al., 2001), and those metabolism pathways might be associated with the mechanisms of sulphate-reduction and reductive dechlorination that occur in anoxic environments. Wackett and Hershberger (2001) suggested benzenic ring reduction via carboxilation in anaerobic conditions could be one of the general rules of biodegradation, thus it is necessary to gather more information about CTN anaerobic degradation and metabolism. However, a little information has been reported for CTN degradation under anaerobic conditions. To author's best knowledge, only Carlo-Rojas et al. (2004) have studied the anaerobic degradation of CTN in a banana plantation and their response to simulation by different carbon/nitrogen ratios. In most area of East Asia, CTN is often applied to control rice fungal diseases in paddy fields, where anaerobic-like conditions dominantly occur. Thus, it is important to study the anaerobic degradation of CTN in soil.

In this study, we investigated the CTN degradation behaviors in four different paddy soils under laboratory anaerobic conditions, and

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evaluated the effects of the soil properties, initial CTN concentrations, and the main intermediate (HTI) on anaerobic degradation of CTN. The information on the anaerobic degradation behaviors of CTN in paddy soils obtained in this study is useful for the assessment of CTN contamination in environment and mechanism study of its bioremediation under anaerobic conditions.

2. Materials and methods

2.1. Chemicals

Chlorothalonil (CTN, purity 99.4%) was purchased from Promochem (Wesel, Germany) and 4-OH-chlorothalonil (HTI, purity 99.5%) was obtained from ISK-Biotech (New York, USA). The structures of CTN and HTI are shown in Fig. 1. The solubility of CTN in water is only 0.6 mg L^{-1}, while it reaches as many as 2000 mg L^{-1} in acetone. The standard stock solutions of CTN and HTI were separately prepared in acetone at a concentration of 50 mg L^{-1}. The stock solutions were prepared freshly every two month and stored in amber bottles at −20 °C until use. Except where noted, all solvents were of HPLC-grade and all reagents were of reagent grade. The distilled water was purified with a Mill-Q-Plus system (Millipore, Molsheim, France) before use.

2.2. Soils

Two of the studied paddy soils (designated as HB1 and HB2), without CTN application history, were collected from rice fields of two places in Huaibin County, Henan Province, China. The other two paddy soils (named as QJ1 and QJ2), which were applied CTN twice yearly at 375 g a.i ha^{-1} each time in the middle of May and August of past three years (2004–2006), were collected from two rice fields in Qianjiang City, Hubei Province, China. In the four paddy soils, CTN was not detected in HB1 and HB2 soil samples, meanwhile CTN was found at 0.89 and 0.71 ng g^{-1} d.w. in QJ1 and QJ2 soil samples, respectively. The major CTN metabolite (HTI) was also detected (0.45 ng g^{-1} d.w.) in QJ2 soil. All paddy soil samples were collected in October 2006, and their physical–chemical characteristics and contamination status are summarized in Table 1. The collected soil samples were air-dried, ground, sieved (< 2 mm mesh), and stored submerged under water at room temperature. Before starting the anaerobic degradation experiment, the soil samples were equilibrated under submerged conditions at 22 °C for two weeks (Shibata et al., 2007). The experimental constant temperature was guaranteed because the operation was conducted under the thermostatic incubation room.

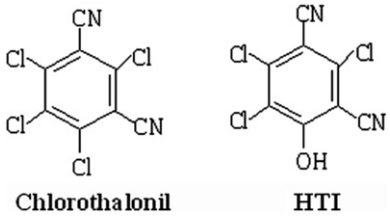

Fig. 1. Structural formulas of chlorothalonil (CTN) and 4-hydroxychlorothalonil (HTI).

Table 1
Physical–chemical characteristics of the four paddy soils investigated in this study.

Kind of sediment	HB1	HB2	QJ1	QJ2
pH	4.7	5.1	6.6	6.3
Water content (%)	22.6	19.3	26.9	17.4
TC (%)	1.37	1.53	3.27	4.12
TN (%)	0.11	0.05	0.04	0.19
SO_4^{2-} (mg/kg)	9.67	25.68	13.76	14.26
NO_3^- (mg/kg)	0.89	1.41	2.17	3.12
Soil texture	Light clay	Silty loam	Silty loam	Sandy loam
Chlorothalonil (ng g^{-1}.d.w)	ND	ND	0.89	0.71
Metabolite A (ng g^{-1}.d.w)	ND	ND	ND	0.45

ND indicates the abbreviation of "not detectable".
TC and TN indicate the abbreviation of "total carbon" and "total nitrogen", respectively.

