Journal of Hazardous Materials 186 (2011) 1438–1444

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Potential for biodegradation of polychlorinated biphenyls (PCBs) by *Sinorhizobium meliloti*

Chen Tu[a,b], Ying Teng[a,b], Yongming Luo[a,b,*], Xiuhua Li[a], Xianghui Sun[a,b], Zhengao Li[a], Wuxing Liu[a], Peter Christie[c]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] Agri-Environment Branch, Agri-Food and Biosciences Institute, Belfast BT9 5PX, UK

ARTICLE INFO

Article history:
Received 20 May 2010
Received in revised form 29 October 2010
Accepted 6 December 2010
Available online 10 December 2010

Keywords:
Biodegradation
Intermediate
Polychlorinated biphenyls
Rhizobia

ABSTRACT

Resting cell assay and soil microcosms were set up to investigate the biodegradation capability and metabolic intermediate of polychlorinated biphenyls (PCBs) by a rhizobial strain *Sinorhizobium meliloti*. Biodegradation was observed immediately after 2,4,4′-TCB was supplied as a sole source of carbon and energy in liquid cultures. After 6 days, the percent biodegradation of 2,4,4′-TCB was 77.4% compared with the control. The main intermediate was identified as 2-hydroxy-6-oxo-6-phenylhex-2,4-dienoic acid (HOPDA) for 2,4,4′-TCB as determined by gas chromatography–mass spectrometry (GC–MS). Inoculation with *S. meliloti* greatly enhanced the degradation of target PCB mixtures in the soil. Moreover, soil culturable bacteria, fungi and biphenyl degrading bacteria counts showed significant increase after inoculation of *S. meliloti*. This study suggests that *S. meliloti* is promising in PCB bioremediation.

1. Introduction

Polychlorinated biphenyls (PCBs) are a family of chlorinated aromatic hydrocarbons comprising 209 different congeners. They represent a serious environmental problem due to their low degradability, high toxicity, and strong bioaccumulation. The microbial degradation of PCBs has been extensively studied in recent years [1–4]. Ahmed and Focht [5] were the first to describe two *Achromobacter* strains capable of degrading several PCB congeners to chlorobenzoic acids. Since then several microorganisms belonging to the genera *Burkholderia*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Norcardia*, *Arthrobacter*, *Acinetobacter*, *Bacillus* and *Corynebacterium* have been isolated that can aerobically degrade PCBs [6,7].

Besides the PCB degraders above, *Rhizobia*, as soil nitrogen fixing bacteria, has now emerged as a promising candidate. As they are naturally exposed to a range of aromatic exudates of roots and thus may prove to possess interesting aromatic catabolic pathways and capabilities [8–11]. Compared to the extensive studies towards their application in agricultural inoculum, relatively few studies have investigated the aromatic degrading function in *Rhizobia*. Damaj and Ahmad [12] reported the capability of PCB degradation by a rhizobial strain *Rhizobium meliloti* Zb57 in resting-cell cultures for the first time. Then the indigenous rhizobial strains were isolated from soils contaminated with PAHs and PCBs [13]. Mehmannavaz et al. [14] investigated the effects of plant–*Rhizobia*–soil interactions on the biotransformation of PCBs in the soil. Furthermore, a genetically modified rhizobial strain (*S. meliloti* USDA 1936), with added genes for PCB degradation in conjunction with the symbiotic plant alfalfa, was proved to enhance PCB degradation [15]. However, little information is available on the metabolic pathway and intermediates of PCBs transformed by *Rhizobia*. Moreover, there is a large data gap surrounding the soil microbial effects on *Rhizobia* inoculation during the bioremediation of PCB-contaminated soil.

The aims of the present study were to investigate the degradation capability of PCBs by a wild-type *Sinorhizobium meliloti* (strain ACCC17519) in both liquid cultures and contaminated soil, to identify the metabolic intermediates of 2,4,4′-TCB by rhizobial resting cells, and to describe the microbial effects of *S. meliloti* bioaugmentation in PCB contaminated soil.

2. Materials and methods

2.1. Bacteria and chemicals

The rhizobial strain used was *S. meliloti* from the Agricultural Culture Collection of China (strain ACCC17519). 2,4,4′-TCB

* Corresponding author at: Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, P.O. Box 821, 71 East Beijing Road, Nanjing 210008, Jiangsu, China. Tel.: +86 25 86881101; fax: +86 25 86881126.
E-mail address: ymluo@issas.ac.cn (Y. Luo).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.12.008

(PCB28, Accustandard, USA) was selected for the resting cell assay as it was one of the widely studied model PCB congeners [16,17]. The vapor pressure, octanol/water partition coefficient, and Henry's law constant of PCB28 are 0.0277 Pa, log K_{ow} 5.5, and 33.11 Pa m^3 mol^{-1}, respectively [18]. PCB-Congener-Mix (including di-CB: PCB8; tri-CB: PCB18, PCB28; tetra-CB: PCB44, PCB 52, PCB66, PCB77; penta-CB: PCB101, PCB105, PCB118, PCB126; hexa-CB: PCB128, PCB138, PCB153; hepta-CB: PCB170, PCB180, PCB187; octa-CB: PCB195, PCB200; nona-CB: PCB206; and deca-CB: PCB209) ranged from lower chlorobiphenyls to higher chlorobiphenyls was used for soil microcosm experiment. Numbers in the PCB congeners indicate different quantity and position of chlorine atoms in the biphenyl backbone according to the International Union of Pure and Applied Chemistry (IUPAC). n-Hexane was purchased from Tedia (USA). All other chemicals were analytically pure obtained from China.

