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Augmenting atrazine and hexachlorobenzene degradation under different soil redox conditions in a bioelectrochemistry system and an analysis of the relevant microorganisms

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ABSTRACT

Soil microbial fuel cells (MFCs) are a sustainable technology that degrades organic pollutants while generating electricity. However, there have been no detailed studies of the mechanisms of pollutant degradation in soil MFCs. In this study, the effects of external resistance and electrode effectiveness on atrazine and hexachlorobenzene (HCB) degradation were evaluated, the performance of soil MFCs in the degradation of these pollutants under different soil redox conditions was assessed, and the associated microorganisms in the anode were investigated. With an external resistance of 20 Ω, the degradation efficiencies of atrazine and HCB were 95% and 78%, respectively. The degradation efficiency, degradation rate increased with decreasing external resistance, while the half-life decreased. There were different degradation trends for different pollutants under different soil redox conditions. The fastest degradation rate of atrazine was in the upper MFC section (aerobic), whereas that of HCB was in the lower MFC section (anaerobic). The results showed that electrode effectiveness played a significant role in pollution degradation. In addition, the microbial community analysis demonstrated that *Proteobacteria,* especially *Deltaproteobacteria* involved in current generation was extremely abundant (27.49%) on soil MFC anodes, although the percentage abundances of atrazine degrading *Rhodocyclaceae* (8.77%), *Desulfitobacterium* (0.64%), and HCB degrading *Desulfuromonas* (0.73%), were considerably lower. The results of the study suggested that soil MFCs can enhance the degradation of atrazine and HCB, and bioelectrochemical reduction was the main mechanism for the pollutants degradation.

1. Introduction

Pesticides are an important pollution source in agricultural environments. Generally, pesticides persist in soil, with long degradation half-lives (Accinelli et al., 2001; Adrian et al., 2000). Most studies of pesticide degradation in soil have focused on surface soil (Brejda and Waller, 1988; Topp et al., 1994; Winkelmann and Klaine, 1991). Nevertheless, the results obtained from surface and subsurface soil are significantly different (Chung et al., 1995b; Ro and Chung, 1995). Pesticides can infiltrate aquifers from surface soil, with rainfall making the removal of pollutants more difficult.

Atrazine and hexachlorobenzene (HCB) are two commonly used pesticides. Atrazine (6-chloro-N-ethyl-N′-isopropyl-1,3,5-triazine-2,4-diamine) is a moderately persistent chemical in agricultural soils, with a half-life ranging from a few days to years (Accinelli et al., 2001; Albright et al., 2013; Chan-Cupul et al., 2016) depending on the soil properties, environmental factors, and microorganism species. The degradation of atrazine in the environment involves three processes: dechlorination, dealkylation, and deamination (Albright et al., 2013; Kumar and Singh, 2016). Many studies have considered atrazine degradation under aerobic conditions (Issa and Wood, 1999; Ro and Chung, 1995) and in surface soil (Miller et al., 1997; Sde and Hofman, 2000). However, the degradation rate of atrazine is very slow in subsurface soil, while there is a modest transformation of atrazine under anaerobic conditions (Accinelli et al., 2001; Chung et al., 1995a; Delaune et al., 1997).

The production of HCB has been banned in the United States and Europe; however, it is an important byproduct or intermediate material during the manufacture of some pesticides (Bailey, 2001). Due to its physico-chemical properties, and to its persistence in the environment, HCB can be transported over long distances and is bioaccumulated. Degradation of HCB in the environment takes place under anaerobic conditions (Adrian et al., 2000; Beurskens et al., 1992). A reductive dechlorination reaction is the main transformation process under anaerobic conditions (Chang et al., 1998; Nowak et al., 1996). In this reaction, the higher chlorinated benzenes are reductively dechlorinated

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to lower chlorinated benzenes, while a chlorine is replaced by a hydrogen. Chlorobenzene is present as an electron acceptor, and hydrogen as an electron donor, in the process of reductive dechlorination under anaerobic conditions (Adrian et al., 2000).

The anaerobic degradation of organic pollutants is an important natural process, with many microorganisms capable of degrading a variety of organic compounds under anoxic conditions. However, some organic pollutants persist in waterlogged soil or sediment because of a lack of suitable electron acceptors (Carmona et al., 2009; Foght, 2008; Reddy et al., 2002). Both atrazine and HCB can be degraded under anaerobic conditions and via dechlorination. Soil microbial fuel cells (MFCs) are devices that enables organic matter (e.g., soil organic matter, acetate) to be oxidized to electrical energy at the anode and reduce electron acceptance at the cathode (Dunaj et al., 2012; Wang et al., 2016). There are favorable anaerobic conditions at the anode and oxygen-deficient or aerobic conditions at the cathode. These conditions meet the requirements for atrazine and HCB degradation under aerobic or anaerobic conditions. Although organic pollution can be degraded by soil MFCs (Cao et al., 2015; Huang et al., 2011), no detailed studies have been conducted to determine whether cathode effectiveness and different soil redox conditions have an influence on the degradation of pollutants.

