Journal of Hazardous Materials 179 (2010) 709–714

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Hexachlorobenzene dechlorination as affected by nitrogen application in acidic paddy soil

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ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form 8 March 2010
Accepted 13 March 2010
Available online 19 March 2010

Keywords:
Hexachlorobenzene
Biodegradation
Nitrogen
Methane
Paddy soil

ABSTRACT

Batch incubation experiments were conducted to study the effects of different nitrogen (N) fertilizers (NH_4HCO_3, $CO(NH_2)_2$, and $NaNO_3$) on hexachlorobenzene (HCB) dechlorination in an acidic paddy soil. Results showed that NH_4HCO_3 and $CO(NH_2)_2$ had similar effects on HCB dechlorination, and their application amount was a crucial factor on reductive dechlorination. The addition of a proper amount of 0.14 g NH_4HCO_3- or $CO(NH_2)_2$-N to 500 g soil promoted HCB dechlorination, however, the application of a high amount (0.84 g) of NH_4HCO_3- or $CO(NH_2)_2$-N inhibited HCB dechlorination. Additional $NaNO_3$ served as an electron acceptor and led to lower soil pH, thus inhibited HCB dechlorination. Detected dechlorinated products showed that the dominant pathway of HCB dechlorination was HCB → pentachlorobenzene (PeCB) → 1,2,3,5-tetrachlorobenzene (TeCB) → 1,3,5-trichlorobenzene (TCB), and PeCB was the main metabolite. The role of methanogenic bacteria in HCB dechlorination was uncertain and conditions-dependent.

1. Introduction

Although the commercial production of hexachlorobenzene (HCB), one of the persistent organic pollutants (POPs), has been banned in most countries, it is still used as an intermediate in several countries [1,2]. The global production of HCB exceeded 100,000 tones before 1997 [2], and existing field data indicated that soil was an important reservoir for POPs [3]. Elimination of HCB from soil is of great concern for its persistence, accumulation, diffusivity and toxicity in food chain [2].

Anaerobic reductive dechlorination is a crucial pathway for HCB degradation, because six electrophonic chlorine atoms on the benzene ring make aerobic oxidative degradation of HCB difficult [4,5]. In natural environment, dechlorination can occur under anaerobic condition, such as in submerged paddy soil, sludge, and sediment [5–7], and the resultant lower chlorinated products can be sequentially degraded until completely mineralized under aerobic conditions [7,8]. Reductive dechlorination process is mainly achieved by anaerobic microbes in the presence of electrons. Therefore, the process of electrons-transfer, such as nitrogen (N) transformation including nitrification and denitrification, is likely to influence dechlorination. However, the effect of N application on the degradation of organic compounds is poorly known. Some studies confirmed that N addition accelerated the degradation of organic contaminants owing to stimulating microbial activity [9–11]. Some studies showed that N addition inhibited the degradation of organic pollutants, because N sources could alter enzymatic systems responsible for the degradation and inhibit specific degrader's ability [12–14]. In addition, some other studies suggested an optimal *C*/*N* ratio for POPs biodegradation, and N sources were commonly added when carbon sources were excessive in the environment [15–17]. However, the effects of different N forms, such as nitrate N (NO_3^--N) or ammonium N (NH_4^+-N), on HCB degradation, and the relationship between N transformation and HCB dechlorination remain unclear.

Under anaerobic condition, methanogenic bacteria are typical anaerobic microbial community and dechlorination is potentially affected by methane (CH_4) production according to previous results, but their findings were conflicting. Some studies showed that methanogenic bacteria were involved in dechlorination process effectively [7,18,19]; while other studies suggested that methanogenic bacteria were not involved in dechlorination [5,20]. Therefore, more studies are necessary to elucidate the influence of CH_4 production on reductive dechlorination.

Paddy rice is the staple food for South-East Asian countries, and in southern China acidic paddy soil is cropped to rice. In addition, food crop serves as the first link of food chain process and can be susceptible to contamination by pollutants in soil [21]. During rice growing season the soil is submerged, with prevalence of anaerobic condition. It is therefore of interest to study how to accelerate HCB anaerobic reductive dechlorination in acidic paddy soil. In this study, batch incubation experiments were conducted to evaluate the effect of different forms and amounts of N fertilizers on HCB dechlorination, so as to estimate the dominant dechlorination

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0304-3894/$ – see front matter.
doi:10.1016/j.jhazmat.2010.03.059

pathway of HCB, and to elucidate the relationship between HCB dechlorination and CH_4 production.

