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Rapid degradation of 2,4-dichlorophenoxyacetic acid facilitated by acetate under methanogenic condition

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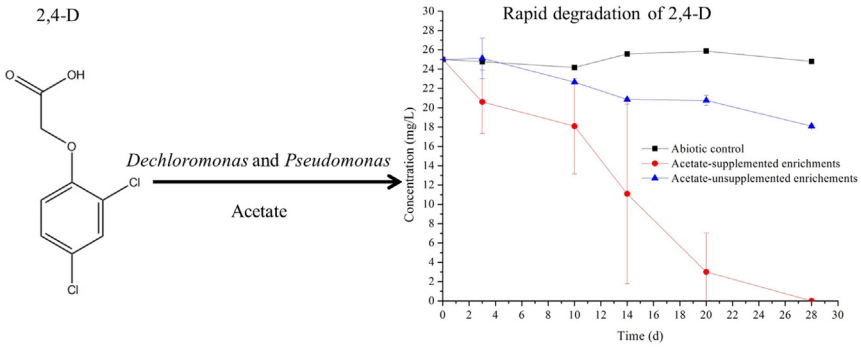
HIGHLIGHTS

- The acetate addition facilitated 2,4-D degradation by the enrichments.
- The addition of acetate and 2,4-D altered the bacterial community composition.
- *Dechloromonas* and *Pseudomonas* were the dominant 2,4-D degraders.

GRAPHICAL ABSTRACT



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ABSTRACT

Acetate can be used as an electron donor to stimulate 2,4-dichlorophenoxyacetic acid (2,4-D), which has not been determined under methanogenic condition. This study applied high-throughput sequencing and methanogenic inhibition approaches to investigate the 2,4-D degradation process using the enrichments obtained from paddy soil. Acetate addition significantly promoted 2,4-D degradation, which was 5-fold higher than in the acetate-unsupplemented enrichments in terms of the 2,4-D degradation rate constant. *Dechloromonas* and *Pseudomonas* were the dominant 2,4-D degraders. Methanogenic inhibition experiments indicated that the 2,4-D degradation was independent of methanogenesis. It was proposed that the accelerated 2,4-D degradation in the acetate-supplemented enrichment involved an unusual interaction, where members of the acetate oxidizers primarily oxidized acetate and produced H_2. H_2 was utilized by the 2,4-D degraders to degrade 2,4-D, but also partially consumed by the hydrogenotrophic methanogens to produce methane. The findings presented here provide a new strategy for the remediation of 2,4-D-polluted soils.

1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) is globally used in modern agriculture to control weeds and increase agricultural productivity. The intensive and continuous use of 2,4-D has resulted in its continuous discharge into the environment. 2,4-D has been frequently detected in surface and ground water as well as soil, posing the potential threats to the environment ecosystem and human health (Celis et al., 2008; Wang et al., 2009). Thus, it is imperative to develop strategies that eliminate the 2,4-D residues in the environment. Biodegradation has been regarded as a promising and cost-effective strategy for complete removal of 2,4-D (Greer and Shelton, 1992).

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Anaerobic biodegradation is among the key routes of 2,4-D removal from the soils and sediments (Wang et al., 2009). It is known that the 2,4-D removal takes place by reductive dehalogenation processes under anaerobic conditions. This process is implemented by anaerobic bacteria via co-metabolism or dehalorespiration (Boyle et al., 1999; Wang et al., 2009), which was regulated by the availability of organic substrates that acted as electron donors (Robles-González et al., 2006). This highlighted the importance of organic matter in the soils. However, previous studies showed that the high-organic-matter soils disabled the rapid degradation of 2,4-D, most likely due to a tenacious binding of 2,4-D to the organic matter that resulted in decreased availability to microorganisms (Greer and Shelton, 1992). Previous studies have shown that the addition of carbohydrate can promote the 2,4-D removal in the sequencing batch reactors (SBRs) and soil slurry reactors (Celis et al., 2008; Robles-González et al., 2006). These findings indicated the importance of adding carbon sources to the soils. It was observed that volatile fatty acids (VFAs) were produced and then degraded in anaerobic SBR amended with 2,4-D and glucose (Celis et al., 2008), indicating that the produced individual VFAs might make a contribution to the 2,4-D degradation. Several works have shown that the acetate addition can accelerate the reductive dehalogenation of 2,4-dichlorophenol (2,4-DCP) (Warner et al., 2002) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Gibson and Suflita, 1990). The use of H_2 and acetate as electron donors to promote the 2,4-D dechlorination rate has also been well documented under the sulfate-reducing conditions (Boyle et al., 1999). Thus, in the light of this cited work, the 2,4-D removal might be facilitated in the presence of an electron donor (e.g. acetate) under the condition of methane production.