2.2. Resting cell assay

Resting cell assay was performed according to Ahn et al. [19] with modification. The bacteria were grown individually in 100 mL of sterile pH 7.0 yeast mannitol broth medium at 28 °C for 2 days. Cells were harvested by centrifugation at 8000 rpm for 5 min, washed twice with sterile pH 7.0 sodium phosphate buffer (PBS), and resuspended in the same buffer to an O.D. of 1.0 at 600 nm. 500 μL of 2,4,4′-TCB stock solution (50 mg kg^{-1} in acetone) was added to sterile 50 mL screw-cap glass vials prior to 2 mL of *S. meliloti* suspension culture. Cells inactivated by autoclaving at 121 °C for 20 min were used as control. To avoid volatilization and photodegradation of PCB28, vials in triplicate were closed with aluminum foil lined caps, and incubated at 28 °C on a rotary shaker at 200 rpm for 6 days in dark. Biodegradation was assayed by measuring 2,4,4′-TCB disappearance in resting cell cultures. Percent biodegradation of 2,4,4′-TCB was calculated by the equation:

$$\text{Percent biodegradation}\,(\%) = \frac{\text{PCB}_{\text{Control}} - \text{PCB}_{S.meliloti}}{\text{PCB}_{\text{Control}}} \times 100 \qquad (1)$$

2.3. Soil microcosms

Microcosm experiments were designed to investigate the potential of *S. meliloti* in bioremediation of PCB contaminated soil. PCB-free soil sample was collected from the suburban of Nanjing city. Soil samples were thoroughly homogenized and sieved with a 2 mm sieve. Two hundred grams (dry weight) of the soil was added to a previously sterilized pot. The soil was artificially contaminated with PCB-Congener-Mix (containing 21 PCB congeners) to a total concentration of 400 μg kg^{-1} dry weight soil (2 mL of 40 mg L^{-1} PCB acetone stock was added by syringe while the soil was continuously mixed) and the water content was adjusted to 45% of the soil water holding capacity (WHC). The soil microcosms were allowed to equilibrate for 3 months before inoculating with 10% or 20% (v:w) of *S. meliloti* suspension culture (3×10^8 cfu mL^{-1}). The inocula were prepared as per the resting cell assay. The control soil microcosms were inoculated only with sterilized culture medium. All treatments were run in triplicate. Soil samples were collected for PCB analysis on Days 0, 5, 10, 20 and 30, respectively.

2.4. PCB extraction

Following incubation of the resting cell assays, each vial was stopped by the addition of 100 μL perchloric acid. Three milliliters of hexane was added to the cell suspension for the extraction of 2,4,4′-TCB, with 0.4 g ammonium sulfate as an emulsion-breaker. Samples were shaken vigorously for 10 min on a vortex, followed by a 1-h standing. The phases were allowed to separate and the hexane phase was analyzed by gas chromatography (GC).

For extraction of PCBs from soil microcosms, a modified method of Gao et al. [20] was used. Briefly, 10 g soil sample was thoroughly extracted with 30 mL hexane/acetone (1:1, v/v) overnight, sonicated at 600 W, 25 °C for 15 min with a Model KQ-600B sonicator (Kunshan Ultrasonic Instruments). Extraction and ultrasonication were repeated twice using 20 mL of the same solvent mixture each time. The extracts were combined and collected in 120 mL glass vials. Extract volumes were condensed to 5 mL using a rotary evaporator. Each extract was then transferred to a 25 cm × 1 cm purification column filled with silicon gel, Al_2O_3, acidic silicon gel and Na_2SO_4 powder in sequence. The column was eluted with 25 mL of n-hexane, the eluate was concentrated and dissolved in 5 mL of hexane before GC analysis.

2.5. Analysis of PCBs by gas chromatography (GC)

A Varian 3800 gas chromatography, equipped with an electron capture detector (ECD) and a Varian CP-Sil 24CB column was utilized. The injector and detector temperatures were 260 °C and 300 °C, respectively. The column was initially held at 180 °C for 0.5 min, then ramped at 30 °C min^{-1} to 260 °C, held for 18 min, then 10 °C min^{-1} to 270 °C and held for 2 min. PCB congeners were identified by matching the retention time to the standard and were quantified using peak area integration. All samples were analyzed in triplicate. Certified standards were used to prepare standard curves for gas chromatography. Concentrations of PCBs were quantified by an external standard. The recoveries of PCBs from spiked samples were in the range of 76.3–98.5%. The detection limits ranged from 1.33 to 3.45 μg kg^{-1}, with a linear range from 0.5 to 50 ng mL^{-1}. The coefficient of determination was 0.9995.

2.6. Analysis of PCB intermediates by gas chromatography–mass spectrometry (GC–MS) and UV–visible scanning spectrophotometer

Following PCB extraction from the resting cell assay, after the hexane phase containing 2,4,4′-TCB was extracted and analyzed by GC, the aqueous phase comprising PCB intermediates was acidified to pH 2.0 with 3 M HCl and extracted twice with ethyl acetate. The ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated by N_2 flow to dryness. The extracts were dissolved in 1 mL hexane and incubated with 100 μL bis (trimethylsilyl) trifluoroacetamide (BSTFA) for derivatization at 60 °C for 20 min prior to analysis by GC–MS.