There have been few investigations of the atrazine and HCB degradation and transformation rates in subsurface soil. Despite several published studies of atrazine and HCB degradation, little is known about the characteristics of their degradation by soil MFCs. In this study, the atrazine and HCB degradation rate was analyzed under different external resistances and soil redox conditions in soil MFCs. It was considered whether the mixture of pollutants (atrazine + HCB) affected these metabolic products and the relationship between degradation rate and current was evaluated. Furthermore, the influence of electrodes on the degradation of organic pollutants was investigated and the microorganisms in the anode were identified.

2. Materials and methods

2.1. Soil MFC construction

Atrazine (purity > 97%) and HCB (purity > 98%) were obtained from Energy Chemicals (China) and AccuStandard (USA), respectively. All organic solvents were HPLC-grade or pesticide analysis grade.

A schematic diagram of the soil MFC device and soil properties were presented in a previous study (Fig. S1, SI) (Cao et al., 2015; Wang et al., 2016). Each MFC in this soil contained contaminated soil that was saturated with nutrient medium (Klass, 1998). The atrazine or HCB was dissolved in acetone solution and sonicated for 5 min until dissolution was complete. Fifty-milliliters of a pesticide-acetone solution was added to the soil and stirred thoroughly to create a homogeneous mixture. The soil/acetone/pesticide mixing process was conducted in a fume cupboard under aseptic conditions to avoid the microbial contamination of soil and to allow for complete evaporation of acetone. The final concentrations of atrazine and HCB were 100 mg/kg for both pollutants. The experiment was performed in an incubator at 30 ± 1 °C in the dark and was conducted in triplicate using three soil MFCs (Chan-Cupul et al., 2016; Yuan et al., 2007).

2.2. Batch experiments

A series of batch experiments were performed to determine the degradation of atrazine in the soil MFCs at five different external resistances and with an open-circuit as a control. The soil in the MFCs was only contaminated with atrazine. Triple replicates for each treatment and the control were then constructed, denoted as R1 (20 Ω), R2 (300 Ω), R3 (620 Ω), R4 (1000 Ω), R5 (2000 Ω), and CK (open-circuit), and installed in the soil MFCs. Another experiment involving atrazine degradation was conducted on the pollution mixture (atrazine and HCB) in the soil MFCs for the 20 Ω treatments (R1′). Samples were taken to evaluate the progress of pesticide degradation every 7 days.

The degradation of HCB and HCB in a pollution mixture (atrazine and HCB) was evaluated in soil MFCs. The external resistance was 20 Ω in these experiments. An open-circuit with soil containing the pollutant mixture was established as a control. There were three treatments, marked as R1-HCB, R1′-HCB, and CK', and three replicates for each treatment and the control. Samples were taken to evaluate the progress of pesticide degradation every 7 days.

To analyze atrazine and HCB degradation under different soil redox conditions, the soil MFC was divided into three sections: upper, middle, and lower (Fig. S1). All of the soil samples were frozen to dryness to avoid loss by evaporation. These samples were sieved and stored at 4 °C prior to use.

2.3. Extraction and analysis

2.3.1. Atrazine

Atrazine was extracted from soil (1 g) by adding 5 ml acetone, 5 ml *n*-hexane (pesticide analysis grade), and anhydrous sodium sulfate (0.5 g), and shaking for 24 h in the dark at room temperature. The soils were then placed in an ultrasonic bath for 30 min. The tubes were centrifuged for 3 min (6000 rpm) at 4 °C and the supernatants were further filtered through a 0.45 μm ultra-filtration membrane (Generay Biotech (Shanghai) Co., Ltd., China). The extracts were transferred to a 10 ml graduated test tube with a stopper, blown with nitrogen to near dryness, and diluted with hexane to 1 ml for analysis.

The atrazine concentration was measured by gas chromatography-mass spectrometry (GC-MS) (Thermo Fisher Scientific Co., Ltd., USA), with a DB-5 quartz capillary column (30 mm × 0.25 mm × 0.25 μm). The carrier gas was high purity helium, with a flow rate of 1 ml/min. The injector and detector temperatures were 240 and 300 °C, respectively. The initial temperature was set at 40 °C and held for 3 min, increased by 30 °C/min to 190 °C and maintained for 5 min, and then increased again by 30 °C/min to 250 °C, where it was maintained for the final 5 min. The injection volume was 1 μL in the split mode (10:1). The flow rates of carrier gas, hydrogen, and air were 1.0, 3.0, and 60.0 ml/min. The extraction efficiency of this method was 95–105%, and this value was taken into account in the final quantification. The metabolites of atrazine were analyzed according to Papadakis and Papadopoulou-Mourkidou (Vanderheyden et al., 1997).

2.3.2. Hexachlorobenzene

On each sample day, a 1 g sample of soil containing HCB was added to 10 ml *n*-hexane and 0.5 g of anhydrous sodium sulfate. The samples were taken in the same way as the atrazine samples. The quantification and identification of HCB was conducted using GC-MS. The analysis of HCB was conducted using methods described by Cao et al. (2015) and Wang et al. (2017).

2.4. Microbial community analysis

The microbial community structures of the anode in soil MFCs were analyzed using high throughput pyrosequencing and a clone library. The DNA was extracted from the anode biomass in the soil MFCs at the end of the experiment. The V4–V5 region of the bacteria 16S ribosomal RNA gene was amplified by polymerase chain reaction (PCR) (95 °C for 5 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 5 min) using primers 515F 5′-GTGCCAGCMGCCGCGG-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′, where the barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate with a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.