2. Materials and methods

2.1. Chemicals and reagents

Hexachlorobenzene, pentachlorobenzene (PeCB), monochlorobenzene (MCB), all isomers of tetrachlorobenzene (TeCB), trichlorobenzene (TCB) and dichlorobenzene (DCB), purity >99.5%, were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Sodium sulphate (Na_2SO_4, Nanjing Chemical Reagent Co.) was oven-dried at 400 °C for 4 h before use. Silica gel (Nanjing Chemical Reagent Co.) was activated at 130 °C for 18 h, deactivated by 3.3% deionized water, according to EPA Method 3630 [22]. Other chemical reagents were of analytical grade as required.

2.2. Soil preparation

The upper 20 cm arable paddy soils were sampled at Experimental Station of Red Soil Ecology, Chinese Academy of Sciences, located in Yingtan, Jiangxi Province, China (28°15′N, 116°55′E). The dry soil was ground to pass a 2 mm sieve and stored at room temperature for 1 week for the incubation experiment. The main soil properties were: 1.43% organic C, 0.09% total N, 18.63 mg kg^{-1} NO_3^--N, 5.26 mg kg^{-1} NH_4^+-N, 7.62 cmol kg^{-1} CEC, 26% clay, 35% silt, 39% sand, and pH 4.0. The background HCB concentration in soil was 6.24 ng g^{-1}.

The contaminated soil was prepared as follows: 10 mg HCB was dissolved in 100 ml hexane and added to 50 g quartz sand powder (100 mesh), then mixed thoroughly until the solvent evaporated completely. The HCB concentration of contaminated quartz sand powder was 200 μg g^{-1}. Individual portions of 5 g contaminated quartz sand powder and 500 g soil were homogenized thoroughly and then transferred to the incubation flask. The initial concentration of HCB added to each soil sample was 1.98 μg g^{-1}, but the detected initial concentration of HCB by gas chromatograph in each contaminated soil sample was 1.82 μg g^{-1} as a result of HCB recovery during the analysis process.

2.3. Experimental design

The seven treatments conducted with 500 g contaminated soil containing 1.82 μg g^{-1} HCB were: (1) control (no N addition), (2) 0.84 g NH_4HCO_3-N, (3) 0.14 g NH_4HCO_3-N, (4) 0.84 g $CO(NH_2)_2$-N, (5) 0.14 g $CO(NH_2)_2$-N, (6) 0.84 g $NaNO_3$-N, (7) 0.14 g $NaNO_3$-N. All the treatments were replicated three times. We used 0.84 and 0.14 g N per 500 g incubation soils, so as to adjust soil *C*/*N* ratio at 10/1 and 15/1, respectively, considering that the *C*/*N* ratio of soil tested was 16/1. Each N source was added with 277 ml deionized water according to 100% of water holding capacity (WHC) of soil and an excess of 10 ml water to keep soil submerged during the whole incubation time. The incubation flasks were equipped with a gas tight inlet to collect gas samples to monitor CH_4 production. The incubation experiment was in a closed system under N_2 gas in the headspace of flasks, and the flasks were incubated at 25 °C in the dark for 7 weeks. After each week, soils and gas samples were sampled for analysis of chlorobenzene compounds, N contents and CH_4 concentrations.

2.4. Analytical methods

2.4.1. Soil pH, NH_4^+-N and NO_3^--N

Soil pH was measured *in situ* with a pH meter (HANNA, pH 211). The NH_4^+-N and NO_3^--N contents of soil were determined by extracting 5 g soil with 50 ml 2 M KCl, and analyzing soil extracts with the indophenols blue spectrophotometric method and ultraviolet spectrophotometric method, respectively [23,24].

2.4.2. Hexachlorobenzene and its dechlorinated products

Hexachlorobenzene and its dechlorinated products (PeCB, TeCB, TCB, DCB and MCB) in soils were extracted by accelerated solvent extraction (Dionex, ASE 200). Soil samples (5 g) were homogenized with 5 g diatomaceous earth. The extraction conditions were at a temperature of 90 °C and a pressure of 10 MPa. Hexane/acetone (3:1, v/v) was used as extraction solvent. The extracts were concentrated to about 2 ml in a rotary vacuum evaporator at 45 °C, and then cleaned by passing them through solid phase extraction (SPE) cartridges containing 1 g silica gel and 2 g Na_2SO_4, eluted with 15 ml hexane/dichloromethane (9:1, v/v). Finally, the eluate was concentrated to about 2 ml, and then diluted with hexane to 10 ml for further analysis.