GC–MS was performed on an Agilent 7890A GC system linked 5975C mass selective detector and a DB-5MS column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 80 °C with a 2.0-min hold, and a 8 °C min^{-1} increment to 160 °C, then 4 °C min^{-1} to 270 °C with a 25-min hold. The injector, ion source, and detector temperatures were 250 °C, 250 °C, and 270 °C, respectively. Helium was used as carrier gas, with a linear rate of 1.0 mL min^{-1}. A 70-eV electron impact mode was also selected, with a mass scan scope from 45 to 550 amu. One microlitre of the sample was injected.

Quantitative detection for yellow intermediate HOPDA (2-hydroxy-6-oxo-6-phenylhex-2,4-dienoic acid) was determined by measuring A_{430} of the culture on a UV–visible scanning spectrophotometer (T6, PGENERAL, China) [21].

2.7. Enumeration of microorganisms in soil microcosms

Culturable soil bacteria and fungi were counted at the end of the experiment using plate count techniques [22]. Ten grams of soil

samples were diluted in a 500 mL flask containing 100 mL sterile water and sterile glass beads. The flask was then shaken on a rotary shaker at 200 rpm for 10 min to make microbes desorb from soil particles and distribute evenly throughout the extractions. Ten-fold serial dilutions from 10^{-1} to 10^{-9} were performed for plate count with nutrient broth agar (NBA) for bacteria and potato dextrose agar (PDA) for fungi. Biphenyl degrading bacteria were counted in 96-well microplates with three replicates per dilution using a modified most probable number (MPN) method [23]. The biphenyl stock solution (0.4 g mL^{-1}) used as sole carbon source was aliquoted (10 μL) to each well and the acetone was allowed to evaporate prior to the addition of 72 μL Bushnell Haas (BH) mineral salts medium (per liter: 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 1.0 g KH_2PO_4, 1.0 g $(NH_4)_2HPO_4$, 1.0 g KNO_3, 0.05 g $FeCl_3 \cdot 6H_2O$; pH 7.0). Each plate was inoculated with 10^{-1} to 10^{-7} serial dilutions (8 μL per well, one dilution per row) of soil extracts. All plates were incubated in the dark at 28 °C. After two weeks, 20 μL of filter-sterilized p-iodonitrotetrazolium violet (3 g L^{-1}) was added to each well and the microplates were incubated overnight. Positive wells as indicated by colour development were counted, and the MPN was calculated according to the published MPN tables.

2.8. Statistical analysis

Statistical analysis was carried out using the SPSS 14.0 software package. The chemical and microbiological data were analyzed by one-way ANOVA. Treatment means were compared using Duncan's multiple range test at the 5% level.

3. Results

3.1. 2,4,4′-TCB biodegradation by S. meliloti resting cells

The degradation dynamics of 2,4,4′-TCB by *S. meliloti* in liquid culture is presented in Fig. 1. After 6 days of incubation, the concentration of 2,4,4′-TCB decreased in both treatments. However, significant difference was observed between *S. meliloti* treatment (0.20 ± 0.02 mg L^{-1}) and the autoclaved control (0.91 ± 0.03 mg L^{-1}, $p < 0.05$). Calculated percent biodegradation of 2,4,4′-TCB by *S. meliloti* was also described in Fig. 1. The percentage of 2,4,4′-TCB biodegradation received 34.6%, 52.4%, and 77.4% on Day 1, Day 3, and Day 6, respectively. Loss of 2,4,4′-TCB in the control was possibly caused by non-biological processes such as volatilization and photodegradation during the extracting process due to the volatile physicochemical property of 2,4,4′-TCB.

3.2. Identification of metabolic intermediates

The presence of yellowish brown colour in the organic extract of culture does indicate the presence of metabolic products and was confirmed by GC–MS. A novel peak was produced in the *S. meliloti* resting cell treatment as shown in Fig. 2A and B. The mass spectrum for peak 1 of Fig. 2B showed a molecular-ion peak at *m*/*z* 217 and other peaks at *m*/*z* 210 and 195 (Fig. 2C). Ion fragment *m*/*z* 120 and 75 corresponded to hypnone and benzene rings, respectively, which were the fractions of the intermediate attacked by the ion source in MS. On the basis of mass spectrum comparison, the intermediate was identified as 2-hydroxy-6-oxo-6-phenylhex-2,4-dienoic acid (HOPDA, *m*/*z* 218). The kinetics of degradation of 2,4,4′-TCB and the production of yellow metabolite HOPDA were investigated simultaneously. In the first day, HOPDA accumulated with approximately the same rate as the decrease of 2,4,4′-TCB and turned up to the maximum level. Then the degradation of HOPDA started slowly (Fig. 2D).

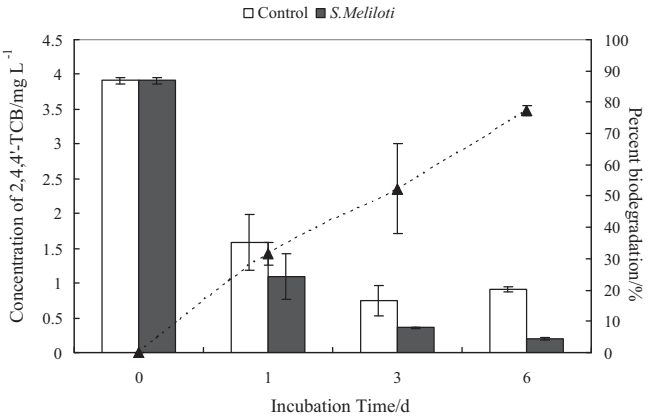

Fig. 1. Time course of 2,4,4′-TCB concentration and percent biodegradation in *S. meliloti* 1021 resting cells.