2.3. Anaerobic CTN degradation

All experiments were performed triplicately in 125-mL bottles, in which 10 g of soils, 30 ml of deionized water, and 5 μg g^{-1} of CTN were added under a gentle nitrogen flow. The small amount of acetone solvent, which was used for dissolving CTN, in soil sample was proved to be no adverse effect on anaerobic microbial activity by our previous study. All bottles were flushed with pure nitrogen gas (99.999%) for 15 min to remove any trace of oxygen in containers, tightly capped with butyl rubber stoppers, sealed with aluminum crimps, and wrapped in aluminum foil to prevent from photolysis. All the sample bottles were incubated for two months at 30 °C, and the residual CTN concentrations, oxidation–reduction potential (ORP) values, and methane concentrations in samples were measured at intervals (0, 5, 10, 20, 40 and 60 days) of incubation. All experiments were conducted in an anaerobic glove box (Forma Scientific, model 1025S/N, USA).

In order to study the contribution of anaerobic microorganisms to CTN degradation, the sterilized (ST) control samples were prepared in four paddy soils. All 125-mL vials containing 10 g soil were capped slightly, wrapped in aluminum foil, and autoclaved for 3 h (three separate 1 h treatments at 121 °C). Then, 50 μg of CTN was added, followed by adding 30 ml of the ST deionized water. The vapor-phase in vials was removed by nitrogen gas to produce the anaerobic conditions and the samples were incubated as described above.

2.4. Effect of metabolite HTI on CTN degradation

HTI standards were spiked into the samples at a series of concentrations (0.1, 0.5 or 1 μg g^{-1}) to test the effects of HTI on anaerobic CTN degradation. The tested concentrations of HTI were referred to the report by Lu et al. (2008). The non-sterile (NST) controls were prepared without addition of HTI and incubated without shaking at 30 °C in darkness.

2.5. Extraction and clean-up of soil samples

Because the solubility of CTN in water is relative low, we did not measure its water equilibrium concentration. Referring to the report by Kwon and Armbrust (2006), soil suspension samples (10 ml) were collected at intervals, adjusted pH to 3 by HCl because the acidic conditions contribute to the high recoveries for most of analytes, and followed by adding 30 ml of water/dichloromethane (1/3, v/v) solution. After shaking for 30 min, the samples were centrifuged for 5 min at 4000 rpm, and the supernatants were pipetted into a 100-mL vial. The soil samples were extracted twice following the procedure described above, and the combined supernatants were filtered through a polytetrafluoroethylene (PTFE) filter membrane (30 mm diameter, 0.2 μm pore size) to remove any soil particles. And then the dichloromethane layer was collected and evaporated to dryness under a gentle nitrogen flow. The residue was redissolved in 2 ml of water/acetonitrile solution (1/1, v/v) before injected into high-performance liquid chromatography (HPLC).

2.6. HPLC analysis

An Agilent 1100 model HPLC equipped with photodiode array detector was used for all experiments. The analytical column used was a YWG-C_{18} reversed-phase column (250 × 4.6 mm ID, 5-μm particle size) and column temperature was controlled at 30 °C. The mobile phase was made up of acetonitrile (ACN) and 0.5% phosphoric acid in water. The gradient elution started with 20% ACN for 3 min, linearly increased to 90% ACN within 20 min and then remained at 90% ACN for an additional 10 min. The injection volume was 20 μl and the flow rate was 0.8 ml min^{-1}. The detection wavelength was set at 232 nm.

2.7. Recovery study

A recovery study was performed by spiking four paddy soils with standard CTN stock solution at a series of concentrations (0.1, 1, 5 and 10 μg g^{-1}). The residual extraction and analysis were conducted as the method described above. The average recoveries for CTN in anaerobic NST soils were from 81.2% to 94.5% and the relative standard deviations (RSDs) were from 4.6% to 8.9%. In the case of anaerobic ST soils, the average recovery was in the range of 83.7–91.2% and RSDs was from 3.5% to 9.2%. As a result, the adopted method could meet the requirements for residual analyses of pesticides (the required recovery ranged from 80% to 120%).

2.8. Determination of ORP (oxidation–reduction potential) and methane production rate

ORP value of supernatant was measured by Ultrameter II™ 6 P (Myron L Company, USA) to ensure the anaerobic conditions. The pH value was measured with a pH meter (PHS-3C model, Shanghai Aiyite CO., Ltd., Shanghai, China).