The concentrations of HCB and its dechlorinated products were measured by gas chromatograph (Agilent 6890) equipped with a DB-5 capillary column (30 m length × 0.32 mm inside diameter × 0.25 μm film thickness), a ^{63}Ni electron capture detector and an HP 7683 auto-sampler. Nitrogen was used as the carrier gas. Temperature program: 60 °C for 2 min, 5 °C min^{-1} to 190 °C, 20 °C min^{-1} to 280 °C, hold for 7 min. The injector and detector temperature were 240 and 290 °C, respectively. The injection volume was 1 μl in a splitless mode. The method detection limits were as follows: 250 pg μl^{-1} for MCB, 18–30 pg μl^{-1} for DCBs, 6–12 pg μl^{-1} for TCBs, 5 pg μl^{-1} for TeCBs, 1 pg μl^{-1} for PeCB and 0.5 pg μl^{-1} for HCB.

Chlorobenzenes were identified by comparing their retention times with reference substances. Quantification was performed by using linear calibration curves ($r^2 = 0.99$) of each chlorobenzene. Recovery experiments were conducted at concentrations of 500 ng g^{-1} for HCB and 50 ng g^{-1} for the other chlorobenzenes. The recoveries of HCB, PeCB, TeCBs and TCBs were between 82% and 93%, and those of DCBs and MCB were between 66% and 72%.

2.4.3. Methane

Every week, 20 ml gas samples were collected by a gas tight needle which was introduced through the inlet of incubation flasks; then the sample was injected into 18 ml pre-evacuated vials fitted with butyl rubber stoppers before the analysis of CH_4. Methane concentration in the gas sample was measured by Shimadzu (GC-12A) with a FID and a 2-m Porapak Q (80/100 mesh) column. The oven, injector and detector temperatures were 80, 200 and 200 °C, respectively. The carrier gas (N_2) flow rate was 30 ml min^{-1}. Flame gases (H_2 and O_2) were set at 20 and 30 ml min^{-1}, respectively. Methane reference gas (Nanjing Special Gas Factory) was used for calibration. The reference gas was calibrated against standard gas supplied by the National Institute for Agro-Environmental Sciences (Tsukuba, Japan).

2.5. Statistical analysis

In terms of statistics, the algebraic average was used as the final value. Analysis of variance (ANOVA) was performed using Windows-based SPSS 13.0 (significance level at 0.05).

3. Results and discussion

3.1. Soil pH, NH_4^+-N and NO_3^--N concentrations

Soil pH in the treatments with 0.14 and 0.84 g $NaNO_3$-N was lower than in all other treatments (Table 1), especially within the first 3 weeks. It was probably because the additional Na^+ exchanged more H^+ and Al^{3+} ions which were electrostatically adsorbed in the

Table 1
Soil pH, NH_4^+-N and NO_3^--N contents of different treated soils during the incubation.

Treatments		pH				NH_4^+-N (mg kg^{-1})				NO_3^--N (mg kg^{-1})			
		1 week	3 weeks	5 weeks	7 weeks	1 week	3 weeks	5 weeks	7 weeks	1 week	3 weeks	5 weeks	7 weeks
Control		6.0c	6.5c	6.8b	6.6b	82.0ab	120.4b	77.7a	100.0a	15.7a	20.0a	19.4a	18.5a
NH_4HCO_3 (g N)	0.84	6.9f	7.0e	7.0c	7.1d	175.4d	380.6d	449.5d	518.2c	17.2a	15.2a	17.3a	18.0a
	0.14	6.5e	6.6d	6.7b	6.7c	101.5b	183.4c	285.2c	246.7b	17.4a	15.7a	12.4a	15.0a
$CO(NH_2)_2$ (g N)	0.84	6.9f	7.0e	7.2d	7.2e	192.1d	396.7d	473.7d	545.2c	18.8a	16.9a	17.7a	18.9a
	0.14	6.3d	6.6cd	6.8b	6.8c	138.5c	186.5c	149.7b	174.1b	15.2a	19.4a	14.6a	17.2a
$NaNO_3$ (g N)	0.84	4.5a	4.8a	5.1a	5.3a	66.8a	63.9a	69.7a	79.3a	373.6b	345.3b	83.1b	128.1b
	0.14	5.4b	6.2b	6.7b	6.7bc	66.4a	102.0b	85.0a	89.1a	10.1a	14.4a	16.1a	17.5a

All values are means of three replicates. Different lower cases within a column denote the significant difference (LSD test) at $p < 0.05$.

diffuse layer of soil colloid, and increased soil acidity [25]. The application of NH_4HCO_3 or $CO(NH_2)_2$ increased soil pH significantly at the 1st week ($p < 0.05$), and the highest soil pH value was obtained in the treatment with 0.84 g NH_4HCO_3- or $CO(NH_2)_2$-N. It was possibly because the release of HCO_3^-, formed by NH_4HCO_3 or $CO(NH_2)_2$ hydrolysis, could produce OH^-.