3.3. Bioremediation of PCB-contaminated soil by S. meliloti

Total concentrations of 21 PCB-Congener-Mix in the soil microcosms are presented in Table 1. After 30 days of bioremediation, total PCB concentrations across all treatments ranged from 126.7 to 198.0 μg kg^{-1} dry soil. Inoculation with *S. meliloti* significantly reduced soil PCB concentrations compared with the uninoculated control ($p < 0.05$). A remarkable enhancement in total PCB degradation was also observed between 20% and 10% inocula treatments, except for the data from Day 30.

Percent biodegradation of single congener from the 21 PCB mixtures was calculated for each treatment. Inoculation of *S. meliloti* significantly increased the percent biodegradation of all the 21 PCB congeners at the end of the experiment (Fig. 3). In the uninoculated control, the percent biodegradation of 21 PCB congeners varied from 7.8% to 100%, with only 5 of them got a percentage higher than 50%. While in the *S. meliloti* inocula treatments, 14 of the 21 PCB congeners received more than 50% degradation. Moreover, *S. meliloti* inoculation was proved to shorten the time for PCB 126 and PCB 200 depletion from 20 days to less than 10 days.

Table 1
Total concentration of 21 PCB-Congener-Mix in soil microcosms under different treatments.

Times (days)	Control		10% inocula		20% inocula	
	Concentration (μg kg^{-1})	Biodegradation (%)	Concentration (μg kg^{-1})	Biodegradation (%)	Concentration (μg kg^{-1})	Biodegradation (%)
Initial	335.9 ± 9.1	0	335.9 ± 9.1	0	335.9 ± 9.1	0
0	328.1 ± 9.1	2.5 ± 2.3b	317.8 ± 9.9	5.4 ± 3.0b	296.4 ± 11.6	11.8 ± 3.5a
5	293.9 ± 1.8	12.5 ± 0.5c	270.0 ± 2.8	19.6 ± 0.8b	246.5 ± 5.2	26.6 ± 1.5a
10	238.2 ± 4.6	29.1 ± 1.4c	182.5 ± 3.8	45.7 ± 1.1b	156.0 ± 7.3	53.5 ± 2.2a
20	206.9 ± 3.3	38.4 ± 1.0c	178.4 ± 1.5	46.9 ± 0.4b	146.0 ± 4.4	56.5 ± 1.3a
30	198.0 ± 17.6	41.1 ± 5.2b	135.1 ± 14.3	59.8 ± 4.3a	126.7 ± 3.9	62.3 ± 1.2a

Data are based on the sum of 21 congener concentrations, and are presented as the mean ± SD. Means in the same row with the same letter are not significantly different ($p > 0.05$).