However, it still is unclear whether the addition of an electron donor (e.g. acetate) in soil has also a stimulatory effect on the 2,4-D degradation during methanogenesis and what the relationship of 2,4-D removal with electron donors and methane production is. To answer these questions, the paddy soil enrichments obtained in the presence of acetate and 2,4-D were firstly established. Using the enrichments, the metabolites, 2,4-D removal, and microbial community structure were subsequently investigated. The mechanism for 2,4-D removal was then determined via methanogenic inhibition experiments.

2. Materials and methods

2.1. Incubation conditions

Paddy soil samples were collected from a rice field (Hainan, China). Total solids (TS) content of the soil samples was 51% (w/w), 2.3% of TS were volatile solids (VS). All batch experiments were conducted at 30 °C in 60 mL of bottles with a working volume of 30 mL in the presence of 25 mg/L of 2,4-D. Each test was performed in duplicate under an anaerobic condition. The major components of medium (pH 7.0) contained a final concentration of 25 mg/L of 2,4-D. The other components of medium were prepared as described previously (Yang et al., 2016a).

In test 1, acetate was tested as an electron donor, where 0.05 g-VS/L of soils were used. The experimental treatments included acetate (10 mM)-supplemented and unsupplemented bottles as well as an abiotic control (medium). The resulted enrichments were used to carry out subsequent tests for the 2,4-D degradation.

Test 2 was performed to investigate the effect of methanogenic inhibitor 2-bromoethane sulfonate (BES) on the 2,4-D degradation. The obtained acetate-supplemented enrichment was inoculated (15%, v/v) to fresh medium and tested under the following conditions: (1) 0 mM acetate; (2) 10 mM acetate; and (3) 10 mM acetate and 100 mM BES. The obtained acetate-unsupplemented enrichments were inoculated (15%, v/v) to fresh medium free of acetate.

Test 3 was conducted to investigate the effect of chloroform and/or H_2 on 2,4-D degradation. The obtained acetate-supplemented enrichment (15%, v/v) was used under the following conditions: (1) 10 mM acetate and 0.1% (v/v) chloroform; (2) 10 mM acetate, 0.1% chloroform and 10% (v/v) H_2; (3) 10 mM acetate, 100 mM BES and 10% H_2; (4) 10% H_2; and (5) 10 mM acetate. The parallel incubation free of 2,4-D (named as CK) was carried out with 10 mM acetate as a control.

2.2. Analytical methods

Methane, hydrogen, TS, VS and acetate were determined as described previously (Yang et al., 2016a). 2,4-D and its metabolites in samples were determined with high performance liquid chromatography (HPLC, Waters 1525) equipped with UV detection (226 nm) and ZORBAX SB-C18 column (Agilent). 0.05% H_3PO_4 (A) and 80% methanol (B) were used as the mobile phase at a flow rate of 1 mL/min at 30 °C. A linear gradient was performed as follows: 10 min, 20% A; 1 min, 70% A; 9 min, 20% A.

Solid phase extraction (SPE) of the samples was performed using a SPE Vacuum Manifold (Supelco, Bellefontem, PA, USA) equipped with 500 mg of ENVI™-18 (reversed phase packing made of a silica-based gel). Briefly, conditioning was performed with methanol and Milli-Q water prior to the extraction. 2 mL of sample was then added into the SPE cartridge which was subsequently eluted with methanol.

The 2,4-D and its metabolites were determined using a gas chromatography-mass spectrometry (GC–MS, Agilent 7890A-5975C) equipped with an electrospray ionization (ESI) source and a HP-INNOWAX Polyethylene Glycol column. Helium gas was used as the carrier gas at a flow rate of 1.0542 mL/min. Programmed column temperatures were performed as follows: 50 °C held for 2 min; 10 °C/min to 250 °C, held for 10 min. Injection volume was set to 1 μL. The mass spectrometer was operated in EI mode at 230 °C with a Quadrupole temperature of 150 °C. Identification and analysis of compounds were carried out with the help of a list database.

The 2,4-D degradation rate constants were calculated using an apparent first order reaction equation:

$$\ln Ct = -kt + \ln C0$$

where C0 represents the initial concentration of 2,4-D, Ct represents the concentration of 2,4-D at time t, k represents the degradation rate constant (d^{-1}) and t represents the cultivation time (d).

The genomic DNA extraction and quantification, PCR amplification, construction of amplicon library targeted for V3-V5 region of 16S rDNA were carried out as described previously (Fu et al., 2016). Illumina sequencing was carried out at GENEWIZ, Inc. (Suzhou, China). Sequence analysis and taxonomic classifications were conducted as previously described (Yang et al., 2016b).

3. Results and discussion

3.1. 2,4-D degradation

The profiles of methane production and 2,4-D degradation are shown in Figs. 1 and S1. As shown in Fig. 1, the results from the abiotic control showed that 2,4-D was stable in the medium. When acetate was added to the bottles, the 2,4-D degradation in the acetate-supplemented enrichments were nearly completely degraded, which was 2.6-fold higher than that in the acetate-unsupplemented enrichments at the end of incubation. In addition, the 2,4-D degradation rate constant of 0.067 d^{-1} in the