Soil NH_4^+-N contents at different sampling times in various treatments are presented in Table 1. The applications of NH_4HCO_3 and $CO(NH_2)_2$ had similar effect on increasing NH_4^+-N concentrations, and the highest NH_4^+-N concentration was observed in the treatment with 0.84 g NH_4HCO_3- or $CO(NH_2)_2$-N. The $NaNO_3$ supply tended to decrease soil NH_4^+-N concentrations, and no significant difference of NH_4^+-N contents was observed between the treatments with 0.14 and 0.84 g $NaNO_3$-N. In control and the treatments with 0.14 g $CO(NH_2)_2$- or $NaNO_3$-N, soil NH_4^+-N concentrations increased markedly within the first 3 weeks, and decreased from the 3rd to 5th week, then increased again from the 5th to 7th week. In the treatment with 0.14 g NH_4HCO_3-N, soil NH_4^+-N concentrations increased dramatically within the first 5 weeks, and then decreased from the 5th to 7th week. In the treatments with 0.84 g NH_4HCO_3- or $CO(NH_2)_2$-N, soil NH_4^+-N concentrations increased during the whole incubation time, but in the treatment with 0.84 g $NaNO_3$-N, soil NH_4^+-N contents decreased slightly within the first 3 weeks and then increased after the 3rd week. In the treatment with 0.84 g $NaNO_3$-N, soil NO_3^--N content was significantly higher than in all other treatments within the 1st week attributed to the hydrolysis of $NaNO_3$, and then soil NO_3^--N concentration decreased dramatically with time due to denitrification under the anaerobic conditions. In the other treatments, denitrification was inapparent during the incubation time.

3.2. Effect of different N compounds and amounts on HCB dechlorination

3.2.1. Hexachlorobenzene

The changes of extractable HCB residues in soils are shown in Fig. 1. During the first 5 weeks, no significant difference of HCB residues was obtained among treatments except that in the treatment with 0.14 g $CO(NH_2)_2$-N was significantly lower than in both treatments with 0.84 g $CO(NH_2)_2$-N and 0.84 g $NaNO_3$-N at the 3rd week. At the 6th week, HCB residues in the treatment with 0.14 g NH_4HCO_3-N was significantly lower than in the other treatments except for 0.14 g $CO(NH_2)_2$-N and 0.84 g NH_4HCO_3-N. At the end of incubation, HCB residues in the two treatments with $NaNO_3$ were higher than in control and the other four treatments, and in the treatments with 0.14 g NH_4HCO_3- or $CO(NH_2)_2$-N were lower than in all other treatments. These results suggested that $NaNO_3$ addition probably inhibited HCB dechlorination, and 0.14 g NH_4HCO_3- or $CO(NH_2)_2$-N application promoted HCB degradation in the soil.

For $NaNO_3$ addition treatments, HCB residues decreased slowly during all 7 weeks. In control, HCB residues decreased by 13.4% of