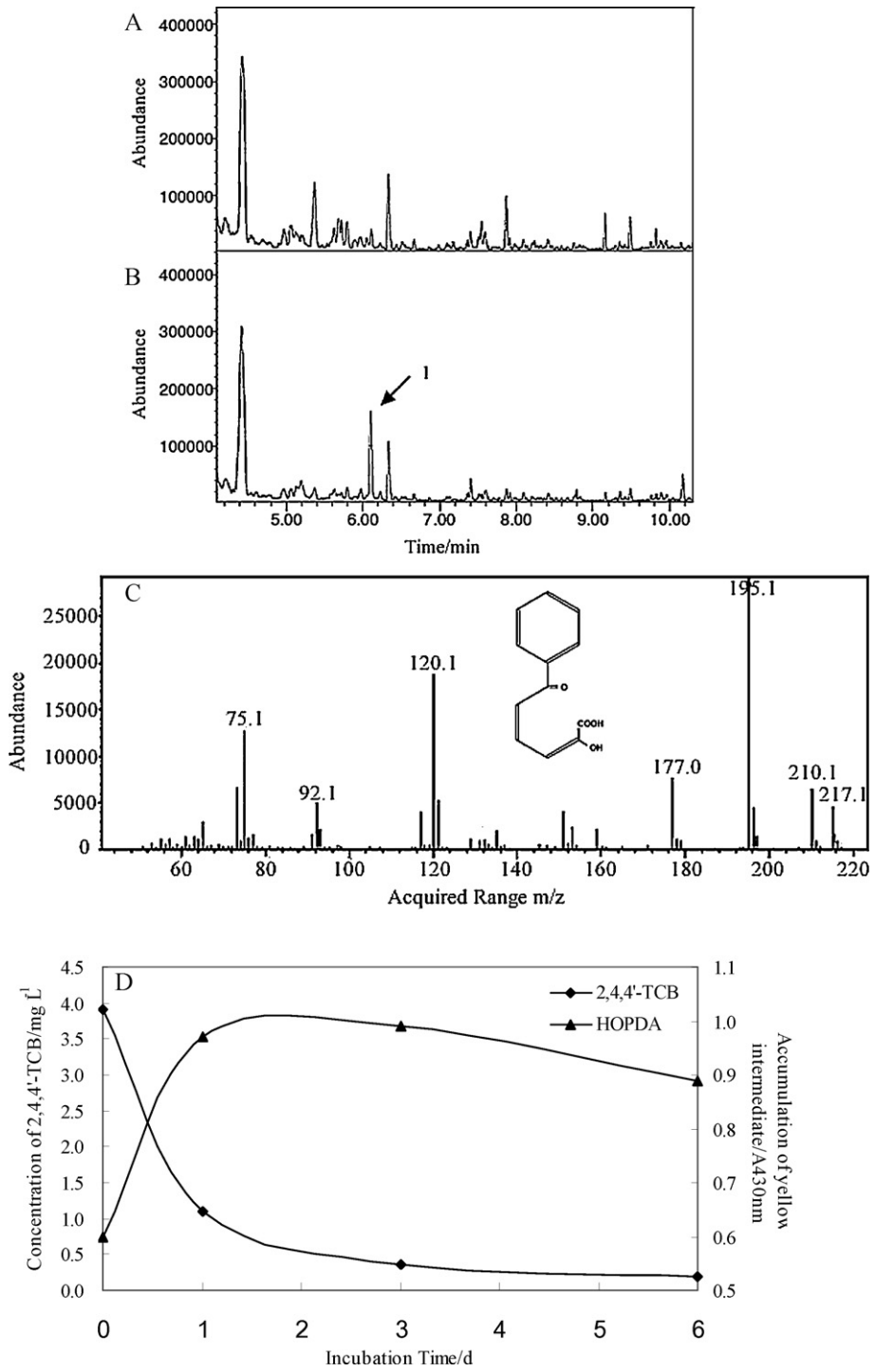

Fig. 2. Identification of the main intermediate from 2,4,4′-TCB by *S. meliloti* 1021 resting cells (A) GC of the control reaction. (B) GC of the products after 6 days of incubation. (C) Mass spectrum of peak 1 in (B). (D) Mass balance of 2,4,4′-TCB and yellow intermediate (HOPDA).

3.4. Effect of S. meliloti inoculation on soil microorganisms

Soil culturable bacteria, fungal and biphenyl-degrading bacterial counts from different treatments are presented in Table 2. Counts of culturable bacteria, fungi and biphenyl degrading bacteria in the soil were increased from all the inoculated treatments than from the uninoculated control. Moreover, the bacterial counts in 20% inocula were significantly higher than in corresponding 10% inocula treatment ($p < 0.05$) but there was no significant differences in counts of fungi or biphenyl degrading bacteria between the two inocula treatments.

Table 2
Soil culturable bacterial, fungal and biphenyl-degrading bacterial counts.

Soil microbial counts	Control	10% inocula	20% inocula
Bacteria (10^7 cfu g^{-1})	0.7 ± 0.6b	2.7 ± 0.6b	7.3 ± 3.1a
Fungi (10^6 cfu g^{-1})	0.4 ± 0.1b	1.4 ± 0.3a	1.5 ± 0.3a
BD Bacteria (10^5 MPN g^{-1})	0.4 ± 0.1b	1.8 ± 0.4a	2.5 ± 0.7a

Data are presented as mean ± SD. Means in the same row with the same letter are not significantly different ($p > 0.05$).

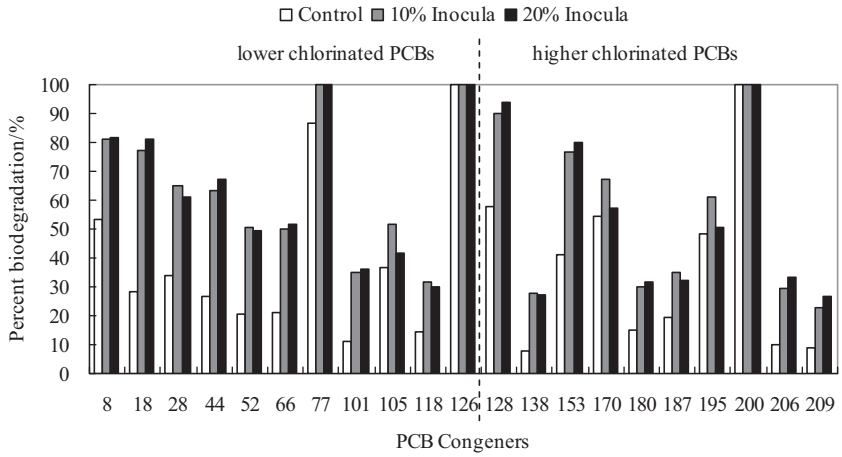

Fig. 3. Mean percent biodegradation of PCB congeners in the soil under different treatments.

4. Discussion

Rhizobia, by definition and classification, are soil N_2-fixation bacteria associated symbiotically with plant roots and the rhizosphere. The hallmark of rhizobial studies has always been focused on their agricultural significance, e.g. in competition and survival as inocula, in N_2-fixation and nodulation, in cross-talk between microbe and plant symbiont [13]. Expecting *Rhizobia* to inhabit environments with aromatic pollutants is not surprising since they have long been known to metabolize simple aromatic compounds like flavones and other phenolic root exudates [9–11], and to possess ring-cleavage enzymes such as catechol 1,2-dioxygenase and protocatechuate 3,4- or 4,5-dioxygenase [8,9,24,25]. Recent studies suggest that free-living *Rhizobia* can also tolerate and biotransform soil contaminants, such as PCBs, PAHs, TNT, atrazine and halobenzoates [13,15,26–28].