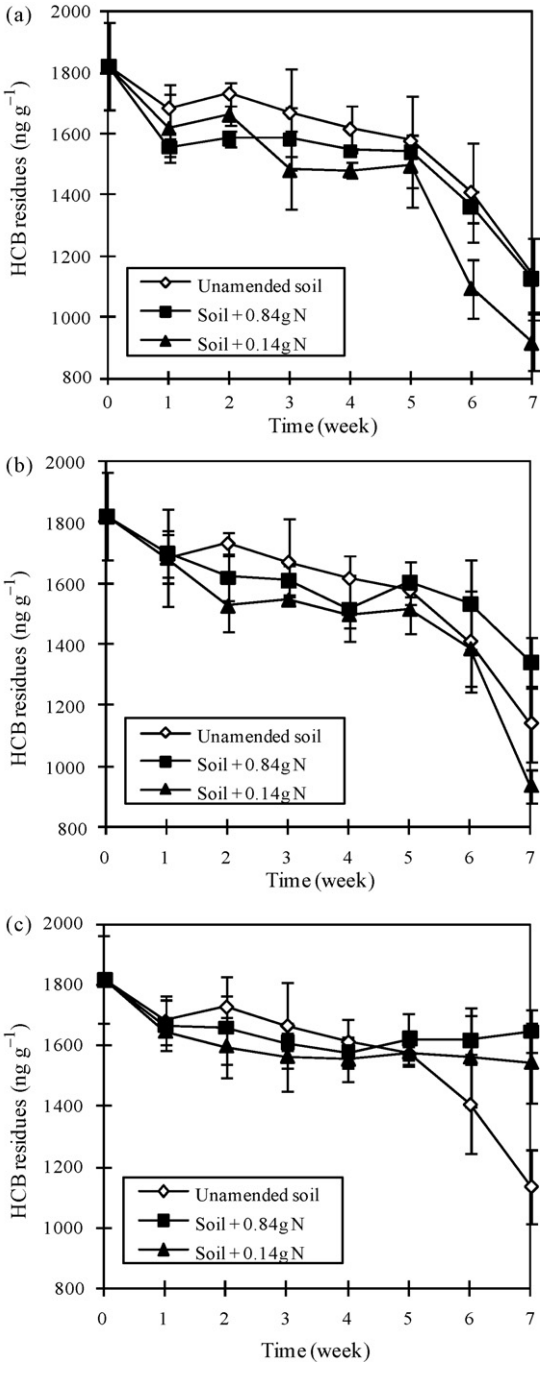

Fig. 1. Time development of extractable HCB residues in soils as affected by different forms and amounts of N fertilizers: (a) NH_4HCO_3, (b) $CO(NH_2)_2$, and (c) $NaNO_3$.

the initial quantity before the 5th week, and then dropped dramatically by 24.1% in the last 2 weeks. In the treatments with 0.14 and 0.84 g NH_4HCO_3-N, HCB residues decreased by 17.9% and 15.5% during the first 5 weeks, and then dropped rapidly by 31.8% and 22.8% in the last 2 weeks, respectively. Similar results were obtained in treatments with 0.14 and 0.84 g $CO(NH_2)_2$-N, HCB residues decreased by 16.8% and 12.0% during the first 5 weeks, and then dropped by 31.8% and 14.4% in the following 2 weeks, respectively. The results indicated that removal of HCB occurred during the incubation period. This removal can be due to HCB dechlorination and probably formation of bound residues whereby these processes exhibited a lag phase. Different acclimation periods, ranging from 2 days to 2 months, for HCB dechlorination were reported [5,7,26,27]. The observed lag time was 5 weeks in this study. It was not only attributed to the fact that the arable soils were artificially contaminated by HCB, thus it took time for the acclimation of native degrading microbial community, but was also due to the fact that native microbial community needs some time to adapt to the reductive conditions after submerging.

3.2.2. Dechlorinated metabolites

The main anaerobic dechlorinated product of HCB observed in this study was PeCB (Fig. 2). The concentrations of PeCB increased slowly during the first 3 weeks, and then increased considerably from the 3rd to 7th week except for the treatment with high amount of $NaNO_3$. After 4 weeks, PeCB concentrations in the treatments with low amount of NH_4HCO_3 or $CO(NH_2)_2$ were higher than in control, and in the treatment with 0.14 g $CO(NH_2)_2$-N was more pronounced. Thus we guessed that $CO(NH_2)_2$ was more effective than NH_4HCO_3 for stimulating degraders to dechlorinate HCB, which needs further study. Whereas PeCB concentrations in the treatments with high amount of NH_4HCO_3 or $CO(NH_2)_2$ were both lower than in control. From Fig. 2a and b, it seems that proper amount of NH_4^+-N application (0.14 g NH_4HCO_3- or $CO(NH_2)_2$-N) accelerated HCB dechlorination, which was attributed to the following two reasonable explanations. Firstly, NH_4^+-N supply can stimulate biodegradation when soil organic carbon is excessive [15,27,28], and the addition of a proper amount of NH_4^+-N probably adjusted soil *C*/*N* ratio to a better level for the degraders' activity than the original *C*/*N* value. Secondly, there were microbes which could involve in anaerobic ammonium oxidation process in paddy soil [29,30], thus the additional NH_4^+-N, as a reductant, might accelerate the reductive dechlorination of HCB. However, high amount of NH_4^+-N supply (0.84 g NH_4HCO_3- or $CO(NH_2)_2$-N) in soils decreased HCB dechlorination rates. It was firstly because high amount of NH_4^+-N supply accelerated reductive decomposition of soil organic carbon and promoted CH_4 production (Fig. 3a and b), and CH_4 production competed for electrons with HCB dechlorination. Secondly, it has been reported that excessive N addition could alter enzymatic systems of soil responsible for the degradation [12,13], and inhibit specific degraders' ability [14]. The application of NO_3^--N decreased PeCB production rates significantly (Fig. 2c). It was firstly because dechlorination mainly occurred in alkalescent [31], and the $NaNO_3$ application resulted in a lower soil pH (Table 1) which inhibited HCB dechlorination. Secondly, 0.84 g $NaNO_3^-$-N supply promoted denitrification process (Table 1), which accepted electrons and competed with reductive dechlorination [7,18]. Thirdly, NO_3^--N supply might alter enzymatic systems responsible for the degradation and inhibit degrader's activity. Our results indicated that N supply had multifunctional effects on dechlorination by influencing dechlorinating microbes, and by serving as electron donor or electron acceptor.