In the present study, the potential role of PCB biodegradation by *S. meliloti* strain ACCC17519 was evaluated both in liquid cultures and soil microcosms. Results from the resting cell assay of this study and our published work [29] showed that *S. meliloti* could take 2,4,4′-TCB (PCB 28) as a sole carbon and energy source, without using biphenyl as the co-metabolic substrate. *S. meliloti* transformed 2,4,4′-TCB immediately upon entering the culture vials. At the end of the 6-day incubation, more than 70% of 2,4,4′-TCB had disappeared (Fig. 1), which was more efficient than other rhizobial strains ever reported [12]. Results from the soil microcosms also indicated *S. meliloti* playing an important role in the bioremediation of PCB-contaminated soils. Regardless of the initial amount of *S. meliloti* inocula, soil PCB concentrations in both two inoculation treatments declined significantly over the whole incubation period when compared with the uninoculated control (Table 1). Moreover, 20% inocula of *S. meliloti* does show a significant increase in the biodegradation of soil PCBs than the 10% inocula treatment. Furthermore, *S. meliloti* also harbors a broad specificity towards a series of PCB homologous. Inoculation with *S. meliloti* could significantly enhance the depletion of all the 21 PCB congeners, especially for less chlorinated biphenyls (Fig. 3). Among the 11 lower chlorinated PCB congeners, 6 of them (PCB18, 44, 52, 66, 101, and 118) received more than 2-fold increase in the PCB degradation percentage after inoculation, while the ratio in higher chlorinated PCBs was 4/10. This result was consistent with our previous study that *Rhizobia* inoculation could enhance the depletion of the lower chlorinated biphenyls [30].

PCBs are subject to both aerobic and anaerobic metabolism by microorganisms [4,16]. Under the aerobic conditions, lower chlorinated PCBs can be co-metabolized through 2,3-dioxygenase encoded by *bph* gene operon in a well known PCB degrader *Pseudomonas pseudoalcaligenes* KF707 [31]. Briefly, PCB is oxidized by biphenyl-2,3-dioxygenase (encoded by *bphA* gene) leading to the formation of the corresponding *cis*-dihydriol. The *cis*-dihydriol is subsequently oxidized by a dehydrogenase (encoded by *bphB* gene) to a 2,3-dihydroxy intermediate. Then the 2,3-dihydroxybiphenyl is oxidized by an oxygenase (encoded by *bphC* gene) to the *meta* cleavage product, HOPDA, which is hydrolysed by a hydrolase (encoded by *bphD* gene) to the corresponding benzoic acid [3,4].

In this study, HOPDA was identified as the principal intermediate by GC–MS during biotransformation of 2,4,4′-TCB by *S. meliloti* (Fig. 2). As intermediates are always unstable and would be further degraded in a few hours or days by microbes in the culture, no commercial HOPDA could be purchased as standards except for chemical synthesis from few laboratories in the world. Consequently, there is no model MS pattern of HOPDA in the GC library. The comparison of MS spectrum profile for HOPDA in this study with the one reported by Catelani et al. [32] showed slightly differences in the ion peaks. These differences may be due to the MS conditions. Mass balance with the initial PCB28 and HOPDA (Fig. 2D) confirmed our inference. However, further identification of the 2,4,4′-TCB intermediate by *S. meliloti* is needed via MS–MS or nuclear magnetic resonance (NMR) analysis.

Studies concerning the metabolic pathway of PCBs by rhizobial strain are few in number. Damaj and Ahmad [12] used total cellular DNA dot-blots to determine whether the BP/PCB degradation system in rhizobial strains shares genetic homology with the well known biphenyl dioxygenase or the catechol 2,3-dioxygenase systems. All four rhizobial strains including *R. meliloti* hybridized strongly with *C. testosteroni* derived *bphABC* gene probe, as well as the *xylE* gene from the toluene degradation plasmid *pTOL*, which indicating a high degree of homology. Recently, as breakthroughs have been made in genomic DNA sequencing technology, the genome of *S. meliloti* strain 1021 is publicly available on the internet (NCBI Reference Sequence: NC_003047.1). Several dioxygenases such as catechol 1,2-dioxygenases, protocatechuate 3,4-dioxygenases and some putative dioxygenase sequences have been annotated. However, there is no clear evidence that biphenyl dioxygenase *bphABC* gene cluster was listed in the strain 1021 genome. It is possible that the rhizobial DNA hybridizes with the *bphABC* probe is not involved in PCB degradation at all, but instead functions in some other capacity, and the PCB degradation observed is a secondary or unrelated phenomenon. Alternatively, other annotated or putative dioxygenase genes inherently bestowed in the rhizobial genome may be important for the utilization of PCBs as well as certain aromatic plant compounds [13]. In this study, it is

interesting that no residual chlorine was detected in the intermediate of 2,4,4′-TCB metabolite, HOPDA, indicating a dechlorinating process was occurred during the PCB biotransformation. This phenomenon was further confirmed by free-chlorine releasing assay (our unpublished data). Bioinformatical analysis suggests that there is putative dehalogenase located in the *S. meliloti* 1021 genomic DNA. Therefore, PCB degradation observed in *S. meliloti* strain of this study is probably a cumulative result of more than one pathway present, or a completely different pathway. Further studies on enzymes and molecular physiology should be taken to clarify the PCB metabolic pathway in *S. meliloti*.