After 5 weeks, 1,2,3,4-TeCB was slightly detected in control and the treatments with NH_4HCO_3 or $CO(NH_2)_2$, and there was low amount of 1,3,5-TCB produced at the last week in control (Table 2). Furthermore, 1,2,3,5-TeCB and 1,3,5-TCB concentrations in soils

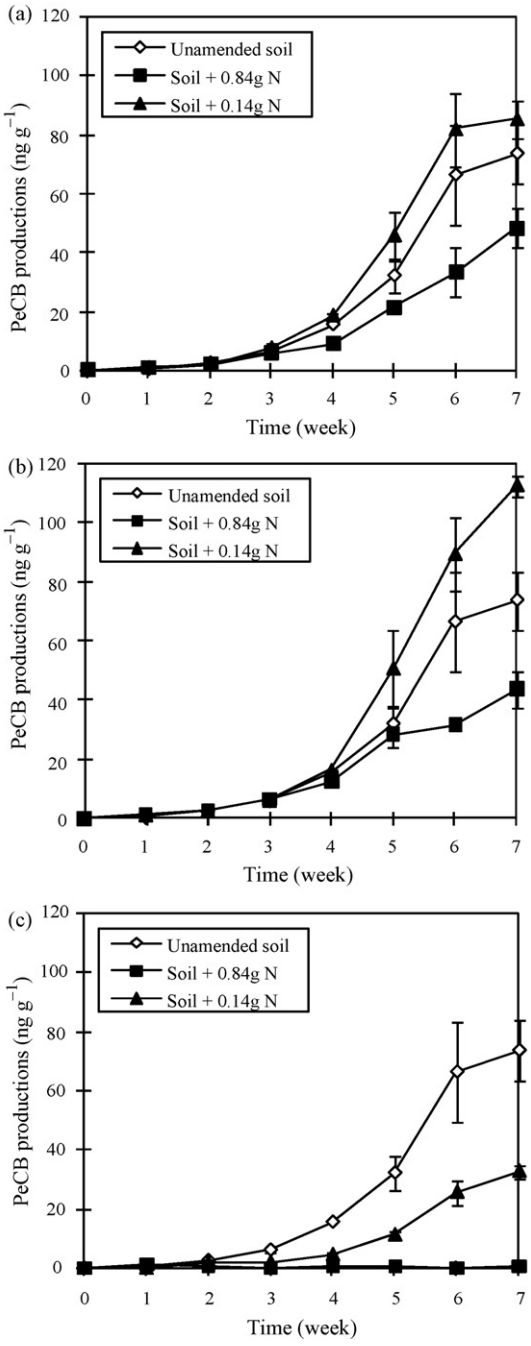

Fig. 2. Time development of PeCB productions in soils as affected by different forms and amounts of N fertilizers: (a) NH_4HCO_3, (b) $CO(NH_2)_2$, and (c) $NaNO_3$.

treated with low amount of NH_4HCO_3 or $CO(NH_2)_2$ increased significantly in the last 2 weeks (Table 2). Because there was no other dechlorinated product except PeCB in the treatments with $NaNO_3$, Table 2 did not present them. These results also proved that the most effective dechlorination was obtained in the treatment with low amount of NH_4HCO_3 or $CO(NH_2)_2$ and dechlorination process was accelerated significantly within the last 2 weeks. It was reported that the volatile HCB and its metabolites from saturated soil with water were negligible [5], thus the main reduction of HCB in soil was degradation or bound residues. Since the sum of dechlorinated products cannot quantitatively explain the disappearance of HCB from soil, it can be concluded from our results that the disappearance of HCB could also be due to the formation of bound residues. The application of 0.14 g NH_4HCO_3-N resulted

Table 2
Detected dechlorinated products (μg kg^{-1}) except for PeCB in soils from the 5th to 7th week.

Treatments		1,2,3,4-TeCB			1,2,3,5-TeCB			1,3,5-TCB		
		5 weeks	6 weeks	7 weeks	5 weeks	6 weeks	7 weeks	5 weeks	6 weeks	7 weeks
Control		1.8 ± 0.6	1.6 ± 0.2	–	–	–	–	–	–	1.7 ± 0.7
NH_4HCO_3 (g N)	0.84	–	4.4 ± 1.4	3.8 ± 2.8	–	–	–	–	–	–
	0.14	–	5.4 ± 3.8	–	–	11.0 ± 7.6	21.3 ± 7.2	–	29.6 ± 0.9	77.4 ± 15.3
$CO(NH_2)_2$ (g N)	0.84	1.1 ± 0.5	–	–	–	–	–	–	–	–
	0.14	1.0 ± 0.5	–	5.6 ± 3.8	–	12.7 ± 8.8	23.7 ± 0.4	–	22.8 ± 5.7	77.2 ± 10.2

All values are means ± standard deviations of triplicate samples. "–" means no dechlorinated product was detected.

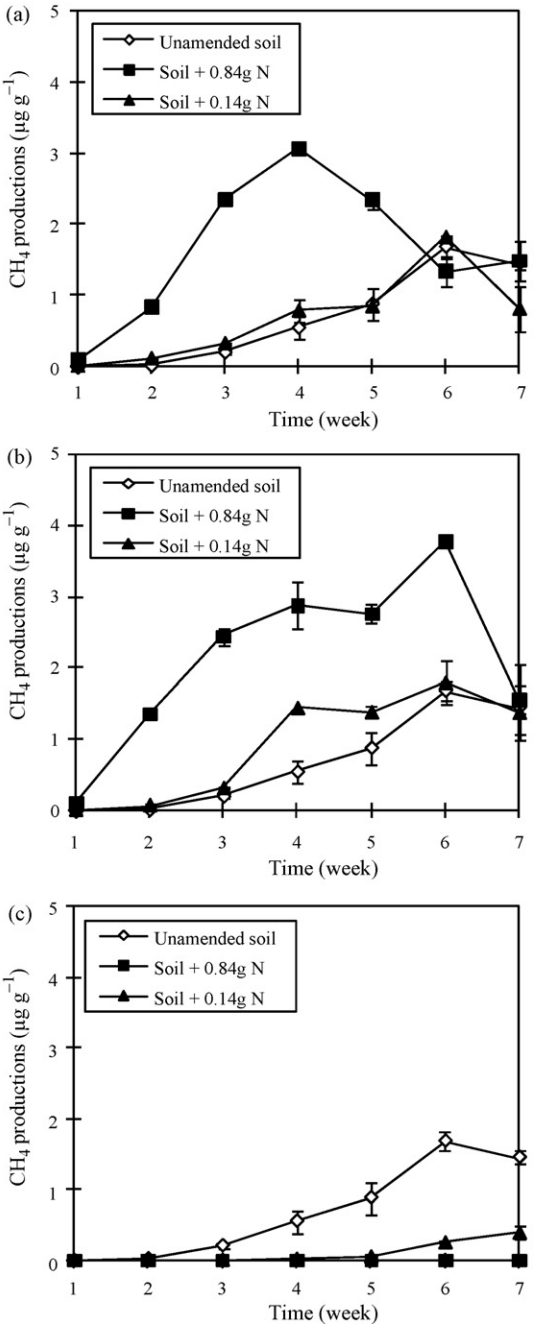

Fig. 3. Time development of methane productions as affected by different forms and amounts of N fertilizers: (a) NH_4HCO_3, (b) $CO(NH_2)_2$, and (c) $NaNO_3$.

in a lower HCB concentration after 7 weeks than 0.14 g $CO(NH_2)_2$-N, but the total concentration of dechlorinated metabolites in the treatment with 0.14 g NH_4HCO_3-N was lower than in the treatment with 0.14 g $CO(NH_2)_2$-N. It indicated that bound HCB residues in the treatment with 0.14 g NH_4HCO_3-N might be higher than in the treatment with 0.14 g $CO(NH_2)_2$-N. Moreover, the results showed that 1,2,3,5-TeCB and 1,3,5-TCB were the main dechlorinated products of PeCB, and the dominant dechlorination pathway of HCB was HCB → PeCB → 1,2,3,5-TeCB → 1,3,5-TCB. This dechlorination pathway occurred because the Cl atoms in *meta*-positions were removed more easily, attributed to their lower values of Gibbs free energy than Cl in other positions (*para*- and *ortho*-) of benzene ring, which confirming what have already been reported [5,7,18,32].