Bioremediation of PCB contaminated soil can be performed by native organisms or exogenous organisms that are added to supplement the native population (i.e., bioaugmentation). The rationale underlying bioaugmentation is simple: the augmentation of catabolically relevant organisms to hasten remediation [33]. However, exotic catabolic bacterial inocula may have some negative effects on indigenous microorganism community [34], which limit their application as a bioremediation strategy. *Rhizobia* are ubiquitous soil N_2-fixation bacteria that have been widely used in agricultural inocula. In the present study, *S. meliloti* inoculated to the soil microcosm could significantly enhance the depletion of soil PCB congeners. Explanations to this result could be varied. *S. meliloti* inoculation may degrade soil PCBs directly, or stimulate the proliferation of soil indigenous microbes especially for biphenyl-degrading bacteria, which may play an important role in the soil PCB removal. The microbial effects of *S. meliloti* inoculation were evaluated by enumeration of soil culturable bacteria, fungal and biphenyl-degrading bacteria counts at the end of the bioremediation. Soil culturable bacteria, fungal and biphenyl-degrading bacteria counts showed a significantly increase in *S. meliloti* inoculated microcosms than in the uninoculated control (Table 2). This may be due to the change of soil econiche after inoculation. Our previous work showed that the inoculation of *Rhizobia* could increase the numbers of biphenyl-degrading bacteria (BDB), such as *Pseudomonas* sp., *Burkholderia* sp., *Bordetella* sp., and other uncultured bacteria [35]. The increased BDB could degrade or transform more PCBs from the soil and decrease the soil ecotoxicity, which contribute to the increased numbers of soil bacteria and fungi. Thus, *S. meliloti* may enhance the degradation of soil PCBs in a more complex and synergetic way.

In conclusion, *S. meliloti* plays an important role in the biodegradation of PCBs in liquid cultures and soil. The biotransformation product of 2,4,4′-TCB by *S. meliloti* was 2-hydroxy-6-oxo-6-phenylhex-2,4-dienoic acid (HOPDA). Inoculation with *S. meliloti* may greatly increase the counts of soil culturable biphenyl-degrading microbes. However, further studies are needed to investigate the metabolic pathway of PCB degradation by *S. meliloti* and the genes that encoding the key enzymes in the pathway. These results may provide evidence for the potential application of *Rhizobia* in bioremediation of PCB-contaminated soil.

Acknowledgements

This research was funded by grants from the National Natural Science Foundation of China (Projects 40921061 and 40701080), the Intellectual Innovation Program of the Chinese Academy of Sciences (Project KZCX2-YW-404), the National Agricultural Department Public Benefit Research Foundation (Project 200803034), and the K.C. Wong Education Foundation (Hong Kong).