3.3. Methane production and HCB dechlorination

The time courses of CH_4 emission from soils are presented in Fig. 3. In the treatment with 0.84 g NH_4HCO_3-N, CH_4 production increased up to 3.1 μg CH_4 g^{-1} soil at the 4th week, and then decreased to 1.5 μg CH_4 g^{-1} soil at the 7th week. In the treatment with 0.14 g $NaNO_3$-N, CH_4 production only reached 0.1 μg CH_4 g^{-1} soil at the 7th week, and in the treatment with 0.84 g $NaNO_3$-N, CH_4 production was negligible. In the treatment with 0.84 g $CO(NH_2)_2$-N, CH_4 production reached the highest rate of 3.8 μg CH_4 g^{-1} soil at the 6th week, and the other three treatments showed similar time courses. Application of high amounts of $CO(NH_2)_2$ or NH_4HCO_3 increased CH_4 production markedly. It was because the decomposition of $CO(NH_2)_2$ and NH_4HCO_3 in flooded conditions could liberate CO_2, and methanogenic bacteria can easily produce CH_4 from CO_2 reduction [33]. However, $NaNO_3$ supply inhibited CH_4 production. It was firstly because NO_3^--N supply resulted in extended oxidative conditions and inhibited CH_4 production, which has been reported by Banik et al. [34]. Furthermore, the inhibition of CH_4 formation by toxic denitrification intermediates might be a crucial factor according to previous findings [35].

In control and the treatment with 0.14 g NH_4HCO_3-N, both dechlorination and methanogenesis rates increased continuously within the first 6 weeks, and then both methanogenesis and dechlorinated rates decreased within the last week. In addition, in the treatments with 0.14 or 0.84 g $NaNO_3$-N, methanogenesis rates were lower than in control, and HCB dechlorination was also inhibited. These results indicated that methanogenic bacteria could involve in dechlorination process, which agreed with Nowak et al. [26] and Chen et al. [19]. While for the treatment with 0.84 g NH_4HCO_3-N, dechlorination rate increased during all incubation time, but methanogenesis rates increased before the 4th week and decreased from the 4th to 6th week, then increased in the last week. In the treatments with $CO(NH_2)_2$ or $NaNO_3$, dechlorination and methanogenesis rates were also in a different trend. Furthermore, the applications of 0.14 g NH_4HCO_3 or $CO(NH_2)_2$-N accelerated HCB dechlorination, but had no significant effect on CH_4 production. The 0.84 g NH_4HCO_3 or $CO(NH_2)_2$-N supply stimulated CH_4 production but inhibited HCB dechlorination. These results suggested that methanogenic bacteria and HCB dechlorination were not positively

correlated. Furthermore, it indicated that a high quantity of CH_4 production had a negative effect on HCB dechlorination, which was because CH_4 production combined with anaerobic degradation of organic carbon competed for electrons with reductive dechlorination. Therefore, the role of methanogenic bacteria in dechlorination was uncertain and conditions-dependent, which needs further research.

4. Conclusions

In this study, it was proved that HCB could be dechlorinated effectively by the native anaerobic microbial communities in a submerged soil. The process of N transformation accompanied by electrons-transfer influenced reductive dechlorination significantly. The application of NO_3^--N as an electron acceptor had negative effect on HCB dechlorination. However, application of a proper amount of NH_4^+-N stimulated anaerobic microbes to dechlorinate HCB. In contrast, high amount of NH_4^+-N inhibit HCB dechlorination. Therefore, the optimization of HCB dechlorination is possible by applying a proper amount of NH_4^+-N in contaminated soil.

Acknowledgements

This study was financially supported by Knowledge Innovation Program of the Chinese Academy of Sciences (No. KZCX2-YW-404), National Natural Science Foundation of China (Nos. 40921061, 20702028 and 40801117), and Innovative project of Chinese Academy of Sciences (No. ISSASIP0714).

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