References

[1] D.A. Abramowicz, Aerobic PCB biodegradation and anaerobic PCB dechlorination in the environment, Res. Microbiol. 145 (1994) 42–46.
[2] M.R. Natarajan, W.M. Wu, R. Sanford, M.K. Jain, Degradation of biphenyl by methanogenic microbial consortium, Biotechnol. Lett. 21 (1999) 741–745.
[3] D.H. Pieper, Aerobic degradation of polychlorinated biphenyls, Appl. Microbiol. Biotechnol. 67 (2005) 170–191.
[4] J.A. Field, R. Sierra-Alvarez, Microbial transformation and degradation of polychlorinated biphenyls, Environ. Pollut. 155 (2008) 1–12.
[5] M. Ahmed, D.D. Focht, Degradation of polychlorinated biphenyls by two species of *Achromobacter*, Can. J. Microbiol. 19 (1973) 47–52.
[6] M. Seto, M. Ida, N. Okita, T. Hatta, E. Masai, M. Fukuda, Catabolic potential of multiple PCB transformation systems in *Rhodococcus* sp. strain RHA1, Biotechnol. Lett. 18 (1996) 1305–1308.
[7] M. Sakai, S. Ezaki, N. Suzuki, R. Kurane, Isolation and characterization of a novel polychlorinated biphenyl-degrading bacterium, *Paenibacillus* sp. KBC101, Appl. Microbiol. Biotechnol. 68 (2005) 111–116.
[8] Y.P. Chen, A.R. Glenn, M.J. Dilworth, Aromatic metabolism in Rhizobium trifolii-catechol 1,2-dioxygenase, Arch. Microbiol. 141 (1985) 225–228.
[9] Y.P. Chen, C.R. Lovell, Purification, Properties of catechol 1,2-dioxygenase from Rhizobium leguminosarum biovar viceae USDA 2370, Appl. Environ. Microbiol. 56 (1990) 1971–1973.
[10] D. Tepfer, A. Goldmann, N. Pamboukdjian, M. Maille, A. Lepingle, D. Chevalier, J. Denarie, C. Rosenberg, A plasmid of *Rhizobium meliloti* 41 encodes catabolism of two compounds from root exudate of Calystegium sepium, J. Bacteriol. 170 (1988) 1153–1161.
[11] J.R. Rao, J.E. Cooper, *Rhizobia* catabolize nod gene-inducing flavonoids via C-ring fission mechanisms, J. Bacteriol. 176 (1994) 5409–5413.
[12] M. Damaj, D. Ahmad, Biodegradation of polychlorinated biphenyls by rhizobia: a novel finding, Biochem. Biophys. Res. Commun. 218 (1996) 908–915.
[13] D. Ahmad, R. Mehmannavaz, M. Damaj, Isolation and characterization of symbiotic N_2-fixing *Rhizobium meliloti* from soils contaminated with aromatic and chloroaromatic hydrocarbons: PAHs and PCBs, Int. Biodeterior. Biodegrad. 39 (1997) 33–43.
[14] R. Mehmannavaz, S.O. Prasher, D. Ahmad, Rhizospheric effects of alfalfa on biotransformation of polychlorinated biphenyls in a contaminated soil augmented with *Sinorhizobium meliloti*, Process Biochem. 37 (2002) 955–963.
[15] Y. Chen, A. Adam, O. Toure, S.K. Dutta, Molecular evidence of genetic modification of *Sinorhizobium meliloti*: enhanced PCB bioremediation, J. Ind. Microbiol. Biotechnol. 32 (2005) 561–566.
[16] J. Borja, D.M. Taleon, J. Auresenia, S. Gallardo, Polychlorinated biphenyls and their biodegradation, Process Biochem. 40 (2005) 1999–2013.
[17] M. Komancova, I. Jurcova, L. Kochankova, J. Burkhard, Metabolic pathways of polychlorinated biphenyls degradation by *Pseudomonas* sp. 2, Chemosphere 50 (2003) 537–543.
[18] D. Mackay, W.Y. Shiu, K.C. Ma, S.C. Lee, Hand Book of Physical–Chemical Properties and Environmental Fate for Organic Chemicals, CRC Press, Boca Raton, U.S.A., 2006.
[19] Y.B. Ahn, L.A. Beaudette, H. Lee, J.T. Trevors, Survival of a GFP-labeled polychlorinated biphenyl degrading psychrotolerant *Pseudomonas* spp. in 4 and 22 C soil microcosms, Microb. Ecol. 42 (2001) 614–623.
[20] J. Gao, Y.M. Luo, Q.B. Li, H.B. Zhang, L.H. Wu, J. Song, W. Qian, P. Christie, S.M. Chen, Distribution patterns of polychlorinated biphenyls in soils collected from Zhejiang Province, East China, Environ. Geochem. Health 28 (2006) 79–87.
[21] Q. Hong, X.J. Dong, L.J. He, X. Jiang, S.P. Li, Isolation of a biphenyl-degrading bacterium, *Achromobacter* sp. BP3, and cloning of the bph gene cluster, Int. Biodeterior. Biodegrad. 63 (2009) 365–370.
[22] Z.G. LI, Y.M. Luo, Y. Teng, Research Methods of Soil and Environmental Microbiology, Science Press, Beijing, China, 2008 (in Chinese).
[23] B.A. Wrenn, A.D. Venosa, Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure, Can. J. Microbiol. 42 (1996) 252–258.
[24] Y.P. Chen, C.R. Lovell, Purification and properties of a homodimeric protocatechuate 4,5-dioxygenase from Rhizobium leguminosarum, Arch. Microbiol. 161 (1994) 191–195.
[25] Y.P. Chen, M.J. Dilworth, A.R. Glenn, Aromatic metabolism in Rhizobium trifolii-protocatechuate 3,4-dioxygenase, Arch. Microbiol. 138 (1984) 187–190.
[26] C. Bouquard, J. Ouazzani, J. Prome, Y. Michel-Briand, P. Plesiat, Dechlorination of atrazine by a *Rhizobium* sp. isolate, Appl. Environ. Microbiol. 63 (1997) 862–866.
[27] M. Labidi, D. Ahmad, A. Halasz, J. Hawari, Biotransformation and partial mineralization of the explosive 2,4,6-trinitrotoluene (TNT) by rhizobia, Can. J. Microbiol. 47 (2001) 559–566.
[28] B. Song, N.J. Palleroni, M.M. Haggblom, Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments, Appl. Environ. Microbiol. 66 (2000) 3446–3453.
[29] L. Xu, Y. Teng, Y.M. Luo, Z.G. Li, Effects of *Rhizobium meliloti* on PCBs degradation and transformation in solution culture, Environ. Sci. 31 (2010) 255–259 (in Chinese).
[30] Y. Teng, Y.M. Luo, X.H. Sun, C. Tu, X. Li, W.X. Liu, Z.G. Li, P. Christie, Influence of arbuscular mycorrhiza and rhizobium on phytoremediation by alfalfa of an agricultural soil contaminated with weathered PCBs: a field study, Int. J. Phytoremed. 12 (2010) 516–533.
[31] K. Taira, J. Hirose, S. Hayashida, K. Furukawa, Analysis of bph operon from the Polychlorinated biphenyl-degrading strain of *Pseudomonas pseudoalcaligenes* KF707, J. Biol. Chem. 7 (1992) 4844–4853.

[32] D. Catelani, A. Colombi, C. Sorlini, V. Treccani, Metabolism of biphenyl. 2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate: the meta-cleavage product from 2,3-dihydroxybiphenyl by *Pseudomonas putida*, Biochem. J. 134 (1973) 1063–1066.
[33] I.P. Thompson, C.J. van der Gast, L. Ciric, A.C. Singer, Bioaugmentation for bioremediation: the challenge of strain selection, Environ. Microbiol. 7 (2005) 909–915.
[34] B.E. Andersson, K. Tornberg, T. Henrysson, S. Olsson, Three-dimensional outgrowth of a wood-rotting fungus added to a contaminated soil from a former gasworks site, Bioresour. Technol. 78 (2001) 37–45.
[35] L. Xu, Y. Teng, Z.G. Li, J.M. Norton, Y.M. Luo, Enhanced removal of polychlorinated biphenyls from alfalfa rhizosphere soil in a field study: the impact of a rhizobial inoculum, Sci. Total Environ. 408 (2010) 1007–1013.